METABOLIC FLEXIBILITY IN CHILDREN WITH OBESITY

METABOLIC FLEXIBILITY IN CHILDREN WITH OBESITY: EXERCISE AS A DYNAMIC STIMULUS TO ASSESS METABOLIC HEALTH

by

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TITLE: **Metabolic Flexibility in Children with Obesity: Exercise as a Dynamic Stimulus to Assess Metabolic Health**

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ABSTRACT

The research in this thesis was completed to advance our knowledge about metabolic flexibility under exercise conditions in children with obesity. The primary objectives of the studies were to determine the validity and reliability of a novel metabolic flexibility test to screen for risk factors associated with type 2 diabetes, including impaired glucose tolerance, dysglycemia, and insulin resistance (Chapter 3 and Chapter 4), and to investigate the effect of 7 days of exercise training on insulin resistance and metabolic flexibility in children with obesity (Chapter 5). Compared to children with normal glucose tolerance, children identified with impaired glucose tolerance did not have reduced metabolic flexibility, which was measured by calculating exogenous carbohydrate oxidative efficiency during exercise (Chapter 3). Despite greater insulin resistance in the children with impaired glucose tolerance, metabolic flexibility was not significantly different between groups. Bivariate analyses showed that metabolic flexibility was not associated with fasting glucose, insulin resistance or beta-cell function in the entire group of participants (Chapter 4). However, when the children were separated by sex, metabolic flexibility was inversely associated with insulin resistance and directly associated with whole body insulin sensitivity index and beta-cell function in boys but not in girls (Chapter 4). The metabolic flexibility test demonstrated good reliability based on an intraclass correlation coefficient of 0.692 (Chapter 4). After 7 days of exercise training in a group of children with obesity, insulin resistance and metabolic flexibility did not improve

as hypothesized (Chapter 5). An unexpected observation was the large inter-individual variability in these responses in the early stages of exercise therapy. Altogether, the studies presented in this thesis were the first to examine metabolic flexibility under exercise conditions at baseline and in response to exercise training in children. The studies were also the first to investigate the relationship of metabolic flexibility with fasting glucose and insulin resistance in children, and to assess the reliability of test results when repeating the non-invasive metabolic flexibility test on a separate day. By increasing our general knowledge of metabolic flexibility testing in children, we can better evaluate the clinical utility of the test to screen for risk factors associated with type 2 diabetes in the future.

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‰ [δ^{13}C]PDB-1	per mille versus delta 13-Carbon Pee Dee Belemnitella-1
^{13}C	Carbon 13
2-h [GLUC]	2-hour glucose concentration
A1C	glycated hemoglobin
ADA	American Diabetes Association
Akt	akt/protein kinase b
AMP	adenosine monophosphate
AMPK	adenosine monophosphate-activated protein kinase
aPKC	atypical protein kinase c
AS160	akt substrate of 160 kda
ATP	adenosine triphosphate
AUC CHO_{exo}	area under the curve exogenous carbohydrate oxidation
AUCgluc	area under the curve glucose
AUCins	area under the curve insulin
BMI	body mass index
BW	body weight
Ca^{2+}	calcium
CaMKII	calcium/calmodulin-dependent protein kinase 2
CDA	Canadian Diabetes Association
CDC	Centers for Disease Control and Prevention
CHO	carbohydrate
CHO_{endo}	endogenous carbohydrate
CHO_{exo}	exogenous carbohydrate
CHO_{tot}	total carbohydrate
CO_2	carbon dioxide
CVD	cardiovascular disease
EE	energy expenditure
FAT_{tot}	total fat
FFA	free fatty acids

