THE EFFECT OF HIGH-INTENSITY INTERVAL EXERCISE ON GLUCOSE TOLERANCE AND INSULIN SENSITIVITY IN HEALTHY AND DIABETIC YOUTH

Submitted by Emma Joanne Cockcroft, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Sport and Health Sciences, January 2017.

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(Signature) …………………………………………………………………………………………………………………………

1
Abstract

Cardiovascular disease (CVD) and type two diabetes mellitus (T2D) are among the leading causes of death worldwide. Insulin resistance (IR) and hyperglycaemia are risk factors for CVD and T2D and are known to be prevalent in youth. Physical activity (PA) is known to improve IR and glucose tolerance in youth, but current levels of PA are low meaning alternative PA recommendations are needed. The purpose of this thesis is to investigate the effect of low volume high-intensity interval exercise (HIIE) on insulin and glucose health outcomes in male children and adolescents. Additionally, the thesis will explore the potential for HIIE to improve glycaemic control in paediatric patients with type one diabetes mellitus (T1D). Chapter 4 examines the relationship between estimates of insulin sensitivity (IS) based on oral glucose tolerance test (OGTT) and fasted assessment methods, in addition to the day-to-day reliability of these measures in children and adolescents. Results from this chapter advocated the Cederholm index to measure IS in this sample due to the low day to day reliability (coefficient of variation (%CV) of 6.4%). Chapter 5 demonstrates comparable results, reporting moderate improvements to IS and glucose tolerance measured via an OGTT 10 minutes after a single bout of HIIE and work-matched moderate-intensity exercise (MIE) in adolescent boys (13-15 y old). The findings from Chapter 5 are extended in Chapter 6, where changes to OGTT derived IS and glucose tolerance were measured up to 24 h post exercise and fasting measures of IS up to 48 h after exercise. Improvements to IS and glucose tolerance after the OGTT persisted for up to 24 h after HIIE and MIE, but no changes to fasting outcomes were observed over the 48 h period. In contrast to Chapter 5, Chapter 7 reports that a single bout of HIIE but not work-matched MIE resulted in only a small improvement in IS in 8-10 year old boys.
Chapter 8 assesses the efficacy of 6 sessions of HIIE performed over 2 weeks to alter fasting and postprandial (mixed-meal tolerance test) insulin and glucose outcomes in adolescent boys. In contrast to acute exercise (Chapters 5 and 6), HIIE training over 2 weeks did not improve insulin and glucose outcomes in this population. Finally, Chapter 9 presents a case study on three adolescents with T1D to examine the effect of acute HIIE and MIE on glycaemic control. This study indicates that both MIE and HIIE have the potential to improve short-term (24 h) glycaemic control within this clinical population. Taken collectively, the studies from this thesis demonstrate that HIIE offers an effectual and feasible alternative to MIE to improve insulin and glucose health outcomes in healthy children and adolescents, and short-term glycaemic control in adolescents with T1D.
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Publications and conference presentations

Publications

Accepted


Conference presentations

Oral presentations


Poster presentations


# Table of contents

Abstract .................................................................................................................. 2  
Acknowledgements ................................................................................................. 4  
Publications and conference presentations ................................................................ 6  
Table of contents ....................................................................................................... 9  
List of tables ........................................................................................................... 11  
List of figures ........................................................................................................... 13  
List of equations ...................................................................................................... 18  
Glossary of terms .................................................................................................... 19  
1. Introduction .......................................................................................................... 21  
2. Literature review .................................................................................................. 26  
   2.1. Glucose regulation .......................................................................................... 26  
   2.2. Glucose transport ......................................................................................... 30  
   2.3. Glucose dysregulation ................................................................................... 31  
   2.4. Insulin resistance, glucose tolerance and disease development ................. 34  
   2.5. A paediatric problem? .................................................................................. 38  
   2.6. Assessment of insulin resistance .................................................................. 41  
   2.7. Factors affecting insulin resistance in youth ................................................. 47  
   2.8. Physical activity, insulin resistance and glucose tolerance in youth ............. 51  
   2.9. Exercise training studies ............................................................................... 61  
   2.10. Acute exercise, insulin sensitivity and glucose tolerance ......................... 70  
   2.11. Acute exercise and postprandial fat oxidation ............................................ 75  
   2.12. Type one diabetes and cardiovascular risk .................................................. 75  
   2.13. Summary and experimental aims ................................................................. 80  

3. General methods ..................................................................................................... 84  
   3.1. Participant recruitment and inclusion / exclusion criteria ............................. 84  
   3.2. Standardisation of testing conditions ............................................................ 85  
   3.3. Anthropometry .............................................................................................. 87  
   3.4. Pubertal status .............................................................................................. 88  
   3.5. Maximal oxygen uptake and gas exchange threshold .................................... 89  
   3.6. Exercise protocols ....................................................................................... 90  
   3.7. Resting metabolic rate ................................................................................. 93  


3.8. Oral glucose tolerance test and mixed meal tolerance tests .................... 94
3.9. Blood sampling and analyses .................................................................. 96
3.10. Continuous glucose monitoring ............................................................. 101
3.11. Sample size calculation .......................................................................... 103
3.12. Statistical analyses ................................................................................ 103

4. Agreement and reliability of fasted and oral glucose tolerance test derived indices of insulin sensitivity and beta cell function in boys ....................... 105
4.1. Abstract ................................................................................................. 106
4.2. Introduction ........................................................................................... 106
4.3. Methods ................................................................................................. 108
4.4. Results .................................................................................................. 112
4.5. Discussion ............................................................................................. 118
4.6. Conclusion ............................................................................................. 123

5. High-intensity interval exercise is an effective alternative to moderate-intensity exercise for improving glucose tolerance and insulin sensitivity in adolescent boys ......................................................... 124
5.1. Abstract ................................................................................................. 125
5.2. Introduction ........................................................................................... 125
5.3. Methods ................................................................................................. 127
5.4. Results .................................................................................................. 132
5.5. Discussion ............................................................................................. 137
5.6. Conclusions ........................................................................................... 141

6. Acute exercise and insulin sensitivity in boys: a time course study .......... 142
6.1. Abstract ................................................................................................. 143
6.2. Introduction ........................................................................................... 144
6.3. Materials and methods .......................................................................... 146
6.4. Results .................................................................................................. 152
6.5. Discussion ............................................................................................. 159
6.6. Practical implications ............................................................................. 163
6.7. Conclusion ............................................................................................. 164
7. A single bout of high-intensity interval exercise and work-matched moderate intensity exercise has minimal effect on glucose tolerance and insulin sensitivity in 7-10 y old boys

7.1. Abstract

7.2. Introduction

7.3. Methods

7.4. Results

7.5. Discussion

7.6. Conclusion

8. Two weeks high intensity interval training on fasting glucose, glucose tolerance and insulin resistance in adolescent boys: a preliminary study

8.1. Abstract

8.2. Introduction

8.3. Methods

8.4. Results

8.5. Discussion

8.6. Conclusion

9. High intensity interval exercise and glycaemic control in adolescents with type one diabetes mellitus: A case series

9.1. Abstract

9.2. Introduction

9.3. Patient information

9.4. Experimental design

9.5. Outcomes

9.6. Discussion

10. Summary of findings, implications, limitations and future directions

10.1. Summary of experimental Chapters

10.2. Synthesis of the experimental findings

10.3. Practical research implications

10.4. Limitations

10.5. Conclusion

11. REFERENCES

12. APPENDIX
List of tables

Table 2.1. Action of key metabolic hormones in glucose homeostasis..................29

Table 2.2. Diagnostic criteria for impaired fasting glucose, impaired glucose tolerance and diabetes mellitus (ADA, 2010).................................................................33

Table 2.3. Cross-sectional studies on association between physical activity and glucose and insulin health outcomes in youth.................................................................56

Table 2.4. Longitudinal studies on association between physical activity and glucose and insulin health outcomes in youth.................................................................60

Table 2.5. Physical activity and exercise training intervention on glucose and insulin health outcomes in youth.................................................................68

Table 2.6. Acute exercise studies and glucose and insulin health outcomes in youth.................................................................74

Table 3.1. Formulas and references for indices of IS and β-cell function derived from fasting and OGTT measurements of glucose and insulin........................................100

Table 4.1. Relationship between OGTT and fasted measures of IR......................114

Table 4.2. Relationship between OGTT and fasted measures of β-cell function.....115

Table 4.3. Day to day reliability of fasting and OGTT derived measures of IR......116

Table 4.4. Day to day reliability of fasting and OGTT derived measures of β-cell function.................................................................117

Table 5.1. Physiological and perceptual responses to HIIE and MIE..................133

Table 6.1. Participant descriptive characteristics.............................................153
Table 6.2. Daily PA and dietary intake during the 48 h preceding and during each experimental visit ................................................................. 154

Table 6.3. Physiological and perceptual responses to HIIE and MIE ............ 154

Table 7.1. Participant descriptive characteristics ........................................ 174

Table 7.2. 48 h diet and PA prior to each experimental condition .............. 175

Table 7.3. Physiological and perceptual responses to HIIE and MIE ........... 176

Table 8.1. Participant descriptive characteristics ........................................ 195

Table 8.2. PA and dietary intake during the 48 h preceding each experimental visit ..................................................................................... 195

Table 8.3. Physical and biochemical characteristics at PRE, 20 h and 70 h post intervention ................................................................. 196

Table 9.1. Participants descriptive characteristics .................................... 209

Table 9.2. The effects of acute MIE and HIIE on 24-h glycaemic control, postprandial response to MMTT and overnight glycaemia in three adolescents with T1D ................................................................. 215
List of figures

**Figure 2.1.** Glucose homeostasis for non-diabetic individual in a fasted (A) and fed (B) state. In diagram A, blood (plasma) glucose is derived from glycogenolysis (1), stimulated by glucagon, whilst basal levels of insulin control glucose disposal (2), with minimal suppression of gluconeogenesis and glycogenolysis due to low levels of circulating insulin (3). In diagram B, blood glucose is derived from the ingestion of carbohydrate (1). Glycogen secretion is supressed by increased insulin (2, 3). This increase in insulin supresses liver gluconeogenesis and glycogenolysis (4), whilst increasing glucose disposal in peripheral tissue (5). Figure adapted from Aronoff et al. (2004), with permission…………………………………………………………………30

**Figure 2.2.** Type two diabetes development over time. Figure reproduced from Ramlo-Halsted and Edelman (1999) with permission……………………………………………………….32

**Figure 2.3.** Glucose homeostasis for a diabetic individual in both a fasted (A) and fed (B) state. In diagram A, blood (plasma) glucose is derived from glycogenolysis and gluconeogenesis (1), stimulated by glucagon (2). The given rate of peripheral glucose disposal is influenced by exogenous insulin (3, 4). In a diabetic sate, the rate of hepatic gluconeogenesis and glycogenolysis is not appropriately regulated. In diagram B, the fed state plasma glucose is derived from ingestion of carbohydrate, regulated by the injection of exogenous insulin (1). Glucagon secretion is not supressed (2), leading to elevated hepatic glucose production (3) and an imbalance in the overall rate of glucose appearance, disposal, and ensuing hyperglycaemia (5). Figure adapted from Aronoff et al. (2004), with permission……………………………………………………………………………………………………34
**Figure 2.4.** The role of insulin resistance in the development of cardiovascular disease. Both insulin resistance and hyperglycaemia lead to oxidative stress and overproduction of superoxide by the mitochondria. This process activates the damaging pathways that leads to multiple diabetes related complications. ROS; reactive oxygen species, FFA; Free fatty acid, HDL-Cholesterol; high density lipoprotein cholesterol, LDL- Cholesterol; low density lipoprotein cholesterol. Used with permission from Laakso (2010)………………………………………………………… 36

**Figure 2.5.** Changes in insulin resistance and body fatness throughout the life course. Image from Zeitler and Nadeau (2009) with permission………………………………………………………… 50

**Figure 3.1.** Example oxygen uptake ($\dot{V}O_2$) trace from a combined ramp and supramaximal test to exhaustion to determine $\dot{V}O_2$ max in a participant. $\dot{V}O_2$ max was taken as the highest 10 s average $\dot{V}O_2$ during either the ramp or the supramaximal component of the test. In the example above $\dot{V}O_2$ reached 3.42 L.min$^{-1}$ during the ramp part of the test and 3.51 L.min$^{-1}$ during the supramaximal bout. The $\dot{V}O_2$ max was therefore taken as 3.51 L.min$^{-1}$ for this participant…………………………………………………………90

**Figure 3.2.** An example CGM trace over a 24h period from 08:00 to 08:00 during the laboratory testing day in Chapter 9. Exercise started at 10:30, the mixed meal tolerance test (MMTT) 12:00. The target range for glycaemic control are shown in red (above 7.2) and blue (below 3.9)…………………………………………………………103

**Figure 5.1.** Schematic of protocol for experimental visits 2,3 and 4………………130
**Figure 5.2.** Plasma glucose and insulin response following the OGTT displayed over time (A, D), using the incremental AUC (B, E) or the total AUC (C, F). HIIE, high-intensity interval exercise. MIE, moderate intensity exercise. CON, control. A, D shown as mean ± SEM. B,C,E,F shown as mean ± SD. * represents a meaningful beneficial effect (ES>0.2) for exercise compared to control. Please see text for details.

**Figure 5.3.** IS (Cederholm). Values shown as mean ± SD. HIIE, high-intensity interval exercise. MIE, moderate intensity exercise. CON, control. * represents a meaningful beneficial effect (ES>0.2) for exercise compared to control. Please see text for details.

**Figure 5.4.** Energy expenditure (A) and fat oxidation (B) response following the OGTT. HIIE, high-intensity interval exercise. MIE, moderate intensity exercise. CON, control. Please see text for details.

**Figure 6.1.** Fasting glucose, insulin and HOMA-IR, HOMA-S% and HOMA-B at PRE-Ex, 24h-POST and 48h-POST exercise Where; ● CON, ▪ MIE, △ HIIE. Error bars omitted for clarity.

**Figure 6.2.** Changes to tAUC, iAUC glucose, insulin and IS at POST-Ex and 24h-POST after an OGTT. Where; ● CON, ▪ MIE, △ HIIE.* represents a significant difference for HIIE vs. CON and # represents a significant difference MIE vs. CON.
Figure 7.1. Postprandial plasma glucose and insulin response following the OGTT displayed over time (A, B) and using incremental (C, D) and total (E, F) UC. Values shown as mean ± SD. Error bars omitted in A and B for clarity…………………177

Figure 7.2. IS as measured using the Cederholm index. Values shown as mean ± SD…………………………………………………………………………………………178

Figure 8.1. Postprandial plasma glucose and insulin response to the mixed meal tolerance test (MMTT) at baseline and at 20 h and 70 h after the HIIE training intervention. Results shown as mean ± SEM…………………………………………………197

Figure 8.2. Scatter plot showing correlation between change in HOMA-IR after at 20 h POST HIIE training and HOMA-IR at baseline. ** P<0.01 *P<0.05………………….198

Figure 9.1 Schematic of protocol for experimental visits……………………………………210

Figure 9.2. Individual glycaemic response to exercise (A,B and C) and MMTT (D,E and F). Changes in interstitial glucose levels estimated by CGM system during moderate intensity………………………………………………………………………………214
Figure 10.1. Change in insulin sensitivity (A) and glucose tolerance (B) compared to control following moderate-intensity exercise (MIE) and high-intensity interval exercise (HIIE). Data are pooled across Chapters 5-7 .................................226

Figure 10.2. Individual change in insulin sensitivity after acute high intensity interval exercise and moderate intensity exercise compared to control. N=28 with data combined from Chapters 5-7 ........................................................................................................229

Figure 10.3. Change in IS after acute HIIE (A) and MIE (B). White fill bars are younger participants from Chapter 7 (n=11), whilst the black filled bars are adolescents from Chapters 5 and 6 (n=17)........................................................................................................230

Figure 10.4. Relationship between the change in insulin sensitivity and fat oxidation with change to total area under curve fat oxidation from control: moderate intensity exercise and control: high intensity interval exercise using combined data from Chapters 5-7 (n=23)........................................................................................................233
LIST OF EQUATIONS

Equation 3.1. Mechanical work done during cycling exercise………………………….91

Equation 3.2. Fat and oxidation and energy expenditure………………………………….93
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>β</td>
<td>Beta</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>CRF</td>
<td>Cardiorespiratory fitness</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence intervals</td>
</tr>
<tr>
<td>CGM</td>
<td>Continuous glucose monitor</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>ES</td>
<td>Effect size</td>
</tr>
<tr>
<td>ET</td>
<td>Endurance training</td>
</tr>
<tr>
<td>EYHS</td>
<td>European Youth Heart Study</td>
</tr>
<tr>
<td>FGIR</td>
<td>Fasting glucose insulin ratio</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>FSIVGTT</td>
<td>Frequently sampled intravenous glucose tolerance test</td>
</tr>
<tr>
<td>GET</td>
<td>Gas exchange threshold</td>
</tr>
<tr>
<td>GIR</td>
<td>Glucose infusion rate</td>
</tr>
<tr>
<td>GLUT</td>
<td>Glucose transporter</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated haemoglobin</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HFM</td>
<td>High fat meal</td>
</tr>
<tr>
<td>HIIE</td>
<td>High-intensity interval exercise</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostatic model assessment of insulin resistance</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HEC</td>
<td>Hyperinsulinemic-euglycaemic clamp</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
</tr>
<tr>
<td>iAUC</td>
<td>Incremental area under curve</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
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</tbody>
</table>
IS  Insulin sensitivity
kcal  Kilocalorie
LDL  Low density lipoprotein
\overset{\text{\text{\text{\text{\text{max}}}}}}{\text{\text{\text{\text{\text{\text{\text{$v$}}}}}}}}  Maximal oxygen uptake
MVPA  Moderate to vigorous intensity physical activity
MIE  Moderate-intensity exercise
MPA  Moderate-intensity physical activity
MI  Myocardial infarction
NHANES  National Health and Nutrition Examination Survey
n  Number
OGTT  Oral glucose tolerance test
PP  Peak power
%CV  Percent coefficient of variation
PA  Physical activity
QUICKI  Quantitative insulin sensitivity check index
tAUC  Total area under curve
T1D  Type one diabetes mellitus
T2D  Type two diabetes mellitus
TE  Typical error
UK  United Kingdom
VPA  Vigorous-intensity physical activity
WHO  World Health Organisation
y  Year
YFS  Young Finns Study
CHAPTER 1

Introduction

Type two diabetes (T2D) represents a global health burden being the 8th leading cause of death worldwide (WHO, 2016). T2D accounts for ~10% of the total health expenditure in the United Kingdom (UK), which is expected to rise to ~17% and an estimated £39.8 billion by 2035 (Hex et al., 2012). T2D is a well-established risk factor for cardiovascular disease (CVD), and adults with T2D have a 2-3-fold increased risk of CVD compared with non-diabetic individuals (Sanwar et al., 2010). Within the UK, T2D was the cause of 1.5 million deaths in 2012 and the resulting increased risk of CVD in these patients was responsible for an additional 2.2 million deaths (WHO, 2016). Given the burden of T2D and CVD, early prevention is of upmost importance.

There is a growing body of evidence that both T2D and CVD have their origins in childhood (Steinberger et al., 2009). For example, data from the Pathological Determinations of Atherosclerosis in Youth Study reported early atherosclerotic fatty streaks in arteries of children and adolescents (McGill et al., 2000). There have also been cases of overt T2D reported in youth (D’Adamo and Caprio, 2011), with the first case in the UK reported in 2000 (Ehtisham et al., 2000). Several modifiable factors have been suggested to increase the risk of T2D and CVD including overweight/obesity; poor diet, smoking, high blood pressure, low levels of physical activity (PA), impaired glucose tolerance (IGT) and insulin resistance (IR) (Martín-Timón et al., 2014). Furthermore, it is known that certain risk factors, such as high
blood pressure, and physical inactivity, track from childhood through adolescence and into early adulthood (Kemper et al., 1990).

IR is known to be prevalent in both obese and normal weight youth (Aldhoon-Hainerová et al., 2014), and having IR in youth can predict elevated risk of T2D and CVD in early adulthood (Yajnik et al., 2015). Although intervention studies in adults have shown modifying risk factor status can prevent the development of CVD and T2D (Eddy et al., 2009), they do not completely eliminate the elevated disease risk (McGill et al., 2008). This highlights the importance of primary risk factor prevention from an early age.

Physical inactivity is the 4th leading risk factor for all-cause mortality worldwide (WHO, 2016). In adults, strong evidence exists to support regular engagement in PA to protect against the development of CVD (Thompson et al., 2003) and T2D (Shi et al., 2013). The association between PA and cardiometabolic risk is also established in children and adolescents, with observational studies showing a graded dose-response reduction in the clustering of cardiometabolic risk factors for T2D and CVD with increasing levels of PA (Ekelund et al., 2007, Andersen et al., 2006). In addition, it is known from observational studies that children who participate in regular PA have increased IS compared to their less active peers (Schmitz et al., 2002). Due to the established relationship between increased PA participation and a reduction in T2D and CVD risk factors, including reduced IR or elevated IS (Fedewa et al., 2014), the UK government currently recommends that children and adolescents achieve at least
60 minutes of moderate to vigorous physical activity (MVPA) on a daily basis (Department of Health, 2011). It is important to highlight this recommendation is a minimum requirement, as the odds ratio for a clustering of risk factors is 3.29 (95% confidence interval (CI) 1.96 to 5.22) greater for children and adolescents completing 56 minutes of MVPA per day compared to 131 minutes per day (Andersen et al., 2006). Finally, recent UK data indicates that only 21% of boys aged 5-15 y old are meeting the current PA recommendations for health and PA levels are known to decline from childhood into adolescence (Towsend et al., 2015).

Given the above, there has been great interest to investigate whether interventions can increase PA levels in youth. However, a comprehensive meta-analysis of 30 studies including 6,153 participants (3,232 girls, 2,921 boys, age at baseline 1.8 to 13.1 y) with a median intervention duration of 26 weeks, found minimal success, increasing MVPA by only ~ 4 minutes per day (Metcalf et al., 2012). As such, consideration of alternative PA recommendations to improve health in children and adolescents is needed. In this context, it should be noted that a recent observational study reported improved cardiometabolic health status in youth who perform as little as 8 minutes of vigorous physical activity (VPA) a day (Carson et al., 2014). These data suggest that performing just a few minutes of VPA per day may be important for promoting health and reducing cardiometabolic risk factors in youth. As a result of this evidence as well as data from adult literature (Gibala et al, 2012), there has been a proliferation of interest into the beneficial effects of time-efficient, high-intensity interval exercise (HIIE), which combines short period of high-intensity exercise with light recovery, on cardiometabolic health markers (e.g. weight status and cardiorespiratory
fitness) in children and adolescents. HIIE may be an effective approach to increase VPA as the intermittent nature of it matches the habitual PA of youth (Riddoch et al., 2007, Trost et al., 2002) and has also been suggested to be more enjoyable than continuous exercise (Barkley et al., 2009). Research on the health benefits of HIIE training in youth has recently been compiled in published reviews (Logan et al., 2014, Costigan et al., 2015). Research specifically relating to the ability of HIIE to improve glucose and insulin health outcomes is, however, currently limited, yet this area is important since IR and IGT are both risk factors of T2D and CVD.

In a similar manner to T2D, type one diabetes mellitus (T1D) is a disease of glucose dysregulation. However, T1D arises following the autoimmune destruction of insulin producing pancreatic beta (β) cells (Atkinson and Eisenbarth, 2001), requiring the immediate need for exogenous insulin replacement. T1D is one of the most common chronic childhood diseases (Karvonen et al., 2000, Gale, 2005) with a prevalence of ~ 18-20 children in every 100,000 in the UK (Onkamo et al., 1999). One of the major health concerns in T1D is the increased risk of CVD, which is the major cause of mortality within this patient group (Soedamah-Muthu et al., 2006). Evidence from epidemiological studies indicate that hyperglycaemia, as a result of poor glycaemic control, is an independent risk factor of CVD risk and the major cause of patient mortality (Mannucci et al., 2013). The risk of CVD in patients with T1D is not limited to adulthood, as pre-clinical signs of CVD present within the first decade of life (Khan et al., 2000, Jarvisalo et al., 2004).
There is evidence to suggest that PA may provide an effective strategy to manage CVD risk and glycaemic control in children and adolescents with T1D (Herbst et al., 2007, Seeger et al., 2011). From a paediatric perspective, the American Diabetes Association (ADA) recommends that children and adolescents with T1D undertake 30-60 minutes of daily MVPA. However, evidence for these guidelines are equivocal (Salem et al., 2010, Campagne et al., 1984, Landt et al., 1985, Huttunen et al., 1989), and the optimal form, duration and intensity of PA to reduce CVD risk and improve glycaemic control in children and adolescents with type T1D is currently unknown. Much like “healthy” children and adolescents, those with T1D are also not achieving the current UK daily PA guidelines (Cuenca-Garcia et al., 2012), despite the possible health benefits. Therefore, there is potential to explore the use of small volumes of HIIE to improve glycaemic control in youth with T1D. Whilst a single bout of HIIE has been shown to be effective at improving 24 h glycaemic control in adults with T2D (Gillen et al., 2012), this has yet to be investigated in youth with T1D.

To summarise, the primary aim of this thesis is to provide a sequence of novel investigations into the effect of HIIE on insulin and glucose health outcomes in children and adolescents. Additionally, the potential for HIIE to improve glycaemic control in a clinical population of adolescents with T1D will be examined. Nevertheless, before detailing the experimental work conducted throughout this thesis, Chapter 2 will provide a critical review of the related literature, Chapter 3 will provide an overview of the methods and procedures used, collectively presenting a clear rationale and the intended aims of the experimental Chapters (Chapters 4-9).
CHAPTER 2

Literature review

This literature review provides a brief overview of blood glucose regulation and transportation, and how dysregulation of blood glucose is linked to the development of CVD and T2D. This will be followed by a critical review of the methodological approaches used to measure IS and glucose tolerance in children and adolescents, and key literature regarding the effects of PA, exercise training and acute exercise on IR and glucose tolerance in children and adolescents. Finally, the chapter will end with the presentation of key literature around the role of exercise in the management of glycaemic control in children and adolescents with T1D. The overarching aim of this chapter is to provide a compelling rationale as to why performing HIIE may be an effective approach to improve glucose and insulin health outcomes in healthy and diabetic youth.

2.1. Glucose regulation

The regulation of blood glucose is critical for normal physiological function. In a healthy individual, blood glucose is maintained between 4.0 to 6.0 mmol.L\(^{-1}\). The regulation of blood glucose concentration is a function of the rate of glucose entering the circulation balanced by the rate of glucose uptake from the circulation. Circulatory glucose derives from three key sources: intestinal absorption in the postprandial state, glycogenolysis and gluconeogenesis. Glycogenolysis is the breakdown of stored glycogen within the liver and muscle into glucose. Gluconeogenesis is the synthesis
of glucose from smaller molecules, such a lactate and pyruvate, when in a fasted state. The major site of gluconeogenesis is in the liver. The key hormones responsible for these processes are detailed in Table 2.1, and the key organs and hormones are further illustrated in Figure 2.1.

Since glucose is the primary source of energy (synthesis of adenosine triphosphate (ATP)) in cells, there is a constant depletion of blood glucose from the circulation. Therefore, a fine balance between supply and demand is crucial in the maintenance of blood glucose levels. The liver is the predominant metabolic regulatory organ in glucose homeostasis, with approximately 90% of all non-dietary circulatory glucose directed from the liver. This non-dietary glucose arises from stored glycogen which is rapidly available for release into the circulation. Other key organs in glucose homeostasis are peripheral tissues (e.g. skeletal muscle and adipose) and the pancreas. The key hormones and their actions are detailed below:

**Insulin:** This hormone responds to increasing blood glucose, such as following the ingestion of a carbohydrate (CHO) based meal. Insulin is a peptide hormone, which is derived from the larger precursor, proinsulin. Insulin is stored and secreted from pancreatic β-cells. Insulin has direct and indirect actions on liver, kidney, adipose and muscle tissue. The effects of insulin include:

- Translocation of glucose transporter (GLUT)-4 to the cell membrane to increase glucose uptake into the cell (Huang & Czech, 2007). This is most significantly
seen in skeletal muscle, which is an important repository for glucose in the postprandial state.

- Suppression of glucose release from the liver and kidney.
- Inhibition of the release of free fatty acids (FFA) into the circulation and increased rate of clearance from the blood.
- Conversion of glucose to glycogen.

**Glucagon:** This is the major counter-regulatory hormone to insulin. In a similar manner to insulin, glucagon is essential for the moment-to-moment regulation of blood glucose. Glucagon is released from the pancreatic alpha (α) cells when plasma glucose levels are falling, such as in response to catecholamine (adrenaline and noradrenaline) release. Glucagon’s actions oppose those of insulin, thus functioning to maintain glucose levels between meals and during other metabolic stresses (e.g. exercise), stimulating the liver to release glucose into the circulation and inhibiting glycolysis.

**Catecholamine hormones:** In response to hypoglycaemia, the catecholamine hormones adrenaline and noradrenaline are released, playing a significant role in the maintenance of blood glucose levels during exercise by increasing hepatic glucose output and inhibiting insulin release. Catecholamine hormones also have a direct effect on increasing muscle glycolysis and glycogen breakdown, resulting in increased glucose production, as well as decreased glucose clearance by inhibition of tissue glucose uptake (Rizza et al., 1980).
**Growth hormone:** Unlike the effects of glucagon and catecholamine hormones, the effect of growth hormone (and cortisol) can take several hours. Growth hormone is secreted from the anterior pituitary gland when blood glucose levels fall, thus stimulating lipolysis and inhibition of insulin action.

**Cortisol:** This hormone is released from the adrenal cortex in response to a fall in blood glucose, where it inhibits the release and action of insulin.

**Table 2.1. Action of key metabolic hormones in glucose homeostasis**

<table>
<thead>
<tr>
<th></th>
<th>Glucose production</th>
<th>Glucose utilisation</th>
<th>Lipolysis</th>
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<tbody>
<tr>
<td>Insulin</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Glucagon</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Catecholamine</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Cortisol</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

↓: decrease, ↑: increase, ↔: no effect.
**Figure 2.1.** Glucose homeostasis for non-diabetic individual in a fasted (A) and fed (B) state. In diagram A, blood (plasma) glucose is derived from glycogenolysis (1), stimulated by glucagon, whilst basal levels of insulin control glucose disposal (2), with minimal suppression of gluconeogenesis and glycogenolysis due to low levels of circulating insulin (3). In diagram B, blood glucose is derived from the ingestion of carbohydrate (1). Glycogen secretion is suppressed by increased insulin (2, 3). This increase in insulin suppresses liver gluconeogenesis and glycogenolysis (4), whilst increasing glucose disposal in peripheral tissue (5). Figure adapted from Aronoff et al. (2004), with permission.

### 2.2. Glucose transport

Transport of glucose into cells is facilitated by GLUT molecules on the cell membrane. There are a number of different GLUT transporters expressed in different types of tissue. These GLUT transporters are differentially effected by the hormones involved in blood glucose regulation, as detailed below:

**GLUT-1:** This transporter is found in all cell types and is primarily responsible for the maintenance of basal glucose. GLUT-1 is generally in low abundance, especially in muscle tissue.
**GLUT-2:** Predominantly found in the liver and kidney cells, this transporter can both absorb glucose into the cell and transport glucose back into the blood at times of low blood glucose.

**GLUT-4:** This transporter is found within adipose and skeletal tissue and is responsive to hormonal action. When the concentration of blood glucose is low, this transporter is expressed at the cell surface in relatively low amounts, with the majority of GLUT-4 occupying intracellular storage sites. However, when insulin concentrations are high, due to an increase in blood glucose after eating or after performing exercise (Richter and Hargreaves, 2013), GLUT-4 vesicles are translocated to the cell membrane, allowing for an increased rate of glucose uptake into the cell.

### 2.3. Glucose dysregulation

T2D is a disease resulting from glucose dysregulation. This disease progresses from an early asymptomatic stage, where IR is compensated for by an increase in insulin secretion, to overt diabetes requiring pharmacological intervention. Understanding the natural history of developing T2D is important when considering how to develop preventative strategies. The progression from normal glucose tolerance through to T2D in terms of insulin, glucose and IR is shown in Figure 2.2.
IR is a physiological state within which impaired insulin action requires increased insulin levels to exert its biological role(s) on insulin sensitive tissue. This impaired insulin action results in the disinhibition of lipolysis within adipose tissue, reduced glucose uptake into muscle and the impairment of gluconeogenesis in the liver (Mlinar et al., 2007). Thus, the IR state requires an increased concentration of insulin to achieve the required biological state. The pancreas initially compensates for this by increasing insulin production. However, over time the pancreas loses its capacity to produce insulin, leading to both ß-cell failure and hyperglycaemia (Lebovitz, 2001). The inverse of IR is IS, which refers to the sensitivity of cells to the action of insulin. These terms are used interchangeably throughout this thesis with a decrease in IR, or an increase in IS, referring to the same physiological construct.
In an initial IR state, the body is able to compensate by increasing insulin output (hyperinsulinemia). Therefore, blood glucose levels remain normal until the insulin producing β-cells reach a threshold for compensation. This can result in IGT, which is a state of hyperglycaemia between normal glucose tolerance and diabetes (see Table 2.2). IGT represents an intermediate stage in the development of T2D and is an important risk factor in the development of overt T2D. Indeed, 25-75% of those with IGT develop overt T2D within a decade (Saad et al., 1988).

**Table 2.2:** Diagnostic criteria for impaired fasting glucose, impaired glucose tolerance and diabetes mellitus (ADA, 2010).

<table>
<thead>
<tr>
<th></th>
<th>Fasting plasma glucose (mmol.L⁻¹)</th>
<th>2 hr post 75 g oral glucose tolerance test (mmol.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal glucose tolerance</td>
<td>&lt;6.1</td>
<td>&lt;7.8</td>
</tr>
<tr>
<td>Impaired fasting glucose/impaired glucose tolerance</td>
<td>≥ 6.1 to 7.0</td>
<td>7.8 &lt; 11.1</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>≥ 7.0</td>
<td>≥ 11.1</td>
</tr>
</tbody>
</table>

In a diabetic state, normal glucose regulation cannot occur due to the failure of tissues to respond to the effects of insulin. In turn, this can progress towards β-cell failure and the failure of the body to produce insulin. Glucose regulation in the diabetic state is shown in Figure 2.3.
Figure 2.3. Glucose homeostasis for a diabetic individual in both a fasted (A) and fed (B) state. In diagram A, blood (plasma) glucose is derived from glycogenolysis and gluconeogenesis (1), stimulated by glucagon (2). The given rate of peripheral glucose disposal is influenced by exogenous insulin (3, 4). In a diabetic state, the rate of hepatic gluconeogenesis and glycogenolysis is not appropriately regulated. In diagram B, the fed state plasma glucose is derived from ingestion of carbohydrate, regulated by the injection of exogenous insulin (1). Glucagon secretion is not suppressed (2), leading to elevated hepatic glucose production (3) and an imbalance in the overall rate of glucose appearance, disposal, and ensuing hyperglycaemia (5). Figure adapted from Aronoff et al. (2004), with permission.

2.4. Insulin resistance, glucose tolerance and disease development

Section 2.2 has outlined how dysregulation of glucose metabolism results in IR and IGT, contributing to the development of T2D. However, it is also important to outline the association between T2D and CVD, and that IR is not only a risk factor for T2D, but also for CVD.

The chronic hyperglycaemia associated with both IGT and T2D can result in elevated risk of CVD and long-term health complications related to CVD. Indeed, ~40-60% of
deaths in patients with diabetes (T1D and T2D) are attributable to CVD. Furthermore, both T2D and CVD are associated with common causally linked risk factors including tobacco use, physical inactivity, high sugar and fat dietary intake, and excessive alcohol consumption, all of which are associated with adverse metabolic changes, such as hyperglycaemia (Laakso, 2001).

The risk of CVD related mortality in patients with diabetes is almost double that of aged-matched non-diabetic individuals (Laakso, 2010). A 7 y longitudinal study using data from 1,059 patients with T2D and 1,373 non-diabetic patients, revealed that the T2D patients, with no prior history of myocardial infarction (MI), had the same risk of death from coronary heart disease (CHD) as the non-diabetic patients with a history of MI (Haffner et al., 1998). Furthermore, a 18 y follow-up study by Juutilainen and colleagues (2005) described T2D as a CHD equivalent, when the criterion for CHD was a history of MI. However, when alternative definitions of CHD were used (e.g. angina, ischemic electrocardiogram changes, or any evidence of prior CHD), the hazard ratio for those with T2D without prior CHD was worse than that of non-diabetic patients with prior CHD. This emphasises the elevated risk of mortality due to CVD in patients with diabetes.

The close association between T2D and CVD suggests that they may share the same underlying genetic and environmental antecedents. This theory is known as the ‘common soil hypothesis’ (see Figure 2.4). The theory postulates that IR is central to the development of both T2D and to the clustering of CVD risk factors (e.g. central
obesity, IR, glucose intolerance, dyslipidaemia and hypertension) (Alberti et al., 2009, Kassi et al., 2011). This clustering of cardiometabolic risk factors is commonly referred to as the metabolic syndrome (MetS) (Alberti et al., 2009, Kassi et al., 2011, Laakso, 2010). The common soil hypothesis proposes that IR, IGT and overt diabetes are all associated with an increased risk of CVD (Balkau et al., 1999, Lakka et al., 2002), whilst all being associated with an increased oxidative stress (Evans et al., 2003). The commonality of an increased oxidative stress has led to the proposal that this persistent pathogenic factor may act to mediate the appearance of IR, the development of overt diabetes, and the increased risk of CVD (see figure 2.4).

**Figure 2.4.** The role of insulin resistance in the development of cardiovascular disease. Both insulin resistance and hyperglycemia lead to oxidative stress and overproduction of superoxide by the mitochondria. This process activates the damaging pathways that leads to multiple diabetes related complications. ROS; reactive oxygen species, FFA; Free fatty acid, HDL-Cholesterol; high density lipoprotein cholesterol, LDL-Cholesterol; low density lipoprotein cholesterol. Used with permission from Laakso (2010).

IR is thought to be linked with a number of risk factors associated with both T2D and CVD and studies have found associations between IR and asymptomatic
atherosclerosis (Laakso et al., 1991), and coronary artery disease in adults without diabetes mellitus (Bressler et al., 1996). Furthermore, levels of fasting insulin, which is a marker of IR, have also been associated with CVD events in non-diabetic adults (Laakso, 1996). In a longitudinal study with a 5 y follow up, IR and the resultant hyperinsulinaemia were found to be predictors of future CVD, independent of other risk factors such as age, body mass index (BMI), and history of smoking (Yip et al., 1998). More recent work has utilised data from the National Health and Nutritional Examination Survey to estimate, over a period of 60 y, the number of MI events that could be prevented by improving IR and other risk factors (e.g. high density lipoprotein (HDL) cholesterol, BMI and triglycerides). This comprehensive study concluded that IR is likely to be the most important single risk factor for coronary artery disease, and that maintaining IR could prevent ~42% of MIs over a 60 y period (Eddy et al., 2009).

In addition to IR, there is evidence that postprandial glucose is an important contributing factor to the development of atherosclerosis and CVD. The Whitehall Study assessed 18,403 male civil servants aged 40-64 y over a 10 y period. They reported a non-linear relationship between mortality from CHD and stroke, and 2 h postprandial blood glucose levels. This relative risk of mortality from CHD was doubled in those above the 95th centile point with glucose intolerance and diabetes (Fuller et al., 1983). A subsequent meta-analysis confirmed this association, whereby a total of 95,783 participants across 20 studies were followed for a total of 12.4 y. This analysis found that a high fasting glucose, and both 1 h and 2 h postprandial glucose levels, increased the relative risk of CVD events by 1.22 and 1.58, respectively. The relationship between glucose and CVD events was also present below the diabetic
threshold (Coutinho et al., 1999). This association between glycaemia and CVD has also been shown elsewhere (DECODE study group, 2001, Levitan et al., 2004). In the meta-analysis by Levitan and colleagues (2004), 38 prospective studies reported CVD incidence or mortality and blood glucose levels. Those with highest post-challenge glucose (8.3-10.8 mmol.L\(^{-1}\)) had a 27% greater risk of CVD compared to the group with the lowest post-challenge glucose (3.8-5.9 mmol.L\(^{-1}\)). Additionally, Temelkova-Kurktschiev and colleagues (2000) measured the carotid intima-media thickness, a sub-clinical marker of atherosclerosis, in 582 individuals without known diabetes and examined its association with post-challenge blood glucose, post-challenge glucose spikes, fasting glucose, and glycated haemoglobin (HbA1c). It was reported that both the post-challenge glucose and post-challenge glucose spikes were strongly associated with carotid intima-media thickness and therefore the progression of atherosclerosis.

Given the above discussion, evidence supports the notion that IR, IGT and elevated postprandial glucose excursions are all important in the development of T2D and CVD (Nigro et al., 2006). Therefore, understanding the pathogenesis of these diseases and how interventions might attenuate the appearance of insulin and glucose risk factors is an important research avenue for reducing the prevalence of T2D and CVD.

2.5. A paediatric problem?

Although the clinical manifestations of CVD and T2D are not detectable until adulthood, their origins have been evidenced in childhood and are related to changes
in cardiometabolic risk factors including glucose and insulin. The presence of fatty streaks in the large arteries of children and adolescents were first reported in the early 20th century (Klotz, 1911). Since this initial report, research has highlighted that the development of fatty streaks and raised lesions in the right coronary artery in young persons (15-34 y) is positively associated with LDL cholesterol, hypertension, IGT and obesity (McGill et al., 2000). Furthermore, the Pathological Determinants of Atherosclerosis in Youth Study found hyperglycaemia to be strongly associated with the progression of fatty streaks (McGill et al., 1995) and microscopic lesions (McGill et al., 2000) in the coronary artery of participants aged 15-34 y. In a review of the main findings from the prospective Cardiovascular Risk in Young Finns study, the authors concluded that childhood risk factors are associated with subclinical atherosclerosis, as measured using the carotid intima-media thickness, in adulthood. This included protective factors such as high levels of PA and risk factors such as overweight/obesity, elevated blood pressure, smoking, and the metabolic syndrome where IR is grouped with other metabolic risk factors (Juonala et al., 2013). Data taken from the Bogalusa Heart Study also found that elevated insulin levels persisted from childhood though to young adulthood, and were associated with clinically important CVD risk factors in adulthood (Bao et al., 1996). Finally, research by Franks et al. (2010), in a cohort study of 4,857 Pima Indian youth (5-9 y), reported that elevated 2 h glucose levels (below the level of IGT, but above normal) were associated with increased risk of death from endogenous causes, including CVD and T2D, during a mean follow-up of 23.9 y.
Although T2D was once thought of as a metabolic disorder exclusive to adults, it is becoming increasingly prevalent in obese adolescents (Pinhas-Hamiel and Zeitler, 2005). The first case of T2D in youth within the UK was reported in 2000 (Ehtisham et al., 2000). It is currently estimated that 23,000 people under the age of 17 y have diabetes, with 1.5% of the 23,000 being reported as T2D. In those aged 10-14 y, the prevalence of T2D is much higher, reported as 39.1% of the total cases of diabetes (D’Adamo and Caprio, 2011). The emergence of T2D in youth is likely to have implications for CVD development, as pre-diabetic youth have been found to have a clustering of CVD risk factors alongside increased arterial wall thickness and stiffness (Shah et al., 2014). Utilising pooled data from the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study, a large population-based observational study followed a total of 1,781 participants, aged 9-18 y at baseline, for 14-27 y. The results highlighted that youth with MetS (BMI, hypertension, HDL-Cholesterol, triglycerides and glucose) had a 2- to 3-fold greater risk of increased carotid intima-media thickness and T2D in adulthood when compared to those free of MetS (Magnussen et al., 2010). Finally, higher levels of insulin, glucose, and IR in childhood have also been shown to relate to adverse cardiovascular risk in adulthood. In a sample of 357 participants, sampled at 8 y and 21 y, elevated prepubertal glucose and insulin status predicted higher CVD risk and markers of atherosclerosis in early adulthood, independent of obesity (Yajnik et al., 2015).

Importantly, the presence of risk factors in youth, including glucose and insulin, can strongly predict the risk of CVD, T2D and subclinical atherosclerosis in later adulthood. This clearly highlights the importance of primary prevention during childhood and
adolescence (Gidding et al., 2016), including interventions designed to modify established risk factors with the goal of preventing future risk of CVD.

2.6. Assessment of insulin resistance

Previous sections have documented the importance of understanding IR during childhood and adolescence as a risk factor for the development of both T2D and CVD. Thus, it is important to be able to accurately measure and track IR and glucose tolerance in both children and adolescents. Indeed, there are a myriad of proposed methodologies for estimating IR (and IS) in youth. These range from single blood sample methods to more complex, time consuming, and intrusive measures, such as the hyperinsulinemic-euglycaemic (HEC) clamp. This section will present a discussion in relation to available methods to quantify IR/IS in youth using recent reviews on this topic (Brown and Yanovski, 2014, Muniyappa et al., 2015).

2.6.1. Hyperinsulinemic-euglycaemic clamp

The HEC is the “gold standard” technique for the assessment of IS (DeFronzo et al., 1979). The HEC directly measures insulin stimulated glucose uptake at a given level of hyperinsulinemia. Estimation of insulin action can then be made on the basis of the relationship between change in insulin concentration and the change in glucose production. Effectively, the clamp artificially raises insulin concentration above baseline to a steady-state hyperinsulinemic level, thereby increasing glucose disposal in insulin sensitive tissue and suppressing endogenous glucose production in the liver. Glucose is infused at a variable rate to “clamp” glucose in the euglycemic range (4-
6 mmol.L\(^{-1}\)). The participant will reach a steady state of glucose infusion, and this glucose infusion rate (GIR), after controlling for any urine glucose excretion during the test, is equal to the rate of glucose disposal. Individuals with a higher IS have a higher GIR, whilst insulin-resistant individuals will have a lower GIR to maintain euglycaemia.

Although upheld as the gold standard, the HEC has a number of limitations, especially within paediatric research. The technique is invasive, time consuming, labour intensive and expensive. For example, each test requires 2-3 h of time per participant, whilst an experienced technician must always be present. The HEC is therefore not appropriate for large scale studies and investigations where multiple measurement points are needed. Despite these limitations the HEC has previously been used in research with children and adolescents, but this is largely restricted to participant groups who are overweight or obese. For example, Lee et al. (2012) employed the HEC technique to assess the effects of exercise on IS in obese adolescent boys, and other authors have validated surrogate estimates of IS against the HEC in criterion validity studies in obese youth (Schwartz et al., 2008, Brown and Yanovski, 2014).

### 2.6.2. Insulin tolerance test

The insulin tolerance test provides a direct measurement of glucose disappearance in response to an intravenous insulin bolus (0.1 units per kg of body mass). Glucose is measured for a 15 minute period following insulin injection, and the rate of glucose disappearance from 3 to 15 minutes is used as a measure of insulin action (Bonora et al., 2007). Problems with this test include risk of hypoglycaemia, especially in insulin
sensitive individuals. Validity studies of the insulin tolerance test in children are limited, with one study showing no significant correlation to the HEC in 8 adolescents with T1D (Sarnblad et al., 2002). Reliability of this assessment method has been found to be between 6-9 percent coefficient of variation (%CV) in adults (Akinmokun et al., 1992, Bonora et al., 1989) and 18.1 %CV in children with T1D (Sarnblad et al., 2002). Use of this test to measure insulin responses is therefore limited. Albeit, it has been found effective as a clinical test that assesses growth hormone deficiency in children (Lone et al., 2011).

2.6.3. Frequently sampled intravenous glucose tolerance test

In the frequently sampled intravenous glucose tolerance test (FSIVGTT), a bolus injection of glucose (~ 0.3 g per kg of body mass) is infused intravenously, whilst frequent blood samples for glucose and insulin are obtained during a 3 h period (−10, −5, 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 21, 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100, 110, 120, 140, 160, and 180 minutes). Blood samples are then interpreted using minimal model assessment (Bergman et al., 1985), providing the parameters of IS and glucose effectiveness. The latter is a reflection of the ability of glucose to stimulate glucose uptake and inhibit glucose synthesis. The FSIVGTT has previously been used in obese adolescents (Rössner et al., 2008) and shown to correlate moderately ($r=0.69$) with the HEC in 20 healthy children with normal glucose metabolism (Henderson et al., 2011). However this technique involves multiple blood draws as well as specialised software (using the Bergman minimal model) for interpretation and thus is time consuming, invasive and comes with associated cost.
2.6.4. Oral glucose tolerance test

The OGTT is a widely used method to assess glucose tolerance, IS and β-cell function (Reaven and Miller, 1968). This method involves the ingestion of glucose (1.75 g per kg of body mass, up to a maximum of 75 g) in ~ 300 mL of water, typically over a 5-minute period. Blood samples for the determination of glucose and insulin are taken throughout the test (usually at 30 minute intervals), although more frequent measurement points have been advocated, especially during the initial 30 minutes after ingestion (Dalla Man et al., 2005). The OGTT acts to stimulate the insulin-glucose homeostatic feedback process and can provide information on IS in the postprandial period, which has been suggested to play a role in the development of T2D and CVD (Levitan et al., 2004).

The OGTT is routinely used in clinical practice to provide diagnostic information about diabetes status. Additionally, it is frequently used in the paediatric research setting. One of the benefits of the OGTT, when compared to both the HEC and FSIVGTT, is that it more closely mimics the normal physiological meal response to ingesting glucose, including the subsequent digestion and absorption processes. Values obtained from the OGTT can be used to calculate IS using a number of available indices, which fall under two general categories of calculations for IS (Brown and Yanovski, 2014):

1. Theoretically derived equations which are based on the principle that IS during the OGTT is inversely proportional to the product of the mean insulin and glucose values during the test. Equations of this nature include the Belfiore and Matsuda indices (Belfiore et al., 1998).
2. Formulas designed to maximise correlation with the HEC, such as the Stumvoll and Cederholm indices (Stumvoll et al., 2000).

Although the OGTT is much easier to carry out than the HEC and FSIVGTT, details of validity and reliability relating to surrogate estimates of IS derived from a OGTT are generally limited to overweight and obese youth and poorly understood in healthy participant groups (Brown and Yanovski, 2014). The reliability of the OGTT to diagnose IGT has been shown to be poor in a sample of 60 overweight youth (8-17 y old) (Libman et al., 2008). This research found only a moderate correlation between two tests ($r=0.37$, $P<0.01$) when separated by 1-25 days. Additionally there was shown to be only a 27.3% positive agreement between diagnosis of IGT between the two tests (Libman et al., 2008). Along with reliability, the validity of the estimate is also important to consider. This is typically established through correlation analysis with the "gold standard" HEC as the criterion measure. There is a limited body of research into the validly of IR assessment in youth. A recent review by Brown and Yanovski (2014) summarises the validity studies in the assessment of IR in youth. Of the two main OGTT derived estimates of IS (Matsuda, Cederholm), only the Matsuda index has been formally validated against the HEC, albeit in overweight youth with IGT. Large correlation coefficients of 0.77 and 0.78 were provided (Yeckel et al., 2004, George et al., 2011).
2.6.5. Mixed meal tolerance test

The mixed meal tolerance test (MMTT) involves the ingestion of a solid or liquid meal. Blood samples are collected prior to the meal and at set intervals throughout the postprandial period. Like the OGTT, the MMTT method activates the insulin-glucose homeostatic feedback process. Thus, the MMTT can provide information on glucose tolerance from assessment of the glucose response during the postprandial period. However, the MMTT does not allow for the assessment of IS estimates, allowing just the measure of glucose tolerance in the postprandial period. Results from the MMTT assessment method are also influenced by nutrients other than glucose, such as protein and fat, which can slow gastric emptying and stimulate insulin secretion (Brodovicz et al., 2011). However, the MMTT is more reflective of “free living dietary conditions” than other measures such as the HEC and OGTT, making it more ecologically valid.

2.6.6. Fasting methods

Surrogate estimates of IR/IS can also be based on single measurements of glucose and insulin in a fasted state. These estimates are generally based on the principle that during euglycaemia insulin secretion compensates for IR. Due to low insulin levels in the fasted state, insulin primarily acts on adipose tissue and liver, with these estimates being considered to reflect hepatic IR (Wallace, Levy, & Matthews, 2004). A number of different indices are used, but the most commonly used indices include the homeostatic model assessment (HOMA-IR) (Matthews et al., 1985), the quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000) and the fasting glucose insulin ratio (FGIR) (Legro et al., 1998). These fasted estimates of IR/IS are commonly
used in paediatric research due to their ease of assessment and analysis compared to the OGTT.

A limited body of evidence exists in relation to the validity of fasting based estimates (HOMA-IR, QUICKI and FGIR), and validation studies that have been performed have focused on overweight/obese youth with metabolic conditions, such as polycystic ovary syndrome of premature menarche (Brown and Yanovski, 2014). Furthermore, there is a paucity of evidence on the day to day reliability of fasting assessment methods in paediatric groups. Current evidence is limited to a study by Uwaifo and colleagues (2002) who reported a strong correlation (r>0.85) between repeated measurements of QUICKI and HOMA-IR in 31 children aged between 6 and 11 y, and mean BMI of 25.1 ± 4.9 kg/m². Although this study suggests strong agreement between repeated measures of QUICKI and HOMA-IR, at least in children with a high BMI, this study is limited by its statistical approach. The authors did not report the within-participant variation and mean bias of the measures, which are recommended when documenting measurement reliability (Atkinson and Nevill, 1998, Hopkins, 2000). Additionally, no reliability data are available for OGTT measures of IS/IR using recommended reliability statistics.

2.7. Factors affecting insulin resistance in youth

IR has been implicated as an important risk factor in the development of T2D and CVD, and preventing IR in youth is an important primary strategy to reduce the risk of
disease development in later adult life (see section 2.4). Consequently, it is important to understand the factors which may influence IR in children and adolescents.

### 2.7.1. Sex

CVD risk is known to be lower in women than in men, with women having a more favourable risk factor profile than age-matched male counterparts (Ervin, 2009). Conclusions have been made that this female advantage occurs due to an attenuation of the relationship between IR and CVD risk (Kim and Reaven, 2013). However, during childhood, it is apparent that girls have higher levels of IR than boys. Data from the 9 y longitudinal EarlyBird study showed that even after adjustment for PA, body composition and energy expenditure, girls have elevated IR compared to boys at 5, 6, 13 and 15 y of age (Jeffery et al., 2012). Similar findings have been reported by Moran and colleagues (1999) in a cross-sectional study of 10-14 y olds. In a cohort of 350 participants, the authors found that IS (measured using the HEC) was higher in males. However, this was only significant at Tanner stage IV (a method of assessment of maturation using stages of the development of sex characteristics; for more detail see Section 3.4). Further research conducted by this group (Moran et al., 2008) reported rapid increases in IR in males throughout adolescence (11-19 y) despite decreases in body fat. In comparison, girls’ IR decreased despite increases in their body fat. Regardless of their increased IR when compared to females at 11 y, by 19 y of age males were more insulin resistant. Conversely, in Czech adolescents, IGT, which results as a consequence of IR, was evident in 7.0% of the 1,518 participants (13.0 to 17.9 y), with a higher prevalence found in boys (9.7%), compared to girls (4.4%) (Aldhoon-Hainerová et al., 2014). This suggests that boys may develop IGT at a lower
level of IR, suggesting boys to be an important target population for interventions to improve IS and glucose tolerance.

2.7.2. Age and pubertal status

The presence of a physiological increase in IR during puberty was first described by Amiel and colleagues (1986). This study sampled 16 non-diabetic children ranging from Tanner stage I to IV. Using the HEC technique, the researchers identified that pubertal children had higher IR than adults and prepubertal children. This transient increase in IR during puberty has since been confirmed through cross-sectional and longitudinal studies (Ball et al., 2006, Goran and Gower, 2001, Moran et al., 1999). The generally accepted understanding is that IR increases at the onset of puberty, peaks during mid-puberty, before recovering during the final stage of pubertal development (Goran and Gower, 2001) and is detailed in Figure 2.5. However, other studies have shown no difference in IR across Tanner stages and suggest that the changes in IR during development are mediated by changes in body mass and composition, and not solely by pubertal development per se (Hoffman et al., 2000). Additionally, Jeffery and colleagues (2012) conducted a large longitudinal study following 307 healthy children from 5 to 14 y and found IR to increase as many as 4 y prior to any hormonal evidence of puberty, assessed by the first detection of the luteinizing hormone.

Despite inadequate knowledge regarding why a transient IR occurs during adolescence, and how it differs across the different stages of puberty, it is apparent
that the period of adolescence is associated with higher levels of IR. This has led to the suggestion that puberty may, in itself, be a risk factor for the early development of T2D, especially in children who are already overweight or obese (Reinehr et al., 2015). Although, in most cases, this increased IR would not cause T2D, it could be suggested that the transient IR and associated elevated insulin levels may put greater demand on β-cell function and have negative impact on cell function later in adulthood (Metcalf et al., 2015). This may especially be the case for those with elevated insulin levels due to low levels of PA or increased adiposity.

Figure 2.5. Changes in insulin resistance and body fatness throughout the life course. Image from Zeitler and Nadeau (2009) with permission.
2.7.3. Body composition

As alluded to above, there is evidence of an association between IR and adiposity in children and adolescents (see figure 2.5). In a study comparing obese and lean children aged 9-11 y, obese children were found to present with higher IR (HOMA-IR) compared with lean children. Additionally, IR was positively correlated with total body fat. The study also reported that waist circumference and daily PA explained 49% of the variance in HOMA-IR (Krekoukia et al., 2007). In a sample of children and young adults (4-25 y) IR was found to be associated with higher fat mass, in particular high abdominal adiposity (Roemmich et al., 2002). Lee et al. (2012) assessed body composition and IS (HEC method) in 145 healthy black and white youth aged 8 to 17 y. Findings showed that increasing waist circumference and BMI percentile were significantly associated with lower IS and higher fasting insulin. Waist circumference, however, was found to explain greater variance in the metabolic profile than BMI percentiles, suggesting that the relationship between body composition and IR is related to (central) visceral adiposity. Taken together, these data from children and adolescents show that adiposity, in particular visceral adiposity is associated with IR. This relationship has been suggested to be due to excess lipid accumulation in the liver or through systemic inflammation and the production of inflammatory cytokines (Hardy et al., 2012).

2.8. Physical activity, insulin resistance and glucose tolerance in youth

In adults, clear evidence exists showing that regular engagement in PA can protect against the development of T2D (Shi et al., 2013) and CVD (Thompson et al., 2003). As alluded in Chapter 1, the relationship between PA and cardiometabolic risk is also
well established in children, with a graded dose-response relationship identified for PA and CVD risk factors as evidenced in a recent systematic review (Janssen and LeBlanc, 2010). Here a more detailed discussion of the evidence for the association between PA and insulin and glucose health outcomes in children and adolescents will be presented. The evidence from select cross-sectional (Table 2.3) and longitudinal (Table 2.4) studies will be discussed, before detailing the effects of exercise training interventions (Table 2.5). Gaps in the literature regarding PA, IS and glucose tolerance will be addressed.

2.8.1. Cross-sectional studies

Two publications from the European Youth Heart Study (Ekelund et al., 2007; Andersen et al., 2006) have shown independent associations between PA and metabolic risk in children and adolescents from Denmark, Estonia, and Portugal. Firstly, Andersen and colleagues (2006) reported a negative graded association between the clustering of CVD risk factors and PA status, in 1,732, 9 and 15 y olds. They also reported significant but weak associations between total MVPA and single risk factors including fasting glucose, insulin, triglycerides, and cholesterol. However, the strongest association was between MVPA and IR (r =-0.17, P=0.0001), after adjustment for both age and sex. The findings from Andersen et al., (2006) also suggest, based on a clustered z score including systolic blood pressure, triglyceride, cholesterol, IR, sum of skinfolds and aerobic fitness, that as much as 90 minutes of MVPA may be needed prevent IR in youth. This highlights that the current guidelines of 60 minutes of MVPA may be an underestimation of the amount of PA needed to optimise health benefits. In the same cohort, Ekelund et al. (2007) examined the cross-
sectional association of PA and cardiorespiratory fitness (CRF) on metabolic risk factors in 1,709, 9-10 and 15-16 y olds. Results showed an independent association of both PA and CRF on clustered metabolic risk (waist circumference, blood pressure, fasting glucose, insulin, triglyceride and HDL-cholesterol levels). Importantly the association of CRF with clustered risk was partly confounded by adiposity whereas the association with PA was independent, suggesting that CRF and PA affect cardiometabolic risk through different pathways.

In the multi-centre Cardiovascular Risk in Young Finns Study, Raitakari et al. (1997), reported the association with PA and risk factors for coronary heart disease in 2,358 individuals aged between 9 and 24 y. Comparison groups of different PA levels showed significantly lower insulin among physically active males when compared to their less active peers, but this was not evident in females. Interestingly, this sex-dependent effect has been confirmed by Imperatore and colleagues (2006) as part of the National Health and Nutrition Examination Survey. This study included 1,783 adolescents aged 12-19 y. Sex-specific multiple regressions controlling for age, race and BMI showed that in boys only, PA was positively and significantly associated with IS (QUICKI).

The narrative review by Berman et al. (2012) focused on the association of PA and IS, using a range of study designs including interventional, longitudinal and cross-sectional approaches. Seventeen cross-sectional studies were included with ages ranging from 5 to 19 y. Collectively, the studies indicated a significant positive
relationship between PA and IS, which was independent of adiposity. The review also found clear evidence for an association in adolescents, but data in prepubertal children were less clear. For example, using cross-sectional data from two longitudinal studies, no significant relationship was found between PA and IS in 5 and 8 y old children at baseline (Bunt et al., 2003, Telford et al., 2009). Additionally, data from the EarlyBird study, a longitudinal study of 307 children recruited at age 4.9 ± 0.3 y, showed no association between PA and IR at 8 y of age (Metcalf et al., 2009).

As current PA guidelines for health are based on performing at least 60 minutes of MVPA per day, the majority of studies investigate the relationship between MVPA and health outcomes. However, recent evidence suggests that grouping moderate (MPA) and vigorous (VPA) PA into a single exposure variable of MVPA may be misleading. For example, a cross-sectional study on 605 youth aged between 9 and 17 y, reported that cardiometabolic risk clustering reduced in a dose-dependent manner with increasing levels of VPA. This was not the case for light or moderate PA (Hay et al., 2012). Although this study does not report glucose or insulin outcomes, it highlights the importance of VPA for preventing cardiometabolic health in youth. This has also been confirmed within a review paper (Berman et al., 2012), where 10 studies analysed the association of PA by intensity; 100% of the reported studies found that VPA was independently associated with insulin indices, whereas only 83% and 50% found associations with MVPA and MPA respectively.
The reported research highlights that increasing PA among youth is important for IS, but the effect may be stronger in adolescents compared to children. This section also highlights the importance of exercise intensity, with VPA superior to that of light or moderate PA for improving cardiometabolic health outcomes, but specific evidence for insulin and glucose is currently lacking.
Table 2.3. Cross-sectional studies on the association between physical activity and glucose and insulin health outcomes in youth

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Age, y</th>
<th>Sex</th>
<th>Weight status</th>
<th>Measurement methods</th>
<th>PA</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ekelund et al. (2007)</td>
<td>1,709</td>
<td>9 and 15</td>
<td>F/M</td>
<td>All</td>
<td>FI, FG</td>
<td>Accelerometer</td>
<td>MVPA independently associated with FG and FI</td>
</tr>
<tr>
<td>Andersen et al. (2006)</td>
<td>1,732</td>
<td>9 and 5</td>
<td>F/M</td>
<td>All</td>
<td>FI, FG, HOMA-IR</td>
<td>Accelerometer</td>
<td>Significant but weak associated between MVPA and FI, FG and HOMA-IR</td>
</tr>
<tr>
<td>Imperatore et al. (2006)</td>
<td>1,783</td>
<td>15.4 ± 0.1</td>
<td>F/M</td>
<td>All</td>
<td>FI, FG, QUICKI</td>
<td>Questionnaire</td>
<td>MVPA positively associated with IS in boys only (r=0.14, P&lt;0.001)</td>
</tr>
<tr>
<td>Raitakari et al. (1997)</td>
<td>2,358</td>
<td>16.4 ± 5</td>
<td>F/M</td>
<td>All</td>
<td>FI</td>
<td>Questionnaire</td>
<td>FI significantly lower among physically active males</td>
</tr>
<tr>
<td>Schmitz et al. (2002)</td>
<td>357</td>
<td>10-16</td>
<td>F/M</td>
<td>All</td>
<td>FI, HEC</td>
<td>Questionnaire</td>
<td>MVPA correlated with FI (r=-0.17, P=0.03) and IS (r=-0.35, P=0.0001)</td>
</tr>
<tr>
<td>Hay et al. (2012)</td>
<td>609</td>
<td>9-17</td>
<td>F/M</td>
<td>All</td>
<td>N/A</td>
<td>Accelerometer</td>
<td>Only VPA consistently associated with cardiometabolic risk factors in youth (waist circumference, BMI, blood pressure)</td>
</tr>
</tbody>
</table>

2.8.2. Longitudinal studies

In contrast to cross-sectional studies, which are limited to a single measurement time point, longitudinal data provide novel insight into the relationship between PA and IS throughout childhood and adolescence. Several relevant studies have been previously reported in a review article (Berman et al., 2012) and this section will focus on pertinent papers that are summarised in Table 2.4.

Over a 5 y period, in a cohort of 90 boys and girls aged 5 y at baseline, Bunt et al. (2003) showed that PA decreased but that children with the smallest decrease in PA has the smallest decrease in IS independent of body mass and adiposity. These data therefore suggest that maintaining PA may have beneficial effects on IS in children. Similarly, one of the papers from the European Youth Heart Study reported a longitudinal decline in PA from age 9 to 15 y, with the decline in PA significantly associated with adverse changes in participants fasting insulin and HOMA-IR at 15 y after controlling for BMI and waist circumference (Jago et al., 2008). Finally, Telford et al. (2009) conducted a 2 y longitudinal study on a cohort of 498 children aged 8 -10 y. Results showed an increase in HOMA-IR from age 8-10 y which was only associated with PA in boys, with higher PA attenuating the increase in HOMA-IR. However, this study did not control for body mass or body composition within the analysis.

There has been interest into the independent effect of sedentary time on cardiometabolic health in children. A recent meta-analysis of pooled data from 14 studies, comprising data from 20,871 children (ages 4 - 18 y) found that MVPA was
significantly associated with cardiometabolic health outcomes (waist circumference, blood pressure, triglyceride, HDL-cholesterol and insulin) independent of age, sex, sedentary time and waist circumference. However, sedentary time was not associated with these health outcomes regardless of time spent in MVPA. This suggests that PA is more important for targeting health outcomes, including insulin.

In a 2 y prospective cohort study, Carson and colleagues (2014) examined the longitudinal associations between different PA intensities and cardiometabolic risk factors (BMI, waist circumference and systolic blood pressure) in 605 Canadian youth aged 9-17 y at baseline. Notably, differences in risk factors observed across quartiles of VPA were made within a very narrow range (1-8 minutes), compared to the much larger time differences in moderate intensity PA (30-75 minutes). Results from this study suggest that even very short bouts of VPA can result in protective effects on cardiometabolic risk, thus highlighting the potential for VPA to maximise health benefits in youth. Although this study did not specifically report glucose or insulin outcomes, it evidences the importance of performing VPA for modifying cardiometabolic health outcomes in youth.

As described in section 2.6.2, adolescence is associated with a transient increase in IR. Over two longitudinal studies, Metcalf and colleagues (2015, 2009) have recently used data from the EarlyBird study to show that the relationship between PA and HOMA-IR is dependent on age. In these studies, PA was measured annually from age 9 to 16 y in 300 children (151 boys). Metabolic health, including IR (HOMA-IR), was
assessed concurrently. Results showed that the age-related change in IR from 9 to 16 followed an inverted U-shape, with IR being similar at age 9 and 16 y and peaking at age 12-13 y. When split into “more physically active” and “less physically active” groups, the peak of IR was lower in the “more active” group, with a 26.2% lower IR at age 12.5 y. This difference was also reduced by ~7% when body fat was controlled for. This group difference was diminished either side of 12.5 y, and by 16 y of age the difference between the “more active” and “less active” group was only 1.4%. Combined with the findings from Telford et al. (2009) and Bunt et al. (2003), where no relationship was observed between PA and IS at 5 and 8 y respectively, this shows that the relationship between PA and IR can change throughout childhood and adolescence, with attenuated effects of PA during childhood and late adolescents compared to early adolescence.

Collectively, section 2.8 has provided evidence for a decline in PA levels from early to late childhood, and that this decline is associated with adverse changes in IS. This highlights the importance of PA to promote cardiometabolic health in children. Two further studies (Carson et al., 2014, Hay et al., 2012), although not specifically reporting insulin and glucose outcomes, suggest that very short bouts of VPA can result in protective effects on cardiometabolic risk, thus highlighting the potential for VPA to maximise health benefits in youth. Additionally, the current evidence base suggests the effects of PA on insulin and glucose health outcomes may be mediated by age, which is of importance considering current PA recommendations for health group children aged between 5-18 y under the same guidelines.
<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Age, y</th>
<th>Sex</th>
<th>Weight status</th>
<th>Measurement methods</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunt et al. (2003)</td>
<td>90</td>
<td>5-10</td>
<td>F/M</td>
<td>All</td>
<td>Questionnaire</td>
<td>Decrease PA between 5 to 10 associated with increased IR (r=-0.22, P=0.04)</td>
</tr>
<tr>
<td>Jago et al. (2008)</td>
<td>384</td>
<td>9-15</td>
<td>F/M</td>
<td>All</td>
<td>Accelerometer</td>
<td>Change in PA negatively associated with fasting insulin (z=-2.47, p=0.014) and HOMA-IR (z=-2.31, P=0.021)</td>
</tr>
<tr>
<td>Metcalf et al. (2009)</td>
<td>213</td>
<td>5-8</td>
<td>F/M</td>
<td>All</td>
<td>Accelerometer</td>
<td>No significant association between MVPA and HOMA-IR during the 3 y period</td>
</tr>
<tr>
<td>Metcalf et al. (2015)</td>
<td>300</td>
<td>9-16</td>
<td>F/M</td>
<td>All</td>
<td>Accelerometer</td>
<td>Peak IR 17% lower in active adolescents independent of body fat and pubertal status. At 16 y no relationship between PA and IR</td>
</tr>
<tr>
<td>Telford et al. (2009)</td>
<td>498</td>
<td>8-10</td>
<td>F/M</td>
<td>All</td>
<td>Pedometer</td>
<td>Longitudinal relationship between HOMA-IR and PA in boys X-sectional: no sig relationship with PA and IS at baseline</td>
</tr>
<tr>
<td>(Carson et al., 2014)</td>
<td>315</td>
<td>12-14</td>
<td>F/M</td>
<td>N/A</td>
<td>Accelerometer</td>
<td>Time spent in VPA at 12 y associated with decrease BMI and BP at 2 y follow up.</td>
</tr>
</tbody>
</table>

PA: physical activity; FI: fasting insulin; FG: fasting glucose; IS: insulin sensitivity; IR: insulin resistance; HOMA-IR: homeostatic model assessment of insulin resistance; MVPA: moderate to vigorous physical activity
2.9. Exercise training studies

Given the importance of PA, research has focused on exercise interventions to increase PA levels and improve cardiometabolic health outcomes. This section provides evidence, as summarised in Table 2.5, for the effects of exercise training interventions on IS and glucose tolerance in children and adolescents.

A recent meta-analysis (including studies of all weight status') examined the effects of exercise training on fasting insulin (24 studies) and IR (15 studies) in youth was conducted by (Fedewa et al., 2014). Based on cumulative results from all 24 studies, this meta-analysis showed a small to moderate effect of exercise training on fasting insulin (effect size \( \text{ES}=0.48 \)) and improvements to IR (\( \text{ES}=0.31 \)), which was consistent across sex, race and age. However, the majority of available evidence for this meta-analysis was based on studies in overweight/obese youth, which questions the application of such findings to healthy children and adolescents. As the current thesis will focus on healthy children and adolescents, related studies will be outlined below.

Gutin et al. (2011), investigated the effects of a 10 month exercise intervention on cardiometabolic biomarkers including fasting insulin and glucose. Participants (n=142) were 8-12 y old black girls. 21% were overweight and 31% obese. Participants were allocated to the 10 month intervention (n=118) or control group (no intervention, n=83). The intervention consisted of a total of 80 minutes of exercise split into: 25 minutes of skill development, 35 minutes of MVPA and 20 minutes of toning and stretching. The
10 month intervention led to beneficial changes in body composition (% body fat, BMI and waist circumference) as well as total cholesterol and low density lipoprotein (LDL) Cholesterol, but no change to fasting glucose or insulin (Gutin et al., 2011). In another intervention designed to increase PA levels, Macais-Cervantes and colleagues (2009) assessed fasting glucose and insulin and HOMA-IR in 6-9 y old boys and girls. Seventy-four participants were split between a control (n=36) and intervention (n=38) group. Participants who had high PA levels at baseline were excluded (based of PA tertiles for the whole group). Children in the intervention group were simply instructed to increase their PA, with the objective of increasing their steps by 2,500 per day. The intervention group improved PA levels (increase an average 4,581 steps/day), and observed a decrease in HOMA-IR (mean from 2.99 to 2.11) and fasting insulin (mean from 86.1 to 57.6 pmol/L). However, there was no change to fasting glucose. Both of the above studies are limited by reporting only a fasting assessment of IR, which has been suggested to only represent hepatic IS, and thus may have missed the beneficial effects of PA on peripheral IS (Holloszy, 2005).

Van der Heijden et al. (2009) reported the effects of a controlled aerobic exercise programme on both hepatic and peripheral IS in a mixed sex group of 29, sedentary adolescents (Tanner stages IV-V, 14 lean, 15 obese). The exercise programme consisted of 12 weeks of training with two supervised sessions per week of 30 minutes / week at 70% heart rate maximum. Body fat and lean mass were measured by dual-energy x-ray absorptiometry. The exercise programme increased peripheral IS (IVGTT) in both the lean and obese participants (35% and 59% respectively) and hepatic IS (hepatic insulin sensitivity index) was improved in both groups (19% and
23% respectively). In the lean participants, body mass significantly increased (0.8 ± 0.4 kg) due to changes in lean body mass, and in obese participants body mass did not change. However lean mass significantly increased and fat mass decreased to the same extent. Changes to IS observed in the lean group were without changes to fat mass, suggesting that the beneficial effects of exercise are independent of changes in body composition.

These three studies show that limited evidence exists in relation to assessing the effectiveness of exercise training interventions on IS in youth, especially those who are of a healthy weight.

**2.9.1. High-intensity interval exercise training**

The evidence presented in section 2.8 and so far in section 2.9 has shown the beneficial effects of PA on cardiometabolic risk factors, supporting the current guidelines of performing at least 60 minutes of MVPA per day. Albeit, evidence from the European Youth Heart Study suggests that these guidelines may not be sufficient to optimise the risk factor profile in youth (Andersen et al., 2006). Additionally, the majority of children and adolescents are not reaching the current PA guidelines for health (Towsend et al., 2015). Furthermore, a recent meta-analysis of exercise and PA interventions (Metcalf et al., 2012) stated that such interventions are largely ineffective, only increasing levels of MVPA by ~ 4 minutes per day. However, it is proposed that VPA may be more important than MVPA in terms of promoting
cardiometabolic risk and as little as 8 minutes of VPA may be sufficient to elicit health benefits.

Recently, performing HIIE has been used as an approach to increase levels of VPA in children and adolescents. HIIE alters between short periods (4 s to 4 minutes) of high-intensity exercise (ranging from ~ 85% to 250% of $\dot{V}O_2$ max, where the latter is considered ‘all out’) interspersed with periods of light recovery (Buchheit and Laursen, 2013). HIIE training has been shown to be an effective alternative to traditional endurance training to improve aerobic fitness (Milanović et al., 2015) and cardiometabolic health (Batacan et al., 2016) outcomes in adults. The interest in this modality of exercise on glucose and insulin health outcomes in children and adolescents has stemmed from its observed benefits in adults, where, for example HIIE improved health outcomes including IS (Babraj et al, 2009) and has also been suggested to be superior to MIE (Rynders et al 2013). Additionally HIIE has been suggested to be an efficacious approach to increasing VPA levels in children as the intermittent nature of HIIE matches their habitual PA patterns (Riddoch et al, 2007, Trost et al, 2002). Evidence also suggests that children are more motivated to perform interval type exercise over continuous exercise (Barkley et al., 2009), further reinforcing the potential of HIIE to increase VPA and improve health outcomes in youth.

The evidence base for HIIE training and cardiometabolic health outcomes in adolescents has recently been synthesised in a systematic review by Costigan et al
(2015), who showed the effects of HIIE training on CRF and body composition to be large and moderate respectively. Furthermore, a narrative review from Logan et al (2014) presented evidence supporting HIIE training as an efficacious exercise modality to improve metabolic health in adolescents. These reviews collectively suggest HIIE training to be a feasible and time efficient approach to improve cardiometabolic health in children and adolescents. This section will, however, critically discuss studies which have focused on HIIE and glucose and insulin outcomes and are summarised in Table 2.5.

Racil et al. (2013) reported improvements to HOMA-IR of -29.2 ± 5.3% after 12 weeks of HIIE training in obese adolescent females (15.9 ± 03 y) alongside reductions in body mass, waist circumference and percent body fat. The HIIE training consisted of 2 sets of 6-8 x 30 s running sprints (at 100-110% of the running speed corresponding to their maximal oxygen uptake ($\dot{V}O_2$ max)) with 30 s active recovery between each repetition and 4 minutes static recovery between sets. Training took place 3 times per week on non-consecutive days. Additionally, Corte de Araujo and colleagues (2012) compared 12 weeks of endurance training (ET) (30 to 60-minute continuous exercise at 80% of the peak heart rate) and HIIE training (3 to 6 repetitions of 60 s sprints at 100% of peak running speed, interspersed by 3 minutes active recovery at 50% of maximum running speed) in obese females (10.7 ± 0.7 y). Both training types improved fasting insulin (ET- 29.4%; HIIE- training- 30.5%) and HOMA-IR (ET- 42.8%; HIIE training- 37.0%) compared with pre-training. Body mass was significantly reduced in the HIIE training group (2.6%), but not in the ET group (1.2%), although the authors did not report any relationship between the changes in body mass and improved metabolic
outcomes. These studies collectively show the benefits of HIIE training, which appear comparable to traditional type endurance exercise, at least in in obese youth. Both these studies are limited, however, in that they focus on overweight/obese youth. Additionally they only report fasting glucose and insulin outcomes, which limits observation of improvements after exercise training to that of hepatic IS, despite knowledge that exercise may primarily affect peripheral IS (Holloszy, 2005).

As well as in overweight / obese groups, the effects of HIIE training have also been extended to general groups of school-aged adolescents. In a cohort of adolescents (64 boys, 25 girls; aged; 16.7 ± 0.6 y, overweight n=20, healthy weight n=69), Buchan et al. (2013) reported no change to fasting insulin or glucose variables after 7 weeks of training (4-6 x maximal sprint running with 30 s active recovery, 3 x per week) when compared to the control group. Additionally, there were no changes to other biochemical markers of CVD risk (total cholesterol, HDL-Cholesterol, LDL-Cholesterol, high-sensitivity C-reactive protein, interleukin-6, adiponectin and triglycerides). In an earlier study by the same group, MIE training (15 boys, 2 girls) (~ 20 minutes of continuous running at ~ 70% of $\dot{V}O_2$ max) decreased fasting insulin, but no changes were observed following HIIE training (13 boys, 4 girls) (4-6 x maximal sprint running with 30 s active recovery, 3 x per week) (Buchan et al., 2011). This suggests that MIE may be superior at reducing fasting insulin compared to HIIE. In a more recent study, Weston et al. (2016) reported no change to non-fasted glucose after a 10 week school–based HIIE intervention. This intervention was conducted with a mixed sex group of 101 adolescents (14.0 ± 0.2 y), consisting of 4-7 repetitions of 45 maximal effort exercise with 90 s recovery 3 times per week. Finally, recent work has
investigated the dose response of HIIE training on cardiometabolic health outcomes (Logan et al., 2016). In this study, 26 low PA male adolescents (age 16 ± 1 y) were assigned into 5 treatment groups performing different doses of HIIE training over an 8 week period. Groups 1-5 reflected the number of sets of HIIE performed in each of the twice weekly sessions. One set consisted of 4 x 20 s “all out” exercise with 10 s rest with 2 minutes in between each set. For example, group five completed 5 sets of 4 x 20 s “all out” exercise with 10 s rest. The mode of exercise was chosen by the participants and consisted of rowing, cycling, treadmill running, cross-training, shuttle runs, repeated box jumps, or non-contact boxing. In this study changes in glucose, insulin and HOMA-IR were non-significant and showed no clear dose response to the different HIIE groups. However there were significant improvements to visceral fat mass (~10%), and $\dot{V}O_2\text{max}$ (~6%), but no clear HIIE dose response.

The limited evidence base for HIIE training in youth shows that HIIE training may be more effective if targeted at an ‘at risk’ population, with interventions in overweight / obese groups showing significant changes to glucose and insulin outcomes which were not observed in the healthy groups. However, the evidence presented here is limited as few studies have examined HIIE training interventions in adolescents not specifically recruited for being overweight/obese. Finally, the studies discussed are limited in their inclusion of only fasting measures of insulin, glucose and IS, and not including dynamic (e.g. OGGT) assessment of IS. Fasting and dynamic tests reflect different type of IS (peripheral and hepatic) highlighting that the measurement of both, where possible, is important in order to understand the benefits of PA on IS.
### Table 2.5. Physical activity and exercise training intervention on glucose and insulin health outcomes in youth

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Participant characteristics</th>
<th>Measurement methods</th>
<th>Intervention</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gutin et al. (2011)</td>
<td>142</td>
<td>9.5 ± 0.9 F All FG, FI</td>
<td>FG, FI</td>
<td>10 months 5 x week 80 minutes, 25 minutes skill, 35 minutes VPA and 20 minutes “toning” and stretching, HR above 150 bpm during VPA</td>
<td>No effect of HIIE on FG (P=0.63) and FI (P=0.69)</td>
</tr>
<tr>
<td>Macias-Cervantes et al. (2009)</td>
<td>76</td>
<td>7.5 F/M All FG, FI, and HOMA-IR</td>
<td>Instructed to increase baseline PA by 2,500 steps</td>
<td>Decrease in FI and HOMA-IR in intervention group but not in control.</td>
<td></td>
</tr>
<tr>
<td>Van der Heijden et al. (2009)</td>
<td>29</td>
<td>15.1 ± 0.3 F/M Lean (n=14) Obese (n=15)</td>
<td>IVGTT (minMod)</td>
<td>3 months 2 x week 30 minutes 70% HR peak</td>
<td>Peripheral (35% and 59%) and hepatic (19% and 23%) IS increased in both lean and obese after exercise programme.</td>
</tr>
<tr>
<td>Buchan et al. (2013)</td>
<td>64</td>
<td>16.7 ± 0.6 F/M All FG and FI</td>
<td>HIIE: 4-6 30-s max sprint running 30s active recovery</td>
<td>No change in FG (P=0.52) or FI (P=0.24) between HIIE and CON</td>
<td></td>
</tr>
<tr>
<td>De Araujo et al. (2012)</td>
<td>30</td>
<td>10.5 ± 0.8 F/M Obese FG and FI and HOMA-IR</td>
<td>3 months 2/week HIIE: 3-6 sets of 60-s at 100% peak velocity (3 minutes active recovery) MOD: 30-60 minutes at 80% peak HR</td>
<td>FI and HOMA-IR lower for both groups post intervention P&lt;0.01. FG unchanged</td>
<td></td>
</tr>
</tbody>
</table>

Continued overleaf
<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Age, y (Mean ±SD)</th>
<th>Sex</th>
<th>Weight status</th>
<th>IS/IR</th>
<th>Intervention</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racil et al. (2013)</td>
<td>34</td>
<td>15.9 ± 0.3</td>
<td>F</td>
<td>Obese</td>
<td>FG and FI and HOMA-IR</td>
<td>3 month 2/week</td>
<td>HIIE: 2 x (6-8 x 30s 100-110% maximal aerobic speed 30s active recovery MIIE: 2 x (6-8 x 30s 70-80% maximal aerobic speed 30s active recovery</td>
</tr>
<tr>
<td>Weston et al. (2016)</td>
<td>101</td>
<td>14.0 ± 0.3</td>
<td>M/F</td>
<td>All</td>
<td>Glucose (non fasting)</td>
<td>10 week: 3 x sessions/week 4-7 reps of 45s max effort activity with 90s recovery</td>
<td>No significant changes to glucose</td>
</tr>
<tr>
<td>Logan et al. (2016)</td>
<td>26</td>
<td>16 ± 1</td>
<td>M</td>
<td>Low active</td>
<td>FG and FI and HOMA%S</td>
<td>8 weeks, 3 x sessions per week (2 x HIIE, 1 x resistance). 5 treatment groups reflecting number of HIIE sets completed during each session. 1 set = 4 x 20s &quot;all out&quot; with 10s recovery</td>
<td>No significant changes to FI, FG or HOMA%S in group and no quadratic trend for dose response.</td>
</tr>
</tbody>
</table>

2.10. Acute exercise, insulin sensitivity and glucose tolerance

In adults, improvements in IS can be seen after just a single session of exercise, highlighting the rapid accrual of benefit, which can persist for ~ 48 h post exercise (Mikines et al., 1988, Koopman et al., 2005). It has also been suggested that the metabolic benefits of training are due to the most recent bout of exercise rather than a chronic adaptation of training per se (Devlin et al., 1987). Knowledge of the potential for a single session of exercise to alter IS and glucose tolerance in youth is important in order to make recommendations on the frequency and intensity of exercise to promote health benefits. In adults an acute bout of HIIE has been shown to improve health outcomes including IS (Babraj et al, 2009). Additionally, HIIE may afford superior improvements in IS and glucose tolerance compared to MIE (Rynders et al, 2013). However, studies assessing the acute effects of a single exercise bout on IS and glucose tolerance in youth are sparse compared to exercise training studies. Details of the limited studies reporting the effects of acute exercise on glucose and insulin health outcomes in children and adolescents are summarised in Table 2.6 and will be discussed below.