FFM	fat free mass
FPG	fasting plasma glucose
GLUT-4	glucose transporter protein isoform-4
HOMA-IR	homeostasis model assessment of insulin resistance
HR	heart rate
HR_{max}	maximal heart rate
ICC	intraclass correlation coefficient
IFG	impaired fasting glycemia
IFG+IGT	impaired fasting glycemia + impaired glucose tolerance
IGF-1	insulin-like growth factor-1
IGT	impaired glucose tolerance
IR	insulin resistance
IRS-1	insulin receptor substrate-1
ISSI-2	insulin secretion-sensitivity index-2
MetFlex	metabolic flexibility
Na^+/K^+ ATPase	sodium-potassium adenosine triphosphatase
NGT	normal glucose tolerance
OGTT	oral glucose tolerance test
PDH	pyruvate dehydrogenase
PHV	peak height velocity
PI3-kinase	phosphatidylinositol-3 kinase activity
POST	after exercise training
PRE	before exercise training
SGLT1	sodium-glucose transporter 1
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TCA cycle	tricarboxylic acid cycle
$\dot{V}CO_2$	carbon dioxide production
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_{2max}$	maximal oxygen uptake
WBISI	whole body insulin sensitivity index

FORMAT AND ORGANIZATION OF THIS THESIS

This thesis was prepared in the “sandwich thesis” format outlined in the McMaster University School of Graduate Studies Guide for the Preparation of Master’s and Doctoral Theses, published in November 2014. Chapter 1 includes an introduction and literature review, which sets the context for the body of scientific research. Chapter 2 is a brief description of important study design and methodological details. Chapters 3, 4, and 5 consist of three original research papers that are published or under peer-review. These chapters are formatted in accordance with the requirements of the journal in which they were published or submitted for publication. A concluding chapter (Chapter 6) summarizes and discusses the main findings of the thesis, challenges and limitations, and future research directions. Appendices are included at the end of the document to supplement Chapters 2 and 6.

CONTRIBUTIONS TO MULTI-AUTHORED PAPERS

CHAPTER 3

Publication: Chu L, Morrison KM, Riddell MC, Raha S, Timmons BW. No difference in exogenous carbohydrate oxidation during exercise in children with and without impaired glucose tolerance. *J Appl Physiol* 121: 724 –729, 2016.

Contributions: The data collection, experiments and statistical analyses were completed by L. Chu. B.W. Timmons, K.M. Morrison, and M.C. Riddell contributed to the conception and design of the research study and obtained funding. B.W. Timmons, K.M. Morrison, and S. Raha supervised the study. The initial draft of the manuscript was written by L. Chu. All authors interpreted results of the experiments, critically reviewed and edited the manuscript, and approved the final version of the manuscript. The primary supervisor for the study was B.W. Timmons.

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CHAPTER 1

1 INTRODUCTION

The rise in childhood obesity over the past few decades has led to an increased number of children being diagnosed with type 2 diabetes mellitus (T2DM), a disease once diagnosed primarily in adults. The progression from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) to T2DM ensues gradually over time in adults, but appears to occur more rapidly in children with obesity (Weiss & Caprio, 2005). This emphasizes the need for earlier screening, diagnostic and intervention strategies. Current clinical tests for diagnosing T2DM include fasting blood work and/or an oral glucose tolerance test (OGTT), which may be quite invasive for children. Therefore, an innovative, non-invasive test that may provide an alternative method for screening for risk factors associated with T2DM, such as IGT, dysglycemia and insulin resistance (IR), would be beneficial for improving clinical care.

In children with obesity, several key metabolic consequences are already present, including IR, a common precursor in the progression from NGT to IGT to developing T2DM. Evidence in adults (Corpeleijn, Saris, & Blaak, 2009; Galgani, Moro, & Ravussin, 2008) suggests the development of IR is closely linked to impaired metabolic flexibility (MetFlex). MetFlex refers to the capacity of the system to match substrate utilization with substrate availability. To date, very little is known about the relationship between MetFlex and IR in children with obesity.