In both non-overweight and overweight girls aged between 9-14 y, Zakrzewski and Tolfrey (2012) found that a single bout of MIE (~500 kcal) at 50% of participants $\dot{V}O_2$ max did not significantly increased HOMA-IR the following day (~16 h), but did improve fasting insulin and total area under curve (tAUC) insulin in response to a high glycaemic index breakfast. However, IR was measured in the fasting state in this study using the HOMA method which is known to reflect hepatic IS, and may overlook any improvements to peripheral IS after acute exercise (Muniyappa et al., 2015).
Additionally, it cannot be overlooked that any acute benefits from the exercise may have been negated by ~16 h post exercise.

In contrast, IS has been assessed using estimates (c-peptide minimal model) from a dynamic MMTT (consisting of a chocolate milkshake made from milk powder, milk, cream and chocolate syrup; 2,803 kJ, 45/40/15% of energy from CHO/fat/protein, respectively) after a single bout of MIE lasting 45 minutes at 75% of peak heart rate in adolescents selected based on low levels of PA and aerobic fitness (Short et al., 2013). IS was shown to be augmented by 78% 40 minutes after MIE. A novel feature of this study was that an additional assessment of IS was made ~ 17 h after exercise and showed a 45% improvement (Short et al., 2013). Therefore, when compared to the work of Zakrzewski and Tolfrey (2012), the different findings may be due to the dynamic nature of the assessment method (fasted vs. MMTT) reflecting a peripheral measure of IS, or the pre-selection of participants based on low levels of habitual PA and cardiorespiratory fitness. However, the study conducted by Short et al. (2012) does not include a comparison with other exercise intensities, meaning the optimal intensity of exercise required to improve glucose and insulin in youth is still unknown.

The research reported above shows there is evidence to suggest that acute MIE may have beneficial effects on IS and glucose tolerance in youth. However, little is currently known about how these potential benefits are influenced by exercise intensity, and specifically the use of HIIE protocols. The evidence base for the effect of acute HIIE on glucose and insulin health outcomes is restricted to studies employing a high fat
meal (HFM) challenge to measure postprandial lipaemia (Bond et al., 2015b, Bond et al., 2015d, Thackray et al., 2013, Tolfrey et al., 2014). Several of these studies also make comparison with a bout of MIE and will be discussed below.

Bond and colleagues (2015d) found no acute changes in the area under curve (AUC) glucose response to a HFM (~ 1.50 g of fat (70% total energy), 1.20 g CHO (25%) and 0.21 g of protein (5%) per kg of body mass) following HIIE (8 x 1 minute bouts of cycling at 90% of the participants peak power (PP)) and MIE (90% of gas exchange threshold (GET) for a duration work matched to HIIE) in adolescent boys and girls (14.3 ± 0.3 y). However when the same HIIE exercise was spread throughout the course of a day (i.e. HIIE: 4 x 2 x 1 minute cycling at 90% peak power), significant reductions in the tAUC glucose response to two HFM challenges were observed but not in response to a work-matched condition of MIE in adolescent males and females (Bond et al., 2015b). This suggests that changes may be intensity dependent, when exercise is spread throughout the course of the day. Similarly, Thackary and colleagues (2013) found no effect of 10 x 1 minute running at 100% of max aerobic running speed on fasting glucose, fasting insulin, and their AUC responses to a HFM (consisting of croissants, chocolate spread, whole milk, double cream, and milkshake powder, providing 1.5 g fat (60% of total energy), 1.8 g CHO (33%), 0.4 g protein (7%), and 93 kJ energy per kg body mass) the day following exercise in 15 boys (11.8 ± 0.4 y). Finally, neither vigorous (6 x 10 minutes at 70% \( \dot{V}O_2 \) max) or moderate (6 x 10 minutes at 53% \( \dot{V}O_2 \) max) intensity running, were shown to affect fasting glucose or AUC glucose in response to a HFM (providing 1.5 g of fat (70% of total energy), 1.22
g of CHO (25%) and 0.22 g of protein (5%) the day after exercise in 8 health adolescent (13.0 ± 0.3 y) boys (Tolfrey et al., 2008).

This section highlights clear limitations with regard to the current evidence base surrounding acute exercise, specifically HIIE, on changes to IS and glucose tolerance in children and adolescents. Current studies are limited to protocols assessing only MIE, or protocols where HIIE is assessed but in response to a HFM not allowing assessment of IS. The benefits of MIE have been shown to persist up to ~17 h post exercise (Short et al., 2013) but no studies have reported this for HIIE, meaning it is unknown whether improvements to IS after exercise is intensity dependant. Studies directly comparing HIIE to MIE are also needed to isolate the effect of exercise intensity thus providing evidence of optimal intensity of exercise to improve IS and glucose tolerance in youth. Additionally there is a clear lack of evidence surround the effects of acute exercise in younger prepubertal children, which based on previously reviewed observational data (sections 2.8.1 and 2.8.2), may have a limited scope to improve IS and glucose tolerance following exercise (Metcalf et al., 2015, Metcalf et al., 2009).
**Table 2.6. Acute exercise and glucose and insulin health outcomes in youth**

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Participant characteristics</th>
<th>Measurement methods</th>
<th>Intervention</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short et al. (2013)</td>
<td>12</td>
<td>Age, y (Mean ±SD)</td>
<td>F/M</td>
<td>Low PA</td>
<td>OGTT (MinMod)</td>
</tr>
<tr>
<td>Thackray et al. (2013)</td>
<td>15</td>
<td>11.8 ± 0.4</td>
<td>M</td>
<td>Healthy/active</td>
<td>FI, FG, HFM</td>
</tr>
<tr>
<td>Tolfrey et al. (2008)</td>
<td>8</td>
<td>13 ± 0.3</td>
<td>M</td>
<td>Healthy</td>
<td>FG, HFM</td>
</tr>
<tr>
<td>Bond. et al. (2015d)</td>
<td>20</td>
<td>14.3 ± 0.3</td>
<td>F/M</td>
<td>Healthy</td>
<td>AUC glucose, HFM</td>
</tr>
<tr>
<td>Bond et al. (2015b)</td>
<td>19</td>
<td>13.7 ± 0.4</td>
<td>F/M</td>
<td>Healthy</td>
<td>AUC glucose, HFM</td>
</tr>
<tr>
<td>Zakrzewski and Tolfrey (2012)</td>
<td>27 (12 OW)</td>
<td>12 ± 1</td>
<td>F</td>
<td>Overweight and healthy</td>
<td>FG, FI, HOMA-IR, 500 kcal of treadmill exercise at Fat max</td>
</tr>
</tbody>
</table>

2.11. **Acute exercise and postprandial fat oxidation**

Fat oxidation is thought to be related in IS (Samuel et al., 2010), and also has implications for exercise induced fat loss (Barwell et al., 2009). There is a growing body of literature on the effects of acute exercise on fat oxidation in youth. For example, Zakrzewski and Tolfrey (2012) found a single bout of MIE (~ 500 kcal) at 50% of participants V0₂ max) to increase fat oxidation, the day following exercise in normal weight girls. Additionally, Bond. et al. (2015d) found an increase in fat oxidation following acute bouts of both HIIE (23%) and MIE (16%), suggesting a possible intensity dependent change. Despite clear evidence of the effects of both MIE and HIIE on improvements to fat oxidation in youth the relationship of changes to insulin and fat oxidation following both MIE and HIIE and yet to be investigated in youth.

2.12. **Type one diabetes and cardiovascular risk**

In the UK, the incidence of paediatric T1D is recorded as the 5th highest in the world (Patterson et al., 2009). T1D is associated with a heightened risk of early atherosclerotic complications, including a 4 - 8 fold increased risk of coronary heart disease when compared to the general population (Swerdlow and Jones, 1996). Furthermore, it has been shown that the development of atherosclerosis begins during childhood, with an asymptomatic increase in the intima-media thickness of the common carotid artery being reported in children with T1D who died of ‘unnatural death’ (Järvisalo et al., 2002). Recently, a study investigating the prevalence of CVD risk factors within a cohort of 27,358 children and adolescents with T1D found that 69% of patients had ≥ 1 CVD risk factors (Schwab et al., 2006). Additionally, a study
investigated ideal cardiovascular health, consisting of seven modifiable health factors / behaviours (smoking, BMI, PA, cholesterol, blood pressure, and HbA1c) and their association with measures of arterial structure and function (Alman et al., 2014). This study included 190 adolescents with T1D, and reported that the number of ideal cardiovascular health factors was significantly associated with lower arterial stiffness and improved brachial artery distensibility. Notably, no participants in the study met all seven modifiable factors for ideal cardiovascular health, thereby highlighting the importance of targeting this population with interventions to minimise the risk of future CVD.

One important modifiable risk factor related to CVD risk in T1D is glycaemic control (Lind et al., 2014). An increment of 1 unit (%) of HbA1c has been shown to increase the risk of CVD mortality by 52.5% in patients with T1D (Juutilainen et al., 2008). The prevalence of poor glycaemic control in youth with T1D has been reported in a number of cross-sectional studies ranging from 60% to 91% (Steigleder-Schweiger et al., 2012, Margeirsdottir et al., 2008, Tulloch-Reid et al., 2009) making it a key target for interventions to improve CVD risk.

2.12.1. Physical activity in children with type one diabetes

Much like non-diabetic children and adolescents, it is important for children with T1D to take part in PA. Not only is PA important for the management of diabetes related complications, but it is also an important part of other aspects of development, such as the maintenance of bone mass and body composition (Roberts and Taplin, 2015).
The therapeutic effects of PA in the management of CVD risk in children with T1D have recently been reviewed (Tully et al., 2016). For example, Herbst and colleagues (2007) reported longitudinal data on 23,251 children and adolescents (3-18 y) across 209 centres situated in Germany and Austria. Risk factors for CVD included plasma lipids, blood pressure and HbA1c. PA data were self-reported. Results from this study highlighted that increased PA was associated with a beneficial CVD risk profile, including lower lipoprotein levels, diastolic blood pressure and glycaemic control. Similarly, Bernardini and colleagues (2004) found that children who self-reported their PA as less than 60 minutes per week had significantly higher HbA1c (8.9 ± 0.5) than children performing 120-360 (8.3 ± 0.4%) or 360-480 (7.4 ± 0.6%) minutes of PA per week. Additionally, Seeger and colleagues (2011) found significant improvements to brachial artery endothelial function after an 18-week exercise training programme in 7 children (10.9 ± 1.5 y). The programme consisted of running twice per week, consisting of one session of 30 minute group based activities (e.g. ball games) and one session involving 30 minutes interval running. The benefits of exercise training as a therapeutic tool to improve CVD risk in adolescents with T1D has also been investigated in a group of 196 adolescents. Participants were randomly assigned to either a control group (no exercise training), or training groups of 1 session/week or 3 sessions/week, for a 6 month period. The exercise training programme improved glycaemic control from 8.9% to 8.1% (Salem et al., 2010), with the authors suggesting that exercise is an indispensable tool for the management of children with T1D.

In a recent systematic review of PA and sedentary behaviour interventions in youth with T1D, MacMillan et al. (2014) reported pooled data from 10 interventions reporting
HbA1c. The meta-analysis showed an overall significant reduction in HbA1c of 0.85%, suggesting that PA can positively affect HbA1c in youth with T1D. However, this report also indicated a high degree of bias in all reported studies, with a key methodological limitation related to not reporting changes in insulin dose and diet, which are known to affect HbA1c. This review also highlighted the lack of research designed to investigate the “optimal” intensity and duration of exercise, meaning there is limited evidence of “what” should be encouraged for this patient group.

2.12.2. Levels of PA in children with T1D.

Due to the established benefits of PA in decreasing CVD risk, children and adolescents’ with T1D are currently encouraged by the ADA to undertake 30-60 minutes of MVPA per day (Silverstein et al., 2005). However, much like non-diabetic youth, many fail to achieve this target and data on the PA status of children with T1D is less comprehensive than in healthy children. In females with T1D (11 to 19 y), Schweiger and colleagues (2010) measured PA by self-report questionnaires. PA data were categorised as the number of days participants had accumulated 60 minutes of MVPA during a typical week. Results from this study showed that only 10 out of the 213 (4.7%) adolescent females met the recommended 60 minutes of MVPA per day. An additional study using objectively measured PA (Actigraph GT3X), found no difference in PA levels in children with T1D compared to their disease free siblings (27.6 ± 21.4 vs 20.1 ± 11.4, respectively), with both groups achieving less that the recommended 60 minutes of MVPA on a daily basis (Cuenca-Garcia et al., 2012). However, this study did not report the number of children achieving or not achieving the recommendations. Collectively, these studies highlight that much like non-diabetic
children and adolescents, many children with T1D are also not meeting PA guidelines for health regardless of the implications for their cardiovascular health and glycaemic control. As a result, there is a need to investigate other forms of exercise and the optimal type of exercise to improve health outcomes in youth with T1D.

**2.12.3. HIIE in children with type one diabetes**

Sections 2.11.1 and 2.11.2 highlight the importance of risk factor reduction in youth with T1D and the benefits of PA. However due to low levels of PA it is important to establish the “optimal” PA recommendations and different strategies to increase PA in this population. An approach which has gathered recent attention is HIIE. This type of exercise has been shown to promote cardiometabolic health and has potential superiority to MIE across a range of clinical populations (Tjonna et al., 2009, Jung et al., 2015). Specifically, it has been shown to improve glucose control in adults with pre-diabetes (Little et al., 2014) and T2D (Little et al., 2011, Gillen et al., 2012). In the study by Little et al. (2011), 6 sessions of HIIE training, consisting of 10 x 60 s at 90% of maximal heart rate with 60 s recovery, were performed over a two week period. The training intervention improved 24 h glucose concentration (as measured by a continuous glucose monitoring system (CGM)), and the postprandial response to a test meal in the 24 h following the final training session, suggesting that this type of exercise has the potential to improve glycaemic control in adults with T2D. From an acute exercise perspective, Gillen et al. (2012) investigated the acute effect of HIIE on 24 h blood glucose response, using CGM, in adults with T2D. Findings showed HIIE to reduce the 3 h postprandial glucose AUC, decrease time spent in hyperglycaemia and a trend for lower blood glucose over a 24 h period. These studies highlight the
potential benefits of performing HIIE on glycaemic control, at least in adults with T2D, but no studies have reported this in children and adolescents with T1D.

As well as the potential benefits of HIIE, the risk associated with this form of exercise should be considered. PA poses a risk of increased hypo- and hyper-glycaemic excursions, which are established barriers to exercise in patients with T1D (Brazeau et al., 2008). For example, following 60 minutes of MIE running, 83% of children with T1D experience a 25% drop in blood glucose from baseline, with 30% being classified as hypoglycaemic during or immediately following exercise, and 28% developing nocturnal hypoglycaemia (Tansey et al., 2006, Tsalikian et al., 2005). Knowledge of such excursions in blood glucose during and following exercise is crucial, as an important aspect of diabetes management is the adjustment of exogenous insulin and/or CHO intake prior, during or following exercise to maintain euglycaemia. However, changes in glycaemic control during and following a bout of HIIE in paediatric patients with T1D is currently unknown.

2.13. Summary and experimental aims

The importance of glucose and insulin in the aetiology of both CVD and T2D make it an important target for interventions to reduce the burden of these chronic conditions. This is particularly pertinent in males, due to the increased risk of CVD compared to females, as well as observations that despite lower levels of IR, boys have higher prevalence of IGT. With evidence to show that the natural history of these diseases begin in youth, it is logical to explore primary prevention strategies in childhood and
adolescence. Available data evidence that exercise can modulate IS in youth, however, the optimal intensity, frequency and duration to improve health outcomes are currently unknown. Given that few children and adolescents are meeting current PA recommendations for health, it is important to establish new approaches such as HIIE and contrast this against traditional approaches such as MIE. Current knowledge of the potential for exercise to improve IS is also limited from a methodological perspective, with most studies focusing on fasted measures which may not be sensitive to changes in peripheral IS following exercise. Also, how long potential benefits to IS persist after exercise and how the potential beneficial effects of exercise are dependent on age are poorly understood.

Given the points above, the primary purpose of this thesis is to undertake a number of novel experimental studies aimed to identify the effects of HIIE on glucose and insulin health outcomes in healthy boys. A second purpose is to investigate the acute effects of HIIE on glycaemic control in adolescents with T1D. The specific aims of each experimental chapter are provided below:

1. Chapter 4 presents the inter-method agreement and day to day reliability of commonly used fasting and postprandial measures of IS in youth.

2. Chapter 5 investigates the potential of HIIE to improve IS and glucose tolerance in adolescent boys in contrast to work-matched MIE, using indices shown to be reliable in Chapter 4. Furthermore, this chapter will establish the
effect of HIIE and MIE on resting fat oxidation, and whether this is related to changes in IS.

3. Chapter 6 builds upon the aims of Chapter 5 and looks to establish whether the effects of an acute bout of HIIE and work-matched MIE on IS and glucose tolerance are realised up to 48 h after exercise in adolescent boys. This chapter includes the use of both fasting and OGTT derived estimates of IS, which enables speculation into possible mechanisms for the effects of exercise on IS.

4. Chapter 7 replicates the aims of Chapter 5, however, this is conducted in a group of pre / peri-pubertal boys, to examine if the benefits of acute HIIE and work-matched MIE on IS and glucose tolerance can be observed in this group. This chapter also establishes the effect of HIIE and MIE on resting fat oxidation, and whether this is related to changes in IS.

5. Chapter 8 aims builds on Chapters 5 and 6 where acute HIIE was shown to improve IS and aims to establish whether two weeks of HIIE training can enhance IS in adolescent boys. Due to reliability data reported in Chapter 4 this chapter uses a range of fasting measures alongside glucose and insulin responses following a MMTT.
6. Finally, given the increased CVD risk in T1D, alongside the importance of glycaemic control in the management of the condition, Chapter 9 aims to assess the effectiveness and feasibility of an acute bout of HIIE to improve short-term glycaemic control in adolescents with T1D in contrast to MIE.
CHAPTER 3

General methods

This chapter will describe the methods used in the experimental Chapters presented in the thesis. The studies received ethics approval by either the Sport and Health Sciences Ethics Committee at the University of Exeter (Chapters 4-8) or the National Health Service Research Ethics Committee (Chapter 9).

3.1. Participant recruitment and inclusion/exclusion criteria

In Chapters 4-8 participants were recruited from local primary or secondary schools. After initial discussion with school contacts describing the research questions to be addressed and the associated study requirements, potential participants were initially approached using a class assembly to describe the research in more detail. After the assembly children were given the opportunity to ask questions, provided with a study information pack, and asked to discuss the project with their parents/guardians. Example parent and participant information sheets, child assent and parental consent forms are provided in Appendices 4-8. Children and adolescents interested in taking part in the project were asked to return the completed parental consent and child assent form and the health questionnaire to a contact within the school. Parents/guardians were also contacted to discuss any further questions or concerns they might have about their child's involvement in the project. Participants were recruited onto the study if they met the inclusion/exclusion criteria. Exclusion criteria included the presence of any known disease or contraindications to exercise, as well
as the use of medications that are known to influence glucose metabolism. Participants were recruited from different schools for Chapters 5-8. Participant data presented in Chapters 5-8 were combined for Chapter 4.

In Chapter 9, participants were recruited from the diabetes clinic at the Royal Devon and Exeter Hospital. Suitability to take parts was assessed using clinical information from routine hospital appointments in relation to the inclusion and exclusion criteria. Inclusion criteria for this study included, participants aged 12-17 y, a clinical diagnosis of T1D of more than 3 y duration, HbA1c between 53 mmol/mol (7.0%) and 97 mmol/mol (11.0%) and a BMI between the 5th and 95th centile for age and sex. Exclusion criteria were any episode of severe hypoglycaemia within the past 3 months, any other medical conditions which may influence glycaemic control or ability to undertake exercise.

3.2. Standardisation of testing conditions
During a scheduled familiarisation visit for each study, participants were given verbal instructions on the importance of controlling for diet, PA and the overnight fast, and the requirements for recording diet and PA. Participants and their parents/guardians were also given written instructions and reminders (e.g. phone calls, SMS text messages) before each experimental visit.
3.2.1. *Dietary intake*

In Chapters 4-7 and 9, participants recorded dietary intake for a 48 h period prior to each experimental condition, as well as during each 48 h experimental condition in Chapters 6 and 9. With supervision from their parents/guardians, participants were asked to record all food and drink they consumed using household measures (e.g. tablespoon, cupful, large bowl) or product measures (e.g. slice of white bread) and also include the cooking method where possible. After the first experimental visit, the food diaries were returned to participants and they were asked to replicate the same diet in the 48 h prior to the subsequent experimental visits. This method was preferred to using a prescribed diet in order to ease burden on participants and increase compliance with the protocol. In Chapter 9, participants also recorded dietary intake in the same manner, however this took place in the 48 h preceding and during the 48 h of each experimental condition. For each study, food diaries were assessed for total energy intake and macronutrient composition (absolute and relative) using commercially available software (Chapters 4-8: Comp Eat Pro, Nutrition Systems, UK, Chapter 9: Nutritics, Nutrictics LTD, Ireland). For all experimental studies, participants arrived at the laboratory in a fasted state and refrained from food and drink (other than water) from ~ 10 pm the night before each visit.

3.2.2. *Physical activity*

In Chapters 4-9 participants wore a triaxial accelerometer (GeneActiv GENEA, UK) on their non-dominant wrist for measurement of PA at 100 Hz. The accelerometers were selected to increase compliance due to their lightweight and waterproof characteristics. Participants were asked to continuously wear the device during the
day and night time. If the device was removed, participants were asked to make note of the type, intensity and duration of any PA undertaken in the food diary. Prior to each experimental condition participants were also instructed to avoid any organised PA. Data from the accelerometer were downloaded and converted into 60 s epochs using GENEActiv PC software (version 1.2.1). Using software specific macros and validated paediatric cut points for this device (Phillips et al., 2013), time spent sedentary and performing light, moderate and VPA were calculated. Minimum wear time for analysis was set at 10 h per day (Rich et al., 2013).

3.3. Anthropometry

In all experimental Chapters body mass and stature was measured to the nearest 0.1 kg and 0.01 m respectively, using established procedures (Nagy et al., 2008). In Chapters 4-8, percentage body fat was estimated using the subscapular and triceps skinfold sites, using validated age and sex specific equations (Slaughter et al., 1988). Skinfold thickness was measured to the nearest 0.2 mm on the right hand side of the body by the same investigator using skinfold callipers (Holtain Limited, UK). The median of three measurements of each site was used to estimate body fat. This skinfold method has been shown to be both valid and reliable for predicting % body fat in children and adolescents (Silva et al., 2013).

In Chapter 9 percentage of body fat was estimated using air displacement plethysmography (BodPod®, Body Composition System, Life Measurement Instruments, Concord, California, USA). The BodPod® is a double chamber unit, with
the chambers separated by a diaphragm which is electronically controlled and measures perturbations in pressurisation of the chamber for volumetric measurement. Prior to testing, the system was calibrated following the manufacturer’s guidelines and using a cylinder of known volume (49.887 L). After briefing on the procedure, and wearing appropriate clothing (a swimsuit and swim cap), participants sat still in the chamber to calculate body volume. This measurement took place twice, and if the difference between the first two measures was more than ± 75 mL, a third measurement was taken. The mean of the two closest measurements used for calculating body density. Lung volume was then estimated using age and sex specific prediction equations provided as part of the software, and used to estimate body composition using the Siri equation (Siri, 1993). The BodPod® has been shown to accurately predict visceral adipose tissue and total body fat in children (Winsley et al., 2005).

3.4. Pubertal status
Pubertal status was obtained by self-assessment according to the five stages (Tanner, 1962) of pubic hair development using adapted drawings (Morris and Udry, 1980). After verbal explanation from the primary investigator, the drawings were presented on an information sheet (see Appendix 9). The participant returned home with the form and circled the most appropriate number before signing and returning the form in a sealed envelope during their next visit to the laboratory. This method of assessment was chosen because of its simplicity and practicality, along with its widespread use in the paediatric literature.
3.5. Maximal oxygen uptake and gas exchange threshold

Using an electronically braked cycle ergometer (Lode Excaliber Sport, Groningen, Netherlands) a combined ramp incremental and supramaximal test to exhaustion was used to establish participants maximal oxygen uptake (\(\dot{V}O_2\) max) and GET in all experimental Chapters (Barker et al., 2011). Participants were initially briefed on the test protocol and instructed to maintain a self-selected cadence between 70 and 80 revolutions per minutes (rpm) throughout the test. The incremental ramp rate was set at 25 W·min\(^{-1}\) in Chapters 5-6 and 8-9 and at 7.5 W·min\(^{-1}\) in Chapter 7. Exhaustion was reached when participants failed to hold a cadence < 60 rpm for 5 consecutive seconds despite encouragement. After a 5 minute cool down at 20 W, participants received a 5-10 minute seated rest period before commencing the supramaximal validation exercise bout. During this test, participants cycled at 20 W for 2 minutes before a step transition to 105% of the peak power achieved during the initial incremental test. Participants were asked to keep cadence between 70-80 rpm throughout and continue to cycle until they were no longer able to hold a cadence < 60 rpm despite verbal encouragement. At exhaustion, the power output was lowered to 20 W and participants allowed to cool down for at least 5 minutes. This protocol has been validated for determining \(\dot{V}O_2\) max in children (Barker et al., 2011). When measured using a single ramp protocol, the measurement of \(\dot{V}O_2\) max in children has a CV of 4.1% (Welsman et al., 2005).

Pulmonary gas exchange (Cortex Metalyzer III B, Germany) and heart rate (Polar, Finland) were measured throughout the tests. The metabolic cart was calibrated before each measurement, using standard calibration gas (5% CO\(_2\), 17% O\(_2\), Cranlea,
Birmingham, UK) and a 3.0 L calibration syringe (Hans Rudolph, USA). Participants \( \dot{V}O_2 \) max was accepted as the highest 10 s average \( \dot{V}O_2 \) during the ramp or supra-maximal test (see Figure 3.1). The GET was estimated at the point where the first disproportionate increase in CO\(_2\) production compared to \( \dot{V}O_2 \) during the ramp incremental test. This method for determining GET has been shown to be reliable in a paediatric population (Fawkner et al., 2002).

![Figure 3.1](image)

**Figure 3.1.** Example oxygen uptake (\( \dot{V}O_2 \)) trace from a combined ramp and supramaximal test to exhaustion to determine \( \dot{V}O_2 \) max in a participant. \( \dot{V}O_2 \) max was taken as the highest 10 s average \( \dot{V}O_2 \) during either the ramp or the supramaximal component of the test. In the example above \( \dot{V}O_2 \) reached 3.42 L.min\(^{-1}\) during the ramp part of the test and 3.51 L.min\(^{-1}\) during the supramaximal bout. The \( \dot{V}O_2 \) max was therefore taken as 3.51 L.min\(^{-1}\) for this participant.

### 3.6. Exercise protocols

In Chapters 5-7 and 9, participants completed two exercise conditions in a counterbalanced order on a cycle ergometer (Lode Excalibur Sport, Groningen,
Netherlands). The conditions were HIIE and continuous MIE. The HIIE protocol consisted of 3 minute warm up cycling at 20 W (10 W in Chapter 7) followed by eight repeated bouts of 1 minutes cycling at 90% of the peak power determined during the ramp-incremental test, interspersed with 1.25 minutes recovery at 20 W (10 W in Chapter 7), followed by a 3 minute cool down at 20 W (10 W in Chapter 7). This HIIE protocol was implemented as it has been shown to be a practical and feasible in youth (Bond et al., 2015a, Bond et al., 2015b, Bond et al., 2015d). This reduced exertion HIIE protocol was chosen over the use of shorter 'sprint' based interval protocols (e.g. 6 s) due to concerns about participant acceptability and compliance. For example, it has been shown that only one third of participants were able to tolerate a 6 s sprint interval training exercise protocol in youth (Sedgwick et al., 2015).

The MIE exercise protocol was performed at an intensity equivalent to 90% of the previously determined GET for a duration which was set to match the work performed during the HIIE using the equation:

\[
kJ \text{ min}^{-1} = \frac{\text{power output} \times 60}{1000}
\]

\textbf{Equation 3.1.} Calculation of mechanical work done during cycling exercise

Work-matching was performed to isolate the effect of exercise intensity between the two exercise conditions. In Chapter 9 the MIE protocol, was performed for 30 minutes and not worked matched to reflect the lower limit of the ADA recommendation for exercise in children with T1D (American-Diabetes-Association, 2015)
Throughout the exercise protocols in Chapters 5-7 and 9, gas exchange (Cortex Metalyzer III B, Germany) and heart rate (Polar, Finland) were measured. Participants also provided a rating of perceived exertion (RPE) using a validated 1-10 scale (Yelling et al., 2002, see Appendix 11). RPE was recorded by the supervising researcher every 5 minutes during MIE and after each 1 minute interval during HIIE. The scale was explained to participants before the start of the exercise. Participants were asked to point to the step that reflected their perceived effort level. Within 5 minutes of exercise completion, exercise enjoyment was assessed using a 16 point physical activity enjoyments scales (PACES) validated for use in adolescents (Motl et al., 2001, see Appendix 10). This scale consists of 16 elements which begin with the stem “when I completed high intensity interval exercise / moderate intensity exercise…” The 16 bipolar statements on a 5-point Likert-type scale (1=”disagree a lot” to 5=”agree a lot”). Enjoyment was calculated with the 16 responses after 8 “negative” items were reversed.

In Chapter 8 participants completed two weeks of HIIE training in a school-based laboratory using a friction braked cycle ergometer (Monark 827e, Monark exercise AB, Sweden) with adjustments made to the handle bar and seat height for each participant. Three supervised HIIE sessions took place per week over a two week period. Each session started with a 3 minute warm up of unloaded pedalling, followed by 8-10 one minute intervals at 90% of the peak power achieved during the incremental ramp test. Each interval was interspersed with 1.25 minute of unloaded pedalling. Sessions 1 and 2 consisted of 8 x 1 minute bouts, sessions three and four 9 x 1 minute bouts and sessions five and six 10 x 1 minute bouts. Participants were asked to maintain a self-
selected cadence (70-95 rpm) given that the power output of the friction braked ergometer is dependant of cadence. Participants were reminded before each session of their selected cadence and this was monitored during all exercise sessions. The progression of the two week HIIE training was based on previously published studies (Whyte et al., 2010, Little et al., 2011).

3.7. Resting metabolic rate

In Chapters 5 and 7 resting metabolic rate (RMR) was estimated using indirect calorimetry. Expired air measurements were taken during 10 minutes of supine rest whilst wearing a mask covering mouth and nose (Silicone V2™ oral-nasal, Hans Rudolph Inc). Participants were asked to remain still throughout measurement and were observed throughout to ensure this. An online gas analysis system (Metalyzer 3B, Cortex, Leipzig, Germany) was used to determine the gas exchange data. Total energy expenditure (EE), and the rate of fat oxidation were estimated using the average $\dot{V}O_2$ and $\dot{V}CO_2$ over the 10 minute period using the calculations proposed by Frayn (1983):

$$\text{Fat oxidation (g·min}^{-1}) = (1.67 \times \dot{V}O_2 \text{ (L·min}^{-1}) - (1.67 \times \dot{V}CO_2 \text{ (L·min}^{-1})$$

$$\text{EE (kJ·min}^{-1}) = 4.1855 \times ((\text{fat (g·min}^{-1}) \times 9) + (\text{CHO (g·min}^{-1}) \times 4))$$

Equation 3.2. Calculation of energy expenditure and fat oxidation via indirect calorimetry

Form these calculations, the relative contribution of fat and CHO oxidation to the total energy expenditure was calculated using the Atwater factors of 9 kcal·gram and 4 kcal·gram, respectively.
Although the ventilated hood system and longer measurement periods are recommended for the assessment of RMR, the use of long protocols (e.g. 30 minutes) in children has been shown to increase restlessness and decrease the reliability of measurements (Ventham and Reilly, 1999). In the absence of access to the ventilated hood system, the use of the mask based system for the assessment of RMR has been deemed acceptable for use in children (Mellecker and McManus, 2009). Additionally, the measurement of RMR over a 10 minute period has been shown to be no different from a 30 minute measurement period (Mellecker and McManus, 2009). Pooled data from Chapters 5 and 7 demonstrate a between-day CV of 11.4% for RMR, which given a day to day variability of RMR of ~ 5% in adults (Compher et al., 2006) appears acceptable.

### 3.8. Oral glucose tolerance test and mixed meal tolerance tests

The OGTT has long been used as a tool to examine glucose tolerance and IS. The OGTT is a simple, low risk test designed to active the glucose homeostatic process and provide information on IS and glucose tolerance in the postprandial period. Alternative methods for the assessment of IS such as the “gold standard” HEC (DeFronzo et al., 1979) was not feasible in the current thesis due to the high cost and invasive nature of the measurement method.

In Chapters 5 and 7 the OGTT involved ingestion 1.75 g / kg of glucose in 300 mL of water, up to a maximum of 75 g. In Chapter 6 a glucose load of 75 g was delivered
using 394 mL of commercially available Lucozade original. Seven capillary blood samples were drawn (0, 10, 20, 30, 60, 90 and 120 minutes) post ingestion of the fluid as it has been shown to provide an accurate estimate of insulin secretion and action (Dalla Man et al., 2005). Values of plasma glucose and insulin during the OGTT were used to calculate IS and glucose tolerance. Depending of the estimate used, the OGTT have been shown to be a valid alternative to the HEC and FSIVGTT methods for determining IS (Avignon et al., 1999, Matsuda and DeFronzo, 1999) and been indicated as appropriate for use in children and adolescents (Conwell and Batch, 2004). Data presented in Chapter 4 indicate a day to day reliability for the responses to the OGTT with a 5.9% CV for tAUC glucose and a 14.3% CV for tAUC insulin.

In Chapter 8 a mixed meal tolerance test (MMTT) was used to determine plasma glucose and insulin responses to a test meal which is more reflective of day to day living. Within a 15 minute period, participants consumed a breakfast meal which consisted of a fruit smoothie, chocolate croissant with chocolate spread and a chocolate muffin (80 g of glucose, 68 g of fat, 7134 kJ), with the blood samples taken pre and 30, 60, 90 and 120 minutes post ingestion.

Chapter 9 involved the use of a MMTT in children with type one diabetes using a liquid meal consisting of 6 mL\*kg (maximum 360 mL) of per 100 mL: CHO 15.9 g, protein 7.9 g, fat 3.3 g) (Ensure Plus HP Vanilla, Abbott). This formulation was used in line with previous work in paediatric patients with T1D designed to examine c-peptide
function (Oram et al., 2014). The glucose response to this MMTT was quantified every 5 minutes using a CGM (see section 3.10 for details).

3.9. Blood sampling and analyses

In Chapters 4-9, capillary blood samples (~ 600 µL) were taken from a pre-warmed hand into heparin fluoride coated and lithium heparin coated microvettes (CB 300 tubes, Sarstedt Ltd, Leicester, UK) for determination of plasma glucose and insulin respectively. After collection, both microvettes were centrifuged at 1300 g for 10 minutes. Plasma was separated and either analysed immediately (plasma glucose) or stored at -80°C for later determination of plasma insulin.

3.9.1. Plasma glucose

Plasma glucose was determined using a commercially available glucose analyser (YSI 2300 Stat Plus, Yellow Springs, OH, USA). The YSI STAT 2300 uses steady state measurement methodology, where membrane based glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide. The difference between the sample and baseline current are proportional to the glucose concentration. The YSI measures glucose within the range of 0-30 mmol.L⁻¹ from a 25 µL sample. Samples were measured in duplicate during all studies presented in the thesis with an average intraassay % CV of < 1%.
3.9.2. **Plasma insulin**

After study completion, stored samples were thawed for determination of plasma insulin using an enzyme-linked immunosorbent assay (ELISA) (DRG Diagnostics, Germany). Briefly 25 μL of sample was placed in microtiter wells, which were coated with a monoclonal antibody directed towards insulin. Samples were combined with enzyme conjugate. After incubation, the bound conjugate remains in the well. During a second incubation step Streotavin Peroxidase Enzyme complex binds to the biotin-anti-insulin antibody, where the amount of bound enzyme complex is proportional to the concentration on insulin in the sample. Finally a substrate solution was added, with the intensity of the developed colour proportional to the concentration on insulin. The DRG insulin ELISA kit can determine insulin between 1.76 – 100 μIU/mL. All samples were run in duplicate and with at variability of < 10 % CV.

3.9.3. **Assessment of IS and β-cell function**

In Chapters 4-8 IS/IR were estimated from measures of blood glucose and insulin obtained in the fasted state or after the OGTT. A number of different calculation methods are presented in the Chapters, which are described below and summarised in Table 3.1.

3.9.4. **Fasting**

In Chapters 4 and 6-8, fasting plasma glucose and insulin were used to calculate IR, IS and β-cell function using HOMA (Matthews et al., 1985). This relationship between glucose and insulin in the basal, fasted state reflects the balance between hepatic
glucose output and insulin secretion, which is maintained by the homeostatic feedback loop between the liver and β-cells (Wallace et al., 2004). This method of assessment has been validated for use in healthy children and adolescents against the HEC ($r^2 = 0.82, P<0.001$) (Gungor et al., 2004). In Chapters 4 and 8 IR/IS was also estimated by the QUICKI (Mather et al., 2001) and FGIR (Legro et al., 1998) methods, the validity of which has been determined in youth against the HEC method (Brown and Yanovski, 2014).

In Chapter 4 β-cell function was assessed in the fasted state using the HOMA-β index (Matthews et al., 1985).

3.9.5. OGTT

In Chapters 4-8, plasma insulin and glucose over the 2 h OGTT were used to calculate IS using the Cederholm index. This index was used as it represents peripheral IS and muscular glucose uptake due to the dominant role of the peripheral tissue after an oral glucose load (Cederholm and Wibell, 1990). This test was included in studies as it was hypothesised that peripheral IS would be the primary site of improvements after exercise. The Cederholm index has also been used in previous exercise based studies in adults (Babraj et al., 2009) and children (Elsedfy et al., 2014). The Cederholm index was found to have a high degree of day to day reliability with a CV of 6.4% (see Chapter 4).
In Chapter 4 the Matsuda index (Matsuda and DeFronzo, 1999) was used as an additional estimate of IS from the OGTT. The Matsuda index has not been validated in a normal weight paediatric population, with validation data only available in obese groups (Brown and Yanovski, 2014).

In Chapter 4 β-cell function was also assessed from OGTT data using the Stumvoll (Stumvoll et al., 2000) and insulinogenic 30’ and 120’ (Seltzer et al., 1967) indices.
Table 3.1 Formulas and references for indices of IS and β-cell function derived from fasting and OGTT measurements of glucose and insulin

<table>
<thead>
<tr>
<th>Index</th>
<th>Formula</th>
<th>Chapter</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OGTT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cell function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stumvoll</td>
<td>1283 + 1.829 × ins30’ − 138.7 × G30’ + 3.772 I0’</td>
<td>4</td>
<td>Stumvoll et al. (2000)</td>
</tr>
<tr>
<td>Insulinogenic 30’</td>
<td>( \frac{I30’ - I0’}{G30’ - G0’} )</td>
<td>4</td>
<td>Seltzer et al. (1967)</td>
</tr>
<tr>
<td>Insulinogenic 120’</td>
<td>( \frac{\Delta AUC , G}{\Delta AUC , I} )</td>
<td>4</td>
<td>Seltzer et al. (1967)</td>
</tr>
<tr>
<td>IS/IR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matsuda index</td>
<td>( \frac{1000}{\sqrt{(G0’ - I0’)(\text{mean} , G , \text{OGTT} \times \text{mean} , I , \text{OGTT})}} )</td>
<td>4</td>
<td>Matsuda and DeFronzo (1999)</td>
</tr>
<tr>
<td>Cederholm index</td>
<td>( 75000 + (G0’ - G120’ \times 1.15 \times 180 \times \frac{\text{BW}(120)}{\text{Gmean} \times \log \text{Imean}}) )</td>
<td>4-6, 8</td>
<td>Cederholm and Wibell (1990)</td>
</tr>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-B</td>
<td>20 × ( \frac{\text{ins0’}}{\text{glu0’} - 3.5} )</td>
<td>4</td>
<td>Matthews et al. (1985)</td>
</tr>
<tr>
<td>IS/IR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>( \frac{\text{ins0’} \times \text{glu0’}}{22.5} )</td>
<td>4-8</td>
<td>(Matthews et al., 1985)</td>
</tr>
<tr>
<td>QUICKI</td>
<td>( \frac{1}{\log \text{ins0’} + \log \text{glu0’}} )</td>
<td>4</td>
<td>Mather et al. (2001)</td>
</tr>
<tr>
<td>FGIR</td>
<td>( \frac{G0’}{I0’} )</td>
<td>4</td>
<td>Legro et al. (1998)</td>
</tr>
</tbody>
</table>

3.10. Continuous glucose monitoring

During Chapter 9, blood glucose was estimated in adolescents with T1D using a CGM (iPro2, Medtronic MiniMed, Northridge, USA). The CGM uses a sensor (Enlite, Medtronic MiniMed, Northridge, USA) to measure glucose concentration in the subcutaneous interstitial fluid. The monitor uses an electro enzymatic sensor which reacts in the presence of glucose using the glucose oxidase reaction which generates an electrical current. Using an algorithm and assumptions about the equilibrium of glucose levels between the interstitial fluid and blood, the sensor estimates circulating blood glucose levels every 5 minutes. Sensor readings require calibration against capillary blood glucose measures which are recorded by participants using conventional capillary blood glucose measures at four points during the day.

CGM has been shown to have acceptable clinical accuracy (Sachedina and Pickup, 2003) and has been validated against glucose measures obtained from glucometers (Gross et al., 2000) as well as capillary and venous blood samples (Sachedina and Pickup, 2003). The use of CGM in children and adolescents with T1D has also been shown to be acceptable and feasible (Buckingham et al., 2007, Patton et al., 2011) with similar reliability between different devices (Damiano et al., 2014). The Enlite sensor has been shown to provide accurate data at different glucose concentrations and rates of change for up to 6 days (Bailey et al., 2014) and has previously been used in a paediatric population (Evans et al., 2014).
The glucose sensor was fitted to the participants’ subcutaneous tissues of the abdomen or upper buttocks using a spring-loaded device (ENLiTE Serter, Metronic, MiniMed, Northridge, USA) two days before the start of each experimental condition. The device was fitted at the participants home in order to limit travel time and burden. Participants were talked through the fitting procedure during the familiarisation visit and again prior to fitting the device. Before insertion, the surrounding skin was cleaned using an alcohol swap. Next, the sensor was inserted using the ‘Serter’ provided by the manufacturer, which uses a retractable needle to place the reactive strip of the sensor in the subcutaneous tissue. The sensor was adhered to the skin using an adhesive dressing which is integral to the sensor. After insertion of the sensor, the iPro 2 transmitter was connected with a flash of green light on the device indicating communication with the sensor. The transmitter was secured to the sensor using an adhesive tab. Once fitted, the sensor and iPro2 device remained in place over the 4 day measurement period. Participants were asked to record at least four blood glucose readings per day to facilitate calibration of the device. This was completed 1 h and 3 h after insertion and before breakfast, lunch, dinner and bed on all other days, when blood glucose levels are quasi-stable. Whilst wearing the CGM device, the participant received no feedback about their blood glucose data.

After each experimental condition, the CGM was removed by the researcher at the participants home. Data were downloaded using CareLink therapy management software (Medtronic, MiniMed, Northridge, USA) and data used to identify blood glucose values over a 24 h period, changes during exercise, MMTT and sleep, according to guidelines for CGM analysis (Clarke and Kovatchev, 2009) (see Figure 102).
3.3). Data were assessed from time spent hypo- (0-3.9 mmol.L\(^{-1}\)) eu- (3.9-7.2 mmol.L\(^{-1}\)) and hyper- (7.2 mmol.L\(^{-1}\) and above) glycaemic according to targets set by the ADA.

**Figure 3.2.** An example CGM trace over a 24h period from 08:00 to 08:00 during the laboratory testing day in Chapter 9. Exercise started at 10:30, the mixed meal tolerance test (MMTT) 12:00. The target range for glycaemic control are shown in red (above 7.2) and blue (below 3.9).

### 3.11. Sample size calculation

For all experimental Chapters, an *a priori* sample size calculation was performed using G-power software (Faul et al, 2007). Reference values from the literature were used to inform effect size statistics and sample size was calculated using 80% statistical power (1-\(\beta\)) and a significance (\(\alpha\)) level of 5%. This was based on the ability to detect a large effect size (>0.8) between conditions, and was based on previously published data in adolescents (Short et al, 2012).

### 3.12. Statistical analyses

Data for all experimental studies were analysed using SPSS (version 20, Chicago, USA). Results are presented as mean ± SD, unless otherwise stated. Total and
incremental AUC analyses were performed to characterise the magnitude of response and change over time respectively. AUC analysis which was performed using GraphPad prism (version 6, GraphPad software, CA, USA) using the trapezium rule.

Mean differences in the dependent variables each chapter were compared between experimental conditions using one-way within-measures analysis of variance (ANOVA) in Chapters 4, 5 and 7 and a two–way within-measures ANOVA in Chapter 6. Post hoc pairwise comparisons were conducted to follow up significant effects and interpreted using P-values and standardised effect size (ES) to detail the magnitude of the effect using the thresholds of: trivial (<0.2), small (>0.2), moderate (>0.5), large (>0.8) and very large (>1.0) (Cohen, 1992). The alpha level was set at 0.05 for all analyses.

In Chapter 4 day to day reliability of the fasting and OGTT derived outcomes of IR and β-cell function was quantified using paired sample t-tests (mean bias), Pearson’s correlation coefficient (r), typical error (TE) and TE expressed as percentage CV (%CV) (Hopkins, 2000).
CHAPTER 4

Agreement and reliability of fasted and oral glucose tolerance test derived indices of insulin resistance/sensitivity and beta cell function in boys
4.1. Abstract

Background: Assessment of plasma insulin and glucose outcomes is important in paediatric studies aimed at reducing future risk of T2D and CVD. The aim of this study is to determine the between method agreement and the day to day reliability of fasting and OGTT derived estimates of IS and β-cell function in healthy boys. Methods: Fasting and OGTT assessment of IR and β-cell function were performed on 28 boys (12.3 ± 2.9 y). Measurements were repeated after 1 week (fasting, n=28) and 1 day (OGTT, n=8). Agreement between estimates of IR and β-cell function was examined using Pearson’s correlation coefficient. Reliability was assessed using change in the mean, Pearson’s correlation coefficient, and typical error expressed as a CV. Results: The Matsuda index was positively related with QUICKI (r=0.88, P<0.001) and negatively related to HOMA-IR (r=-0.76, P<0.001). The Cederholm index was not significantly related with fasting estimates of IR (all r<0.40, P>0.05). For reliability, QUICKI had the lowest CV% for the fasting (4.7%) and the Cederholm index for the OGTT (6.4%) estimates. The largest CV% was observed in fasting insulin (30.8%) and insulinogenic index 30' (62.5%). Conclusion: This study highlights differences in between method agreement and day to day reliability for estimates of insulin resistance/sensitivity in youth. The low CV supports the use of the FGIR (fasting) and Cederholm (OGTT) indices in this population.

4.2. Introduction

The origin of T2D and CVD may lie in childhood (Steinberger et al., 2009). Early dysfunction of β-cell activity and cellular IR appear long before signs and symptoms of overt T2D (Zeitler, 2009, Kahn, 2003). IR is common in obese youth but also present
in those who are normal weight (Aldhoon-Hainerová et al., 2014), and higher levels of IR in youth predict increased risk of CVD and T2D in early adulthood (Yajnik et al., 2015). Research has focused on the primary prevention or management of IR through lifestyle modification, such as increasing PA in children and adolescents (Imperatore et al., 2006, Fedewa et al., 2014). It is, therefore, important to be able to accurately and reliably measure IR and β-cell function indices in youth.

The “gold standard” technique for measuring IR and β-cell function is the HEC (Muniyappa et al., 2015, DeFronzo et al., 1979). This technique is highly invasive, time consuming, labour intensive and expensive, and therefore not appropriate for large scale studies or experimental investigations where multiple measurements may be needed, especially in paediatric groups. In contrast, surrogate estimates of IR and β-cell function are frequently used in studies involving children and adolescents. Estimates of both β-cell function and IR indices can be obtained from fasting or OGTT protocols and have been validated with reference methods in children (Brown and Yanovski, 2014). Surprisingly though, few studies have assessed the agreement between different fasting and OGTT estimates of IR and β-cell function, with data currently only available in overweight youth (Atabek and Pirgon, 2007) or young girls with premature adrenarche (Silfen et al., 2001). With primary prevention studies recruiting predominantly healthy youth, the majority of which are non-obese (Weston et al., 2016, Buchan et al., 2013, Sun et al., 2013), knowledge of the agreement of fasting and OGTT derived IR measures in a “healthy” population is important to establish.
Another issue yet to be clarified in the existing literature is the reliability of IR and \( \beta \)-cell function indices from both fasting and OGTT based measurements in youth. Knowledge of measurement reliability will have implications for estimating study sample sizes and their use by researchers within a given study. Of the limited research to date, Uwaifo and colleagues (2002) reported a strong correlation \((r > 0.85)\) between repeated measurements of fasting indices (QUICKI, HOMA-IR and fasting insulin) in 31 children aged 6 to 11 y. This study is however limited, as the authors did not report the within-participant variation which is recommended when documenting measurement reliability (Atkinson and Nevill, 1998, Hopkins, 2000), and no reliability data were reported for OGTT outcomes.

The aim of the current study was to examine, in healthy male children and adolescents: 1) the relationship between commonly used surrogate markers of IR and \( \beta \)-cell function derived from fasting and OGTT measures; 2) the day to day reliability of IR and \( \beta \)-cell function indices from fasting and OGTT measures using recommended reliability statistics.

4.3. Methods

4.3.1. Participants

Twenty-eight boys \((12.3 \pm 2.9 \text{ y})\) were recruited from local primary and secondary schools. All participants were deemed eligible to participate in the study by completing an initial health questionnaire to exclude any medical conditions, or any medications that are known to effect glucose metabolism. Following an explanation of the study
protocol, written informed parental consent and participant assent were obtained. All protocols were approved by the institutional ethics committee.

4.3.2. Experimental design

The study consisted of three experimental visits, including a familiarisation visit and two metabolic assessments separated by 1 week.

Visit 1: Descriptive characteristics

During the initial familiarisation visit, stature (Harpenden, Holtain Ltd, Crymych, UK) and body mass (Seca 877, Seca Ltd, Birmingham, UK) were measured to the nearest 0.01 m and 0.1 kg respectively. Body mass index (BMI) was calculated and validated using age and sex specific BMI cut points were used to classify participants as overweight and obese (Cole et al., 2000). Skinfold thickness was measured by the same investigator to the nearest 0.2 mm on the right hand side of the body using skinfold callipers (Holtain Limited, UK). The median of three measurements at each anatomical site was used to estimate body fat percentage using validated maturation, race and sex specific equations (Slaughter et al., 1988). Pubertal status was determined by self-assessment of secondary sexual characteristics using adapted drawings of the five Tanner stages of pubic hair development (Morris and Udry, 1980). Cardiorespiratory fitness was directly measured as maximal oxygen uptake ($\dot{V}O_2\text{max}$) using a validated combined ramp and supramaximal exercise protocol (Barker et al., 2011).
Visits 2 and 3: Metabolic assessment

Participants were transported to the laboratory by car at 08:00 following a 12 h overnight fast. All participants (n=28) provided a capillary blood sample for the assessment of fasting plasma glucose and insulin and then completed an OGTT. Participants ingested a glucose load of 1.75 g/kg of body mass, up to a maximum of 75 g. Capillary blood samples were taken at 0, 10, 20, 30, 60, 90 and 120 minutes for the assessment of plasma glucose and insulin (Dalla Man et al., 2005). During the 2 h postprandial period, no other food was consumed but water was available ad libitum. Participants remained in the laboratory throughout this period completing sedentary activities such as reading, playing computer games or watching DVDs. In a subsample (n=8), the OGTT assessment was repeated on the following day using the procedures outlined above. All participants (n=28) had their fasting measures repeated 1 week later.

4.3.3. Blood analysis

Plasma glucose was determined in duplicate using a commercially available glucose analyser (YSI 2300 Stat Plus, Yellow Springs, OH, USA), with an inter-assay coefficient of variation (%CV) of <1%. Plasma insulin was determined in duplicate using an ELISA (DRG Diagnostics, Germany) with an inter-assay CV of <10%.

4.3.4. Calculations

Plasma glucose and insulin were used to calculate IR and β-cell function in the fasted state and during the OGTT using procedures commonly reported in the paediatric
literature (see Table 3.1 in Chapter 3). β-cell function was measured in the fasted state using the HOMA-β (Matthews et al., 1985) index and during the OGTT using the Stumvoll (Stumvoll et al., 2000) and insulinogenic 30’, and 120’ indices (Seltzer et al., 1967)

Fasted plasma glucose and insulin were used to estimate IR using HOMA-IR (Matthews et al., 1985), QUICKI (Mather et al., 2001) and FGIR (Legro et al., 1998). Plasma glucose and insulin responses to the OGTT were used to estimate IS based on the Matsuda (Matsuda and DeFronzo, 1999) and Cederholm (Cederholm and Wibell, 1990) methods. Finally, changes in plasma glucose and insulin during the OGTT were also quantified using tAUC by employing the trapezium rule (GraphPad Prism, San Diego, CA).

HOMA-IR was used to classify participants as “at risk” according to age-specific cut-points (Shashaj et al., 2015). Plasma glucose two h post the OGTT was used to examine normal glucose tolerance according to the American Diabetes Association (<7.8 mmol.L⁻¹) (ADA, 2010).

4.3.5. Control measures
PA was measured for 48 h before each visit using a wrist worn accelerometer (GENEActiv, Activinsights, UK). Accelerometers were worn on the non-dominant wrist and data sampled at 100 Hz. Data were converted into 60 s epochs and used to estimate the time spent during sedentary, light, moderate and VPA using cut points
validated in children (Phillips et al., 2013). Prior to the metabolic assessment, participants were asked to avoid any vigorous or organised PA. Participants were asked to include all food and drink consumed during this period in household measures and also include cooking methods. The food diaries were assessed for total energy and macronutrient content using commercially available software (CompEat Pro, Nutrition systems, UK). Participants were asked to replicate their diet during each metabolic assessment, and if appropriate to document any discrepancies.

4.3.6. Statistical analyses

Following initial analyses to test for a normal distribution (Sharpiro-Wilk test), the association between the fasting and OGTT indices of IR and β cell function was assessed using Pearson’s correlation coefficient (r). Reliability of the fasting and OGTT derived outcomes of IR and β-cell function was quantified using paired sample t-tests (mean bias), Pearson’s correlation coefficient (r), typical error (TE) and TE expressed as percentage CV (Hopkins, 2000). The alpha level was set at 0.05 for all analyses. Data are reported as mean ± standard deviation (SD) unless otherwise stated.

4.4. Results

Twenty eight boys took part in the study (age: 12.3 ± 2.9 y, BMI: 21.8 ± 2.2 kg.m², \( \bar{V}O_2 \) max: 44.9 ± 8.4 mL.kg\(^{-1}\).min\(^{-1}\)). Tanner stage ranged from 1 to 5 (stage 1: n= 7; stage 2: n= 5; stage 4: n= 14; stage 5: n= 2). Fourteen participants were classified as “at risk” according to their HOMA-IR. According to BMI, seven participants (25% of the
sample) were classified as overweight or obese. All participants had normal glucose tolerance. Based on 24 h accelerometer data, participants achieved an average MVPA per day of 123 ± 42 min. Data for habitual physical activity were not collected.

There were no significant differences in total energy intake, individual macronutrient contribution or time spent performing MVPA during the 24 h preceding each laboratory visit ($P=0.10$ to 0.62, data not reported).

### 4.4.1. Relationship between fasting and OGTT outcomes

**Insulin resistance**

The agreement between fasting and OGTT derived measures of IR is shown in Table 4.1. The Matsuda index was significantly and negatively correlated with fasting insulin, HOMA-IR, and significantly and positively correlated with QUICKI and FGIR. The tAUC insulin was significantly and positively correlated with fasting insulin and HOMA-IR and significantly and negatively correlated with QUICKI and FGIR. The Cederholm index was not significantly correlated with any fasting indices (all $P>0.05$).
Table 4.1. Relationship between oral glucose tolerance test and fasted measures of insulin resistance

<table>
<thead>
<tr>
<th>Fasting → OGTT</th>
<th>Fasting glucose</th>
<th>P</th>
<th>Fasting insulin</th>
<th>P</th>
<th>HOMA-IR</th>
<th>P</th>
<th>QUICKI</th>
<th>P</th>
<th>FGIR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsuda</td>
<td>-0.36</td>
<td>0.06</td>
<td>-0.75</td>
<td>&lt;0.001</td>
<td>-0.76</td>
<td>&lt;0.001</td>
<td>0.88</td>
<td>&lt;0.001</td>
<td>0.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cederholm</td>
<td>0.06</td>
<td>0.76</td>
<td>0.27</td>
<td>0.16</td>
<td>0.27</td>
<td>0.16</td>
<td>-0.27</td>
<td>0.17</td>
<td>-0.36</td>
<td>0.06</td>
</tr>
<tr>
<td>2 h glucose (mmol.L⁻¹)</td>
<td>-0.21</td>
<td>0.28</td>
<td>0.02</td>
<td>0.90</td>
<td>0.01</td>
<td>0.95</td>
<td>0.10</td>
<td>0.60</td>
<td>-0.07</td>
<td>0.73</td>
</tr>
<tr>
<td>2 h insulin (µU.mL⁻¹)</td>
<td>-0.27</td>
<td>0.16</td>
<td>0.28</td>
<td>0.15</td>
<td>0.26</td>
<td>0.19</td>
<td>-0.23</td>
<td>0.24</td>
<td>-0.44</td>
<td>0.020</td>
</tr>
<tr>
<td>tAUC glucose (mmol.L⁻¹.min⁻¹)</td>
<td>-0.11</td>
<td>0.56</td>
<td>-0.07</td>
<td>0.73</td>
<td>-0.07</td>
<td>0.73</td>
<td>0.11</td>
<td>0.58</td>
<td>0.13</td>
<td>0.51</td>
</tr>
<tr>
<td>tAUC insulin (µU.mL⁻¹.min⁻¹)</td>
<td>0.21</td>
<td>0.28</td>
<td>0.62</td>
<td>&lt;0.001</td>
<td>0.63</td>
<td>&lt;0.001</td>
<td>-0.54</td>
<td>0.003</td>
<td>-0.62</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

OGTT, oral glucose tolerance test. Relationship between variables expressed as Pearson’s correlation coefficient and P-value. Significant correlations are shown in bold.
\( \beta \)-cell function

The agreement between fasting and OGTT derived measures of \( \beta \)-cell function is shown in Table 4.2. The OGTT derived insulinogenic index 30’ was not significantly correlated with fasting HOMA-\( \beta \). Both insulinogenic index 120’ and Stumvoll index had a significant positive correlation with HOMA- \( \beta \). tAUC insulin and 2 h insulin were significantly correlated with HOMA- \( \beta \).

4.4.2. Reliability

Test-retest reliability for the fasting and OGTT measures of IR and \( \beta \)-cell function are presented in Tables 4.3 and 4.4 respectively.

| Table 4.2. Relationship between OGTT and fasted measures of \( \beta \)-cell function |
|---------------------------------|----------|--------|
| Fasting → OGTT ↓               | HOMA-\( \beta \) | \( P \) |
| Insulinogenic index 30’        | 0.20     | 0.30   |
| Insulinogenic index 120’       | 0.51     | 0.006  |
| Stumvoll index                 | 0.45     | 0.017  |
| 2 h glucose (mmol.L\(^{-1}\))  | 0.18     | 0.35   |
| 2 h insulin (\( \mu \)IU.mL\(^{-1}\)) | 0.51     | 0.006  |
| tAUC glucose (mmol.L\(^{-1}.\)min\(^{-1}\)) | -0.02    | 0.93   |
| tAUC insulin (\( \mu \)IU/mL.min\(^{-1}\)) | 0.46     | 0.015  |

Relationship variables expressed as Pearson’s correlation coefficient and \( P \)-value. Significant correlations are shown in bold.
Table 4.3. Day to day reliability of fasting and OGTT derived measures of IR

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Assessment 1</th>
<th>Assessment 2</th>
<th>Change in mean</th>
<th>P-value</th>
<th>TE</th>
<th>TECV%</th>
<th>Pearson’s correlation coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>28</td>
<td>5.2 ± 0.4</td>
<td>5.3 ± 0.3</td>
<td>-0.04</td>
<td>0.55</td>
<td>0.3</td>
<td>6.5%</td>
<td>0.17</td>
<td>0.37</td>
</tr>
<tr>
<td>Fasting [insulin ]</td>
<td>28</td>
<td>17.3 ± 5.7</td>
<td>18.8 ± 9.5</td>
<td>-1.47</td>
<td>0.41</td>
<td>6.6</td>
<td>30.8%</td>
<td>0.34</td>
<td>0.08</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>28</td>
<td>2.2 ± 0.7</td>
<td>2.4 ± 1.2</td>
<td>-0.18</td>
<td>0.43</td>
<td>0.8</td>
<td>30.6%</td>
<td>0.33</td>
<td>0.09</td>
</tr>
<tr>
<td>QUICKI</td>
<td>28</td>
<td>0.32 ± 0.02</td>
<td>0.31 ± 0.01</td>
<td>-0.01</td>
<td>0.22</td>
<td>0.01</td>
<td>4.7%</td>
<td>0.37</td>
<td>0.05</td>
</tr>
<tr>
<td>FGIR</td>
<td>28</td>
<td>6.1 ± 2.1</td>
<td>6.2 ± 2.5</td>
<td>-0.14</td>
<td>0.33</td>
<td>0.5</td>
<td>6.5%</td>
<td><strong>0.96</strong></td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td><strong>OGTT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h glucose (mmol.L⁻¹)</td>
<td>8</td>
<td>5.8 ± 0.7</td>
<td>5.5 ± 0.5</td>
<td>0.32</td>
<td>0.24</td>
<td>0.4</td>
<td>6.9%</td>
<td>0.60</td>
<td>0.12</td>
</tr>
<tr>
<td>2 h insulin (µIU/mL)</td>
<td>8</td>
<td>33.3 ± 12.6</td>
<td>34.2 ± 13.1</td>
<td>0.92</td>
<td>0.8</td>
<td>7.2</td>
<td>28.4%</td>
<td>0.69</td>
<td>0.06</td>
</tr>
<tr>
<td>tAUC glucose (mmol.L⁻¹.min⁻¹)</td>
<td>8</td>
<td>849.1 ± 94.9</td>
<td>848.9 ± 86.0</td>
<td>0.11</td>
<td>1.00</td>
<td>51.7</td>
<td>5.9%</td>
<td>0.67</td>
<td>0.07</td>
</tr>
<tr>
<td>tAUCinsulin (µIU/mL.min⁻¹)</td>
<td>8</td>
<td>7702.0 ± 1540.9</td>
<td>7702.1 ± 1544.4</td>
<td>-0.13</td>
<td>1.00</td>
<td>1069.8</td>
<td>14.3 %</td>
<td>0.52</td>
<td>0.18</td>
</tr>
<tr>
<td>Cederholm</td>
<td>8</td>
<td>50.0 ± 4.3</td>
<td>49.6 ± 6.6</td>
<td>-0.43</td>
<td>0.77</td>
<td>2.9</td>
<td>6.4%</td>
<td><strong>0.74</strong></td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Matsuda</td>
<td>8</td>
<td>2.5 ± 0.5</td>
<td>2.4 ± 0.5</td>
<td>-0.12</td>
<td>0.68</td>
<td>0.6</td>
<td>26.7%</td>
<td>0.15</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Values reported as means ± SD. TE, typical error; TECV%. TE expressed as percentage of the coefficient of variation. Significant results are shown in bold.
Table 4.4. Day to day reliability of fasting and OGTT derived measures of \( \beta \)-cell function

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Assessment 1</th>
<th>Assessment 2</th>
<th>Change in mean</th>
<th>( P )-value</th>
<th>TE</th>
<th>TE (_{CV%} )</th>
<th>Pearson correlation coefficient</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA- ( \beta )</td>
<td>28</td>
<td>204.4 ( \pm ) 66.4</td>
<td>210.9 ( \pm ) 88.1</td>
<td>-6.5</td>
<td>0.66</td>
<td>54.5</td>
<td>27.1</td>
<td>0.53</td>
<td>0.004</td>
</tr>
<tr>
<td>OGTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stumvoll</td>
<td>8</td>
<td>1902.8 ( \pm ) 247.3</td>
<td>1704.7 ( \pm ) 415.1</td>
<td>198.1</td>
<td>0.4</td>
<td>306.27</td>
<td>20.1</td>
<td>0.22</td>
<td>0.60</td>
</tr>
<tr>
<td>Insulinogenic index 30'</td>
<td>7</td>
<td>189.1 ( \pm ) 168.3</td>
<td>209.1 ( \pm ) 164.1</td>
<td>-20.0</td>
<td>0.32</td>
<td>34.49</td>
<td>62.5%</td>
<td><strong>0.96</strong></td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Insulinogenic index 120'</td>
<td>8</td>
<td>61.4 ( \pm ) 12.8</td>
<td>54.0 ( \pm ) 13.3</td>
<td>7.4</td>
<td>0.23</td>
<td>11.14</td>
<td>19.7%</td>
<td>0.27</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Values reported as means \( \pm \) SD. TE, typical error; TE \(_{CV\%} \), TE expressed as percentage of the coefficient of variation. Insulinogenic index 30', n= 7 due to negative value in one participant. Significant results are shown in bold.
4.5. Discussion

This study shows for the first time in a group of healthy male children and adolescents, the agreement between fasting and OGTT derived estimates of IR and β-cell function as well as day to day reliability of these measurements. This information will help inform researchers on the selection of appropriate indices in studies designed to evaluate IR and β-cell function in youth.

Data from the current study show that the Matsuda index from the OGTT has a strong and significant correlation with a number of fasting indices including a positive correlation with QUICKI and FGIR and a negative correlation with HOMA-IR and fasting insulin. These findings are in agreement with Silfen and colleagues (2001) who in 25 young girls with premature adrenarche, also found the Matsuda index to be significantly correlated with fasting indices including FGIR (r=0.79), QUICKI (r=0.80) and insulin (r=-0.67). Furthermore, a strong correlation between the Matsuda index and HOMA-IR has been reported in healthy active adults, although the magnitude of Pearson’s correlation was not provided (Brestoff et al., 2009). Interestingly, the present study found the Cederholm index, unlike the Matsuda index, to not significantly correlate with any of the fasting measures of IR (all r<0.40, P>0.05) in healthy boys. Previous work has shown that both the Cederholm and Matsuda indices correlate with different magnitudes (r=0.26 and r=0.63, respectively) to the glucose infusion rate during a HEC in 20 children aged 9 ± 2 y (Henderson et al., 2011). Additionally the Matsuda index has been shown to positively correlate with the HEC in two validation studies in obese children, with correlation coefficients of r=0.77 (George et al., 2011) and r=0.78 (Yeckel et al., 2004).
The different agreement between the Matsuda and Cederholm indices with fasting estimates of IS/IR in the present study may be due the physiological foundations of the calculations for these indices. IR formula are generally placed into two categories, those which are theoretically derived based on the principle that IS during the OGTT is inversely proportional to the product of the mean insulin and glucose values during the OGTT (e.g. the Matsuda index), and those designed to maximise the correlation with the HEC (e.g. the Cederholm index) (Brown and Yanovski, 2014, Gutch et al., 2015). It is worth noting that these formulas were originally derived for use with adults and may not be replicated in children. The HEC and Cederholm index primarily reflects “whole body” peripheral IR. Conversely the Matsuda index reflects both hepatic and peripheral IR (Patarrão et al., 2014). Since surrogate indices based on fasted glucose and insulin primarily reflect hepatic IR (Muniyappa et al., 2008), this is likely to account for the strong significant correlations between the fasting IR indices and the Matsuda index but not with the Cederholm index.

Results from the present study highlight agreement between estimates of β-cell function when measured from fasted samples or during an OGTT. A previous study has shown agreement for both OGTT and fasting derived estimates of β-cell function with the acute insulin response to glucose, calculated from a FSIVGTT in 29 children (Henderson et al., 2012) but have not directly compared fasted and OGTT estimates. The agreement of the fasted estimate of β-cell function with OGTT estimates shown in this study imply that fasted samples may be adequate when assessing β-cell function in youth.
The secondary aim of this study was to establish the day to day reliability of both fasting and OGTT derived measurements of IR and β-cell function in healthy male youths. Although we found no evidence of a mean bias between the two measurements points for all the indices, there was considerable variation at the participant level with the TE ranging from 4.7% (QUICKI), 6.4% (Cederholm), 26.7% (Matsuda), 30.6% (HOMA-IR) to 62.5% (insulinogenic index 30’). Detail of the reliability of IR estimates in children are limited, with previous work reporting reliability for both HOMA-IR and QUICKI based on Pearson’s correlation coefficient (r>0.85, P<0.001) with sparse data on the variation at the within participant level (Uwaifo et al., 2002). The reliability of the OGTT has previously been investigated in 60 overweight youth aged 8-17 y with 1-25 days between the tests. Results showed poor reliability of the OGTT for establishing IGT, with 10/60 participants classified as having IGT on the first OGTT and only 3/60 on the second, suggesting large variability in the glucose measurement 2 h post OGTT (Libman et al., 2008). However much like studies assessing reliability of fasting IR, the study by Libman and colleagues (2008) do not report variation at the within participant level, nor do they report measures on insulin or IR outcomes from the OGTT.

In the current study we found a CV of 26.7% for the Matsuda index derived from the OGTT. Previous work in healthy adults with normal glucose tolerance have shown CV of 14-16% for the Matsuda index with the OGTT repeated 5-14 days apart (Utzschneider et al., 2007, Ortega et al., 2014). Our data shows a CV of 6.4% for the Cederholm index, indicating superior reliability over the Matsuda index in male youth. Conversely, although not reporting CV, previous work on normoglycaemic women has
shown the Cederholm and Matsuda indices to have similar interclass correlations ($r=0.84$ and $0.90$ respectively) (Cakir et al., 2006). From a practical point of view this difference in within subject variation ($\%CV$) for the Matsuda and Cederholm indices derived from the current study would have a significant impact on a study sample size for determining a given effect. Using a formula proposed by Hopkins (Hopkins, 2000),

$$\frac{8s^2}{d^2},$$

where $d$ is the smallest worthwhile change and $s$ is the within-subject standard deviation) to detect $\sim 5\%$ change in IS would require a sample size of $\sim 13$ participants using the Cederholm index and $\sim 228$ participants for the Matsuda index. This would have implications on cost and resourcing for studies, highlighting the importance of understanding measurement reliability.

The present work also demonstrated a higher CV in a paediatric population across a range of indices, including HOMA-IR, insulinogenic index 30', 2 h glucose, fasting insulin and fasting glucose when compared to adult data where agreement was assessed over two repeat measurements separated by 5-14 days (Utzschneider et al., 2007). However there are similarities with adult work in terms of which indices have the largest variability. In the current study the insulinogenic index 30' had the highest CV at 62.5% which is in agreement with data (41.1%) from adults with normal glucose tolerance (Utzschneider et al., 2007).

Data from the present study suggest a larger variability in insulin measurements compared to glucose, which may be due to the ten-fold higher analytical variability of
the insulin assay compared with glucose (inter-sample CV of ~ 10% vs. 1% respectively). In adult studies, the increased variability of insulin has also been attributed the early insulin response to a glucose load (Utzschneider et al., 2007). This is reflected in the present work with the highest degree of variability found for the insulinogenic index 30’ (62.5%) which captures changes in insulin over the first 30 minutes of the OGTT. Also, formula where the insulin response is integral to the equation (e.g. the Matsuda index) exhibit poorer reliability compared to the Cederholm index where the insulin response is averaged over the 2 h OGTT. However, our data also show high between day variation for insulin in the fasted state, which is not explained by variation in response to the glucose load via an OGTT. The present study controlled for two main confounders, PA and diet, prior to each repeated test, suggesting the variability in fasting insulin may reflect natural biological variability, possibly due to its short serum half-life, the cyclicity of its secretion and rapid response to change to both hormonal and metabolic environment (Matthews et al., 1983). Additionally the variability in OGTT outcomes could be a result of variance in gastric emptying and gut metabolism.

A number of limitations to this study should be acknowledged. Firstly the inclusion of only healthy “normal weight” boys, and thus limits extrapolation beyond this population. In addition, there is limited research into the validity of IS measures in normal weight, healthy youth.
4.6. Conclusion

This study has demonstrated differences in the agreement between a range of commonly used surrogate estimates of IR and β-cell function using fasting and OGTT protocols in children and adolescents. Additionally we demonstrate varied reliability of the OGTT and fasting estimates of IS. These factors are important to consider when measuring IR in youth and have implications for samples size estimates and detecting the relevant physiological change following intervention. From the point of view of minimising the within subject variability, the use of the FGIR (fasting) and Cederholm (OGTT) indices are recommended in this population.
CHAPTER 5

High-intensity interval exercise is an effective alternative to moderate-intensity exercise for improving glucose tolerance and insulin sensitivity in adolescent boys
5.1. Abstract

Background: HIIE may offer a time efficient means to improve health outcomes compared to MIE. This study examined the acute effect of HIIE compared to a work-matched bout of MIE on glucose tolerance, IS, resting fat oxidation and exercise enjoyment in adolescent boys. Methods: Nine boys (14.2 ± 0.4 y) completed three conditions on separate days in a counterbalanced order: 1) HIIE; 2) work matched MIE, both on a cycle ergometer; and 3) rest (CON). An OGTT was performed after exercise or rest and the iAUC and tAUC responses for plasma glucose and insulin were calculated, and IS estimated (Cederholm index). Energy expenditure and fat oxidation were measured following the OGTT using indirect calorimetry. Exercise enjoyment was assessed using the PACES. Results: The iAUC for plasma glucose was reduced following both MIE (-23.9%, \( P=0.013, \ ES=-0.64 \)) and HIIE (-28.9%, \( P=0.008, \ ES=-0.84 \)) compared to CON. The iAUC for plasma insulin was lower for HIIE (-24.2%, \( P=0.021, \ ES=-0.71 \)) and MIE (-29.1%, \( P=0.012, \ ES=-0.79 \)) compared to CON. IS increased by 11.2% after HIIE \( (P=0.03, \ ES=0.76) \) and 8.4% after MIE \( (P=0.10, \ ES=0.58) \). There was a trend for an increase in fat oxidation following HIIE \( (P=0.097, \ ES=0.70) \). Both HIIE and MIE were rated as equally enjoyable \( (P>0.05, \ ES<0.01) \). Conclusion: A single bout of time efficient HIIE is an effective alternative to MIE for improving glucose tolerance and IS in adolescent boys immediately after exercise.

5.2. Introduction

IR and glucose tolerance are important components of the metabolic syndrome and implicated in the etiology of T2D and CVD (Reaven, 2005). As the origin of such
diseases may lie in childhood (McGill et al., 2008), the prevention of IR and glucose intolerance in this age group is an important strategy for public health. PA can play a major role in this and a recent meta-analysis found a small to moderate effect for exercise training performed over several weeks to improve fasting insulin and IR in youth (Fedewa et al., 2014). However, the optimal form, duration and intensity of exercise to improve glucose tolerance and IR in youth is currently unknown. Furthermore, current research is largely based on overweight/obese children and adolescents despite observations that metabolic abnormalities are present in normal weight individuals (Voulgari et al., 2011).

Recent work in adults has shown time efficient, low volume, HIIE to improve health outcomes, including IS (Babraj et al., 2009). Furthermore, a study on prediabetic adults found a single bout of high-intensity exercise to afford either comparable or superior improvements in IS and glucose tolerance compared to MIE (Rynders et al., 2013). Evidence suggests IS remains elevated up to 17 h after 45 minutes of aerobic exercise in adolescents with low levels of PA and aerobic fitness (Short et al., 2013), but the impact of a single bout of HIIE on IS and glucose tolerance in youth is currently unknown. This is important to establish as less than a third of boys and girls aged 2-15 y currently meet the recommended daily level of PA within the UK (Riddoch et al., 2007) and interventions designed to raise the PA levels in youth only have a small effect (Metcalf et al., 2012). The health benefits that can be gained through alternative forms of exercise, such as HIIE, should be considered.
The primary aim of the study is to test the hypothesis that a single bout of HIIE would result in superior improvements in IS and glucose tolerance in normal weight adolescents when compared to a work-matched bout of MIE. The secondary aim was to examine the effect of HIIE and MIE on RMR, fat oxidation and exercise enjoyment.

5.3. Methods

5.3.1. Participants

Nine pubertal boys (age: 14.2 ± 0.40 y, weight: 55.9 ± 12.4 kg, stature: 1.67 ± 0.13 m; body fat: 17.1 ± 4.2 %), were recruited from a local secondary school. Following an explanation of the study procedures and the associated risks and benefits, parental consent and participant assent were obtained. Participants completed an initial health questionnaire and were free from any metabolic or medical conditions. Ethics approval was granted by the institutional ethics committee.

5.3.2. Experimental design

This cross-over study consisted of four laboratory visits, each separated by approximately 1 week. Visits included an initial familiarisation session and three experimental conditions in a temperature controlled laboratory. All procedures were identical during the experimental conditions apart from the exercise or rest period undertaken. All exercise was performed on a cycle ergometer (Lode Excilibur Sport, Groningen, Netherlands) with appropriate adjustments made for each participant.
During visit 1 stature and body mass were measured to the nearest 0.1 cm and 0.1 kg. Body fat percentage was estimated using skinfold measurements from the triceps and subscapular anatomical sites (Slaughter et al., 1988). Pubertal status was determined by self-assessment of the five Tanner stages of pubic hair development (Tanner and Whitehouse, 1976). Participants were familiarised with the cycle ergometer and completed a combined ramp-incremental and supramaximal test to exhaustion to determine maximal O$_2$ uptake ($\dot{V}O_2$ max) and the (Barker et al., 2011). Pulmonary gas exchange and heart rate were measured (Cortex Metalyzer III B, Germany) and $\dot{V}O_2$ max was accepted as the highest 10 s average $\dot{V}O_2$ during the ramp or supra-maximal test. The GET was estimated at the point where the first disproportionate increase in CO$_2$ production compared to $\dot{V}O_2$ and verified using the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$.

For visits 2-4 participants arrived at the laboratory at ~ 08:00 following a 12 h overnight fast. After 10 minutes of seated rest, participants provided a capillary blood sample for plasma glucose and insulin. Baseline resting metabolic rate (RMR) was determined via indirect calorimetry (Cortex Metalyzer II, Leipzig, Germany) over a 10 minute period to determine RMR and fat oxidation.

At ~ 08:30 participants undertook one of the following conditions in a counterbalanced order: 1) HIIE, consisting of 3 minute warm up at 20 W followed by eight repeated bouts of 1 minutes cycling at 90% of the peak power, interspersed with 1.25 minutes recovery at 20 W, followed by a 3 minutes cool down at 20 W; 2) MIE, consisting of continuous cycling at 90% GET, the duration of which was determined to match the mechanical work-done during HIIE; and 3) rest (CON). Throughout the exercise
conditions gas exchange and heart rate were monitored. Participants provided a RPE to a supervising researcher every 5 minutes during MIE and immediately following each 1 minute interval during HIIE, using the Pictorial Children’s Effort Rating Table (Yelling et al., 2002). Upon completion of each exercise condition participants completed the 16-item PACES (Motl et al., 2001).

Ten minutes after the completion of each experimental condition, an OGTT took place. Participants consumed 75 g glucose in 300 mL of water, after which capillary blood samples were taken at 0, 10, 20, 30, 60, 90 and 120 minutes for assessment of plasma glucose and insulin. RMR was assessed at 60, 120 and 180 minutes post OGTT. During the 3 h postprandial measurement period, no other food was consumed although water was available ad libitum. This was recorded for the first experimental condition and subsequently replicated for the remaining conditions. Participants remained in the laboratory throughout the visit, completing sedentary activities such as reading, watching DVDs or playing computer games. Participants left the laboratory at ~ 13:00.

5.3.3. Control measures

PA was measured during the 48 h period prior to each condition using a wrist worn accelerometer (GENEActiv, GENEA, UK). Data were converted into 1 minute epochs and used to estimate the time spent during sedentary, light, moderate and VPA using cut points validated in children and adolescents (Phillips et al., 2013). Participants were asked to avoid any organised sport during this period.
With supervision from their parents/guardians, a food diary was completed by each participant during the 48 h period preceding each experimental condition. Food diaries were assessed for total energy and macronutrient content using commercially available software (CompEat Pro, Nutrition systems, UK). Participants were asked to replicate their diet during the 48 h preceding each experimental condition, and if appropriate, to document any discrepancies. The experimental protocol is shown in figure 5.1

![Experimental Protocol Diagram](image)

Figure 5.1. Schematic of protocol for experimental visits 2, 3 and 4. RMR, resting metabolic rate; HIIE, high intensity interval exercise; MIE, moderate intensity exercise; CON, Control; OGTT, Oral glucose tolerance test.

### 5.3.4. Blood analyses

Fingertip capillary blood samples (~600 µL) were taken from a pre-warmed hand into a heparin fluoride coated and lithium heparin coated microvette (CB 300 tubes, Sarstedt Ltd, Leicester, UK) for plasma glucose and insulin determination respectively.
Both microvetttes were centrifuged at 1300 g for 10 minutes. Plasma was separated for immediate analysis of glucose (YSI 2300 Stat Plus Glucose analyser, Yellow Springs, OH, USA) or stored at –80°C for later analysis of plasma insulin using an ELISA enzyme immunoassay kit (DRG Diagnostics, Germany). The within batch coefficient of variation for the plasma insulin and glucose analyses was < 5%.

5.3.5. Calculations

Changes in plasma glucose and insulin during the OGTT were quantified using tAUC, iAUC analyses employing the trapezium rule (GraphPad Prism, SanDiego, CA). In line with previous HIIE studies (Babraj et al., 2009, Metcalfe et al., 2012), the Cederholm index was used to estimate IS, which represents peripheral IS (Cederholm and Wibell, 1990). RMR and the absolute fat and CHO oxidation were estimated using the mean $\dot{\text{V}}\text{O}_2$ and respiratory exchange ratio for each 10 minutes measurement (Frayn, 1983). AUC was used to document changes in RMR and fat oxidation following the OGTT.

5.3.6. Statistical analyses

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Mean differences in the physiological and perceptual responses during HIIE and MIE were analysed using paired samples t tests. Analysis of fasting measures, IS and the AUC analyses across conditions was performed using a repeated measures ANOVA. Pairwise comparisons between means were interpreted using P-values and standardised ES to detail the magnitude of the effect.
The magnitude of the difference between variables of interest were explored using ES thresholds of trivial (<0.2), small (>0.2), moderate (>0.5), large (>0.8), and very large (>1.0) (Cohen, 1992). Results are presented as ($P$ value, $ES$), unless stated otherwise.

5.4. Results

The participant’s Tanner stage ranged between 2 and 5 (stage 5: $n=1$, stage 4: $n=7$, stage 2: $n=1$). The combined ramp-incremental and supramaximal test elicited a peak power of $225 \pm 42$ W, $\dot{V}O_2$ max of $46.5 \pm 9.6$ mL·kg$^{-1}$·min$^{-1}$, and a GET of $1.42 \pm 0.36$ L·min$^{-1}$ (55.4 ± 7.0% $\dot{V}O_2$ max).