Past studies have examined MetFlex using a hyperinsulinemic-euglycemic clamp in combination with indirect calorimetry under resting conditions (Apostolopoulou et al., 2016; Galgani, Heilbronn, et al., 2008). However, assessing MetFlex under exercise conditions is arguably more appropriate because of the greater stress on the system to coordinate fuel supply and oxidative machinery when exercise is used as a dynamic stimulus to increase energy demand (Galgani, Moro, et al., 2008).

The research studies in this thesis used an innovative methodology to study MetFlex under exercise conditions by measuring carbohydrate (CHO) oxidation of an orally-ingested CHO drink enriched with Carbon 13 (^{13}C) stable isotopes. We first examined MetFlex during exercise in children with obesity and IGT compared to children with obesity and NGT (Chapter 3). Next, we examined the validity and reliability of the MetFlex test in children with obesity, and its potential to be used as a clinical tool to screen for risk factors associated with T2DM (Chapter 4). Validity was assessed by examining the association of MetFlex during exercise with glycemia and IR. Reliability was assessed by repeating the MetFlex test on a separate day in the same participants and determining the intraclass correlation coefficient (ICC). In the final study, the effect of 7 consecutive days of exercise on MetFlex and IR was investigated (Chapter 5). Our overall aim was to obtain a better understanding of MetFlex in children and evaluate the clinical utility of MetFlex testing to be used for children with an increased risk of developing T2DM. In the future, the test may help

identify why some children are at a greater risk for T2DM compared to others, and elucidate the benefits of exercise therapy.

1.1 **Childhood obesity**

1.1.1 *Prevalence, metabolic consequences and co-morbidities*

The prevalence of childhood overweight and obesity has increased in Canada and other countries worldwide over the past several decades. According to the 2007 World Health Organization growth references, approximately 34.7% of Canadian children were overweight or obese in 2004. This is 11.5% greater than the estimated Canadian prevalence in 1978/79 (Shields & Tremblay, 2010). The global prevalence of overweight and obesity in pre-school aged children is also increasing. In 2010, 43 million children under the age of 5 years were classified as overweight or obese, including 35 million children living in developing countries. The trend is predicted to increase to approximately 60 million in 2020, which translates to an increase from 6.7% in 2010 to 9.1% in 2020 (De Onis M, Blössner, & Borghi, 2010). These data provide great concern for an increased risk of developing metabolic complications in childhood, leading to earlier morbidity and mortality.

Metabolic disturbances that contribute to health complications later in life may already be present in many children with obesity. The metabolic syndrome consists of a cluster of conditions, including high blood pressure, central adiposity, dyslipidemia and/or hyperglycemia, which increase an individual's risk

for cardiovascular disease (CVD) (Alberti & Zimmet, 1998). Adapting adult criteria for defining the metabolic syndrome, Weiss and colleagues (2004) found a strong association between degree of obesity and the metabolic syndrome in children, after adjustment for race and gender. Similarly, an elevated body mass index (BMI) in childhood is associated with an increased risk of coronary heart disease (Baker, Olsen, & Sørensen, 2007; Tirosh et al., 2011). Another consequence of the rise in childhood obesity is its influence on increasing rates of T2DM early in life. T2DM, once thought of as an adult disease, is becoming more prevalent in children, especially within certain ethnic populations (Amed et al., 2010; American Diabetes Association, 2000; Fagot-Campagna et al., 2000). From a population of 7.3 million children in Canada <18 years old, the minimum incidence of T2DM was 1.54 per 100,000 children per year, which included 44.1% of children with Aboriginal heritage, 25% Caucasian, 10.1% African or Caribbean and 10.1% Asian (Amed et al., 2010). The remaining percentage was accounted for by Hispanic, Middle Eastern or mixed ethnicity (Amed et al., 2010). However, regardless of ethnicity, Sihna et al. (2002) reported children with severe obesity had a high prevalence of IGT, which is a strong risk factor for the development of T2DM (Santaguida et al., 2005; Sinha et al., 2002). Consequently, complications associated with obesity form a metabolic profile that contributes to an increased risk for T2DM. The primary focus of this thesis is on risk factors associated with developing T2DM, though it is evident that obesity is associated with a plethora of co-morbidities, even at a young age.