Time spent in light, moderate and VPA in the 48 h preceding each condition was similar across conditions ($P>0.05$, data not reported). Likewise, CHO, fat and protein intake was similar in the 48 h prior to each visit ($P>0.05$, data not reported).

The physiological and perceptual responses during the exercise conditions are presented in Table 5.1. HIIE elicited greater physiological and perceptual stress compared to MIE which is reflected in the elevated $\dot{V}O_2$, heart rate and RPE responses. PACES data showed both exercise conditions were equally enjoyable.
Table 5.1. Physiological and perceptual responses to HIIE and MIE

<table>
<thead>
<tr>
<th>Variable</th>
<th>MIE</th>
<th>HIE</th>
<th>P</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power output, W</td>
<td>78 ± 25</td>
<td>220 ± 49</td>
<td>&lt;0.001</td>
<td>3.04</td>
</tr>
<tr>
<td>Power output, % peak</td>
<td>31 ± 8</td>
<td>90 ± 0</td>
<td>&lt;0.001</td>
<td>9.33</td>
</tr>
<tr>
<td>( \dot{\text{V}}\text{O}_2 ), max, mL·kg(^{-1})·min(^{-1} )</td>
<td>25.6 ± 4.0</td>
<td>46.6 ± 6.8</td>
<td>&lt;0.001</td>
<td>3.18</td>
</tr>
<tr>
<td>( \dot{\text{V}}\text{O}_2 ),% max</td>
<td>55 ± 9</td>
<td>101 ± 12</td>
<td>&lt;0.001</td>
<td>3.72</td>
</tr>
<tr>
<td>HR, beats·min(^{-1} )</td>
<td>136 ± 9</td>
<td>183 ± 9</td>
<td>&lt;0.001</td>
<td>4.33</td>
</tr>
<tr>
<td>RPE</td>
<td>4 ± 2</td>
<td>8 ± 2</td>
<td>&lt;0.001</td>
<td>2.09</td>
</tr>
<tr>
<td>PACES</td>
<td>61 ± 6</td>
<td>61 ± 7</td>
<td>1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Duration, min</td>
<td>28.9 ± 8.1</td>
<td>22.8 ± 0</td>
<td>0.047</td>
<td>0.93</td>
</tr>
<tr>
<td>Work done, kJ</td>
<td>123 ± 24</td>
<td>123 ± 24</td>
<td>-</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Energy expenditure, kcal</td>
<td>183.1 ± 20.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results shown as mean ± SD, probability (P), and effect size (ES).
Abbreviations: HR, Heart rate; RPE, ratings of perceived exertion; PACES, physical activity enjoyment scale. P, probability. ES, effect size.

Fasting plasma glucose was not different between conditions (\( P=0.86 \)). Changes in plasma glucose following the OGTT are presented over time and using the AUC analyses in Figure 5.1. A significant condition effect was found for the plasma glucose iAUC (\( P<0.001 \)). HIIE was 28.9% lower (\( P=0.008, \ ES=-0.84 \)) and MIE 23.9% lower (\( P=0.013, \ ES=-0.64 \)) compared to CON. No difference was found between HIIE and MIE (\( P=0.22, \ ES=-0.24 \)). For the plasma glucose tAUC there was a significant effect of condition (\( P<0.001 \)) with both HIIE (-7.7%, \( P=0.012, \ ES=-0.89 \)) and MIE (-6.2%, \( P=0.039, \ ES=-0.69 \)) lower than CON. The difference between HIIE and MIE for the plasma glucose tAUC was trivial (\( P=0.34, \ ES=-0.19 \)).
Figure 5.2. Plasma glucose and insulin response following the OGTT displayed over time (A, D), using the incremental AUC (B, E) or the total AUC (C, F). HIIE, high-intensity interval exercise. MIE, moderate intensity exercise. CON, control. A, D shown as mean ± SEM. B, C, E, F shown as mean ± SD. * represents a meaningful beneficial effect (ES>0.2) for exercise compared to control. Please see text for details.

Fasting plasma insulin was similar across conditions (P=0.40). Changes in plasma insulin are presented over time and using AUC analyses in Figure 5.1. A significant
effect of condition was found for the plasma insulin iAUC ($P=0.013$). Plasma insulin iAUC was lower for HIIE (-24.2%, $P=0.021$, $ES=-0.71$) and MIE (-29.1%, $P=0.012$, $ES=-0.79$) compared to CON. No difference was found between HIIE and MIE ($P=0.79$, $ES=0.08$). A trend was found for the effect of condition on plasma insulin tAUC ($P=0.09$), with a 12.7% reduction after HIIE ($P=0.07$, $ES=-0.53$) and a 12.3% reduction after MIE ($P=0.07$, $ES=-0.50$) compared to CON. The difference between HIIE and MIE was trivial ($P=0.90$, $ES=-0.03$).

IS data are shown in Figure 5.2. A significant effect for condition on IS was found ($P=0.04$). HIIE increased IS by 11.2% ($P=0.03$, $ES=0.76$) compared to CON, and there was a trend for an 8.4% increase after MIE compared to CON ($P=0.10$, $ES=0.58$). The difference between HIIE and MIE was trivial ($P=0.42$, $ES=0.18$).

![Figure 5.3. IS (Cederholm). Values shown as mean ± SD. HIIE, high-intensity interval exercise. MIE, moderate intensity exercise. CON, control. *, represents a meaningful beneficial effect ($ES>0.2$) for exercise compared to control. Please see text for details.](image-url)
Changes in postprandial RMR and fat oxidation between conditions are shown in supplementary Figure 5.3. (n=7 due to data loss in two participants). No condition effect was found for the tAUC RMR (P=0.94). However, there was a trend towards an effect for the fat oxidation tAUC with a moderate elevation following HIIE (P=0.097, ES= 0.70) compared to CON. A non-significant but small difference was observed following MIE compared to CON (P=0.19, ES= 0.38) or HIIE (P=0.36, ES=0.32).

Figure 5.4. Energy expenditure (A) and fat oxidation (B) response following the OGTT. HIIE, high-intensity interval exercise. MIE, moderate intensity exercise. CON, control. Please see text for details.
5.5. Discussion

The primary finding from this study was that a single bout of HIIE and MIE resulted in: 1) moderate to large reductions in the plasma glucose and insulin responses following an OGTT performed 10 minutes post exercise, and 2) a moderate increase in IS. In addition, HIIE and MIE had no effect on EE but a moderate increase in fat oxidation during the postprandial period was observed following HIIE. Finally, HIIE was more time efficient and perceived to be as equally enjoyable compared to MIE. These novel findings document the potential for time efficient HIIE to improve glucose tolerance, IS and fat oxidation after a single bout of exercise and should be considered as an effective alternative to MIE to improve health markers in adolescent boys.

Postprandial glucose, hyperinsulinaemia and IR are implicated in the development of T2D and CVD (Reaven, 2005), the origin of which may reside in childhood (McGill et al., 2008). It is, therefore, pertinent to note that in the current study just a single bout of HIIE and MIE attenuated the rise in postprandial plasma glucose and insulin and improved IS in adolescent boys. Although the present study provides novel data for HIIE, our MIE data corroborates well with previous investigations. Short and colleagues found a reduced iAUC for plasma glucose and insulin, as well as a 78% increase in IS, after 45 minutes of aerobic exercise (~ 230 kcal) in adolescents with low PA and aerobic fitness (Short et al., 2013). Furthermore, undertaking aerobic exercise (500 kcal) ~16 h before a high glycaemic index test meal lowered postprandial plasma insulin but not glucose in normal weight girls (Zakrzewski and Tolfrey, 2012). Our findings extend this limited body of work by showing that improvements in glucose tolerance and IS can be achieved after just ~ 28 minutes of
MIE (~ 180 kcal) or just 8 minutes of HIIE (22.8 minutes including recovery) in adolescent boys, and that these beneficial effects are exercise intensity-independent. Furthermore, our data cohere well with a recent study in pre-diabetic adults, where a single bout of high-intensity exercise and an isoenergetic bout of MIE (~ 180 kcal) had a beneficial effect on IS and glucose tolerance following an OGTT (Rynders et al., 2013).

It is interesting to note that the improvement in IS in the current study (MIE: 8.4% HIIE: 11.2) is smaller in magnitude than that previously reported in adolescents following acute exercise (~ 45-78%) (Short et al., 2013). The adolescents in the study by Short et al. (Short et al., 2013) were recruited based on low levels of PA and fitness, with \( \dot{V}O_2 \) max being \( \sim 30 \) mL.kg\(^{-1}\).min\(^{-1} \) on average, which is markedly lower than the current study. As reduced aerobic fitness is an independent predictor of cardiometabolic risk in children and adolescents (Andersen et al., 2006), the participants in the study by Short et al. (2013) were likely to have greater capacity for improvement in IS. However, using recently published \( \dot{V}O_2 \) max cut-off values for cardiometabolic risk (Adegboye et al., 2011), five out of the nine adolescents in the present study would be identified as being ‘at risk’, despite being of a normal weight. Another possibility for the smaller improvements in IS in the current study, may be the shorter duration and hence energy expenditure (28.9 ± 8.1 minutes, \( \sim 180 \) kcal) of the MIE condition compared to Short et al.’s study (45 minutes, \( \sim 230 \) kcal) (Short et al., 2013). Finally, the timing of the OGTT post exercise may impact IS due to the influence of counter-regulatory hormones (Rynders et al., 2013), which may explain
discrepancies between the present (10 minutes post exercise) and Short et al.’s (40 minutes post exercise) study.

Mechanistic data for the reduced plasma glucose and insulin responses and improved IS following HIIE and MIE is not available for the current study. However, it has been reported that following acute exercise, contractile induced activation of muscle glucose uptake is elevated due to increased GLUT-4 translocation to the sarcolemma membrane (Henriksen, 2002). Interestingly, it has been postulated that HIIE may be superior to MIE at improving glucose tolerance due to the rapid depletion of glycogen in high-order muscle fibres during HIIE along with increased circulating catecholamines and subsequent increase in glucose utilization (VØllestad and Blom, 1985). We, however, found work-matched bouts of HIIE and MIE to have similar beneficial effects on glucose tolerance and IS following exercise, suggesting exercise intensity per se is not an important factor, at least in healthy adolescent boys.

Although not significant, the present study reported a moderate effect (ES=0.70) for an increased fat oxidation following HIIE. This findings may be important as increased fat oxidation at rest can predict exercise induced fat loss (Barwell et al., 2009), and may have implications for IS (Samuel et al., 2010). However, no correlation was found between the increased fat oxidation following HIIE and the improvement in IS in the present study. Interestingly, a recent study in adolescents failed to observe any changes in fat oxidation over 90 minutes following 2 x 30 s ‘all out’ sprints (Burns et
al., 2012), suggesting a threshold of work done is required for improvements in fat oxidation following HIIE.

In this study adolescent boys showed similar enjoyment levels for HIIE and MIE, despite HIIE eliciting greater physiological and perceptual stress. This is consistent with a recent study showing 8-12 y old boys to prefer short sprint-interval exercise superimposed onto MIE than MIE alone (Crisp et al., 2012). The present study also highlights the time efficient nature of HIIE, with participant’s exercising 28.9 ± 8.1 for MIE compared to 22.8 minutes for HIIE of which only 8 minutes consisted of high-intensity exercise. Although a 5 minute difference may seem minimal it may enhance the applicability of this type of exercise into a school based setting, where physical education lessons are becoming increasingly limited on time. These factors may also have implications for exercise adherence, which requires further research in the form of exercise training studies.

There are a number of strengths in the current study: 1) the comparison HIIE to a work matched bout of MIE to isolate the effect of exercise intensity; 2) the control for diet and PA 48 h prior to each condition; 3) the use of an OGTT to determine glucose tolerance and IS which is deemed more physiologically valid than the clamp method, and acceptable to paediatric patients. A number of limitations, however, should be acknowledged including the lack of mechanistic data to explain the effect of HIIE and MIE on glucose disposal and insulin action. Furthermore, it is known that OGTT derived estimates of IS are highly variable in response to exercise (Ortega et al., 2014)
and there is limited research on the reliability and validity of IS indices in normal weight, healthy youth. Further limitations include a small sample size, and focus solely of males. However the n=9 is in line with previous studies of this type (Burns et al., 2012, Crisp et al., 2012, Short et al., 2013) as well a single sex focus (Burns et al., 2012, Crisp et al., 2012). The varied body mass of the participants may have influenced their response the constant glucose load of 75 g, although no correlation was observed between body mass parameters of glycaemic control (data not reported), this 75g glucose lode in also in line with clinical guidelines.

5.6. Conclusions
The novel finding of this study is that both MIE and HIIE are effective at improving glucose tolerance and IS in adolescent boys, and that these beneficial effects are exercise intensity independent. HIIE was equally enjoyable as MIE and more time efficient, highlighting its potential as a feasible form of exercise prescription for adolescents, who have a well-documented low level of habitual PA and fitness. However the long term effects of this type of exercise of glycaemic control are not yet known.
CHAPTER 6

Acute exercise and insulin sensitivity in boys: a time course study
6.1. Abstract

Background: To examine the time course of improvements in IS in adolescent boys after acute HIIE and MIE. Methods: Eight boys (15.1±0.4 y) completed three 3-day experimental trials in a randomised order: 1) 8 x 1 minute cycling at 90 % peak power with 75 s recovery (HIIE); 2) cycling at 90% of gas exchange threshold for duration to match work during HIIE (MIE) and 3) rest (CON). Capillary blood samples were taken to measure plasma glucose and insulin before (PRE-Ex), and 24 and 48 h post (24h-POST, 48h-POST) in a fasted state, and 40 minutes (POST-Ex) and 24 h (24h-POST) post in response to an OGTT. IS was estimated using the Cederholm (OGTT) and HOMA (fasted) indices. Results: There was no change to fasting plasma glucose and insulin or HOMA at 24h or 48h-POST (all P>0.05). IS from the OGTT was higher POST-EX for HIIE compared to CON (P=0.010, effect size (ES) =1.06, 17.4%), and a non-significant increase in IS after MIE compared to CON (P=0.14, ES=0.59, 9.0%). At 24h-POST, IS was higher following both HIIE and MIE compared to CON (HIIE: P=0.019, ES=0.88, 13.2%, MIE: P=0.024, ES=0.63, 9.7%). Conclusion: Improvements to IS after a single bout of HIIE and MIE persist up to 24h after exercise when assessed by OGTT. However, augmented IS was not seen using HOMA up to 48h post exercise, suggesting peripheral and not hepatic mechanisms underlie the improved IS.
6.2. Introduction

The origins of CVD and T2D are thought to begin in youth (Steinberger et al., 2009). Therefore, modification of related risk factors such as IR and glucose tolerance in this population through PA may play a crucial role in prevention of these conditions (Magnussen et al., 2013). Children and adolescents are currently recommended to undertake at least 60 minutes of MVPA on a daily basis (Janssen and LeBlanc, 2010). However, data from the UK suggest only 21% of boys aged 5-15 y old are meeting this recommendation (Towsend et al., 2015) and PA levels decline during adolescence, making this stage of development a target for interventions (Corder et al., 2013). School based intervention studies have, however, failed to increase levels of PA in youth, with a meta-analysis showing only a small (~ 4 minutes) increase in levels of MVPA per day (Metcalf et al., 2012). New approaches to promote health through PA in children and adolescents are therefore critical. One approach may be to focus on increasing levels of VPA, since small daily amounts of MVPA (~ 7 minutes) are associated with improvements in metabolic health and cardiorespiratory fitness, independent of total MVPA (Carson et al., 2014).

Time efficient, HIIE has been shown to improve cardiorespiratory fitness and cardiometabolic risk factors such as IS, fasting glucose and insulin in youth (Costigan et al 2015; Logan et al 2014). Furthermore, recent research has shown improvements in glucose and insulin health outcomes after a single bout of HIIE (Cockcroft et al., 2015, Bond. et al., 2015b, Thackray et al., 2013) and are either superior (Bond et al., 2015b) or comparable (Cockcroft et al., 2015) to work-matched MIE. However, before the optimal prescription of HIIE to improve glucose and insulin health outcomes can
be recommended, the minimum frequency of bouts needs to be determined by understanding the time course of adaptation to exercise. In adolescents characterised by low levels of PA and cardiorespiratory fitness, a single 45 minute bout of continuous MIE has been shown to increase IS post exercise, with improvements persisting, although slightly reduced, ~ 17 h post exercise (Short et al., 2013). However, unlike after MIE, the time course of changes in IS and glucose tolerance after a single bout of HIIE is currently unknown in adolescents. Understanding the time course of improvements in glucose tolerance and IS would provide useful information to inform the frequency of exercise that is needed to maintain health improvements.

The majority of youth based exercise interventions assessing metabolic health outcomes, such as IS, are judged on fasting measures (De Araujo et al., 2012, Racil et al., 2013), which predominantly reflect hepatic IS (Muniyappa et al., 2015). In contrast, the addition of a dynamic assessment in the form of an OGTT can provide detail on peripheral IS at the skeletal muscle (Muniyappa et al., 2015). Combining both fasting and OGTT indices could therefore enable an indirect insight in potential mechanisms by which HIIE and MIE alters IS in youth, where the use of more invasive procedures is difficult due to ethical issues.

The primary aim of this study was to extend the work of Chapter 5 (Cockcroft et al., 2015) by testing the following novel hypotheses in healthy adolescent boys: 1) that both HIIE and work-matched MIE would improve IS and glucose tolerance for up to 24 h after exercise; and 2) that health improvements would only be found using
dynamic OGTT outcomes and not fasting, reflecting the effect of exercise on enhancing peripheral IS.

6.3. Materials and methods

6.3.1. Participants

Eight adolescent boys (15.1 ± 0.4 y) were recruited from a local secondary school. All participants were deemed able to participate in the study by completing an initial health questionnaire. Exclusion criteria included the presence of any known disease or contraindications to exercise, as well as the use of medications that are known to influence glucose metabolism. Following an explanation of the study procedures and the associated risks and benefits, written informed parental consent and participant assent were obtained. Ethics approval was granted by the University of Exeter, Sport and Health Sciences ethics committee (141203/B/11).

6.3.2. Experimental design

This cross-over study consisted of 10 experimental visits over a 4 week period for each participant. Visits included an initial familiarisation visit and a further 9 visits for 3 experimental conditions with each condition consisting of 1 visit to the research laboratory and 2 visits to a satellite laboratory at the school. All procedures were identical during the experimental conditions with the only difference being the exercise or rest (CON) undertaken. These conditions took place in a counterbalanced order. All exercise was performed on a cycle ergometer (Lode Excalibur Sport, Gronigen,
Netherlands) with appropriate adjustment made to the ergometer handle bar and seat height for each participant.

Visit 1: Anthropometry and aerobic fitness

Stature and body mass were measured to the nearest 0.01 m (Harpenden, Holtain Ltd, Crymych, UK) and 0.1 kg (Seca 877, Seca Ltd, Birmingham, UK). Skinfold thickness was measured by the same investigator to the nearest 0.2 mm on the right hand side of the body using skinfold callipers (Holtain Limited, UK). The median of three measurements at each anatomical site was used to estimate body fat percentage using validated maturation, race and sex specific equations (Slaughter et al., 1988). Body mass index was calculated (body mass/height$^2$) and used to identify overweight and obese participants using validated age specific centiles (Cole et al., 2000). Pubertal status was determined by self-assessment of the five stages of pubic hair development as described by Tanner (Tanner and Whitehouse, 1976).

Participants were then familiarized with the cycle ergometer and completed a combined ramp-incremental and supramaximal test to exhaustion to determine maximal O$_2$ uptake ($\dot{V}O_2$ max) and the GET (Barker et al., 2011). Pulmonary gas exchange and heart rate were measured throughout the test (Cortex Metalyzer III B, Leipzig, Germany). $\dot{V}O_2$ max was accepted as the highest 10 s average $\dot{V}O_2$ during either the ramp or supra-maximal test. The GET was estimated from the disproportionate increase in CO$_2$ production compared to O$_2$ and verified using the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$. 
Visits 2-10: Experimental conditions

Each of the three experimental conditions took place over a 3 day period consisting of 3 visits. On day one participants were transported by car to the laboratory at ~08:00 following a 12 h overnight fast. After 10 minutes of seated rest a capillary blood sample for plasma glucose and insulin (PRE-Ex) was taken. At ~08:30, participants undertook one of the following conditions in a counterbalanced order: 1) HIIE, consisting of a 3 minute warm up at 20 W followed by eight repeated bouts of 1 minute cycling at 90% of peak power, interspersed with 75 s recovery at 20 W, followed by 3 minutes cool down at 20 W; 2) MIE, consisting of continuous cycling at 90% GET, the duration of which was determined to work-match the HIIE condition; and 3) seated rest (CON). These exercise protocols were selected based on previous research in this area showing improved cardiometabolic health outcomes in youth (Bond. et al., 2015b, Bond. et al., 2015d, Cockcroft et al., 2015). Throughout the exercise conditions, gas exchange and heart rate were monitored. Participants provided a rating of perceived exertion (RPE) using the validated Pictorial Children’s Effort Rating Table (Yelling et al., 2002) every 5 minutes during MIE and immediately following each 1 minute interval during HIIE. Upon completion of each exercise condition, participants completed the validated 16-point PA Enjoyment Scale (PACES) to examine exercise enjoyment (Motl et al., 2001). Immediately after the experimental conditions a further capillary blood sample was taken for the assessment of plasma glucose and insulin.
Forty minutes after the completion of each experimental condition, an OGTT took place. After collection of a baseline capillary blood sample (designated as 0 minutes) participants consumed a glucose drink (75 g of glucose from 394 mL of Lucozade original) within a 5 minute period, after which capillary blood samples were taken at 10, 20, 30, 60, 90 and 120 minutes for assessment of plasma glucose and insulin (POST-Ex). During the 2 h postprandial period, no other food was consumed, although water was available ad libitum. This was recorded for each participant in the first experimental condition and subsequently replicated for the remaining conditions. Participants remained in the laboratory throughout the visit, completing activities such as reading, watching DVDs or playing computer games involving minimal movement. Participants left the laboratory at approximately 13:00 and were returned to school.

On day 2 (24h-POST), following a 12 h overnight fast, participants were transported by car to a satellite laboratory within the school at ~ 8:00 am. Participants provided a capillary blood sample for plasma insulin and glucose prior to commencing a further OGTT using the same methodology as outlined above. Participants remained in the laboratory for a 2 h period after the OGTT for blood measures, after which they returned to their scheduled school activities.

On day 3 (48h-POST), again after a 12 h overnight fast, participants were transported by car to the satellite laboratory at ~ 8:00 am where a single capillary blood sample was taken for plasma insulin and glucose determination. Participants then returned to their scheduled school activities.
6.3.3. Control measures

PA was measured during the 48 h period prior to, and during the 48 h follow up period of each condition, using a wrist worn accelerometer (GENEActiv, GENEa, UK). Accelerometers were worn on the non-dominant wrist and data sampled at 100 Hz. Data were converted into 60 s epochs and used to estimate the time spent during sedentary, light, moderate and VPA using cut points validated in children (Phillips et al., 2013). Participants were asked to avoid any strenuous PA during the 48 h period before and during each experimental condition.

With supervision from their parents/guardians, a food diary was completed by each participant during the 48 h period preceding, and during the 48 h follow up period of each condition. Participants were asked to include all food and drink consumed during this period in household measures and also include cooking methods. The food diaries were assessed for total energy and macronutrient content using commercially available software (CompEat Pro, Nutrition systems, UK). Participants were asked to replicate their diet during each experimental condition, and if appropriate to document any discrepancies.

6.3.4. Blood analyses

Fingertip capillary blood samples (~ 600 µL) were taken from a pre-warmed hand into a heparin fluoride coated and lithium heparin coated microvetttes (CB 300 tubes, Sarstedt Ltd, Leicester, UK) for plasma glucose and insulin determination respectively. Both microvetttes were centrifuged at 1300 g for 10 minutes. Plasma was separated
for analysis of glucose (YSI 2300 Stat Plus Glucose analyser, Yellow Springs, OH, USA) or stored at –80°C for later analysis of plasma insulin using an ELISA enzyme immunoassay kit (DRG Diagnostics, Germany) The within batch coefficient of variation for the plasma insulin and glucose analyses was < 5%.

6.3.5. Calculations

Changes in plasma glucose and insulin during the OGTT were quantified using area under the curve (AUC) analyses using the trapezium rule (GraphPad Prism, GraphPad, SanDiego, CA). Both tAUC and iAUC analyses were undertaken to characterise the magnitude of the response and changes over time respectively.

The plasma glucose and insulin responses during the OGTT were used to calculate IS using Cederholm index (Cederholm and Wibell, 1990) in line with previous studies (Cockcroft et al., 2015, Babraj et al., 2009). Fasting plasma glucose and insulin measures were used to calculate IR (IR), IS (%IS) and β-cell function (%B) using homeostatic model assessment (HOMA) 2 programme (Levy et al., 1998), downloaded from the University of Oxford Diabetes Trials Unit website. The HOMA method has been validated for use in an adolescent population against the euglycaemic clamp technique (Gungor et al., 2004).

6.3.6. Statistical analyses

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Mean differences in the physiological and perceptual
responses during HIIE and MIE were analysed using paired samples t tests. Analysis of fasting plasma glucose and insulin, HOMA indices, IS and AUC analyses for plasma glucose and insulin were performed using a two-way repeated measures ANOVA. Normality of distribution for each variable was checked using the Shapiro-Wilk test. Pairwise comparisons between means were interpreted using P-values and standardised effect sizes (ES) to detail the magnitude of the effect. The magnitude of the mean difference between variables of interest were explored using ES thresholds of trivial (<0.2), small (>0.2), moderate (>0.5), large (>0.8), and very large (>1.0) (Cohen, 1992). Results are presented as (P value, ES), unless stated otherwise.

6.4. Results

The participant descriptive characteristics are shown in Table 6.1. Tanner stage ranged between 4 and 5 (stage 4: n= 7, stage 5: n=1). According to BMI, six participants were classified as “normal weight” and 2 “overweight”. Two participants were classified as “at risk” according to their HOMA-IR.
Table 6.1. Participant descriptive characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>15.05 ± 0.37</td>
<td>14.60 to 15.60</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>65.6 ± 9.1</td>
<td>52.55 to 84.60</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.73 ± 0.08</td>
<td>1.58 to 1.82</td>
</tr>
<tr>
<td>BMI (kg·m²)</td>
<td>21.77 ± 2.19</td>
<td>19.33 to 26.08</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>18.13 ± 3.03</td>
<td>13.43 to 23.65</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.91 ± 0.75</td>
<td>2.19 to 4.10</td>
</tr>
<tr>
<td>Fasting glucose (mmol·L⁻¹)</td>
<td>5.31 ± 0.21</td>
<td>4.91 to 5.55</td>
</tr>
<tr>
<td>Fasting insulin (µIU·mL)</td>
<td>22.80 ± 6.06</td>
<td>16.89 to 32.75</td>
</tr>
<tr>
<td>Peak power (W)</td>
<td>275± 22</td>
<td>241 to 305</td>
</tr>
<tr>
<td>$\dot{V}O_2$ max,L·min⁻¹</td>
<td>3.23 ± 0.38</td>
<td>2.58 to 3.76</td>
</tr>
<tr>
<td>$\dot{V}O_2$ max,mL·kg⁻¹·min⁻¹</td>
<td>49.5 ± 4.9</td>
<td>40.6 to 55.6</td>
</tr>
<tr>
<td>GET (L·min⁻¹)</td>
<td>1.48 ± 0.21</td>
<td>1.13 to 1.80</td>
</tr>
<tr>
<td>GET (% $\dot{V}O_2$ max)</td>
<td>45.88 ± 4.50</td>
<td>40.12 to 52.90</td>
</tr>
</tbody>
</table>

Results shown as mean ± SD and range. Abbreviations: BMI, body mass index; GET, gas exchange threshold; HOMA-IR, Homeostatic model of insulin resistance, Blood variables expressed as average of Pre_Ex values across all three conditions: % body fat determined from skin fold measurements.

The amount of light, moderate and VPA before and during each condition was similar across conditions (n=6 due to accelerometer data loss, all P>0.05). Dietary intake of CHO, fat and protein were similar for each condition (all P>0.05). Dietary intake and PA data are shown in Table 6.2.

The physiological and perceptual responses to the exercise conditions are shown in Table 6.3. HIIE elicited higher physiological and perceptual stress than MIE, with significantly higher $\dot{V}O_2$, HR and RPE responses compared to MIE (all P<0.05). PACES data found HIIE to be more enjoyable than MIE and 7 out of the 8 participants reported a preference for HIIE.
### Table 6.2. Daily PA and dietary intake during the 48 h preceding and during each experimental visit

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON</th>
<th>MIE</th>
<th>HIIE</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dietary intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI, (kcal)</td>
<td>1474 ± 280</td>
<td>1747 ± 388</td>
<td>1476 ± 356</td>
<td>0.48</td>
</tr>
<tr>
<td>% CHO</td>
<td>48 ± 5</td>
<td>50 ± 8</td>
<td>48 ± 6</td>
<td>0.66</td>
</tr>
<tr>
<td>% fat</td>
<td>32 ± 6</td>
<td>35 ± 6</td>
<td>37 ± 5</td>
<td>0.16</td>
</tr>
<tr>
<td>% Protein</td>
<td>19 ± 7</td>
<td>16 ± 5</td>
<td>16 ± 3</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Physical activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary (min.day⁻¹)</td>
<td>515 ± 164</td>
<td>419 ± 170</td>
<td>462 ± 195</td>
<td>0.62</td>
</tr>
<tr>
<td>Light (min.day⁻¹)</td>
<td>247 ± 120</td>
<td>249 ± 163</td>
<td>295 ± 187</td>
<td>0.84</td>
</tr>
<tr>
<td>Moderate (min.day⁻¹)</td>
<td>111 ± 24</td>
<td>78 ± 41</td>
<td>110 ± 11</td>
<td>0.18</td>
</tr>
<tr>
<td>Vigorous (min.day⁻¹)</td>
<td>14 ± 5</td>
<td>12 ± 17</td>
<td>16 ± 10</td>
<td>0.84</td>
</tr>
<tr>
<td>MVPA (min.day⁻¹)</td>
<td>125 ± 27</td>
<td>91 ± 56</td>
<td>126 ± 8</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Results shown as mean ± SD, probability (P), MVPA: moderate to vigorous physical activity. MVPA includes the MIE and HIIE exercise sessions on day 3 (~ 22-30 min). Due to data loss physical activity data are reported for n=6.

### Table 6.3. Physiological and perceptual responses to HIIE and MIE

<table>
<thead>
<tr>
<th>Variable</th>
<th>MIE</th>
<th>HIE</th>
<th>P</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power output , W</td>
<td>81 ± 15</td>
<td>247 ± 20</td>
<td>&lt;0.001</td>
<td>7.83</td>
</tr>
<tr>
<td>( \dot{V}O_2 ) (L.min⁻¹)</td>
<td>20.7 ± 3.4</td>
<td>38.1 ± 10.0</td>
<td>&lt;0.001</td>
<td>1.90</td>
</tr>
<tr>
<td>( \dot{V}O_2 % \text{max} )</td>
<td>42.1 ± 8</td>
<td>77.4 ± 20</td>
<td>&lt;0.001</td>
<td>1.86</td>
</tr>
<tr>
<td>HR, beats.min⁻¹</td>
<td>130 ± 12</td>
<td>179 ± 10</td>
<td>&lt;0.001</td>
<td>3.35</td>
</tr>
<tr>
<td>HR, % peak</td>
<td>68 ± 6</td>
<td>95 ± 3</td>
<td>&lt;0.001</td>
<td>4.13</td>
</tr>
<tr>
<td>RPE</td>
<td>3 ± 1</td>
<td>5 ± 1</td>
<td>&lt;0.001</td>
<td>1.62</td>
</tr>
<tr>
<td>PACES</td>
<td>47 ± 7</td>
<td>54 ± 7</td>
<td>&lt;0.05</td>
<td>0.73</td>
</tr>
<tr>
<td>Duration, min</td>
<td>28.7 ± 4.6</td>
<td>22.8 ± 0</td>
<td>&lt;0.05</td>
<td>1.47</td>
</tr>
<tr>
<td>Work done, kJ</td>
<td>136 ± 94</td>
<td>136 ± 94</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Energy expenditure, kcal</td>
<td>206 ± 31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results shown as mean ± SD, probability (P), and effect size (ES). Abbreviations: HR, Heart rate; RPE, ratings of perceived exertion; PACES, physical activity enjoyment scale. P, probability. ES, effect size.
Fasting outcomes

Fasting plasma insulin, glucose and the HOMA indices are shown in Figure 6.1. For fasting plasma glucose and insulin there was no main effect for time (all $P>0.29$) or condition (all $P>0.39$) nor a condition by time interaction (all $P>0.42$). Likewise, there was no main effect for time (all $P>0.20$) or condition (all $P>0.47$) or a condition by time interaction (all $P>0.25$) for HOMA-IR, HOMA-S% and HOMA-β.

**Figure 6.1.** Fasting glucose, insulin and HOMA-IR, HOMA-S% and HOMA-B at PRE-Ex, 24h-POST and 48h-POST exercise Where; • CON, • MIE, △ HIIE. Error bars omitted for clarity.
OGTT outcomes

OGTT outcomes at POST-Ex and 24h-POST are shown in Figure 6.2. The 2 h iAUC plasma glucose showed a trend for a significant main effect of condition \( (P=0.051) \) and a significant main effect of time \( (P=0.019) \), but no significant interaction effect \( (P=0.73) \). Exploratory comparisons of main effect for condition found a large reduction in iAUC plasma glucose after HIIE compared to CON \( (P=0.033, \text{ES}=-0.81, -25\%) \), whilst MIE was moderately but not significantly reduced compared to CON \( (P=0.15, \text{ES}=-0.58, -18.6\%) \). There was a small non-significant reduction in HIIE compared to MIE \( (P=0.27, \text{ES}=-0.33, -10.0\%) \). The main effect of time found a significant reduction in iAUC plasma glucose at 24h-POST compared to POST-Ex \( (P=0.019, \text{ES}=-0.36, -15.7\%) \).

The 2 h tAUC for plasma glucose showed a significant condition effect \( (P=0.011) \), but no effect of time \( (P=0.934) \) nor condition by time interaction \( (P=0.65) \). Follow up analysis of the condition main effect indicates significant, large and very large reductions in tAUC for plasma glucose after MIE and HIIE compared to CON \( (P=0.038, \text{ES}=-0.93, -7.7\% \) and \( P=0.015, \text{ES}=-1.12, -9.2\% \), respectively). There was a small, non-significant reduction in HIIE compared to MIE \( (P=0.36, \text{ES}=-0.33, -7.9\%) \).

The iAUC for plasma insulin showed a trend for a condition main effect \( (P=0.074) \) with no main effect of time \( (P=0.92) \) or a condition by time interaction \( (P=0.95) \). In contrast, there was a significant condition effect for tAUC insulin \( (P=0.033) \) with no main effect of time \( (P=0.11) \) or condition by time interaction \( (P=0.95) \). Follow up analysis of the
condition main effect found a significant and large reduction after HIIE \( (P=0.005, \text{ES}=-0.81, -21.4\%) \) but not MIE \( (P=0.18, \text{ES}=-0.48, -14.6\%) \) compared to CON, and no differences between MIE and HIIE \( (P=0.27, \text{ES}=-0.33, -7.9\%) \).

There was a significant main effect for condition \( (P=0.006) \) and time \( (P=0.027) \) but no condition by time interaction \( (P=0.61) \) for IS (Cederholm). Follow up analysis of the main effect for condition highlighted a very large and significant increase in IS after HIIE compared to CON \( (P=0.004, \text{ES}=1.07, 15.3\%) \) but only a moderate trend for an increase in IS after MIE compared to CON \( (P=0.054, \text{ES}=0.68, 9.3\%) \). There was no difference in IS between MIE and HIIE \( (P=0.23, \text{ES}=0.39, 5.5\%) \). The main effect for time found a significant decrease in IS (Cederholm) from 24h-POST compared to POST-Ex \( (P=0.027) \). At POST-Ex there was a very large, significant reduction in HIIE compared to CON \( (P=0.010, \text{ES}=1.06, 17.4\%) \) with only a non-significant and moderate reduction in MIE compared to CON \( (P=0.14, \text{ES}=-0.59, 9.0\%) \) and HIIE and MIE \( (P=0.15, \text{ES}=-0.47, 7.8\%) \). At 24h-POST, IS after MIE and HIIE was significantly higher than CON \( (P=0.024, \text{ES}=0.65, 9.7\% \text{ and } P=0.019, \text{ES}=0.88, 13.2\% \), respectively) with no difference between MIE and HIIE \( (P=0.54, \text{ES}=0.23, 3.2\%) \).
Figure 6.2. Changes to tAUC, iAUC glucose, insulin and IS at POST-Ex and 24h-POST after an OGTT. Where; ● CON. • MIE, △ HIIE.* represents a significant difference for HIIE vs. CON and # represents a significant difference MIE vs. CON.
6.5. Discussion

The primary finding from this study was that a single bout of HIIE is effective at improving OGTT derived estimates of IS, glucose tolerance and insulin for up to 24 h after exercise. Additionally the magnitude of improvements for HIIE compared to CON were larger than that of MIE. However, neither HIIE nor MIE elicited a change to fasting plasma glucose and insulin or HOMA derived estimates measured up to 48 h post exercise. Seven out of the 8 participants expressed a preference to perform HIIE over MIE, which is reflected in significantly higher PACES score following HIIE compared to MIE. These novel findings further document the potential of HIIE to modulate glucose and insulin outcomes for up to 24 h after a single bout of exercise in youth, as well as highlighting the importance of using dynamic tests to observe the benefits of exercise on glucose and insulin outcomes in comparison to fasting indices.

In agreement with the results from the current study, we have previously shown in a separate sample of adolescent boys a single bout of both HIIE and MIE improves IS and glucose tolerance in response to an OGTT performed immediately after exercise (Cockcroft et al., 2015). Results from the present study extend this evidence base, by showing that these effects persist for up to 24 h after exercise when examined using an OGTT and that the magnitude of the effects are greater after HIIE suggesting an exercise intensity dependence on these health outcomes. In the present study only HIIE significantly improved IS and had a consistently higher magnitude of change as judged by the ES compared to MIE when evaluated against the control condition (ES of 1.07 vs 0.68 for IS after HIIE and MIE compared to CON respectively).
Our data corroborate the work of Short and colleagues (2013), who measured IS after a single bout of MIE (45 minutes at 75% peak HR). They showed improvements in IS of 78% and 45% when exercise was performed either 1 h or 17 h prior to an OGTT respectively. Results from the present study also show a trend (9.3 %) for an improvement in IS after MIE which maintained up to 24 h post exercise. The present study also adds to the work of Short and colleagues showing that alongside MIE, HIIE can also improve IS immediately after exercise, with improvements (15.3 %) also lasting through to the next day and little evidence of a decline over time. However, when expressed as a % change, the magnitude of change in IS in the present study is lower than that of Short and colleagues which could be explained by their sample characteristics who were preselected based on low levels of PA and cardiorespiratory fitness ($\dot{V}O_2$ max of 29.4 ± 7.4 vs. 49.50 ± 4.88 mL·kg⁻¹·min⁻¹ in the current study). This suggests that children with low levels of habitual PA and cardiorespiratory fitness are likely to benefit the most in terms of augmenting IS after an acute bout of exercise. Alternatively, the lower magnitude of change in the current study could be due to the lower energy expenditure during MIE (136 ± 94 kJ vs. 971 ± 92 kJ). However, our earlier work (Cockcroft et al., 2015), and the present study show that improvements in glucose and insulin outcomes immediately after exercise and these persist up to 24 h post exercise in physically active adolescent boys.

A novel and interesting finding in the current study was that HIIE and MIE had no effect on fasting plasma glucose and insulin or HOMA derived indices, despite significant improvements to dynamic measures of IS and glucose tolerance. This finding for fasting plasma glucose and insulin is consistent with earlier work in adolescents. For
example, using a two day protocol Thackeray and colleagues (2013) found no changes to fasting glucose or insulin ~ 15 h after completing 10 x 1 minute running HIIE in adolescent boys (13 ± 0.3 y). Similarly, Tolfrey et al (2008) found no changes to fasted insulin and glucose measured ~ 17 h after moderate (6 x 10 minute blocks at 53% V̇O₂peak) or vigorous intensity (6 x 10 minute blocks at 75% V̇O₂peak) exercise in a group of healthy active boys (11.8 ± 0.4 y). Unlike the present study, both Thackery et al (2013), and Tolfrey et al (2008) also found no significant change to postprandial tAUC measures of insulin and glucose, however this was over a 6 h postprandial period and in response to a high fat meal challenge, as opposed to an OGTT in the present work. Conversely, in adults, a single 30-120 minutes bout of cycling or running exercise at 60 % V̇O₂peak has been shown to reduce fasting glucose, insulin and HOMA-IR measured ~ 12 h later (Magkos et al., 2008). This study found a negative correlation between exercise energy expenditure and changes in fasting plasma glucose and insulin and HOMA-IR, with no apparent effect of exercise when energy expenditure was below 900 kcal. Therefore, the lack of observed changes to fasting outcomes after an acute bout of exercise in the current study and previous paediatric work (Thackray et al., 2013) may be due to low energy expenditure. In the present study, MIE had a mean energy expenditure of 206 ± 31 kcal, and in the research by Tolfrey and colleagues (Tolfrey et al., 2008) this ranged from 367 to 522 kcal; both below this proposed “threshold”. Unfortunately, a dose-response curve for energy expenditure and plasma glucose and insulin outcomes are not currently available in adolescents following a single bout of exercise.
Due to ethical considerations, mechanistic data for the changes in IS and glucose tolerance not available in the current study. However, the varied responses in fasting and dynamic outcomes suggest possible differences in mechanisms after exercise. For example, it has been suggested that fasting measures are more representative of hepatic glucose output and insulin secretion (Muniyappa et al., 2015), implying that a single bout of HIIE and MIE do not alter hepatic glucose regulation. Conversely, IS is estimated using the Cederholm index is proposed to reflect peripheral IS and muscular glucose uptake (Cederholm and Wibell, 1990). This suggests that the acute exercise in the current study augmented IS at the peripheral level. Indeed, it is known that a single bout of exercise leads to depletion of skeletal muscle glycogen stores (Thompson et al., 2001), which may enhance post exercise glucose uptake in order to replenish glycogen (Henriksen, 2002). This glycogen repletion is thought to have two phases: 1) a rapid phase up to 1 h post exercise cessation, which is likely to reflect the changes seen in the current study at POST-EX; and 2) a slow phase lasting 1-2 days post exercise (Price et al., 1999), which may be responsible for the observed maintenance of heightened IS at POST-24 h. The slow phase for glycogen repletion is facilitated by increased translocation of the GLUT-4 skeletal muscle glucose transporter to the cell membrane (Hansen et al., 1998). The increased GLUT-4 translocation in muscle is thought to have a short life and be reversed within 40 h of the last exercise bout (Host et al., 1998), which is consistent with our improvements persisting up to 24 h post exercise. The improvements to IS following HIIE but not MIE compared to CON in the present study may be due to greater glycogen depletion during HIIE, and increased GLUT-4 translocation compared to MIE (Malin et al., 2016).
This study is the first to assess the time course of changes to IS and glucose tolerance after an acute bout of HIIE and MIE, as well as comparing changes in fasting and dynamic tests, in adolescent boys. A strength is that this study included a direct comparison of two different exercise intensities, which were work-matched to isolate the effect of exercise intensity. Secondly, the control of diet and PA in the 48 h preceding and during each experimental condition is important to limit any confounding effect. As with all studies, a number of limitations are noted. Firstly, the small sample size, of healthy adolescent boys, limits extrapolation of the results. Additionally due to the invasive nature of the “gold standard” hyperinsulinemic euglycaemic clamp to assess IS, this was not be feasible in this study on healthy adolescent boys. Therefore, IS was indirectly examined using the OGTT. Finally, it was not possible to conduct a further OGTT at 48 h post exercise due to concerns about time and commitment burden on participants.