1.2 **Type 2 diabetes mellitus in children**

1.2.1 *Clinical diagnosis*

Diabetes is clinically diagnosed using plasma glucose cut-points. These cut-points include values for fasting plasma glucose (FPG) and 2-h glucose during an OGTT to identify diabetes (American Diabetes Association, 2014). In 2009, the International Expert Committee recommended adding glycated hemoglobin (A1C) as one of the criteria to diagnose diabetes in adults (International Expert Committee, 2009). In a patient with classic symptoms of hyperglycemia or hyperglycemia crisis, a random plasma glucose measurement can also be collected for diagnosis (American Diabetes Association, 2014; Goldenberg & Punthakee, 2013). Criteria for the diagnosis of diabetes supported by the Canadian Diabetes Association (CDA) are summarized in **Table 1.1**. The current criteria used for diagnosing diabetes in children are the same as those for adults. One exception is the use of A1C, which is not recommended as a method to diagnose T2DM in children by CDA (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Panagiotopoulos, Riddell, & Sellers, 2013; Goldenberg & Punthakee, 2013).

Early identification of prediabetes is critical because it is linked with an elevated risk for developing T2DM and other health complications such as microvascular disease, stroke and CVD (Santaguida et al., 2005). The same diagnostic tests for diabetes, but with lower cut-points, are used to diagnose prediabetes. The term prediabetes refers to patients with impaired fasting

glycemia (IFG), IGT or both (IFG+IGT) (Goldenberg & Punthakee, 2013). Similar to diabetes, criteria for diagnosing prediabetes are the same in children and adults. At the present time, there is no worldwide consensus on IFG cut-points. CDA defines IFG with a FPG of 6.1 to 6.9 mmol/l, and the American Diabetes Association (ADA) uses a slightly lower cut-point of 5.6 mmol/l to 6.9 mmol/l (American Diabetes Association, 2016a). IGT is defined as a 2-h glucose of 7.8 mmol/l to 11.0 mmol/l during an OGTT by both CDA and ADA (American Diabetes Association, 2016a; Goldenberg & Punthakee, 2013). The interpretation of A1C levels in children for identifying prediabetes remains unclear.

Table 1.1 Criteria for diagnosing diabetes

FPG ≥ 7.0 mmol/L (no caloric intake for at least 8 hours)
or
A1C ≥ 6.5% (adults only, not applicable in children)
or
2-h plasma glucose ≥ 11.1 mmol/L (measured during 75g OGTT)
or
Random plasma glucose ≥ 11.1 mmol/L (taken anytime during the day)

In the absence of asymptomatic hyperglycemia, if one of these laboratory test results are in the diabetes range, a repeat confirmatory laboratory test is required on another day. In the case of symptomatic hyperglycemia, the diagnosis has been made and a confirmatory laboratory test is not required before treatment is initiated. Abbreviations: FPG, fasting plasma glucose; A1C, glycated hemoglobin; OGTT, oral glucose tolerance test. Adapted from Goldenberg and Punthakee (2013).

1.2.2 *Risk factors*

Risk factors for T2DM in children include obesity (Amed et al., 2010), prediabetes, family history, high-risk ethnic populations (i.e., Indigenous groups in Canada, the United States and Australia) (Nadeau & Dabelea, 2008), puberty, maternal diabetes during pregnancy (Dabelea et al., 2008) and other conditions associated with IR, such as polycystic ovarian syndrome (Arslanian, 2000). A common constituent of some of these risk factors for T2DM is their association with IR. In particular, puberty is an important risk factor that warrants discussion in this thesis because of our child population. At the onset of puberty, there is a transient increase in IR, which occurs in healthy, typically developing children (Caprio et al., 1989; Moran et al., 1999). This is usually accompanied by hyperinsulinemia to compensate for the increased IR (Caprio et al., 1989). In children with obesity, there can be a greater stress on the beta-cells to secrete sufficient insulin to maintain euglycemia because of the pre-existing IR before puberty (Pilia et al., 2009). If beta-cell failure occurs, IGT and/or elevated post-prandial glucose levels may ensue, placing the individual at a greater risk of developing T2DM.

1.2.3 *Pathophysiology*

The pathophysiology of T2DM is closely linked with disrupted glucose homeostasis, which relies critically on the coordination between insulin secretion and insulin action (i.e., insulin-mediated peripheral glucose uptake). Disruption of

this balance contributes to glucose dysregulation and T2DM. The transition from NGT to IGT is commonly associated with increasing IR. However, fasting hyperglycemia does not occur solely because of increasing IR. The beta-cells of the pancreas are responsible for secreting adequate insulin to maintain euglycemia in the presence of IR; when beta-cell failure occurs, hyperglycemia persists. This may lead to a transition from a state of euglycemia with hyperinsulinemia to a state of IGT with elevated postprandial glucose, and possibly to the development of T2DM and overt hyperglycemia (Arslanian, 2000). The common pathophysiological state of fasting hyperglycemia in T2DM is characterized by decreased peripheral glucose uptake, decreased beta-cell function, and decreased suppression of hepatic glucose production (Arslanian, 2000; DeFronzo, 1988). **Figure 1.1** summarizes the pathophysiology of T2DM in children.

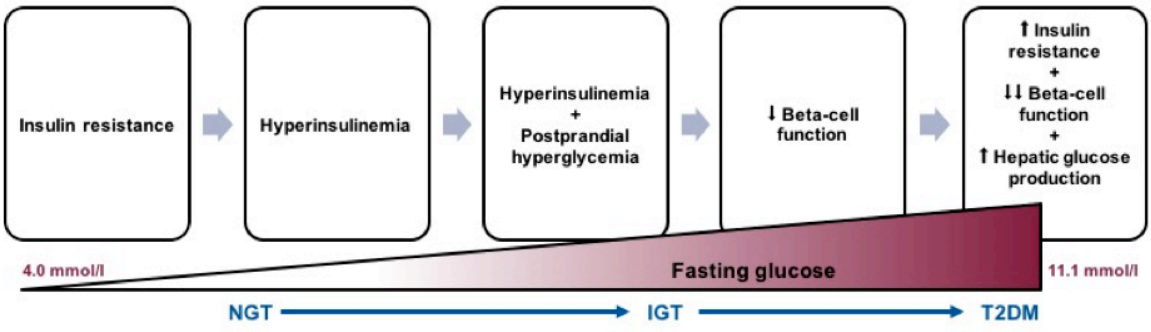

Figure 1.1 Pathophysiology and general progression to type 2 diabetes mellitus (T2DM) in children. Abbreviations: NGT, normal glucose tolerance test; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus. Adapted and modified from Arslanian et al. (2000).

Beta-cell failure can sometimes occur before hyperglycemia is detected. Evidence of this was reported in children who showed signs of impaired beta-cell function but did not have elevated FPG based on criteria for diabetes diagnosis (Burns et al., 2011; Tfayli, Lee, & Arslanian, 2010). Giannini et al. (2012) reported that children with NGT in their study already presented with early IR and defects in insulin secretion. The reduced beta-cell function relative to IR was found in the children with NGT who fell within the higher 2-h glucose range during an OGTT (Giannini et al., 2012). Since disturbances to metabolic health can occur silently before the presence of overt hyperglycemia, children with numerous risk factors should have routine follow-up appointments to be tested for T2DM. One recommendation is to conduct regular screening and diagnosis for diabetes in a clinical setting alongside treatment of other obesity-related comorbidities in children (Zeitler et al., 2014). This could be advantageous for monitoring the development of T2DM, because co-morbidities such as non-alcoholic fatty liver disease, dyslipidemia, and hypertension tend to be more prevalent and occur before the detection of glucose dysregulation (Zeitler et al., 2014).