6.6. Practical implications

This study highlights for the first time that HIIE can improve IS and glucose tolerance up to 24 h after exercise in adolescent boys. This is important as it suggests that metabolic benefits may be maintained if HIIE is performed on alternate days. In addition to the metabolic benefits of exercise, this study also showed that this group found HIIE more enjoyable than the MIE which is consistent with previous work from our laboratory (Bond. et al., 2015d, Cockcroft et al., 2015), and likely to be of practical value since enjoyment and preference may influence adherence to an exercise programme.
Of further importance is the methodological consideration related to the use of fasting and dynamic assessments of IS. OGTT outcomes appear to be more sensitive to the effects of exercise, likely due to augmenting peripheral IS. These findings suggest previous research (Logan et al., 2016) where IS was only measured in a fasted state, may have missed key health benefits. This is of importance since postprandial glucose and insulin outcomes have been shown to be a key risk factor in the development of CVD and T2D, more so than fasting measures (Temelkova-Kurktschiev et al., 2000). This highlights the importance of using dynamic measures of IS and glucose tolerance when assessing the effectiveness of exercise interventions.

6.7. Conclusion

This study shows for the first time that improvements to IS and glucose tolerance after a single bout of HIIE and MIE can last up 24 h after exercise in healthy adolescent boys. However, the magnitude of the effect for HIIE was greater than MIE when compared to CON and participants also showed preference to HIIE and found it more enjoyable. We also show that the beneficial effect of exercise was only found after an OGTT as fasting measures of glucose, insulin and HOMA indices were unaltered after exercise. Evidence that the beneficial effects of acute exercise on IS and glucose tolerance persist for up to 24 h suggests exercise can be performed on alternate days in order to maintain improvements to insulin and glucose outcomes.
CHAPTER 7

A single bout of high-intensity interval exercise and work-matched moderate intensity exercise has minimal effect on glucose tolerance and insulin sensitivity in 7-10 y old boys.
7.1. Abstract

Background: The purpose of this study was to assess the acute effect of HIIE and MIE on glucose tolerance, IS and fat oxidation in young boys. Methods: Eleven boys (8.8 ± 0.8 y) completed three conditions: 1) HIIE; 2) work matched MIE and 3) rest (CON) followed by an OGTT to determine glucose tolerance and IS (Cederholm index). Fat oxidation was measured following the OGTT using indirect calorimetry. Results: There was no effect for condition on plasma glucose and insulin area under the curve (AUC) responses following the OGTT (P>0.09). However, there was a “trend” for a condition effect for IS with a small increase after HIIE (P=0.04, ES=0.28, 9.7%) and MIE (P=0.07, ES=0.21, 6.5%) compared to CON. There was an increase in fat oxidation AUC following HIIE (P=0.008, ES=0.79, 38.9%) compared to CON, but with no differences between MIE and CON and HIIE and MIE (P>0.13). Conclusion: 7-10 y old boys may have limited scope to improve IS and glucose tolerance after a single bout of HIIE and MIE. However, fat oxidation is augmented after HIIE but not MIE.
7.2. Introduction

CVD and T2D are a major global health concern. Both IR and IGT have been implicated in the development of these diseases and are components of the metabolic syndrome (Weiss et al., 2013). The origins of these diseases may lie in childhood (McGill et al., 2008). Therefore, strategies that can modify glucose and insulin during childhood may play an important role in disease prevention in later life.

Higher levels of PA and cardiorespiratory fitness are associated with lower glucose and insulin in youth (Schmitz et al., 2002). As such, children and adolescents are currently recommended to undertake at least 60 minutes of MVPA on a daily basis (Janssen and LeBlanc, 2010). However, recent UK surveillance data indicates only 26% of boys aged 8-10 y of age reach this recommended amount of daily PA (Towsend et al., 2015). Interventions to increase levels of MVPA have also been ineffective (Metcalf et al., 2014), meaning recent research has focused on alternative forms of exercise, such as “time efficient” HIIE. This is because improvements in health outcomes in children and adolescents may be obtained with as little as ~ 7 minutes of VPA per day (Carson et al., 2014).

Previous research has shown HIIE protocols consisting of 8-10 1 minute bouts of high-intensity exercise to be effective at improving both insulin and glucose outcomes in adolescents (Bond et al., 2015d, Cockcroft et al., 2015). However, IR increases with age and pubertal status (Ball et al., 2006, Jeffery et al., 2012) and recent prospective evidence shows the relationship between PA and insulin changes throughout
development (Metcalf et al., 2015). It could, therefore, be hypothesised that the acute effects of exercise on glucose and insulin are dependent on age/pubertal status, with adolescents having a greater scope for improvements compared to younger children. However, the effect of a single bout of HIIE on glucose and insulin has not been examined in younger children.

Therefore, the objective of this study is to elucidate the effects of a single bout of HIIE compared to a work-matched bout of continuous MIE on glucose tolerance, IS and fat-oxidation in 7-10 y old boys.

7.3. Methods

7.3.1. Participants

Eleven boys (age: 8.8 ± 0.8 y old) were recruited from local primary schools. Following an explanation of the study procedures and the associated risks and benefits, parental consent and participant assent were obtained. Exclusion criteria included the presence of any known disease or contraindications to exercise, as well as the use of medications that are known to influence glucose metabolism. Ethics approval was granted by the institutional ethics committee.

7.3.2. Experimental design

This cross-over study consisted of four laboratory visits, each separated by approximately one week. Visits included an initial familiarisation visit and three
experimental conditions in a temperature controlled laboratory. All procedures were identical during the experimental conditions apart from the exercise or rest period undertaken. Exercise was performed on a cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands).

During visit 1 stature and body mass were measured to the nearest 0.01 m (Harpenden, Holtain Ltd, Crymych, UK) and 0.1 kg (Seca 877, Seca Ltd, Birmingham, UK). Skinfold thicknesses were measured by the same investigator to the nearest 0.2 mm and used to estimate percentage body fat using established equations (Slaughter et al., 1988). Body mass index was calculated (body mass /height$^2$) and used to identify overweight and obese participants using validated age specific centiles (Cole et al., 2000). Pubertal status was determined by self-assessment of the five stages of pubic hair development (Tanner and Whitehouse, 1976). Participants were familiarised with the cycle ergometer and completed a combined ramp-incremental and supramaximal test to exhaustion to determine $\dot{V}O_2\text{max}$ and GET (Barker et al., 2011). Pulmonary gas exchange (Cortex Metalyzer III B, Germany) and heart rate (Polar, Finland) were measured throughout the tests. The metabolic cart was calibrated before each measurement, using standard calibration gas and a 3.0 L calibration syringe (Hans Rudolph, USA). $\dot{V}O_2\text{max}$ was accepted as the highest 10 s average $\dot{V}O_2$ during the ramp or supra-maximal test. The GET was estimated at the point where the first disproportionate increase in CO$_2$ production compared to $\dot{V}O_2$ during the ramp incremental test.
For visits 2-4, participants were transported to the laboratory by car and arrived at ~08:00, following a 12 h overnight fast. After 10 minutes of seated rest, participants provided a fingertip capillary blood sample for plasma glucose and insulin. Baseline resting metabolic rate (RMR) was determined via indirect calorimetry (Cortex Metalyzer II, Leipzig, Germany) over a 10 minutes period to determine total energy expenditure (EE) and fat oxidation. At ~ 08:30 participants undertook one of the following conditions in a counterbalanced order: 1) HIIE: 3 minute warm up at 20 W followed by eight repeated bouts of 1 minute cycling at 90% of the peak power, interspersed with 1 minute 15 s recovery at 20 W, followed by a 3 minute cool down at 20 W; 2) MIE: continuous cycling at 90% GET, the duration of which was determined to work-match the HIIE; and 3) seated rest (CON). Throughout the exercise conditions, gas exchange and heart rate were monitored. Participants provided a rating of perceived exertion (RPE) every 5 minutes during MIE and immediately following each 1 minute interval during HIIE (Yelling et al., 2002). Upon completion of each exercise condition participants completed the 16-point PA Enjoyment Scale (PACES) (Motl et al., 2001).

Ten minutes after the completion of each experimental condition, an OGTT took place. Participants consumed 1.75 g·kg⁻¹ body mass (maximum of 75 g) of glucose in 300 mL of water, after which capillary blood samples were taken at 0, 10, 20, 30, 60, 90 and 120 minutes for assessment of plasma glucose and insulin. RMR was measured whilst lying prone at 60, 120 and 180 minutes post OGTT. During the 3 h postprandial measurement period, no other food was consumed although water was available ad libitum. Water intake was recorded for the first experimental condition and
subsequently replicated for the remaining conditions. Participants remained in the laboratory throughout the visit, completing sedentary activities such as reading, watching DVDs or playing computer games. Participants left the laboratory at ~ 13:00.

7.3.3. Control measures
PA was measured during the 48 h period prior to each condition using a wrist worn accelerometer (GENEActiv, GENEA, UK). Data were converted into 1 minute epochs and used to estimate the time spent during sedentary, light, moderate and VPA using cut points validated in children (Phillips et al., 2013). Participants were asked to avoid any organised PA during this period. With supervision from their parents/guardians, a food diary was completed by each participant during the 48 h period preceding each experimental condition. Food diaries were assessed for total energy and macronutrient content using commercially available software (CompEat Pro, Nutrition systems, UK). Participants were asked to replicate their diet during the 48 h preceding each experimental condition and if appropriate, to document any discrepancies.

7.3.4. Blood analyses
Fingertip capillary blood samples (~ 600 µL) were taken from a pre-warmed hand into a heparin fluoride coated and lithium heparin coated microvette (CB 300 tubes, Sarstedt Ltd, Leicester, UK) for plasma glucose and insulin determination respectively. Both micorvettes were centrifuged at 1300 g for 10 minutes. Plasma was separated for immediate analysis of glucose (YSI 2300 Stat Plus Glucose analyser, Yellow Springs, OH, USA) or stored at −80°C for later analysis of plasma insulin using an
ELISA enzyme immunoassay kit (DRG Diagnostics, Germany). The within batch coefficient of variation for the plasma insulin and glucose analyses was < 5%.

7.3.5. **Calculations**

Changes in plasma glucose and insulin during the OGTT were tAUC and iAUC analyses employing the trapezium rule (GraphPad Prism, GraphPad, SanDiego, CA). Plasma glucose and insulin responses during the OGTT were used to calculate IS using the Cederholm index (Cederholm and Wibell, 1990) in line with previous studies (Cockcroft et al., 2015, Babraj et al., 2009). Fasting plasma glucose and insulin were used to calculate IR using homeostatic model assessment (Levy et al., 1998). HOMA-IR was used to classify participants as “as risk” according to age-specific cut-points (Shashaj et al., 2015). Resting energy expenditure (EE) and the absolute fat oxidation were estimated using the mean \( \dot{V}O_2 \) and respiratory exchange ratio for each 10 minutes measurement (Frayn, 1983). AUC analysis was used to document changes in EE and fat oxidation following the OGTT.

7.3.6. **Statistical analyses**

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and are presented as mean ± SD. Mean differences in the physiological and perceptual responses during HIIE and MIE were analysed using paired samples \( t \) tests. Analysis of fasting outcomes, IS and the AUC analyses across conditions were performed using a one way repeated measures ANOVA. The Eta squared thresholds of 0.01, 0.06 and 0.14 were used to identify a small, moderate and large effect from the ANOVA.
analyses. Subsequent pairwise comparisons between means were interpreted using $P$-values and standardised effect sizes ($ES$) to detail the magnitude of the effect (Hopkins et al., 2009). The magnitude of the difference between variables of interest were explored using ES thresholds of trivial (<0.2), small (>0.2), moderate (>0.5), large (>0.8), and very large (>1.0) (Cohen, 1992). Results are presented as ($P$ value, Eta squared) for ANOVA and ($P$ value, $ES$, % change) for pairwise comparisons, unless stated otherwise.

**7.4. Results**

The participants’ descriptive characteristics are shown in Table 7.1. Tanner stage ranged from 1 to 2 (stage 1 $n=7$, stage 2 $n=4$), indicating the participants were pre/peri pubertal. Two participants were classified as obese and three as overweight. Seven participants were classified as “at risk” according to their HOMA-IR. Of the seven “at risk” participants two were obese and two were overweight.
Table 7.1. Participant descriptive characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>8.85 ± 0.8</td>
<td>7.3 to 10.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>32.3 ± 7.1</td>
<td>23.9 to 44.6</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.35 ± 0.1</td>
<td>1.28 to 1.47</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>17.5 ± 2.9</td>
<td>13.6 to 23.0</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>16.8 ± 4.9</td>
<td>9.8 to 23.7</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.79 ± 0.66</td>
<td>0.85 to 3.26</td>
</tr>
<tr>
<td>Fasting glucose (mmol·L⁻¹)</td>
<td>5.14 ± 0.44</td>
<td>4.23 to 5.98</td>
</tr>
<tr>
<td>Fasting insulin (µIU·mL)</td>
<td>13.84 ± 5.08</td>
<td>6.82 to 24.80</td>
</tr>
<tr>
<td>Peak power (W)</td>
<td>95 ± 17</td>
<td>74 to 123</td>
</tr>
<tr>
<td>( \dot{V}_O_2 ) max (mL·kg⁻¹·min⁻¹)</td>
<td>40.2 ± 7.5</td>
<td>30.1 to 50.4</td>
</tr>
<tr>
<td>GET (L·min⁻¹)</td>
<td>0.69 ± 0.10</td>
<td>0.54 to 0.91</td>
</tr>
<tr>
<td>GET (% ( \dot{V}_O_2 ) max)</td>
<td>54.8 ± 5.7</td>
<td>46.3 to 64.2</td>
</tr>
</tbody>
</table>

Results shown as mean ± SD and range. Abbreviations: BMI, body mass index; GET, gas exchange threshold; HOMA-IR, Homeostatic model of insulin resistance; % body fat determined from skin fold measurements.

Time spent sedentary or performing light, moderate or VPA was similar in the 48 h before each condition (n=9 due to accelerometer data loss is 2 participants; P>0.05). Additionally, total energy intake and proportions of CHO, fat and protein were similar in the 48 h period prior to each condition (all P>0.05). The diet and PA data are shown in Table 7.2.
Table 7.2: 48 h diet and physical activity prior to each experimental condition

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON</th>
<th>MIE</th>
<th>HIIE</th>
<th>ANOVA $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI, (kcal)</td>
<td>1563 ± 508</td>
<td>1560 ± 289</td>
<td>1546 ± 155</td>
<td>0.990</td>
</tr>
<tr>
<td>% CHO</td>
<td>51 ± 9</td>
<td>48 ± 6</td>
<td>53 ± 9</td>
<td>0.067</td>
</tr>
<tr>
<td>% fat</td>
<td>33 ± 8</td>
<td>338 ± 3</td>
<td>32 ± 8</td>
<td>0.091</td>
</tr>
<tr>
<td>% Protein</td>
<td>16 ± 4</td>
<td>15 ± 3</td>
<td>15 ± 2</td>
<td>0.164</td>
</tr>
</tbody>
</table>

Physical activity

|Sedentary (min.day$^{-1}$)| 446 ± 70| 436 ± 68| 418 ± 52| 0.554 |
|Light (min.day$^{-1}$)| 221 ± 47| 211 ± 47| 236 ± 51| 0.574 |
|Moderate (min.day$^{-1}$)| 117 ± 52| 106 ± 43| 121 ± 37| 0.660 |
|Vigorous (min.day$^{-1}$)| 19 ± 21| 15 ± 12| 27 ± 28| 0.193 |
|MVPA (min.day$^{-1}$)| 136 ± 66| 121 ± 44| 148 ± 58| 0.453 |

Results shown as mean ± SD, probability ($P$), MVPA: moderate to vigorous physical activity, CHO: carbohydrate. Due to data loss physical activity data are reported for n=9.

The physiological and perceptual responses to the exercise conditions are presented in Table 7.3. HIIE elicited both greater physiological and perceptual stress compared to MIE. According to PACES, HIIE was more enjoyable than MIE.
Table 7.3. Physiological and perceptual responses to HIIE and MIE

<table>
<thead>
<tr>
<th>Variable</th>
<th>MIE</th>
<th>HIE</th>
<th>P</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power output (W)</td>
<td>28 ±8</td>
<td>85 ± 15</td>
<td>&lt;0.001</td>
<td>-4.07</td>
</tr>
<tr>
<td>Power output (% peak)</td>
<td>30 ± 6</td>
<td>90 ± 0</td>
<td>&lt;0.001</td>
<td>-12.47</td>
</tr>
<tr>
<td>Average $\dot{V}O_2$ (L.min$^{-1}$)</td>
<td>0.67 ± 0.15</td>
<td>0.81 ± 0.13</td>
<td>&lt;0.001</td>
<td>-0.94</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (% of max)</td>
<td>53.0 ± 10.0</td>
<td>64.7 ± 9.2</td>
<td>&lt;0.001</td>
<td>-1.07</td>
</tr>
<tr>
<td>HR (beats·min$^{-1}$)</td>
<td>116 ± 10</td>
<td>136 ± 9</td>
<td>&lt;0.002</td>
<td>-1.02</td>
</tr>
<tr>
<td>RPE</td>
<td>4 ± 2</td>
<td>7 ± 2</td>
<td>&lt;0.001</td>
<td>-1.37</td>
</tr>
<tr>
<td>PACES</td>
<td>64 ± 11</td>
<td>67 ± 11</td>
<td>0.04</td>
<td>0.25</td>
</tr>
<tr>
<td>Duration (min)</td>
<td>30.7 ± 6.5</td>
<td>22.8 ± 0</td>
<td>&lt;0.01</td>
<td>-1.50</td>
</tr>
<tr>
<td>Work done (kJ)</td>
<td>50 ± 7</td>
<td>50 ± 7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results shown as mean ± SD, probability (P), and effect size (ES). Abbreviations: HR, Heart rate; RPE, ratings of perceived exertion; PACES, physical activity enjoyment scale.

Fasting plasma glucose was not different between conditions (P=0.84, Eta Squared = 0.01). Changes in plasma glucose following the OGTT are presented over time and using the AUC analyses in Figure 7.1. There was no effect of condition on either the tAUC (P=0.09, Eta Squared = 0.08) or iAUC (P=0.34, Eta Squared = 0.05) for plasma glucose.
Figure 7.1. Postprandial plasma glucose and insulin response following the OGTT displayed over time (A, B) and using incremental (C, D) and total (E, F) UC. Values shown as mean ± SD. Error bars omitted in A and B for clarity.

Fasting plasma insulin was not different between conditions ($P=0.14$, Eta Squared = 0.05). Changes in plasma insulin following the OGTT are presented over time and
using the AUC analyses in Figure 7.1. There was no effect of condition on either tAUC ($P=0.36, \text{Eta Squared} = 0.02$) or iAUC ($P=0.22, \text{Eta Squared} = 0.08$) for plasma insulin.

IS was calculated using the Cederholm index following each condition is shown in Figure 7.2. There was a trend for a small effect of condition ($P=0.07, \text{Partial Eta Squared} = 0.02$) from the ANOVA model. Exploratory follow up analyses indicated a significant but small increase in IS after HIIE compared to CON ($P=0.04, \text{ES}=0.28, 9.7\%$), a non-significant small increase in IS after MIE compared to CON ($P=0.07, \text{ES}=0.21, 6.5\%$) and a non-significant and trivial difference for IS between HIIE and MIE ($P=0.63, \text{ES}=0.07, 3\%$). Neither baseline HOMA-IR nor BMI were not significantly correlated to the change in IS with acute exercise (all $P>0.05$).

![Figure 7.2. IS as measured using the Cederholm index. Values shown as mean ± SD.](image)

Changes in postprandial EE and fat oxidation are only available in eight participants due to data loss. There was no effect of condition on the postprandial tAUC EE
However there was a significant and large effect of condition for tAUC fat oxidation during the postprandial period ($P=0.014$, Eta Squared = 0.14). Follow up analysis showed a significant increase in fat oxidation after HIIE compared to CON ($P=0.008$, ES=0.79, 38.9 %) with only a small to moderate, non-significant difference between MIE and CON ($P=0.13$, ES=0.48) and MIE and HIIE ($P=0.16$, ES=0.31). Changes to tAUC fat oxidation were not significantly correlated to either HOMA-IR or IS (Cederholm index) (both $P>0.05$).

### 7.5. Discussion

The primary findings from this study are: 1) in 7-10 y old boys HIIE but not MIE had a significant but small effect for increasing IS but neither HIIE or MIE had any effect on plasma glucose and insulin AUC responses; and 2) there was a significant increase in postprandial fat oxidation after HIIE but not after MIE compared to CON. Finally although HIIE was associated with higher perceptual and physiological stress than MIE, the enjoyment ratings were higher after HIIE.

Previous research with adolescents has demonstrated a single bout of exercise to reduce postprandial plasma glucose, plasma insulin and increase IS (Zakrzewski and Tolfrey, 2012, Short et al., 2013, Cockcroft et al., 2015). For example, earlier work from our laboratory has shown that immediately post MIE and HIIE a moderate increase in IS (ES=0.76 and ES=0.58) compared to CON is observed respectively. Interestingly, the magnitude of the effects, as judged by the ES statistic, are smaller in the present study for HIIE (ES=0.28) and MIE (ES=0.21) when compared to
previous work in adolescents (Cockcroft et al., 2015). A potential reason for this difference could be because the baseline IR of the participants in the present study on 7-10 y old boys is lower than that of previous research with adolescents, with a mean HOMA-IR of 1.79 ± 0.66 compared to 2.27 ± 0.52 respectively (Cockcroft et al., 2015). This reduced IR in the younger children may limit any acute benefits from exercise. As the HIIE and MIE protocols were identical and levels of MVPA (125 ± 55 vs. 135 ± 40 min.day⁻¹) were similar between previous work in adolescents (Cockcroft et al., 2015) and the present study respectively, this suggests that age/pubertal status of the participants alters the potential for acute exercise to improve plasma glucose and insulin outcomes following an OGTT.

The proposition that age and/or pubertal status may affect the response of glucose and insulin to exercise is supported by recent prospective data on children who were followed-up between ages 9 and 16 y (Metcalf et al., 2015). These authors reported IR to peak at around 12-13 y compared to IR being similar at ages 9 and 16 y. Furthermore, it was demonstrated that physical activity attenuated the adolescent peak in IR. However, the beneficial effects of PA were no longer evident by late adolescence and there was no significant association with levels of PA and insulin in 5-8 y olds (Metcalf et al., 2015). As the participants in the current study were aged between 7-10 y, the lack of an effect of a single bout of HIIE or MIE on plasma glucose and [insulin] shows that younger children are already sufficiently sensitive to the action of insulin and therefore further improvements to IS through exercise may be difficult to achieve.
In the present study demonstrated a 38.9% increase in postprandial fat oxidation compared to CON with no difference observed after the MIE compared to either CON or HIIE. This finding is pertinent as an increased fat oxidation at rest and during exercise may be of practical importance for body weight management, as well as, prevention of T2D and metabolic syndrome (Barwell et al., 2009). Fat oxidation during exercise has previously been shown to be associated with IS in adults (Robinson et al., 2015, Robinson et al., 2016). However, in the current study, we observed no relationship between tAUC fat oxidation following exercise and either baseline HOMA-IR or IS (Cederholm index) following exercise. The increased postprandial fat oxidation after HIIE compared to MIE or CON in the current study is consistent with earlier research in adolescents after an OGTT (Cockcroft et al., 2015) and a high fat meal (Bond et al., 2015d). In contrast, Crisp and colleagues (2012) reported no change in fat oxidation rates after either 30 minutes of MIE or sprint intervals in prepubertal boys (10.1 ± 1.8 y). However, the sprint intervals were of short duration (4 s maximal sprints) that implies longer exercise intervals are needed to observe changes to fat oxidation.

This is the first study to assess the acute effects of different intensities of exercise on insulin and glucose health outcomes in a group of 7-10 y old boys who were pre/peri pubertal. The strengths of the study include: 1) the control of diet and PA over 48 h prior to each experimental visit, which shows these influences were similar prior to each experimental condition in the current study, and 2) the use of an OGTT to determine glucose tolerance and IS, which allows assessment of IS in a dynamic state. However, we acknowledge our findings are limited to a small sample of males,
highlighting the need for further research to confirm these findings in a larger group and the need for the inclusion of female participants. However, based on the magnitude of change observed in the current study for IS (ES ~ 0.25), a sample of ~125 participants would be needed to observe an effect at an alpha of 0.05 and power of 0.80.

Findings from this study indicate higher levels of enjoyment after HIIE and a preference for HIIE compared to MIE. This is despite HIIE eliciting greater physiological and perceptual stress compared to MIE. This higher level of enjoyment following HIIE is consistent with previous research (Cockcroft et al., 2015, Crisp et al., 2012) and is important when considering adherence to an exercise programme, thus supporting HIIE as a feasible and effective form of exercise in this age group.

Our findings in 7-10 y old boys are pertinent since current PA guidelines for health in the UK are the same for young people aged between 5 to 18 y. Findings from the present study and recent experimental work (Cockcroft et al., 2015) suggest that at least in terms of improving glucose and insulin outcomes, younger pre/peripubertal boys respond differently to an acute exercise stimulus compared to their adolescent counterparts. This conclusion is also supported by observational work looking at the relationship between PA and insulin (Metcalf et al., 2015, Metcalf et al., 2009). As improving glucose and insulin in youth may have implications for reducing future risk of CVD and T2D, a ‘one size fits all’ approach to prescribing exercise to improve these
health outcomes between the ages of 5-18 y of age may not be appropriate and further work is needed to investigate this concept.

A number of limitations to this study should be acknowledged. Firstly, the small sample of physically active males, limiting extrapolation and generalisability of these findings. The high levels of physical activity may have limited any improvements to IS from an acute exercise bout, and so future research should investigate this is a group with low levels of physical activity. Additionally the PACES questionnaire used to assess exercise enjoyment has only previously been validated in adolescent girls (Motl et al, 2001).

7.6. Conclusion

The novel finding of this study is that in 7-10 y old boys, acute bouts of MIE and HIIE had minimal effects on IS and no effect on glucose tolerance. However, HIIE was found to increase postprandial fat oxidation, which may have implications for weight management. These findings add to recent observational data (Metcalf et al., 2015, Metcalf et al., 2009), showing minimal effects of PA on insulin and glucose outcomes before puberty. Therefore, future studies should examine whether different PA and exercise recommendations are needed to improve health outcomes in children across different age and maturity groups.
CHAPTER 8

Two weeks high intensity interval training on fasting glucose, glucose tolerance and insulin resistance in adolescent boys: a preliminary study
8.1. Abstract

Background This study examined whether improvements in fasting and postprandial insulin and glucose and aerobic fitness are possible after two weeks of high-intensity interval training (HIIE training) in adolescent boys. Methods: Seven boys (14.3 ± 0.3 y) completed 6 sessions of HIIE TRAINING over a two weeks. HOMA-IR, QUICKI, FGIR and blood glucose and insulin responses to a Mixed Meal Tolerance Test (MMTT) were assessed before (PRE), 20 h and 70 h after (POST) the final HIIE session. Maximal oxygen uptake was assessed PRE and 70 h POST. Results: Compared to PRE, two weeks of HIIE training had no effect on fasting plasma glucose or insulin or HOMA-IR at 20 h and 70 h POST. However, a strong negative correlation between PRE training HOMA-IR, QUICKI and FGIR and change in HOMA-IR, FGIR and QUICKI at 20 h POST (r =-0.96, 0.969 and 0.826 for HOMA-IR, QUICKI and FGIR respectively all P<0.05) was observed. Plasma insulin and glucose area under the curve responses to the MMTT were unchanged 20 h and 70 h POST compared to PRE. Conclusion: Two weeks of HIIE TRAINING did not elicit improvements to fasting or postprandial glucose or insulin health outcomes or aerobic fitness in a group of adolescent boys. Interventions of this type may, however, be effective in adolescents with raised baseline IR.
8.2. Introduction

IR, impaired beta cell function (\(\%\beta\)) and glucose tolerance are all implicated in the development of T2D and CVD (Reaven, 2005). Such risk factors are known to be prevalent in youth (The STOPP-T2D Prevention Study Group, 2006) and can predict future risk of CVD and T2D (Berenson, 2002). The early development of IR begins 10-20 y before disease onset and is thought to be one of the best predictors of future diabetic risk (Shulman, 2000). This makes the pubertal years a prime target for interventions to prevent the onset of T2D and CVD, as well as associated co-morbidities.

PA is an effective intervention to improve risk factors associated with T2D and CVD in youth. A meta-analysis revealed a small to moderate effect of exercise training to improve fasting insulin and IR in youth, especially for those who are overweight or obese (Fedewa et al., 2014). However, despite the known importance of PA in youth, less than one third of school aged children and adolescents meet the minimum UK government recommendation of 60 minutes of MVPA per day (Riddoch et al., 2007). Furthermore, school-based interventions designed to increase levels of PA in youth have been largely unsuccessful, increasing MVPA by \(\sim\) 4 minutes per day on average (Metcalf et al., 2012). Adolescence is associated with declining levels of PA (Dumith et al., 2011) and represents a period in time when PA has the most profound effect on IR (Metcalf et al., 2015), highlighting the importance of exploring alternative forms of PA to improve cardiometabolic health outcomes in this group.
Recent observational data in youth have shown that small amounts (< 7 minutes) of vigorous intensity PA is associated with favourable temporal changes in cardiometabolic risk, including blood pressure, waist circumference and cardiorespiratory fitness in youth (Carson et al., 2014). This suggests that promoting high-intensity PA in this group may help in modifying disease risk. In healthy adolescents, just two weeks of sprint type HIIE training has been shown to improve aerobic fitness (Barker et al., 2014), indicating that short duration HIIE training may have health benefits in youth. However evidence for the metabolic health benefits of HIIE training in youth is currently limited to longer (7-12 weeks) training periods that often target adolescents who are overweight or have low fitness (De Araujo et al., 2012, Racil et al., 2013, Tjonna et al., 2009). In contrast, it has been shown that improvements in IS and glucose tolerance in adolescent boys are possible after just a single bout of HIIE (Chapter 5), suggesting that repeated bouts of HIIE training performed over just two weeks may be a feasible way to improve glucose tolerance and IS in youth.

In adults increased IS from a single session of HIIE has been shown to persist for ~ 48 h (Koopman et al., 2005, Mikines et al., 1988), meaning that any improvements in health outcomes beyond this time frame may be considered a chronic adaption to training. Adult studies on healthy participants or patients with T2D have shown an increase in the expression of skeletal muscle glucose transporters (e.g. GLUT-4) and the activity of mitochondrial enzymes after just 1-2 weeks of HIIE training (Burgomaster et al., 2007, Little et al., 2011), suggesting chronic adaptations are possible. However, a recent study has shown that two weeks of HIIE training in a
mixed-sex group of adolescents (boys = 7; girls=6) has no effect on fasting and postprandial plasma insulin and glucose outcomes when measured 24 and 72 h after the last training session (Bond et al., 2015a). This finding was surprising given previous work showing an acute bout of HIIE improved postprandial outcomes in adolescent boys (Cockcroft et al., 2015). This lack of an effect may, in part, be due to the combined analysis of the adolescent boys and girl in previous work (Bond et al., 2015a) and the use of the HOMA method to estimate IR which known to have poorer measurement reliability (see Chapter 4) compared to other indices such as the QUICKI (Mather et al., 2001) and FGIR (Legro et al., 1998). Additionally establishing the effects of exercise training in boys is important since boys are at an increased risk of developing IR and impaired fasting glucose compared to their female peers (Aldhoon-Hainerová et al., 2014).

Therefore, the aim of this paper was to use a sub-set of data from a previous investigation (Bond et al., 2015a), to examine the effect of two weeks of HIIE training on acute and chronic changes in glucose and insulin outcomes in adolescents boys. It is hypothesised that two weeks of HIIE training will: 1) improve glucose and insulin outcomes the day after the last training session, representing an acute adaption to HIIE training); and 2) improve glucose, insulin and fitness outcomes up to 72 h post the final training session, representing a chronic adaption to HIIE training.
8.3. Methods

8.3.1. Participants

Nine boys were initially recruited from a local secondary school. Following an explanation of the study procedures and the associated risks and benefits, parental consent and participant assent were obtained. Participants completed an initial health questionnaire and were free from any metabolic or medical conditions. Ethics approval was granted by the institutional ethics committee. One boy failed to complete the HIIE training due to an unrelated illness, and one boy could not complete the training due to an unrelated injury. This left a sample of seven participants (14.3 ± 0.3 y) for analysis.

8.3.2. Experimental design

This study consisted of four laboratory visits, and 6 training sessions, which took place over a three week period. Visits included an initial familiarisation visit and three experimental visits. Visits 1 and 2 consisted of baseline measures of fitness and the glucose and insulin response to a mixed meal tolerance test (MMTT) prior to undertaking the HIIE training intervention (PRE). Visits 1 and 2 were separated by 3-5 days. Participants then completed 6 supervised HIIE sessions over a two week period, after which post training measures were assessed 20 h (visit 3; 20 h POST) and 70 h post-intervention (visit 4; 70 h POST).
Visit 1: Familiarisation and baseline fitness assessment

Stature and body mass were measured to the nearest 1 cm and 0.1 kg, and used to calculate body mass index (BMI). BMI was used to classify participants as normal weight, overweight and obese, using validated age-specific percentile cut points (Cole et al., 2000). Pubertal status was determined by self-assessment of the five Tanner stages of pubic hair development (Tanner and Whitehouse, 1976).

Participants were familiarised with the cycle ergometer (Lode Excilibur Sport, Gronigen, Netherlands) and completed a combined ramp-incremental and supramaximal test to exhaustion to determine maximal oxygen uptake ($\dot{V}O_2$ max) and the GET (Barker et al., 2011). Pulmonary gas exchange and heart rate were measured (Cortex Metalyzer III B, Germany) and $\dot{V}O_2$ max was accepted as the highest 10 s average $\dot{V}O_2$ during the ramp or supra-maximal test. Peak power (PP) was taken as the highest power output during the ramp test whilst maintaining a cadence > 60 revolutions min$^{-1}$. The GET was estimated at the point where the first disproportionate increase in CO$_2$ production compared to $\dot{V}O_2$ and verified using the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$.

Visits 2: Baseline metabolic assessment

Participants were driven to the laboratory and arrived at ~ 07:45 following a 12 h overnight fast. After 15 minutes of seated rest, participants provided a capillary blood sample for plasma glucose and insulin. At ~ 08:30 a MMTT was conducted which consisted of a fruit smoothie, chocolate croissant with chocolate spread and a
chocolate muffin (80 g of glucose, 68 g of fat, 7134 kJ). The meal was consumed over a 15 minute period, after which capillary blood samples were taken at 30, 60, 120 minutes for assessment of plasma glucose and insulin. No other food was consumed and water was available ad libitum during visit 2 (PRE). This was recorded and subsequently replicated for the POST measures. Participants remained in the laboratory throughout the visit, completing sedentary activities such as reading, watching DVDs or playing computer games. Participants left the laboratory at ~ 15:00.

High intensity interval exercise training

Participants performed a two week HIIE training programme on a cycle ergometer (Monark 827e, Monark exercise AB, Sweden) with adjustments made to the handle bar and seat height for each participant. Training took place within a local secondary school and consisted of 3 HIIE sessions per week, all sessions were supervised by a researcher. Each session started with a 3 minute warm up of unloaded pedalling, followed by 8-10 one minute intervals at 90 % of the PP achieved during the incremental ramp test performed during visit 1. Each interval was interspersed with 75 s of unloaded pedalling. This HIIE protocol was selected to mimic previous studies from our laboratory (Bond. et al., 2015b, Bond. et al., 2015d, Cockcroft et al., 2015). Sessions 1 and 2 consisted of 8 x 1 minute bouts, sessions three and four 9 x 1 minute bouts and sessions five and six 10 x 1 minute bouts. Participants were instructed to maintain a self-selected cadence (70-95 revolutions min⁻¹) and were reminded of this during each session. The researcher present gave a 10 s warning before the beginning of each bout.
Visit 3 and 4: Post-training

The protocol outlined above for visit 2 was replicated the day after (~ 20 h) and three days (~70 h) after the last training session (20- POST and 70-POST). One h after completion of the MMTT during the 70- POST visit, participants completed a post intervention \( \dot{V}O_2 \) max assessment as described in visit 1.

8.3.3. Control measures

PA was measured during the 48 h period prior to each experimental visit using a wrist worn accelerometer (GENEActiv, Activinsights, UK). Time spent sedentary and performing, light, moderate and VPA was determined using cut points previously validated in a paediatric sample (Phillips et al., 2013). Participants were asked to avoid structured PA outside of the training intervention.

With supervision from their parents/guardians, a food diary was completed by each participant during the 48 h period preceding each experimental visit. Food diaries were assessed to estimate total energy and macronutrient content using commercially available software (CompEat Pro, Nutrition systems, UK). Participants were asked to replicate their diet during the 48 h preceding each experimental visit and if appropriate, to document any discrepancies.
8.3.4. Blood analyses

Fingertip capillary blood samples (~ 600 µL) were taken from a pre-warmed hand into a fluoride heparin coated and lithium heparin coated microvette (CB 300 tubes, Sarstedt Ltd, Leicester, UK) for plasma glucose and insulin determination respectively. Both microvettes were centrifuged at 1300 g for 10 minutes. Plasma was separated for immediate analysis of glucose (YSI 2300 Stat Plus Glucose analyser, Yellow Springs, OH, USA) or stored at −80°C for later analysis of plasma insulin using an ELISA enzyme immunoassay kit (DRG Diagnostics, Germany). Haematocrit and haemoglobin content of samples were assessed from the fasted sample of each visit in order to account for any change in plasma volume following training. In our laboratory, the within batch coefficients of variation for the plasma insulin and glucose analyses were < 5%.

8.3.5. Calculations

Changes in plasma glucose and insulin during the MMTT were quantified using tAUC and iAUC analysed employing the trapezium rule (GraphPad Prism, GraphPad, SanDiego, CA). Fasting plasma glucose and insulin were used to calculate IR, IS and %β using using HOMA-IR (Matthews et al., 1985), QUICKI (Mather et al., 2001) and FGIR (Legro et al., 1998), which have been validated for use in adolescents (Brown and Yanovski, 2014).
8.3.6. Statistical analyses

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Analysis of the HOMA, QUICKI, FGIR, fasting glucose and insulin and AUC calculations across visits was performed using a one-way repeated-measures ANOVA. Changes in fitness parameters were assessed by a paired sample t-test. All results are presented as P value, unless stated otherwise. Pearson correlations were performed between HOMA-IR, QUICKI, FGIR, $\dot{V}O_2$ max and BMI at baseline and change in HOMA-IR after the 2 week training period. A significant correlation was accepted if $P<0.05$.

8.4. Results

The participants' descriptive characteristics are shown in Table 8.1. Tanner stage was provided by 6 participants and ranged between 3 and 4 (stage 4: $n=4$, stage 3: $n=2$). The BMI of participants ranged from 17.8 to 24.0 kg·m$^2$, with 3 participants classified as overweight. Time spent in moderate and VPA in the 48 h preceding each visit highlighted no differences between visits ($P>0.05$). No differences in estimated energy intake or macronutrient contribution to diet were evident prior to each visit (all $P>0.05$). The PA and diet data are shown in Table 8.2.

All participants completed the six HIIE training sessions, with 100% adherence to the protocol, with no adverse effects recorded.
Table 8.1. Participant descriptive characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>14.3 ± 0.3</td>
<td>13.9 to 14.7</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>60.0 ± 7.4</td>
<td>57.7 to 69.9</td>
</tr>
<tr>
<td>Stature, m</td>
<td>1.67 ± 0.81</td>
<td>1.57 to 1.78</td>
</tr>
<tr>
<td>BMI, kg.m²</td>
<td>21.6 ± 2.6</td>
<td>17.8 to 24.6</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD and range. BMI, body mass index; % body fat determined from skin fold measurements.

Table 8.2. Physical activity and dietary intake during the 48 h preceding each experimental visit.

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>20 h POST</th>
<th>70 h POST</th>
<th>ANOVA P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate-vigorous physical activity</td>
<td>45 ± 25</td>
<td>59 ± 42</td>
<td>56 ± 17</td>
<td>0.55</td>
</tr>
<tr>
<td>(min·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy intake (kcal·day⁻¹)</td>
<td>1971 ± 280</td>
<td>1950 ± 294</td>
<td>2052 ± 293</td>
<td>0.71</td>
</tr>
<tr>
<td>Energy from CHO (%)</td>
<td>43 ± 7</td>
<td>47 ± 5</td>
<td>47 ± 9</td>
<td>0.67</td>
</tr>
<tr>
<td>Energy from fat (%)</td>
<td>40 ± 10</td>
<td>36 ± 4</td>
<td>38 ± 5</td>
<td>0.54</td>
</tr>
<tr>
<td>Energy from protein (%)</td>
<td>18 ± 4</td>
<td>17 ± 4</td>
<td>14 ± 4</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Results shown as Mean ± SD. 20 h POST includes the final training session of the HIIE training intervention (~27 minutes).

Fasting and MMTT outcomes and cardiorespiratory fitness data are shown in Table 8.3.
Table 8.3. Physical and biochemical characteristics at PRE, 20 h and 70 h post intervention.