1.2.4 *Overview of diagnostic tests in children*

The main diagnostic tests used for T2DM in children include measuring FPG and 2-h glucose during an OGTT. FPG compared to 2-h glucose during an OGTT is a more convenient, less expensive and less invasive test for diagnosing diabetes. Measuring FPG is also more reproducible compared to 2-h glucose

during an OGTT (Libman, Barinas-Mitchell, Bartucci, Robertson, & Arslanian, 2008). On the other hand, there is a higher detection rate with a 2-h plasma glucose during an OGTT. It allows for the early recognition of prediabetes when IFG may not be present, and for the diagnosis of silent T2DM in children with severe obesity (Sinha et al., 2002). Shaw et al. (2000) found that over 30% of individuals who are diagnosed with diabetes using 2-h glucose during an OGTT have a FPG that is not diagnostic for diabetes.

There are several limitations surrounding the clinical use of the same diagnostic criteria for diagnosing T2DM in children and adults. The cut-points for diagnosing diabetes are based on glycemic thresholds that predict the development of retinopathy in adults (Engelgau et al., 1997; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003; McCance et al., 1994). There is insufficient evidence-based research to support the use of these cut-points in children (Alberti et al., 2004; Hannon & Arslanian, 2015; Kapadia, Zeitler, & Drugs and Therapeutics Committee of the Pediatric Endocrine Society, 2012). Hence, the validity and reliability of measuring 2-h glucose during an OGTT in children requires further investigation, especially because Libman et al. (2008) reported poor reproducibility of the measurement in children with obesity. Data are also lacking to support the recommended glucose load of 1.75 g/kg (maximum dose, 75 g) used for OGTTs in children (Alberti et al., 2004).

Another important measurement to discuss is A1C. There are some discrepancies related to the use of A1C as a diagnostic test. Similar to FPG and

OGTT cut-points, epidemiological studies performed exclusively in adults support the use of A1C cut-points in the diagnosis of prediabetes and diabetes (Buell, Kermah, & Davidson, 2007; Rohlfing et al., 2000). The validity of using A1C as a diagnostic test for diabetes in children is not well supported (Lee, Wu, Tarini, Herman, & Yoon, 2011; Nowicka et al., 2011) or recommended by CDA (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee et al., 2013; Goldenberg & Punthakee, 2013). One of the benefits of measuring A1C for patients is that it does not require fasting overnight. However, if A1C levels are within the diabetes range in adults, the results still require a confirmatory laboratory test on a different day completed within a timely window (Goldenberg & Punthakee, 2013).

Since there is a gap in evidence-based support for applying adult cut-points for diagnosing diabetes to children, it would be valuable to investigate the use of the cut-points in children with more scrutiny, as well as to investigate alternative screening tools to supplement current clinical tests. The chapters in this thesis focus on the latter. The screening test proposed and studied in this thesis was developed based on principles of MetFlex and its association with IR in adults, but tested in children with obesity. Background information and rationale for MetFlex testing is explained in more detail in the following section.

1.3 **Metabolic flexibility, insulin resistance and type 2 diabetes mellitus**

1.3.1 *What is metabolic flexibility?*

MetFlex is characterized by the ability to effectively switch between substrates, such as switching from the oxidation of predominantly lipids under fasting conditions to the oxidation of predominantly CHO under insulin-stimulated conditions (Corpeleijn et al., 2009; Galgani, Moro, et al., 2008; Kelley, Goodpaster, Wing, & Simoneau, 1999). When MetFlex is impaired, as it is found in adults with obesity and IR, there is a blunted response in switching from lipid oxidation to CHO oxidation, and vice versa (Corpeleijn et al., 2009; Galgani, Moro, et al., 2008; Kelley et al., 1999). More specifically, adults with obesity and IR have lower lipid oxidation under a fasting condition compared to healthy adults. During insulin stimulation, lipid oxidation and CHO oxidation do not decrease and increase, respectively, to the same degree in adults with obesity compared to healthy adults (Corpeleijn et al., 2009; Galgani, Moro, et al., 2008; Kelley et al., 1999).