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>20 h post intervention</th>
<th>70 h post intervention</th>
<th>ANOVA P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmo\textperiodcentered L$^{-1}$)</td>
<td>5.05 ± 0.3</td>
<td>5.00 ± 0.3</td>
<td>5.09 ± 0.2</td>
<td>0.86</td>
</tr>
<tr>
<td>Insulin (\textmu U\textmiddot ml.)</td>
<td>19.41 ± 8.4</td>
<td>18.63 ± 3.5</td>
<td>20.60 ± 8.2</td>
<td>0.84</td>
</tr>
<tr>
<td>HOMA-IR (arbitrary units)</td>
<td>2.47 ± 1.04</td>
<td>2.37 ± 0.45</td>
<td>2.61 ± 0.99</td>
<td>0.85</td>
</tr>
<tr>
<td>HOMA-S% (arbitrary units)</td>
<td>45.86 ± 15.44</td>
<td>43.51 ± 8.62</td>
<td>42.27 ± 13.11</td>
<td>0.87</td>
</tr>
<tr>
<td>HOMA- B% (arbitrary units)</td>
<td>170.93 ± 39.86</td>
<td>172.70 ± 18.10</td>
<td>177.26 ± 46.70</td>
<td>0.93</td>
</tr>
<tr>
<td>QUICKI (arbitrary units)</td>
<td>0.311 ± 0.017</td>
<td>0.311 ± 0.010</td>
<td>0.308 ± 0.015</td>
<td>0.89</td>
</tr>
<tr>
<td>FGIR (mg/10$^{-4}$ U)</td>
<td>5.27 ± 1.64</td>
<td>4.96 ± 0.82</td>
<td>4.92 ± 1.43</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>MMTT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tAUC Glucose (mmol\textperiodcentered min\textperiodcentered L$^{-1}$)</td>
<td>91.08 ± 80.26</td>
<td>107.79 ± 77.26</td>
<td>81.06 ± 43.99</td>
<td>0.57</td>
</tr>
<tr>
<td>tAUC Glucose (mmol\textperiodcentered min\textperiodcentered L$^{-1}$)</td>
<td>696.76 ± 74.11</td>
<td>707.67 ± 48.73</td>
<td>690.47 ± 36.96</td>
<td>0.56</td>
</tr>
<tr>
<td>tAUC Insulin (\textmu U\textmiddot ml\textperiodcentered min$^{-1}$)</td>
<td>4499.57 ± 1834.26</td>
<td>4538.14 ± 1882.24</td>
<td>4908.71 ± 1329.51</td>
<td>0.78</td>
</tr>
<tr>
<td>tAUC Insulin (\textmu U\textmiddot ml\textperiodcentered min$^{-1}$)</td>
<td>6807.00 ± 1415.1</td>
<td>6774.29 ± 1661.46</td>
<td>7380.29 ± 1906.95</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Fitness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\dot{V}$\textsubscript{O$_2$} max (mL\textmiddot kg$^{-1}$\textmiddot min$^{-1}$)</td>
<td>40.71 ± 9.80</td>
<td>-</td>
<td>42.08 ± 10.75</td>
<td>0.25</td>
</tr>
<tr>
<td>$\dot{V}$\textsubscript{O$_2$} max(L\textmiddot min$^{-1}$)</td>
<td>2.44 ± 0.70</td>
<td>-</td>
<td>2.52 ± 0.76</td>
<td>0.27</td>
</tr>
<tr>
<td>HR max</td>
<td>192 ± 8</td>
<td>-</td>
<td>193 ± 9</td>
<td>0.65</td>
</tr>
<tr>
<td>GET (L\textmiddot min$^{-1}$)</td>
<td>1.33 ± 0.29</td>
<td>-</td>
<td>1.35 ± 0.28</td>
<td>0.85</td>
</tr>
<tr>
<td>GET (%$\dot{V}$\textsubscript{O$_2$} max)</td>
<td>55.7 ± 7.1</td>
<td>-</td>
<td>54.9 ± 7.5</td>
<td>0.60</td>
</tr>
<tr>
<td>PP (W)</td>
<td>233 ± 58</td>
<td>-</td>
<td>244 ± 66</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Results shown as mean ± SD
Fasting plasma glucose, insulin, QUICKI, FGIR, HOMA-IR, HOMA S% and HOMA β% were unchanged at both 20-POST and 70-POST intervention compared to PRE ($P>0.05$). The plasma glucose and insulin response to the MMTT are shown in Figure 8.1.

**Figure 8.1.** Postprandial plasma glucose and insulin response to the mixed meal tolerance test (MMTT) at baseline and at 20 h and 70 h after the HIIE training intervention. Results shown as mean ± SEM

The tAUC and iAUC analyses for glucose and insulin were unchanged 20 h and 70-POST intervention when compared to PRE ($P>0.05$). Absolute and relative $\dot{V}O_2$ max and PP output were unchanged POST compared to PRE ($P>0.05$). A significant strong negative correlations were found between change in HOMA-IR, QUICKI and FGIR 20-
POST and PRE HOMA-IR, QUICKI and FGIR ($r = -0.96$, $P=0.001$; $r = -0.97$, $P=0.001$; $r = -0.83$, $P=0.022$ for HOMA-IR, QUICKI and FGIR respectively, Figure 8.2). The changes in HOMA-IR, QUICK and FGIR post intervention were not related to $\dot{V}O_2$ max or BMI (both $P>0.05$). There was no correlation between the change in MMTT outcomes at 20-POST and PRE training values (all $P>0.05$).

![Scatter plot showing correlation between change in HOMA-IR after 20 h POST HIIE training and HOMA-IR at baseline. ** $P<0.01$ * $P<0.05$.](image)

Figure 8.2. Scatter plot showing correlation between change in HOMA-IR after 20 h POST HIIE training and HOMA-IR at baseline. ** $P<0.01$ * $P<0.05$. 


8.5. Discussion

The key finding of this preliminary study was that two weeks of HIIE training did not elicit any acute or chronic changes to fasting and postprandial markers of metabolic health in a group of adolescent boys. \( \dot{V}O_2 \) max was also unaffected by the training intervention. However, a strong positive correlation was found between baseline IR and the change in IR 20-POST HIIE training across three different indices, suggesting beneficial effect in participants with the greatest IR at baseline. Short duration HIIE protocols may therefore be a useful exercise strategy for youth with poorer metabolic health profile at baseline and should be a target for future intervention work.

In the present study two weeks of HIIE training (8-10 1 minute intervals at ~ 90% of PP, interspersed with 75 s of unloaded pedalling) was not sufficient to improve IR or fasting and postprandial measures of metabolic health when measured 20-POST or 70-POST the final training session. Interestingly, our findings corroborate those of earlier studies conducted on healthy, asymptomatic adolescents. In two separate studies (Buchan et al., 2013, Buchan et al., 2011), Buchan and colleagues reported no change to either fasting insulin or glucose after a 7 week school-based HIIE training programme (4-6 repeats of 30 s maximal sprints with 20-30 s recovery 3 x per week), but did not report HOMA index of IR, QUICKI or FGIR. However, in these studies moderate intensity PA did improve fasting insulin suggesting this intensity of exercise may be superior to HIIE training. In contrast, studies investigating the effectiveness of HIIE training in overweight or obese participants over 12 weeks (Racil et al., 2013, Tjonna et al., 2009, De Araujo et al., 2012) have shown improvements to fasting glucose, insulin and HOMA-IR. These finding may show that the duration of the HIIE
training programme is important as HIIE training programmes lasting >12 weeks has yet to be conducted in normal weight adolescents to our knowledge. However, it is pertinent to note that in these HIIE training studies on overweight and obese youth (Racil et al., 2013, Tjonna et al., 2009, De Araujo et al., 2012) the participants had a baseline HOMA-IR of ~ 4-5, which is notably higher than the present study (2.5 ± 1.0) and suggests a limited window to improve IR after HIIE training in participants with low baseline IR. Published reference values for HOMA-IR in Caucasian youth suggest a 75th percentile cut-off point for cardiometabolic risk at 3.02 (Shashaj et al., 2015). In our study, analysis of the individual data found three participants appeared to respond positively to two weeks of HIIE training and were characterized by an IR between the 90th and 97th centile. These participants recorded an improvement in IR 20- POST ranging from 59-219%, with the largest improvement occurring in the participant with the highest baseline HOMA-IR. This is reflected by the significant negative correlation between the change in IR 20- POST and PRE IR (Figure 2) which was evident in HOMA-IR, QUICKI and FGIR and suggests that two weeks of HIIE training may be a feasible interventions to improve metabolic health in adolescents with a high IR at baseline. However it is worth noting that this observation may also be a result of regression towards the mean. The divergent nature of individual response to the HIIE may have also been a result of changes to habitual PA pattern. Mean PA was shown to be higher during the post training follow up, however, this does not necessarily reflect change on an individual level, and so controls measures such as asking participants to refrain from routine VPA during the intervention may have meant a number of participants ended up reducing total VPA as a result of the intervention. Finally, it has recently been reported that the ability for PA to attenuate IR is diminished
in adolescents of 16 y of age (Metcalf et al., 2015). The mean age of participants in this present study was 14.3 y with Tanner stages of 3 and 4, which may have influenced the effectiveness of the HIIE training intervention to modify plasma glucose and insulin. Taken collectively, there may be a ceiling to alter IR through just two weeks of HIIE training, especially in those who have a low IR at baseline, are or normal weight and in late adolescence.

In the current study, two weeks of HIIE training had no effect on postprandial plasma glucose and insulin after a MMTT. The inclusion of postprandial measures is a strength of our study because it is known that postprandial hyperglycaemia is a contributor to glycaemic control (e.g. HbA1c), which often precedes any increase in fasting glucose levels and is more harmful to skeletal muscle glucose homeostasis than chronically sustained hyperglycaemia (Monnier et al., 2007). In overweight/obese adolescents reductions in two h postprandial glucose and insulin after an OGTT have been shown after 12 weeks of HIIE training, but not after matched-duration MIE training (Tjonna et al., 2009). In healthy young men (21 ± 2 y), Babraj and colleagues (Babraj et al., 2009) found two weeks of HIIE training (6 sessions of 4-6 30 s sprints) reduced the plasma glucose and insulin AUC response to an OGTT by 12 % and 37 % respectively, 2 to 3 days after the last exercise session. In agreement with the present study, however, the authors found no changes to fasting glucose or insulin (Babraj et al., 2009). These findings suggest that the response to exercise training may differ for fasting and dynamic (postprandial) measures of insulin and glucose. Thus it is possible that the use of the MMTT to examine postprandial changes in glucose and insulin rather than an OGTT in the current study may account for the lack of effect when compared to the
work by Babraj and colleagues. In particular, the MMTT will have a lower glycaemic index which will alter the glucose excursions (Wolever et al., 2006) is likely to have influenced the rate of glucose appearance in the circulation (Cunningham and Read, 1989). That said the MMTT holds better external validity as it is more representative of the habitual nutrient meal composition compared to an OGTT.

One of the aims of this study was to highlight any acute benefits from the HIIE training by measuring the outcomes 20 h post the final training session. Contrary to our original hypothesis, no acute improvements in fasting or postprandial glucose and insulin were present at ~ 20-POST. We have previously shown that a single bout of HIIE can improve both glucose tolerance and IS in adolescent boys (Cockcroft et al., 2015), and it is known that a single bout of MIE can improve glucose and insulin responses to an OGTT up to ~ 17 h post exercise in youth (Short et al., 2013). It is therefore surprising that two weeks of HIIE training did not improve metabolic outcomes the day after the last training session in the current study. However, the aforementioned acute exercise studies used an OGTT and not a MMTT, which may account for the discrepancies in findings. The lack of change to metabolic outcomes 20-POST in the current study may also indicate that improvements after HIIE training in healthy adolescents do not persist into the next day.

Aerobic fitness, as measured using a validated cycle test to exhaustion, was unchanged in adolescent boys after the 2 week HIIE training programme. This result contrasts the outcome of a recent meta-analysis showing that ≥ 4 weeks of HIIE
training to have a large effect on improving cardiorespiratory fitness (ES= 1.05, mean difference = 2.6 mL.kg\(^{-1}\).min\(^{-1}\)) in adolescents (Costigan et al., 2015). A 5% improvement in \(\dot{V}O_2\) max has been shown after two weeks of HIIE training, however this study incorporated 30 s “all out” sprint type HIIE training (Barker et al., 2014), which may have provided a greater stimulus to augment \(\dot{V}O_2\).

This study is the first to assess both fasting and postprandial measures of metabolic health in a healthy adolescent population after short duration HIIE training programme. Previous studies in this area are largely limited to overweight/obese adolescents and longer duration HIIE training programmes. Strengths of this study include the control of PA and diet prior to the experimental measures, which limits any confounding effects these factors may have on results, as well as the inclusion of multiple indices on IR, which in previous work is limited to HOMA-IR which has been shown to have a large variability in this population. Limitations include the lack of a control group, although this is consistent with other short duration HIIE training studies in youth (Barker et al., 2014) and adults (Whyte et al., 2010). The small sample size is also a limitation, which may have influenced the statistical power and limits generalisability of the findings. However, this study was designed as a preliminary study.

8.6. Conclusion

This preliminary study shows that fasting or postprandial measures of insulin and glucose in adolescents were not sensitive to change after two weeks of HIIE training. However, a strong negative correlation between baseline IR and IR 20h POST across
all measurement indices highlights the potential for this type of intervention to promote metabolic health in individuals with elevated baseline IR and is worthy of future investigation.
CHAPTER 9

High intensity interval exercise and glycaemic control in adolescents with type one diabetes mellitus: A case series
9.1. Abstract

Background: Current physical activity guidelines for children with T1D are poorly supported by empirical evidence and the optimal “dose” of physical activity to improve health in children with T1D is unknown. Methods: This case series reports the effect of acute HIIE and moderate-intensity exercise MIE on 24h glycaemic control in three children with T1D using continuous glucose monitoring. Results: individual response to exercise across the participants varied. Both MIE and HIIE resulted in a drop in blood glucose during exercise (38-42% for MIE and 21-46% in HIIE). In the 24h post exercise average blood glucose was lower for all participants after HIIE, and for two after MIE compared to no exercise. In the 24h period following a mixed meal tolerance test, 2 participants had lower average blood glucose after HIIE and MIE compared to CON. All three participants reported HIIE to be more enjoyable than MIE and expressed a preference for HIIE. Conclusion: Findings show both MIE and HIIE have the potential to improve short-term glycaemic control in children with T1D but HIIE may be more enjoyable. Future work with a larger sample size is required to explore the potential for HIIE to improve health markers in this clinical group.
9.2. Introduction

CVD is a major cause of mortality in adults with T1D (Soedamah-Muthu et al., 2006). Glycaemic control is related to CVD mortality, with a 1% increase in HbA1c increasing CVD mortality rates by as much as 52.5% (Juutilainen et al., 2008). The risk of CVD is not only limited to adults with T1D. Subclinical signs of CVD and clustering of CVD risk factors are present in the first decade of life in children with T1D (Snell-Bergeon and Nadeau, 2012). It is therefore important to find interventions which can reduce CVD risk and improve glycaemic control in youth with T1D in order to reduce the risk of CVD mortality in later life.

The therapeutic effects physical activity in the management of CVD risk in youth with T1D are established (Tully et al., 2016). Therefore children and adolescents with T1D are recommended to undertake 30-60 minutes of moderate to vigorous physical activity (MVPA) on a daily basis. However the optimum exercise recommendations for youth with T1D are unknown. In healthy children and adolescents research has focused on the potential for time efficient, HIIE to improve cardiometabolic health outcomes (Costigan et al., 2015). Recently, a single bout of HIIE has been shown in improve glucose tolerance and IS in healthy adolescents (Chapter 5, Cockcroft et al., 2015), highlighting HIIE as a possible strategy to manage glycaemic control in youth with T1D. However, while an acute bout of HIIE has been shown to improve postprandial and 24h glycaemic control in adults with T2D mellitus (Gillen et al., 2012), no data currently exist on the acute effect of HIIE in youth with T1D.
The purpose of this case series is to report the acute effects of HIIE on changes in glycaemic control during exercise in response to meal challenge and over a 24h period in three adolescents with T1D and compare this to the lower limit of the current ADA recommendation for physical activity in youth with type one diabetes, 30 minutes of MIE.

9.3. Patient information

Participant characteristics and HbA1c are shown in Table 9.1. Participants consisted of one female (participant A: 17.1 y) and two males (participant B: 14.8 y, and participant C: 16.6 y) with T1D of at least 3 y duration. All participants were on a basal-bolus insulin regime. Informed parental consent and participant assent were obtained and ethics approval was granted by the National Health Service Research Ethics Committee (14/SW/1028).
Table 9.1: Participants descriptive characteristics

<table>
<thead>
<tr>
<th></th>
<th>Participant A</th>
<th>Participant B</th>
<th>Participant C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>17.1</td>
<td>14.8</td>
<td>16.6</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>62</td>
<td>59</td>
<td>37</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>77.6</td>
<td>50.4</td>
<td>61.8</td>
</tr>
<tr>
<td>Stature, m</td>
<td>1.62</td>
<td>1.72</td>
<td>1.78</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>18.8</td>
<td>23.0</td>
<td>7.6</td>
</tr>
<tr>
<td>Peak power, W</td>
<td>259</td>
<td>173</td>
<td>442</td>
</tr>
<tr>
<td>$\dot{V}O_2$ max (L.min$^{-1}$)</td>
<td>2.69</td>
<td>1.84</td>
<td>3.63</td>
</tr>
<tr>
<td>$\dot{V}O_2$ max (mL.kg.min$^{-1}$)</td>
<td>34.6</td>
<td>36.5</td>
<td>58.7</td>
</tr>
<tr>
<td>GET (L.min$^{-1}$)</td>
<td>1.76</td>
<td>1.01</td>
<td>2.29</td>
</tr>
<tr>
<td>GET (%$\dot{V}O_2$ max)</td>
<td>65%</td>
<td>55%</td>
<td>63%</td>
</tr>
<tr>
<td>MVPA (min/day)</td>
<td>16</td>
<td>21</td>
<td>57</td>
</tr>
</tbody>
</table>

Results shown as individual values. MVPA; moderate to vigorous physical activity, HbA1c, Glycated haemoglobin

9.4. Experimental design

Each participant attended the laboratory on four separate occasions consisting of a familiarisation visit and three experimental conditions. On the familiarisation visit, stature, body mass and body composition (BodPod®, COSMED) were measured before participants undertook a combined ramp-incremental and supramaximal test to exhaustion to determine maximal oxygen uptake ($\dot{V}O_2$ max) and the GET (Barker et al., 2011).

Each experimental condition consisted of four days of data collection and on day 1 participants were visited at home and fitted with a continuous glucose monitoring
system (CGMS) (iPro 2, Medtronic, USA), an accelerometer (GENEA activ, GENE A, UK) and provided with a food intake and insulin administration diary. On day 2, participants continued to wear the CGMS and accelerometer. On day 3 participants attended the laboratory at 08:00 following an overnight fast where they completed an exercise intervention (HIIE, MIE or control) and test meal, as described below. On the afternoon of day 4 the CGMS and accelerometer were removed at the participant’s home. A schematic of the protocol is shown in Figure 9.1.

Figure 9.1 Schematic of protocol for experimental visits CGMS, continuous glucose monitoring system, HIIE, high intensity interval exercise; MIE, moderate intensity exercise; MMTT, mixed meal tolerance test.
Experimental intervention day protocol

At 08:30 participants consumed a standardised breakfast (64 g CHO, 17 g protein, 8 g of fat, 412 kcal of energy) and thereafter rested in the laboratory. At 10:30 participants undertook one of the following conditions in a counterbalanced order: 1) HIIE: 3 minute warm up at 20 W followed by eight repeated bouts of 1 minute cycling at 90% of peak power (as determined by the ramp-incremental test during the familiarisation visit), interspersed with 1.25 minutes recovery at 20 W, followed by a 3 minute cool down at 20 W; 2) MIE: continuous cycling for 30 minutes at 90% GET; and 3) rest in the laboratory (CON). Rating of perceived exertion (RPE) was taken every 5 minutes during MIE and after each interval in HIIE. Following exercise, participants completed the Physical Activity Enjoyment Scale (PACES) (Motl et al., 2001). At 12:00 a mixed meal tolerance test (MMTT) was undertaken where participants consumed a liquid meal (Ensure Plus High Protein, 6 mL per kg (maximum 360 mL), content per 100 mL: CHO 15.9 g, protein 7.9 g, fat 3.3 g, energy 125 kcal) (Oram et al., 2014). Participants remained in the laboratory over a 4h postprandial period. Participants were advised to manage glucose levels as normal throughout each experimental condition, and to record insulin dose, and treatment of hypoglycaemia in the diary provided.

Data analyses

Data are reported for each participant using descriptive statistics (mean ± SD). Time spent performing sedentary, light, moderate and vigorous physical activity was assessed using validated cut-points (Phillips et al., 2013). Food diaries were assessed
for total energy and CHO intake (Nutritics, Nutrictics LTD, Ireland). Mean and peak blood glucose, and time spent in hyper- (> 7.2 mmol·L⁻¹), eu- (3.9-7.2 mmol·L⁻¹), and hypo- (<3.9 mmol·L⁻¹) glycaemia were assessed over a 24h period (08:00 on day 3 to 08:00 on day 4), during exercise, MMTT and night after exercise (23:00-06:00) using the CGMS data (Clarke and Kovatchev, 2009). Dietary CHO intake and insulin use (recorded by participants in the diet and insulin diary) were used to calculate CHO-to-bolus insulin ratio.

9.5. Outcomes

Cardiorespiratory and enjoyment responses to exercise

Mean \(\dot{V}O_2\) was 1.70 ± 0.60 L·min⁻¹ and 1.99 ± 0.98 L·min⁻¹ for HIIE and MIE respectively, Peak. Peak \(\dot{V}O_2\) during HIIE was 2.86 ± 1.04 L·min⁻¹ (98% of \(\dot{V}O_2\) max). RPE was higher in HIIE than MIE (8 ± 1 vs. 6 ± 1) and participants found HIIE more enjoyable than MIE (PACES: HIIE: 68 ± 1 vs. MIE: 57 ± 3).

Glycaemic response to exercise

As shown in Figure 1 (panels A-C), for participants B and C, both MIE and HIIE coincided with a drop in glucose during exercise (MIE: 35% (-4.3 mmol·L⁻¹) and 35% (-1.3 mmol·L⁻¹), HIIE: 43% (-2.4 mmol·L⁻¹) and 8.3% (-0.2 mmol·L⁻¹) respectively. In participant A, blood glucose rose in HIIE (36% + 2.1 mmol·L⁻¹) and MIE (19% + 1.4 mmol·L⁻¹).
24h, MMTT and nocturnal glycaemic responses

Blood glucose data during the MMTT, 24h post exercise period and the night after exercise are shown in Table 2.

Carbohydrate and insulin

Mean CHO intake for day prior to lab visit (CON: 263 ± 42 g, MIE: 267 ± 50 g and HIIE: 269 ± 28 g), the laboratory visit (CON: 278 ± 63 g, MIE: 269 ± 54 g and HIIE: 278 ± 28 g), and the morning post laboratory visit (CON: 93 ± 11 g, MIE: 80 ± 11 g and HIIE: 82 ± 4 g) were similar. Insulin dose varied for each experimental condition (table 9.2). The insulin bolus (units) : CHO (g) ratios for CON, MIE, HIIE were: Participant A: 9, 12 and 14 g; Participant B: 10, 14 and 16 g, Participant C: 12, 12 and 13 g.
Figure 9.2: Individual glycaemic response to exercise (A, B and C). Moderate intensity exercise (MIE), high intensity interval exercise (HIIE) and rest (CON). Exercise was performed 2 h after breakfast.
Table 9.2. The effects of acute MIE and HIIE on 24h glycaemic control, postprandial response to MMTT and overnight glycaemia in three adolescents with T1D.

<table>
<thead>
<tr>
<th></th>
<th>Participant A</th>
<th></th>
<th>Participant B</th>
<th></th>
<th>Participant C</th>
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<tbody>
<tr>
<td></td>
<td>CON</td>
<td>MIE</td>
<td>HIE</td>
<td>CON</td>
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<tr>
<td>24h</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Total 24h bolus insulin</td>
<td>32</td>
<td>28</td>
<td>19</td>
<td>24</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Blood glucose, mmol.L⁻¹</td>
<td>10.8</td>
<td>10.9</td>
<td>7.2</td>
<td>7.2</td>
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<td>% hyperglycaemia</td>
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<td>73%</td>
<td>46%</td>
<td>51%</td>
<td>47%</td>
</tr>
<tr>
<td>% euglycaemia</td>
<td>13%</td>
<td>6%</td>
<td>12%</td>
<td>14%</td>
<td>28%</td>
<td>37%</td>
</tr>
<tr>
<td>% hypoglycaemia</td>
<td>0%</td>
<td>3%</td>
<td>15%</td>
<td>40%</td>
<td>21%</td>
<td>16%</td>
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<td>1</td>
<td>2</td>
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</tr>
<tr>
<td>MMTT</td>
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<td></td>
</tr>
<tr>
<td>Insulin units with meal</td>
<td>7</td>
<td>7.5</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood glucose, mmol.L⁻¹</td>
<td>11.3</td>
<td>8.5</td>
<td>7.9</td>
<td>7.5</td>
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<tr>
<td>tAUC glucose</td>
<td>1360</td>
<td>1026</td>
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<td>Blood glucose, mmol.L⁻¹</td>
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<td>31%</td>
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<td>47%</td>
</tr>
<tr>
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<td>13%</td>
<td>9%</td>
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<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
</tr>
</tbody>
</table>

Results shown as individual values. tAUC; Total area under curve, MMTT; mixed meal tolerance test.
9.6. Discussion

The current study provides unique insight into changes in glycaemic control over a 24h period after an acute bout of HIIE and MIE in three adolescents with T1D. Overall, the data highlights the potential of HIIE to improve 24h glycaemic control and postprandial hyperglycaemia in adolescents with T1D and that they found this form of exercise more enjoyable than MIE.

Our results show reduced 24h glucose levels in all participants for HIIE compared to CON and for two patients for MIE compared to CON, which was partially due to the reduced average postprandial glucose assessed during a MMTT. These findings support previous research in adults with T2D (Gillen et al., 2012), where a similar HIIE protocol (10 x 60 s at 90% maximal heart rate), resulted in lowered average postprandial glucose, and reduced proportion of time spent in hyperglycaemia over the 24h following HIIE. The reduced postprandial hyerglycaemia observed following HIIE in the present study may have important clinical implications given its association with disease development (Ceriello, 2005).

This study highlights glucose perturbations during HIIE and MIE in adolescents with T1D. Blood glucose fell by 38-42% during MIE and 21-46% during HIIE in two participants. These findings concur with previous work by Tsalikian and colleagues (2005) who found that during 60 minutes of MIE 82% of participants experienced at least a 25% decrease compared to pre-exercise glucose level. Conversely, previous research in adults with T1D showed less of a decline in blood glucose following sprint
interval exercise, compared to MIE (Guelfi et al., 2005), which is contradictory to the findings in the present study where the drop in blood glucose during HIIE is more pronounced than the MIE, the disparity with the current study may be due to the “all out” nature of the sprint interval exercise in the study by Guelfi and colleagues (2005) resulting in increased norepinephrine and thus increased hepatic glucose output. The reduced exertion HIIE in the current study may not have been sufficient to have these effects. It is important to note the divergent response of participant A compared to B and C showing an increase in glucose during exercise. This could be down to a number of factors, including sex and maturation status, being the only female as well as the oldest participant.

Results from the present study highlight nocturnal hypoglycaemia following exercise. In two participants, HIIE was associated with an increase in nocturnal hypoglycaemia (35% and 87% compared to 0% in CON), whereas MIE was associated with nocturnal hypoglycaemia in one participant (7% compared to 0%). This increased incidence of hypoglycaemia has been shown previously after MIE in children with T1D (aged 11–17 y), with 22% of participants experiencing hypoglycaemia in the night following 60 minutes of afternoon MIE (Tsalikian et al., 2005). The risk of nocturnal hypoglycaemia after HIIE in children with T1D has not previously been investigated, but is likely due to HIIE having greater insulin sensitising effects (Cockcroft et al., 2015). This is supported by the present study that shows an increase in the insulin:CHO ratio in both exercise conditions but not CON, with a larger increase after HIIE. It is also noteworthy that the highest incidence of hypoglycaemic events throughout the 24h period occurred in participant C, who’s HbA1c was the lowest of the participants. The
observed hypoglycaemic events after exercise may therefore, in part, be due to the low baseline glycaemia in participant C who was also the fittest and most active suggestive of a high IS and is worthy of follow-up.

Besides these novel findings, this study also highlights the difficulty of implementing laboratory controlled studies in children with T1D. Tight control measures over a long postprandial period were affected by the need to correct for hypoglycaemia and with dietary intervention and change in insulin dose. Participants were asked to use their insulin as they would at home, which resulted in, for example subject B, have 0 unit of insulin with the MMTT after both HIIE and MIE. Future research should consider the duration of laboratory visits as this is often cited as reasons for participants not participating, despite appreciating the importance of the research.

Findings from the present study indicate that the participants found HIIE to be more enjoyable than MIE, which may have implications for implementing this type of exercise into an exercise training intervention. Additionally this study also highlights large inter-participant variability in the short-term glycaemic response to acute HIIE and MIE, indicative of the need to personalise glucose management with respect to modifying insulin dose and CHO intake before and after exercise. Despite the obvious limitation of a small samples size, the data from this pilot study highlight HIIE as a potential target for future work in youth with T1D.
Chapter 10

Summary of findings, implications, limitations, and future directions.
The primary aim of this thesis was to undertake a series of novel investigations into the potential for HIIE to improve glucose tolerance and IS in healthy children and adolescents. In addition, the application of HIIE to improve short-term glycaemic control in adolescents with T1D was explored using a case study approach. Each experimental chapter presented in this thesis significantly contributes to the existing literature, as addressed throughout the thesis. Therefore, the purpose of this chapter is to: 1) summarise the findings of each experimental chapter presented in the thesis; 2) synthesise and discuss the broader findings from the thesis; 3) highlight practical implications of the work; and finally 4) thoughtfully consider some of the methodological limitations of the research presented in the thesis. Throughout this chapter, gaps in knowledge and areas of future research will be alluded to.

10.1. Summary of experimental Chapters

10.1.1.1. Chapter 4

Chapter 4 examined the relationship between commonly used surrogate markers of IR in youth, those derived from fasting measures and those from the dynamic OGTT. It also investigated the day to day reliability of these measures. Results show that the Matsuda index had a strong significant correlation with a number of fasting indices (QUICKI, FGIR and HOMA-IR), which was not the case for the Cederholm index. The available fasting and OGTT derived indices showed a varying range of day to day reliability, indicating considerable variation at the participant level, with TE ranging from 4.7% for QUICKI to 62.5% for HOMA-IR. Two commonly used OGTT estimates of IS were found to have large discrepancies in their reliability, with the Cederholm
index shown to be more reliable (6.4%) than the Matsuda index (26.7%). This study is the first to report data surrounding the assessment of IS in healthy children and adolescents and the results have important implications in terms of assisting researchers in the selection of IS estimates and informing sample sizes calculations when designing interventions in this population. This study also highlights the need for further work in this area, especially with regard to validation of IS estimates against the gold standard HEC method.

10.1.2. Chapter 5

The purpose of this chapter was to identify if a single bout of HIIE could result in superior improvements to IS and glucose tolerance compared to work matched MIE in a group of adolescent boys. No previous study had assessed the effects of acute HIIE on IS in adolescents, although evidence existed for the effect of acute MIE in low physically active and low aerobically fit adolescents (Short et al., 2013). This study included control for the main confounders of IS and glucose tolerance, by replicating the participant’s diet 48 h prior to each experimental condition, and by using accelerometers to measure PA during this same time period. The work-matched design of the exercise protocols also allowed for the isolation of any effects of exercise intensity.

The results from this study indicate that when HIIE, consisting of just 8 minutes of exercise at 90% PP, was performed immediately (~ 10 minutes) prior to an OGTT, this resulted in a significant 11.2% improvement in IS (Cederholm index) compared to
CON. In contrast, MIE only resulted in a “trend” for an 8.4% increase in IS compared to CON. Additionally, this study reported that the participants found HIIE and MIE to be equally enjoyable despite the increased physiological and perceptual stress of the HIIE condition.

Therefore, this chapter highlights the possible superior nature of HIIE to improve IS in healthy adolescent boys in contrast to MIE. Since HIIE was equally as enjoyable as MIE, this suggests that HIIE may be a feasible and effectual alternative to MIE in this population to improve IS.

10.1.3. Chapter 6

Chapter 6 extended upon the findings of Chapter 5 by examining whether the acute improvements in IS and glucose tolerance observed after a single bout of HIIE were maintained over a 48 h period. Similar to Chapters 4-5, a methodological strength of this chapter was its control for PA and diet over the experimental conditions. The multi-day measurements in this chapter also allowed for the inclusion of both fasting and OGTT estimates of IS. This provides additional originality to the study and allows for speculation regarding the mechanisms involved in the effects of exercise on IS in this population.

No changes in fasting estimates of IR (HOMA-IR) either 24 or 48 h following both HIIE and MIE were observed. However, IS assessed using an OGTT was significantly increased 40 minutes following only HIIE (17.4%) compared to CON, with only a trend
for an increase following MIE (9.0%). In contrast, 24 h after the exercise bout, IS was significantly augmented following both HIIE (13.2%) and MIE (9.7%).

This study shows for the first time the time course of changes to IS following an acute bout of both HIIE and MIE in adolescent boys. Results show that improvements to IS measures during an OGTT can persist for up to 24 h after exercise but that fasting measurements of IR are insensitive to change over this timeframe. This novel finding allows speculation of the mechanisms by which acute exercise can improve IS in adolescents, with dynamic assessment via the OGTT reflecting peripheral changes in IS compared to the fasted HOMA method which reflects hepatic IS.

10.1.4. Chapter 7
Following on from Chapter 5, Chapter 7 aimed to elucidate if a similar beneficial effect of acute exercise on IS and glucose tolerance could be observed in a pre/peri pubertal boys. Despite using the same exercise protocols and control measures as presented in Chapters 5-6, no differences in IS (Cederholm index) following MIE and only a small increase following HIIE were observed.

This chapter is the first study to examine the acute effect of HIIE and work-matched MIE on glucose and insulin health outcomes in pre/peri pubertal boys. The results indicate that acute exercise in this age group has a small effect on IS immediately after HIIE but not MIE.
10.1.5. **Chapter 8**

Chapter 8 builds upon the findings of Chapters 4-5 and aimed to elucidate if two weeks of HIIE training could improve IS (fasted assessment) and glucose tolerance (assessment by MMTT) in a group of adolescent boys. Drawing upon the findings in Chapter 4 this study reported multiple fasting indices of IR to examine if indices with lower day to day reliability would be more sensitive to change following the intervention. Findings from this study showed that in a healthy group of adolescent boys, 2 weeks of HIIE training did not improve fasting HOMA-IR, QUICKI or FGIR or MMTTT derived glucose tolerance either 24 or 72 h after the last training session. However, results showed a strong negative correlation between baseline IR and change in IR 24 h after HIIE. This suggests that participants with the lowest baseline IR may have potential to improve metabolic health outcomes after just 6 sessions of HIIE training. Thus, this type of training may be a feasible approach to improve IR when targeted at an “at risk” group.

10.1.6. **Chapter 9**

Chapters 5-6 of this thesis demonstrated acute metabolic health benefits after a single bout of HIIE in healthy adolescents. The aim of Chapter 9 was to elucidate whether the same HIIE protocol can be effective at improving short-term glycaemic control in adolescents with T1D. This is important as evidence for the optimum exercise recommendations, in terms of improving glycaemic control, in this population are unknown. This study used a GCM system to measure glucose over a 24 h period incorporating exercise (HIIE and MIE), a MMTT and the nocturnal period, in three adolescents with T1D. Findings from this study suggest that HIIE may be a feasible
form of exercise in adolescents with T1D and an effective means to lowering 24 h blood glucose. However, this study was only a pilot study and, as a result, warrants further research to confirm these findings.

10.2. Synthesis of the experimental findings

10.2.1. Exercise intensity, insulin sensitivity and glucose tolerance

Three experimental Chapters within this thesis (Chapter 5-7) have contrasted a single bout of HIIE and a work-matched bout of MIE on insulin and glucose health outcomes in order to isolate the effect of exercise intensity. Although none of these studies reported significant differences between HIIE in contrast to MIE for IS and glucose tolerance, it is pertinent to note that there was a consistently greater ES for HIIE than MIE when compared to CON (IS: HIIE: 0.76, 1.07 and 0.28 vs. MIE: 0.38, 0.68 and 0.21 for Chapters 5-7 respectively). The larger reduction after HIIE compared to MIE was also observed for the tAUC and iAUC glucose outcomes. Collectively, this is indicative of an exercise intensity dependent effect on the changes in both IS and glucose tolerance, with HIIE being the superior exercise intensity when compared with MIE. In addition, Chapters 5 and 6 found that only HIIE significantly increased IS immediately after exercise compared to CON, with only a “trend” being observed for MIE.

To provide further insight into the possibility of an exercise intensity dependant effect on IS, data from Chapters 5-7 were pooled to illustrate the change in IS after MIE and HIIE compared to CON (n=28). The mean change in IS for MIE compared to CON was
3.19 ± 7.3 and for HIIE compared to CON was 6.04 ± 6.6 (see figure 10.1). Thus, the improvement in IS compared to CON was approximately 2-fold greater after HIIE compared to MIE. Taken as a whole, the evidence from Chapters 5-7 suggests that HIIE may be a more effectual form of exercise to augment IS and improve glucose tolerance in contrast to MIE, but this requires direct confirmation in future research.

![Figure 10.1](image_url)

**Figure 10.1.** Change in insulin sensitivity (A) and glucose tolerance (B) compared to control following moderate-intensity exercise (MIE) and high-intensity interval exercise (HIIE). Data are pooled across Chapters 5-7.

Notably, previous research has also reported that HIIE is superior to MIE at improving glucose responses to a HFM when the exercise was accumulated in four bouts throughout the day (Bond et al., 2015b). In terms of other health outcomes, namely vascular function and blood pressure responses to a HFM, HIIE has also been shown to be superior to MIE (Bond et al., 2015b). A favourable effect of HIIE in terms of IS and glucose tolerance has also been shown in pre-diabetic adults. Rynders et al. (2013) compared isocaloric (~200 kcal) bouts of MIE and HIIE and reported that IS
improved after both exercise bouts by 51% and 85%, for MIE and HIIE respectively. However, only HIIE significantly improved AUC for glucose and insulin during the OGTT.

This apparent superiority of HIIE to increase IS and improve glucose tolerance, when compared to MIE, may be partly influenced by the degree of glycogen depletion during exercise (VØLlestad and Blom, 1985). In adults, it has been suggested that HIIE may hold additional benefits over MIE in augmenting IS. This is largely due to the increased reliance of CHO as a substrate at higher intensity activities, leading to higher levels of glycogen depletion (VØLlestad and Blom, 1985, Malin et al., 2016).

Despite the indications that HIIE might be superior to MIE at improving IS in youth, the HIIE protocol employed within this thesis (8 x 1 minute at 90%) is unlikely to be “optimised”. Additionally, the exercise protocol used was performed on a cycle ergometer and further work into different modalities of exercise, (e.g. running and circuit based exercise) is also worth considering, which might be more transferable to a school based setting. To fully understand the optimal type of HIIE protocol to improve IS, further comparisons need to be made with regard to altering the number, duration and/or intensity of the HIIE work intervals. For example, a HIIE dose response study was recently undertaken by Logan et al. (2016). Twenty-six low physically active male adolescents (16 ± 1 y) were randomly assigned to 5 groups corresponding to the number if HIIE sets completed (4 x 20 s “maximal” 10 s recovery) each session. Sessions were completed 2 x per week for 8 weeks. Results showed improvements
in VO₂ max, body fat percentage and waist circumference when 1 set was completed, with no clear dose response across the groups with increasing number of HIIE sets. However, this study did not include the assessment of IS using from a dynamic test, which would be warranted in future research.

10.2.2. Individual variation

Another consideration of the work within this thesis is the inter–individual response to the exercise stimulus. As can be seen in Figure 10.2, which represents individual change in IS compared to CON in both MIE and HIIE, not all participants responded favourably to the acute exercise stimulus. More often than not, studies report the response to exercise in general terms that represent the “typical response”. However, as highlighted in Figure 10.2 there is a wide range of individual responses, evidencing both “responders” and “non-responders” to exercise. Notably, this ‘responsiveness’ has previously been shown across paediatric work looking at postprandial lipaemia (Thackray et al., 2013, Thackray et al., 2016). Some of the variation, but not all, could be explained by differences in participant age (discussed below in section 10.2.3), but other factors are also likely. This was also eluded to during Chapter 8 of the thesis, where results showed a significant correlation in improvements to HOMA-IR after HIIE training with baseline HOMA-IR, but not in fitness and BMI; suggesting that baseline metabolic health may influence individual response to training. Future research should aim to identify characteristics which correspond to “responders” and “non-responders”, allowing for interventions to be more targeted, whilst minimising the possibility for any adverse response.
10.2.3. Effect of age/pubertal status on changes in IS after exercise

Although not directly assessed, findings from experimental Chapters 5-7 highlight the potential for age and/or pubertal status to influence the acute effects of exercise on the glucose and insulin outcomes. Results of the change in IS after both HIIE and MIE compared to CON from Chapters 5-7 are shown in Figure 10.3. Data has been split to show adolescents (Chapters 5-6) and younger 7-10 y old boys (Chapter 7). The mean improvement for the change after MIE or HIE compared to CON was 1.1 ± 6.6 and 4.4 ± 5.8 in Chapter 7 in 7-10 y old boys (white bars), compared to 4.5 ± 7.1, and 7.1 ± 6.6 in Chapters 5 and 6, in adolescent boys (13-15 y, black bars).
Figure 10.3. Change in insulin sensitivity after acute HIIE (A) and MIE (B). White fill bars are younger participants from Chapter 7 (n=11), whilst the black filled bars are adolescents from Chapters 5 and 6 (n=17).

The attenuated acute change to IS after exercise in younger boys is supported by recent prospective data, where Metcalf and colleagues (2015) found that there was no significant association between PA status and IS in 5-8 y olds, whereas PA attenuated the peak IR during adolescence, until ~ age 16 y when the association was no longer apparent. Reasons for this age effect are currently elusive but may be due to a ceiling of improvements to IS, meaning further improvements are unlikely. However, it is unlikely to be due to the decline in PA during adolescence, since the lack of effect of PA is also observed in older adolescents (Metcalf et al., 2015).

Although not definitive, these data suggest an age and/or pubertal status effect on the ability of exercise to augment IS, and warrants further investigation with direct comparison between well-defined age/pubertal groups. This finding is also important when looking at the effects of previously published exercise interventions. Studies that have shown no improvements in younger (~ 10 y), or older (~16 y) children, may have
been successful during mid-adolescence, and thus these interventions shouldn’t be deemed ineffective until investigated in younger adolescents.

10.2.4. Exercise, fat oxidation and insulin sensitivity

Experimental Chapters 5 and 7 reported changes in fat oxidation alongside measures of IS during the OGTT following HIIE and MIE. Although not the primary outcome of the thesis, discussing this fat oxidation finding further is important in the context of metabolic health as resting fat oxidation is known to predict exercise induced fat loss (Barwell et al., 2009). Additionally, fat oxidation has been reported to be related to IS (Samuel et al., 2010) and has been proposed as a mediator in augmenting IS following exercise (Goodpaster et al., 2003).

Despite the potential link between resting fat oxidation and IS, the results from experimental Chapters 5 and 7 found no significant relationship between changes in IS and fat oxidation following both HIIE and MIE (see Figure 10.4). However, the proposed relationship (Goodpaster et al., 2003) between changes in IS and fat oxidation may be more relevant to chronic exercise training, as reported by Barwell et al. (2009). Unfortunately, resting fat oxidation was not measured in Chapter 8 where HIIE training was undertaken over two weeks and no change in fasting measures of IS were identified. Therefore, future work should consider investigating the potential relationship between resting fat oxidation and IS following exercise training in children and adolescents.
Despite no significant relationship between fat oxidation and changes in IS within this thesis, HIIE augmented fat oxidation to a greater extent than MIE. For example, in Chapter 5 there was a trend and a moderate effect of increased fat oxidation following HIIE compared to CON (ES=0.70), and a non-significant trivial effect following MIE compared to CON (ES=0.19). In Chapter 7, despite limited effect on IS in pre/peri pubetal boys, HIIE significantly improved fat oxidation compared to CON (ES=0.79), whilst no significant difference was observed between MIE and CON (ES=0.48). Therefore, the effect of acute exercise on resting fat oxidation may appear to be dependent on exercise intensity, which is consistent with previous work in adolescents (Bond. et al., 2015d). Mechanistically, it could be hypothesised that this is as a result of the increased catecholamine response to HIIE when compared to MIE (Christmass et al., 1999). Catecholamines, especially adrenaline, have been shown to drive lipolysis and are responsible for fat release from both subcutaneous and intramuscular fat stores (Issekutz, 1978), and thus increased catecholamines in the circulation may result in an increased rate of lipolysis.
Figure 10.4. Relationship between changes in insulin sensitivity and fat oxidation and change to total area under curve fat oxidation following high intensity interval exercise and moderate intensity exercise. Data pooled from Chapters 5-7 (n=23)

10.2.5. Fasted and dynamic measures

Due to methodological and ethical considerations, exploration of the cellular mechanisms accounting for changes to IS and glucose tolerance after exercise in the current thesis was not possible. However, Chapter 6 allowed for speculation on the
potential mechanisms for improved IS after exercise. Specifically, the experimental protocol in Chapter 6 allowed assessment of IS both in the fasted state and after an OGTT. Fasting assessment is thought to measure IS from a hepatic perspective (Muniyappa et al., 2015), whereas dynamic assessment using an OGTT is proposed to be more reflective of peripheral IS (Cederholm and Wibell, 1990). Chapter 6 found that OGTT derived IS was increased after exercise, but no changes were observed in fasting measures. Similar studies have also reported no changes in fasting glucose and insulin the day after a single bout of exercise in adolescents (Thackray et al., 2013, Tolfrey et al., 2008). However, similar to the findings within this thesis, and after a single bout of MIE, Short et al. (2013) showed no change to basal insulin and glucose dynamics despite significant changes in postprandial IS.

The improvements observed in dynamic, but not fasting assessment of IS, suggest that the exercise-related insulin sensitising effects are mainly in peripheral tissues and not at the hepatic level. This is an important point to consider as it suggests that many training studies, which have only reported fasting measures of glucose, insulin and IS, may be missing crucial beneficial effects of the training intervention. These peripheral improvements to IS may also be exercise intensity dependant, as alluded to in section 10.2.1. HIIE is thought to induce higher levels of glycogen depletion due to use of higher order muscle fibres compared to MIE, as well as increased levels of circulating catecholamines (VØLlestad and Blom, 1985).
In Chapter 6, increased dynamic IS was shown to persist for up to 24 h after exercise. This is suggested to be due to the two phases of glycogen repletion after exercise (Henriksen, 2002). The first (rapid) phase takes place up to 1 h following exercise cessation, whilst the second (slow) phase lasts for 1-2 days following the exercise bout (Price et al., 1999). When considering these phases in relation to Chapter 6, where increased IS was shown to persist for 24 h after exercise, the fact that the second (slow) phase of glycogen repletion lasts for 1-2 days following exercise could help to explain these findings.

10.3. Practical research implications

Collectively, the research contained within this thesis shows that both MIE and HIIE can improve IS and glucose tolerance for up to 24 h after exercise in adolescent boys. However, the comparable and possibly superior effects following HIIE suggest that this type of exercise should be considered as a viable alternative to “traditional” MIE in exercise interventions and recommendations for PA in adolescents. Furthermore, knowledge that both MIE and HIIE augment IS for up to 24 h post exercise suggests that exercise could be performed every other day to improve glucose and insulin health outcomes. Whether benefits persist past 24 h, and if there is a difference between MIE and HIIE, is presently unknown, and thereby warrants further study.

Chapter 7 of this thesis found that MIE did not, and HIIE only to a small extent, have an effect on IS and glucose tolerance in 7-10 y old boys. From a practical perspective, this is important since current PA guidelines for health in the UK are identical for young
people aged between 5 – 18 y. These findings show that in terms of improving glucose tolerance and IS, a different relationship exists for the ‘dose’ of exercise required to elicit health improvements in peri/pre pubertal boys, when compared to adolescents. As a result, a “one size fits all” approach to prescribing PA and exercise for health improvements, especially across such a wide age-range (5-18 y), may not be appropriate. Interestingly, similar suggestions for individualising PA recommendations have been made for adults of different ethnic origin (Iliodromiti et al., 2016). Therefore, further intervention work is needed to investigate how age influences the acute effects of exercise on IS.

In addition to the metabolic benefits of exercise, Chapters 5-7 have shown that HIIE is either more enjoyable or maintains similar enjoyment levels when compared to MIE. This is of importance since the enjoyment experienced during exercise has previously been shown to mediate the success of PA interventions and influence long-term PA participation (Dishman et al., 2005). Thus, the similar or higher enjoyment scores for HIIE compared to MIE may have important implications for adherence to a HIIE programme. The preference and enjoyment towards HIIE, combined with its superior health benefits, further evidences its potential use as an alternative strategy to MIE to improve the cardiometabolic health status of youth.

Findings from Chapter 8 suggested that when prescribing HIIE interventions, a more targeted approach may be advisable. In this chapter, the largest changes to IS after 2 weeks of HIIE were observed in those with higher levels of baseline IR, suggesting
Interventions targeted at those most “at risk” may have the most benefit. Indeed, past exercise training interventions have tended to target those who are overweight or obese (Davis et al., 2011, De Araujo et al., 2012), despite evidence that metabolic abnormalities are also apparent in normal weight youth (Weiss et al., 2013). Findings from this chapter suggest there may be merit in interventions targeted at adolescents with raised HOMA-IR. This is logical, if the aim of the intervention is to improve IS or IR.

The improvements to glycaemic control evident after a bout of HIIE, as reported within Chapter 9, may be of clinical value. The observed improvements to 24 h glycaemic control observed after a single bout of exercise, if maintained over a longer period, could lead to improved long term glycaemic control (HbA1c), which is one of the main clinical outcomes in the management of T1D. Importantly, small improvements in HbA1c (1%) are associated with as much as a ~14% reduction in MI (Stratton et al., 2000). This means that improvements to long term glycaemic control after exercise may have an important role in the prevention of CVD in T1D. Additionally, HIIE may have beneficial implications for other CVD risk factors in this patient group such a macro- and micro-vascular function (Khan et al., 2000, Jarvisalo et al., 2004). These risk factors have been shown to be improved in healthy adolescents following HIIE (Bond. et al., 2015c), and it is an important area of future investigation in children with T1D.
Finally, the results from Chapter 4 have methodological implications and should assist researchers in designing studies within which IS and glucose outcomes are sought. Specifically, results from Chapter 4 indicate the day to day reliability of measurements of IR commonly used in paediatric research. Practically, these findings can be used to calculate study sample sizes using the details provided by Hopkins (2000). For example, as previously outlined in Chapter 4, to detect ~ 5% change in IS in a randomised cross-over study you would require a sample size of ~ 13 participants using the Cederholm index and ~ 228 participants for the Matsuda index. This is because the Matsuda index has a day to day reliability that is 4-fold higher than that of the Cederholm index. Understanding this measurement variability will aid researchers in selecting measurement outcomes which require smaller samples to be recruited having implications for resourcing and costing future studies.

10.4. Limitations

There are a number of limitations to this research, as presented throughout each experimental chapter of this thesis. However, a consistent limitation throughout the work (detailed in Chapter 4), is the lack of evidence for validation of the IS measurements in ‘healthy’ children and adolescents, particularly the Cederholm index which had the lowest day to day measurement error. Validating the Cederholm index is of high importance for future work, although such work would have ethical implications due to performing the HEC procedure in a healthy paediatric population. An additional methodological limitation was the lack of a dynamic IS measurement in Chapter 8. The use of a MMTT meant we were unable to report estimates of dynamic IS, meaning that benefits from exercise training may have been missed.
A further limitation of this thesis was the inclusion of only male participants in Chapters 4-8, thereby limiting the extrapolation of findings to female populations. Additionally, the homogeneity of participants in terms of health and PA status (all were healthy and recreationally active) limits the generalisability of the current findings. This was an unavoidable consequence of selection bias, with children volunteering as a result of their interest in sport and activity.

The studies within this thesis were all conducted in a controlled laboratory setting, including Chapter 8, where HIIE training took place in a satellite laboratory in a school. It is of critical importance to see whether this laboratory-based research could be translated into a real world setting. Additionally, each study was limited to cycling based exercise and future research is needed to investigate other modalities, such as running, which maybe more practical to implement outside of the laboratory setting. The cardiometabolic health benefits of school-based HIIE programs have previously been investigated (Weston et al., 2016, Buchan et al., 2013), but these studies did not report dynamic (peripheral) measures of IS. This is likely due to undertaking the work on a larger sample in the school setting. Therefore, future work to establish the health benefits of school-based HIIE programmes would have important implications for the recommendation of HIIE in youth and should focus on implementing a practical and feasible form of HIIE in a “real world” setting, i.e. a school physical education lesson. For this research area to be progressed, large scale randomised control trials will be required.
A further limitation of the work presented in Chapters 5-9 is the use of the PACES scale for the assessment of exercise enjoyment, which has only been previously validated for use in adolescent girls (Motl et al 2001). Exercise enjoyment may also be influenced by a number of factors which should be acknowledged, including past exercise history and experience of HIIE. However this was controlled for by the cross-over study design, and exercise conditions were counterbalanced, meaning that the higher PACES following HIIE is likely to indicate that this type of exercise is more enjoyable, since exercise history and experience of HIIE were consistent across conditions.

Finally, Chapter 9 was limited by a small sample size. This was due to a number of factors, including poor recruitment rates. This is important for future work, highlighting the difficulty of recruiting from paediatric T1D populations, especially when they have to undertake multiple controlled laboratory research visits. Since further work in this area is important, more time and effort should be invested in ensuring experimental protocols are suitable for this population. Input from the paediatric patient group would hopefully ensure better recruitment rates in future studies.

10.5. Conclusion
The work contained within this thesis is the first to investigate the acute effects of different exercise intensities on IS and glucose tolerance in different age groups, as well as adolescents with T1D. Findings show IS improves immediately after a single session of both HIIE and MIE in adolescents boys and persist for up to 24 h after
exercise, showing HIIE to be a feasible and effectual alternative to MIE in this population. Importantly, it is also shown that these acute effects are not as prominent in younger boys. This thesis can be used to inform and direct future research in this increasingly important area of paediatric health, especially given the increasing global burden of both T2D and CVD and the importance of primary prevention in youth.
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APPENDIX
Appendix 1: Example certificates of ethical approval

Certificate of Ethical Approval

Proposal Ref No: 141203/B/11

Title: Time course of changes in glucose tolerance and insulin sensitivity after a single bout of moderate- and high-intensity exercise in adolescent boys

Applicants: Emma Cockerott, Dr Alan Barker, Prof Craig Williams, Amy O’Connor, Hayleigh Weaver, Bert Bond, Owen Tominson, Dimitris Vlachopoulos, Ricardo Santos Oliveira

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: This proposal has been approved until September 2015.

Signature: [Signature]

Date: 7/1/2015

Name/Title of Ethics Committee Reviewer: Dr Melvyn Hillsdon

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.
13 August 2014

Dr Christopher Moudiotis
Consultant Paediatrician
Royal Devon and Exeter NHS Trust
Room G103, Bramble Ward
Royal Devon & Exeter Hospital
Barrack Road
EX2 5DW

Dear Dr Moudiotis,

Study title: The influence of a single bout of high-intensity exercise compared to moderate-intensity exercise on health outcomes in children and adolescents with type 1 diabetes.

REC reference: 14/SW/1028
IRAS project ID: 150912

Thank you for your letter of 8th August 2014, responding to the Committee’s request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Mrs Kirsten Peck, rescommittee.southwest-cornwall-plymouth@nhs.net

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

A Research Ethics Committee established by the Health Research Authority
Appendix 2: Example health screen form

HEALTH SCREEN FOR CHILD VOLUNTEERS (PARENTAL FORM)

Name: ........................................
Height: ........................................ (please provide in cm or feet)
Weight: ........................................ (please provide in kg or stone)

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is:

   i)        To ensure their own continuing well-being
   ii)       To avoid the possibility of individual health issues confounding study outcomes

Your answers to the questions in this questionnaire, on behalf of your child, are strictly confidential.

Please complete this brief questionnaire to confirm your child’s fitness to participate:

1. **At present**, does your child have any health problem for which they are:
   (a) On medication, prescribed or otherwise .......... YES □ NO □
   (b) Attending a general practitioner .................... YES □ NO □
   (c) On a hospital waiting list ............................ YES □ NO □

2. **In the past two years**, has your child had any illness that required them to:
   (a) Consult your family GP............................. YES □ NO □
   (b) Attend a hospital outpatient department ........... YES □ NO □
   (c) Be admitted to hospital............................. YES □ NO □

3. **Has your child 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) Lung problems ..................................... YES □ NO □
(j) Problems with bones or joints .............. YES □ NO □
(k) Disturbance of balance/coordination ....... YES □ NO □
(l) Numbness in hands or feet ................. YES □ NO □
(m) Disturbance of vision ......................... YES □ NO □
(n) Ear/hearing problems ....................... YES □ NO □
(o) Thyroid problems .............................. YES □ NO □
(p) Kidney or liver problems ...................... YES □ NO □
(q) Allergy to nuts ................................... YES □ NO □
(r) Eating disorder ................................... YES □ NO □

4. Do you know of any other reason why your child should not engage in physical activity?

   YES □ NO □

If YES to any question, please describe briefly (for example, to confirm the problem was/is short-lived, insignificant or well controlled).

A member of our research team may contact you if we have any further questions.
Appendix 3: Example parent information sheet

Parent/Guardian Information Sheet

Project Title: How long do improvements in insulin sensitivity after exercise last in boys?

1. What is the purpose of the study?
This study will look at how exercise of different intensities can reduce the amount of sugar in the blood following a sugary drink, both immediately after exercise and up to 48 h after. This is important because as children grow and develop, their body is less able to handle the sugar in the blood, and this may lead to the development of diseases, such as type II diabetes, later in adult life. Knowing how long health improvements last following exercise is important for guiding recommendations regarding how often children should exercise.

2. What does this study involve?
The study will consist of 10 visits over a four week period. The first visit will last no more than two h. The following 9 visits will be split over 3 different experimental tests; each including 1 visit to the laboratory in Exeter, and 2 visits to a temporary laboratory that we have set-up at your child’s school.

Visit 1: Familiarisation and baseline measurements
The first visit will familiarise your child to the laboratory setting and all the equipment that will be used during the study. During this visit we will take measurements of your child’s standing height and sitting height, and estimate their body fatness by gently measuring a fold of skin at different regions of their body. We will also measure your child’s level of pubertal development, as this can influence how the body processes sugar in the blood. We will ask your child to self-asses their pubertal development at
home and in private. This will require your child to look at scientific drawings that show five different stages of pubertal development and to identify which stage best describes their own development. This is a routine procedure which is frequently performed within this population for scientific research.

During this visit your child will be familiarised with the bike that will be used for exercise. They will complete a 10-15 minute exercise test, which involves cycling for as long as possible while the pedals become progressively harder to turn. This is like cycling up a hill that increases with steepness. This test will be followed by a 15 minute rest period and then another very short test (5 minutes) to see whether they can cycle at a higher resistance than that achieved on the first test. These tests are very well tolerated by children but do require a maximal effort. The final moments will therefore be strenuous for your child. During this your child will be wearing a face mask so we can record the amount of oxygen they are using to undertake the exercise.

**Experimental tests:**

This aspect will involve three different exercise tests (see below), each of which will involve 5 consecutive days of data collection, which are outlined below.

**Days 1 and 2:**

Two days prior to the visit to the laboratory in Exeter (Day 3) we ask that your child wears an accelerometer (wrist worn device which measures how active they are) and records the food and drink they consume each day. We will provide these during the 1st visit and explain how to complete the food diary.

**Day 3:**

Your child will need to arrive at the laboratory by 8 am; we will collect you child from home to be transported to our research laboratory. We ask that your child does not eat from 8 pm the night before the visit. This includes not having breakfast in the
morning. Upon arrival we will take a sample of blood from your child’s fingertip to measure their sugar level, and ask them to draw on a scale and fill in short questionnaire to assess appetite. The amount of blood is very small (less than 1 mL) and is a routine procedure, which feels like a small pin prick. This technique will be demonstrated during the first visit. Next your child will perform either; (1) short bouts of high intensity cycling exercise (8 minutes total); (2) continuous moderate intensity cycling exercise, or (3) rest.

1. **High intensity cycling**: This will involve cycling for eight 1-minute intervals at 90% of the resistance that your child obtained during the exercise test on the first visit. Each interval will be separated by 75 s of very light cycling. The exercise will also include a warm-up and cool down period. Your child may find these sessions hard for a short period of time and will be monitored throughout.

2. **Continuous moderate intensity exercise**: During this exercise session your child will be asked to cycle at a light resistance for approximately 20 to 30 minutes. The exact duration will be calculated to match the amount of work they managed to produce during the high intensity exercise session.

3. **Rest**: Your child will rest in the research laboratory.

After the exercise or rest, your child will be asked to fill in two short questionnaires so we can see how much they enjoyed the exercise, and once again look at their appetite. Then they will be asked to drink a small sugary drink within 5 minutes. We will then take a number of small blood samples (less than 1 mL) at 0, 10, 20, 30, 60, 90 and 120 minutes after finishing the drink to measure the amount of sugar in their blood. This procedure is routine and feels like a small pin prick. We will also ask your child to wear a face mask so we can measure the amount of oxygen and carbon dioxide in the air they breathe in and out, at 60, 120 and 180 minutes after the exercise. This will enable us to estimate how much fat and CHO their body is using as fuel. 2 h after exercise your child will fill in the same questionnaire to assess their appetite.
After the measurements are finished your child will be able to have their lunch. Please can they bring along a cold packed lunch. We will then return your child to school for afternoon lessons. During the rest of the day your child will be asked to continue recording the food and drink they consume on the food diary, and to wear the accelerometer to measure how active they are.

Day 4:

The morning after the visit to the laboratory, again after an overnight fast from 8 pm, we will meet your child in school at 8.00 am. If your child normally walks to school we will arrange to pick them up. We will initially estimate how much fat and CHO their body is using as fuel like in the laboratory the previous day. After this your child will fill in the appetite questionnaire and then they will then consume a sugary drink. After the drink we will take a small blood sample at the same time points as the previous day (0, 10, 20, 30, 60, 90 and 120 minutes). Once these measurements are complete, your child will be able to eat their normal lunch.

During the rest of the day your child will be asked to continue recording the food and drink they consume on the food diary, and to wear the accelerometer to measure how active they are.

Day 5:

On the third morning, again after an overnight fast from 8 pm, we will meet your child at school for another blood sample at 8.00 am. If your child normally walks to school we will arrange to pick them up. We will initially estimate how much fat and CHO their body is using as fuel like in the laboratory the previous day. After this your child will fill in the appetite questionnaire. We will then take single blood sample from your child’s fingertip. After this sample we will provide your child with breakfast, so please note any dietary requirements on the contact information sheet.
Controlling for activity and diet:

For studies like this, it is very important that we control for how active your child is and what they eat over the data collection period. This is because exercise and diet has a direct effect on the measurements we are interested in. We are therefore asking your child to wear an accelerometer during their participation in the study, and to record the food they consume each day. After the first exercise experimental test is complete, we will return the original food diary records to your child and kindly ask them to replicate their diet as close as possible. Your assistance in helping your child with this would be much appreciated.

3. What else will my child have to do?

Your child will need to bring suitable kit for the exercise sessions (shorts, t-shirt and trainers), and some schoolwork or a DVD to watch during the rest and observational period. We have a Sony PlayStation 3 and desktop computers with age-appropriate games and educational software which your child may play on during the rest period. We would also encourage your child to ask as many questions as they please. We hope that their visit to the laboratory inspires them to think about their health and the potential of higher education.

4. What are the possible risks of my child taking part?

All the procedures used in the study are regularly used in research in children and adolescents. Prior to completing any exercise an assessment of their health will be made to ensure it is safe for them to take part.

5. What will my child gain from taking part in this study?

This study aims to further understand the health benefits of different types of exercise in children and adolescents. Whilst this may not immediately benefit your child, we hope that your child will enjoy their time in the University laboratories and the chance
to be part of a scientific study. At the Children’s Health and Exercise Research Centre, we pride ourselves on ensuring that each volunteer has an enjoyable and informative experience throughout every research project. We hope that we can inspire your child to take an interest in their health and in the science of exercise, and that this project will be both interesting and fun.

6. What will happen to the results of the study?

Your child’s data will be stored in coded form to protect anonymity and will be completely confidential. This research will form part of a PhD thesis, and this study will also be submitted to relevant scientific journals for publication. Your child’s information and data will not be identifiable in either of these instances. You will be sent a summary of the research findings once all data have been collected and analysed, as well as your child’s individual data with a full explanation of what it represents should you so wish.

7. What should I do if my child would like to take part?

If your child would like to take part in the study you must give your permission by completing the following forms which are included in this information pack:

- The parental consent form
- The child assent form (signed by your child)
- The contact details form
- The health screen questionnaire

You should ask your child to return these forms to the school reception. We will then make contact with you about arranging dates for each visit to the laboratory.

Taking part is entirely voluntary and it is up to you to decide whether or not your child is involved. If you do allow your child to take part you are still free to withdraw your child at any time, without giving a reason. If you have any questions regarding the
nature or purpose of this study, please feel free to contact Miss Emma Cockcroft (the primary investigator).

Thank you for your interest. Please read this information carefully and discuss the study with your child, before deciding whether or not to sign the consent form.
Appendix 4: Example participant information sheet

Participant Information Sheet

Project Title: How long do improvements in insulin sensitivity after exercise last in boys?

1. What is the purpose of the study?
This study will look at how different types of exercise can change the way your body deals with a sugary drink both immediately after exercise and the days following it. This is important because when we eat or drink lots of sugar, the amount of sugar in our blood increases. How well our body processes this sugar is related to the development of a disease called diabetes in later life. How long such health benefits after exercise last is important for recommending how often you should exercise to maintain a good level of health. This study aims to understand how long benefits from different types of exercise last, and may help to recommend how often children your age should exercise.

2. What does this study involve?
The study will consist of 10 visits over a four week period. The first visit will last no more than two hours. The following 9 visits will be split over 3 different experimental tests; each including 1 visit to the laboratory in Exeter, and 2 visits to a temporary laboratory that we have set-up at your child’s school.

Visit 1: Introducing you to the laboratory and fitness test
During the first visit we will show you around the laboratory and let you see and have a practice with all the equipment that will be used during the study. We will also take measurements of your standing height, sitting height and estimate your body fatness.
by gently pinching your skin. We will also ask you to assess your level of pubertal
development, by looking at some scientific drawings that show five different stages.
We will ask you to do this in private at home. This is a routine procedure which is
frequently performed within this population for scientific research.

During this visit you will also be asked to complete a fitness test which involves you
cycling on a bike for as long as possible with the pedals progressively becoming harder
to turn. This is just like cycling up a hill that increases will steepness. You will then be
given 15 minutes to recover and then complete a short cycle test which will be slightly
harder than when you finished the first test. During these tests we ask for lots of effort
and it may be hard in the final few minutes. During this you will be wearing a face mask
so we can record the amount of oxygen you are using to undertake the exercise.

Experimental tests:

This aspect will involve three different exercise tests (see below), each of which will
involve 5 consecutive days of data collection, which are outlined below.

Days 1 and 2:
The two days prior to the visit to the laboratory in Exeter (Day 3) we ask that you wear
an accelerometer (wrist worn device which measures how active you are) and record
the food and drink you consume each day. We will provide these during the 1st visit
and will also explain how to complete the food diary.

Day 3:
You will need to arrive at the laboratory by 8 am, so we will collect you from home to
be transported to our research laboratory. We ask that you do not eat or drink (other
than water) from 8 pm the night before the visit. This includes not having breakfast in
the morning. On arrival we will take a small amount of blood from your fingertip to
measure your blood sugar level and also ask you to draw on a scale and fill in short
questionnaire to assess your appetite. The amount of blood is very small (less than 1
mL) and is a routine procedure, which feels like a small pin prick. We will demonstrate
this to you during the first visit. Next, you will perform either; (1) short bouts of high
intensity cycling exercise (8 minutes total); (2) continuous moderate intensity cycling exercise, or (3) rest.

High intensity cycling: This will involve cycling eight 1-minute intervals at 90% of the resistance that you obtained during the exercise test on your first visit. Each interval will be separated by 75 s of very light cycling. The exercise will also include a warm-up and cool down period. You may find this session hard for a short period of time.

Continuous moderate intensity exercise: During this exercise session you will be asked to cycle at a light resistance for approximately 20 to 30 minutes. The exact duration will be calculated to match the amount of work you managed to produce during the high intensity cycling session.

Rest: You will rest in the research laboratory.

After the exercise or rest, you will be asked to fill in two short questionnaires so we can see how much they enjoyed the exercise, and once again look at your appetite. Then you will be asked to drink a small sugary drink within 5 minutes. We will then take a number of small blood samples (less than 1 mL) at 0, 10, 20, 30, 60, 90 and 120 minutes after finishing the drink to measure the amount of sugar in your blood. This procedure is routine and feels like a small pin prick. We will also ask you to wear a face mask so we can measure the amount oxygen and carbon dioxide in the air you breathe in and out, at 60, 120 and 180 minutes after the exercise, This will enable us to estimate how much fat and CHO you are using as fuel. 2 h after exercise you will fill in the same questionnaire as before to assess their appetite.

After the measurements are finished you will be able to have your lunch. Please can you bring along a cold packed lunch. We will then return you to school for your afternoon lessons. During the rest of the day you will be asked to continue recording the food and drink you consume on the food diary, and we will continue to record how active you are using the wrist worn accelerometer.

Day 4:
The next morning, again after an overnight fast from 8 pm, we will meet you in school at 8.00. If you normally walk to school we will arrange to collect you from your home. We will initially estimate how much fat and CHO your body is using as fuel like in the laboratory the previous day. After this you will again fill in the appetite questionnaire and then they will then consume a sugary drink. After the drink we will take a small blood sample from your finger tip at 0, 10, 20, 30, 60, 90 and 120 minutes. Once these measurements are complete, your will be able to eat your lunch, and then return to lessons.

During the rest of the day you will be asked to continue recording the food and drink you consume on the food diary, and we will continue to record how active you are using the wrist worn accelerometer.

Day 5:

The next morning, again after an overnight fast from 8 pm, we will meet you in school at 8.30 am to drink another sugary drink. If you normally walk to school we will arrange to collect you from your home. We will initially estimate how much fat and CHO your body is using as fuel. After this you will fill in the appetite questionnaire. We will then take single blood sample from the fingertip. After this sample we will provide you with breakfast, so please note any dietary requirements on the contact information sheet.

Controlling for activity and diet:

For studies like this, it is very important that we control for how active you are and what you eat over the data collection period. This is because exercise and diet has a direct effect on the measurements we are interested in. We are therefore asking you to wear and accelerometer during your participation in the study, and to record the food they consume each day. After the first exercise experimental test is complete we will return the original food diary records to you and kindly ask if you can replicate this, as close as possible for the other visits.

3. What else will I have to do?
You will need to bring suitable exercise kit for each visit, such as shorts, t-shirt and trainers. Please also bring some schoolwork or a DVD to watch during the observational period. We also have entertainment available in the laboratory, such as a Sony PlayStation 3 and desktop computers with games and educational software. Please feel free to ask any questions you have during the visits. We hope that you visits will be fun but also give you an insight into what goes on in scientific research.

4. What are the possible risks of taking part?

All the procedures used in the study are regularly used in research in children and adolescents. Prior to completing any exercise we ask that you complete the health assessment questionnaire so we can make sure it is safe for you to take part.

5. What will I gain from taking part in this study?

This study aims to further understand the health benefits of different types of exercise in children and adolescents, and will help to identify how to best promote and protect health in children as they develop into adults. We also hope your time here will be fun and educational and may even inspire you for a future career in science.

6. What should I do if I would like to take part?

If after reading this information sheet you would like to take part and your parents/guardian are happy for you to do so please:

- Complete the attached assent form;
- Ask your parent/guardian to complete consent form and fill in the health questionnaire;
- Complete the contact details form;
- Return all forms to the school reception in the envelope provided.
Appendix 5: Example parent information sheet

Parent/Guardian Information Sheet

Project Title: Exercise and insulin sensitivity in boys.

1. What is the purpose of the study?

This study will look at how exercise of different intensities can reduce the amount of sugar in the blood following a sugary drink. This is important because as children grow and develop, their body is less able to handle the sugar in the blood, and this may lead to the development of diseases, such as type 2 diabetes, later in adult life. This research will help to build future guidelines for exercise in children and adolescents, to help maintain and improve their health into adulthood.

2. What does this study involve?

Participation in this study requires your child to visit a research laboratory at the University of Exeter on four occasions. The first visit should last no more than 2 h and the following three visits will last about 4-5 h each. The purpose of each visit is outlined below.

Visit 1: Familiarisation and baseline measurements

The first visit will familiarise your child to the laboratory setting and all the equipment that will be used during the study. During this visit we will take measurements of your child’s standing height and sitting height, and estimate their body fatness by gently measuring a fold of skin at different regions of their body. We will also measure your child’s level of pubertal development, as this can influence how the body processes sugar in the blood. We will ask your child to self-assess their pubertal development at home and in private. This will require your child to look at scientific drawings that show 5 different stages of pubertal development and to identify which stage best describes
their own development. This is a routine procedure which is frequently performed within this population for scientific research.

During this visit your child will be familiarised with the bike that will be used for exercise. They will complete a 10-15 minute exercise test, which involves cycling for as long as possible while the pedals become progressively harder to turn. This is like cycling up a hill that increases with steepness. This test will be followed by a 15 minute rest period and then another very short test (5 minutes) to see whether they can cycle at a higher resistance than that achieved on the first test. These tests are very well tolerated by children but do require a maximal effort. The final moments will therefore be strenuous for your child.

**Visits 2, 3 and 4: Experimental visits**

Your child will need to arrive at the laboratory by 8:00 am and we can provide transport for you. We ask that your child does not eat from 8pm the night before the visit. This includes not having breakfast in the morning. Upon arrival we will take a sample of blood from your child’s fingertip to measure their sugar level. The amount of blood is very small (less than 1 mL) and is a routine procedure, which feels like a small pin prick. Next your child will perform either; (1) short bouts of high intensity cycling exercise; (2) continuous moderate intensity cycling exercise, or (3) rest. Each of these sessions will be separated by 1 week and will be performed in a random order.

1) **High intensity cycling**

This will involve cycling for eight 1-minute intervals at 90% of the resistance that your child obtained during the exercise test on the first visit. Each interval will be separated by 75 s of very light cycling. The exercise will also include a warm-up and cool down period. Your child may find these sessions relatively hard for a short period of time during each bout and will be monitored throughout.

2) **Continuous moderate intensity exercise**
During this exercise session your child will be asked to cycle at a light resistance for approximately 20 to 30 minutes. The exact duration will be calculated to match the amount of work they managed to produce during the high intensity exercise session.

3) Rest
Your child will rest in the research laboratory with a member of the research team.

After the exercise or rest, your child will be asked to drink a small sugary drink within 5 minutes. We will then take blood samples at 0, 10, 20, 30, 60, 90 and 120 minutes after finishing the drink to measure the amount of sugar in their blood. We will also measure breath samples 60, 120 and 180 minutes after finishing the drink, by asking your child to wear a small mask that covers their mouth and nose.

Before each visit, we will ask your child to wear an accelerometer (a small device that looks like a watch) to estimate how active they are before entering the lab. We will also ask them to record all the food and drink they consume during the two days before each visit using a food diary. We kindly ask that the diet is replicated for each visit as close as possible during this period. Finally we ask that your child refrains from intense exercise the day prior to each visit. These requests are to try and minimise the effect that your child’s diet and exercise before each visit can have on the results.

3. What else will my child have to do?
Your child will need to bring suitable kit for the exercise sessions (shorts, t-shirt and trainers), and some schoolwork or a DVD to watch during the rest and observational period. We have a Sony PlayStation 3 and desktop computers with age-appropriate games and educational software which your child may play on during the rest period. We would also encourage your child to ask as many questions as they please. We hope that their visit to the laboratory inspires them to think about their health and the potential of higher education.
4. **What are the possible risks of my child taking part?**

All the procedures used in the study are regularly used in research in children and adolescents. Prior to completing any exercise an assessment of their health will be made to ensure it is safe for them to take part.

5. **What will my child gain from taking part in this study?**

This study aims to further understand the health benefits of different types of exercise in children and adolescents. Whilst this may not immediately benefit your child, we hope that your child will enjoy their time in the University laboratories and the chance to be part of a scientific study. At the Children’s Health and Exercise Research Centre, we pride ourselves on ensuring that each volunteer has an enjoyable and informative experience throughout every research project. We hope that we can inspire your child to take an interest in their health and in the science of exercise, and that this project will be both interesting and fun.

6. **What will happen to the results of the study?**

Your child’s data will be stored in coded form to protect anonymity and will be completely confidential. This research will form part of a PhD thesis, and this study will also be submitted to relevant scientific journals for publication. Your child’s information and data will not be identifiable in either of these instances. You will be sent a summary of the research findings once all data have been collected and analysed, as well as your child’s individual data with a full explanation of what it represents should you so wish.

7. **What should I do if my child would like to take part?**

If your child would like to take part in the study please fill in the forms provided and return to myself or to someone at the sports centre. I can then contact you and arrange visits.
Taking part is entirely voluntary and it is up to you to decide whether or not your child is involved. If you do allow your child to take part you are still free to withdraw your child at any time, without giving a reason. If you have any questions regarding the nature or purpose of this study, please feel free to contact Miss Emma Cockcroft (the primary investigator).

Thank you for your interest. Please read this information carefully and discuss...
Appendix 6: Example participant assent form

Participant Assent Form (to be completed by the child)

Project title: How long do improvements in insulin sensitivity after exercise last in boys?

Name…………………………………………………………………

I agree to take part in the study as described in the information sheet. The study has been clearly explained to me.

I understand that:

- I will have my height, seated height, weight and body fat measured.
- I will need to assess my pubertal status using scientific drawings. The purpose of this has been made clear to me.
- I will complete a cycle test to exhaustion on my first visit.
- I will participate in a further three exercise trials. Each including one day in the laboratory and two mornings in the school.
- I will have to drink a sugary drink after completing the exercise trial and on the following morning in school.
- Samples of blood will be taken from my fingertip before and after drinking a sugary drink.
- I will be asked to breath into a mask to sample my breath.
- I will be asked to fast the night before test days 3, 4 and 5 for each of the three exercise conditions.
• I will be asked to record dietary information for the 5 days of each exercise condition.
• I will be asked to wear an accelerometer to measure physical activity for the 5 days of each exercise condition.
• I am free to ask any questions at any time.

I know that:
• I can withdraw from the study at any point with no questions asked.

Signed

Date
Appendix 7: Example contact detail form

Contact details

Project title: Exercise and insulin sensitivity in boys.

Child’s name: ________________________________

Parent / guardian name: _________________________

Address: ______________________________________

_________________________________________________________________

_________________________________________________________________

Post code: _______________________________________

Home telephone number: __________________________

Mobile telephone no: ___________________________

Best time to contact you: _______________________

Appendix 8: Maturation assessment form

Maturation Assessment Form

Participant code …………………………

The assessment of your state of puberty is essential for the correct interpretation of your health and exercise performance. All children do not start puberty at the same time and some will progress through puberty more quickly than others. Consequently, two children aged 13 y olds may be the same age, however it is likely that one will be more mature than the other. We therefore require an estimate of your maturation in order to control for the different stages of pubertal development on the outcomes that we will measure. If you have any questions about this assessment, please contact a member of the research team.

Instructions:

On the next page are five drawings showing the changes in pubic hair as a child develops into an adult. Please take your time and read carefully the writing explaining each of the five different stages in pubic hair development. Once you have decided the stage which best describes your present level, please indicate this below by circling the relevant number. Once you have finished, please sign and date this form at the bottom and return it to a member of the research team on your next visit.

ALL INFORMATION WILL BE HANDLED WITH THE STRICTEST OF CONFIDENCE AND ONLY VIEWED BY THE RESEARCH TEAM

Stage: 1 2 3 4 5

Participant signature…………………………………………

Date………………………………..
The drawings on this page show different amounts of male pubic hair. A boy passes through each of the five stages shown by these drawings. Please look at each drawing and read the sentences under the drawing. Then choose the drawing closest to your stage of your hair development. Mark a 1 on the line above that drawing. Then choose the drawing that is next closest to your stage of hair development and mark it a 2. In choosing the right picture, look only at the pubic hair, and not at the size of the testes, scrotum, and penis.

1. Drawing A
   - There is no pubic hair at all.

2. Drawing B
   - There is a little soft, long, lightly colored hair. Most of the hair is at the base of the penis. This hair may be straight or a little curly.

3. Drawing C
   - The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a somewhat larger area.

4. Drawing D
   - The hair is now as dark, curly, and coarse as that of an adult male. However, the area that the hair covers is not as large as that of an adult male. The hair has not spread out to the thighs.

5. Drawing E
   - The hair has spread out to the thighs. The hair is now like that of an adult male. It covers the same area as that of an adult male.
FOOD DIARY

Exercise in children with type 1 diabetes

Name…………………………………………………………………………………………

Diary start
date……………………………………………………………………………………

Diary end
date……………………………………………………………………………………
How to fill in your food diary:

1. Please complete the diary like the example on the following page.

2. Please record all food and drink that is actually eaten or drunk, rather than what is served to you.

4. Remember to include all snacks including drinks, sweets, crisps and biscuits.

5. Please also note down the time of the day you ate each meal / snack.

6. If possible, please state the weight of food eg, 1 bag crisps (30g). If this is not possible, please give quantities in household measures e.g. 3 heaped tbsp cornflakes, 2 level tsp sugar, 1 large thin slice white bread.

7. State cooking method e.g fried, grilled, boiled.

8. Please also include when administered insulin and blood sugar levels at points in the day when you would routinely check.

9. There is also space for you to note down any exercise you did, please include the type of exercise, how hard it was, and the time of day it was done.
<table>
<thead>
<tr>
<th>Date………</th>
<th>Time</th>
<th>Amount and type of food and drink</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>8.00am</td>
<td>3 heaped tablespoons Cornflakes&lt;br&gt;100ml semi-skimmed milk,&lt;br&gt;½ slice large wholemeal bread with&lt;br&gt;margarine,&lt;br&gt;1 level teaspoon jam.</td>
</tr>
<tr>
<td>Mid-morning</td>
<td>10.30am</td>
<td>1 packet (28g) crisps,&lt;br&gt;1 can (330ml) diet coke.</td>
</tr>
<tr>
<td>Lunch</td>
<td>1.00pm</td>
<td>85g chicken&lt;br&gt;2 scoops mashed potato with milk and&lt;br&gt;butler,&lt;br&gt;1 heaped tablespoon peas,&lt;br&gt;1 tablespoon gravy,&lt;br&gt;300ml water.</td>
</tr>
<tr>
<td>Mid-afternoon</td>
<td>3.00pm</td>
<td>2 Bourbon biscuits,&lt;br&gt;250ml orange juice.</td>
</tr>
<tr>
<td>Evening meal</td>
<td>6.30pm</td>
<td>2 fish fingers,&lt;br&gt;10 chips,&lt;br&gt;1 large slice thick white bread with margarine,&lt;br&gt;1 apple.</td>
</tr>
<tr>
<td>Evening snack</td>
<td>8.00pm</td>
<td>1 glass semi skimmed milk,&lt;br&gt;2 digestive biscuits.</td>
</tr>
<tr>
<td>Time</td>
<td>Insulin</td>
<td>Blood sugar</td>
</tr>
<tr>
<td>------</td>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Amount and type of food and drink</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-morning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-afternoon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening meal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening snack</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 10: Example PACES questionnaire

PACES

Participant:

When I completed the high intensity, interval exercise:

. . . . . Agree a lot

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I enjoyed it</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>I felt bored</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>I disliked it</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>I found it pleasurable</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>It wasn't any fun at all</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>It gave me energy</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>It made me depressed</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>It was very pleasant</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>My body felt good</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>I got something out of it</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>It was very exciting</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>It frustrated me</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>It wasn't at all interesting</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>It gave me a strong feeling of success</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>It felt good</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>I felt as though I would rather be doing something else</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
Appendix 11: Children’s effort rating table

The Pictorial Children’s effort rating table (PCERT)