A key component of MetFlex is the capacity to match substrate oxidation with substrate availability (Galgani, Moro, et al., 2008). Past studies have examined both MetFlex to increased lipid availability and MetFlex to increased CHO availability. In a study with adults, impaired MetFlex was associated with an impaired capacity to increase fat oxidation when there was increased fatty acid availability under fasting conditions (Corpeleijn et al., 2009). Because very few studies exist, Galgani and colleagues (2008) recommended that research

examining MetFlex to high-fat diets would be beneficial to understand how lipid accumulation contributes to IR and impaired MetFlex. Therefore, MetFlex to lipid availability may be an area of future research that would be very relevant for elucidating how MetFlex contributes to metabolic complications and the development of T2DM.

To date, most studies have examined MetFlex to CHO. In studies that showed lower CHO oxidation during insulin stimulation in adults with IR compared to healthy adults, findings suggested that the difference was likely due to decreased muscle glucose disposal (Kelley et al., 1999; Kelley & Mandarino, 2000). Impaired MetFlex to CHO is also found in adults with T2DM, and primarily associated with insulin-stimulated glucose disposal rate (Apostolopoulou et al., 2016; Galgani, Heilbronn, et al., 2008). In summary, existing evidence, at least in adults, support an association between impaired MetFlex and IR, which appears to be closely linked with insufficient muscle glucose transport. Therefore, the development of a MetFlex test that is non-invasive would be ideal for children, and may provide information about IR and peripheral glucose disposal. For this reason, the studies in this thesis focused on studying MetFlex to CHO in children with obesity. The following paragraphs will outline what is already known about MetFlex research in children and describe existing MetFlex assessments.

1.3.2 *Overview of metabolic flexibility in children*

Similar to many other areas of scientific research, fewer studies have examined MetFlex in children compared to in adults, and our knowledge is limited due to ethical restrictions and complexities associated with growth and development. In general, children with obesity, IR or IGT have poor glucose disposal. Weiss et al. (2003) showed that peripheral glucose disposal and glucose oxidation during a hyperinsulinemic-euglycemic clamp was lower in children with obesity and IGT compared to children with obesity and NGT. Similarly, Robinson and colleagues (1998) found lower glucose disposal during insulin stimulation in children with obesity compared to lean children and lean adults. Lipolytic regulation and the suppression of fat oxidation with insulin stimulation was also lower in the children with obesity compared to the other groups (Robinson et al., 1998). Based on these studies, IR and IGT in children seems to disrupt normal glucose disposal and substrate metabolism, implying that, much like adults, MetFlex could be impaired in children with obesity and IR.

Studies examining the effects of puberty and substrate metabolism provide insight into the association between MetFlex and IR in children. At the onset of puberty, there is a transient increase in IR (Caprio et al., 1989; Moran et al., 1999), which in turn affects substrate availability and substrate oxidation. Arslanian and Kalhan (1994) showed that the capacity to suppress fat oxidation during high rate insulin stimulation was impaired in pubertal children compared to pre-pubertal children and adults. According to the Randle cycle, elevated fat

oxidation rates may be responsible for decreasing CHO oxidation and insulin-stimulated glucose disposal (Randle, Garland, Hales, & Newsholme, 1963). In support of this, several studies have shown that insulin-stimulated glucose disposal is lower in pubertal children than pre-pubertal children (Amiel, Sherwin, Simonson, Lauritano, & Tamborlane, 1986; Arslanian & Kalhan, 1994; Bloch, Clemons, & Sperling, 1987). Overall, the available literature in children suggests that under resting conditions the normal response of glucose to insulin stimulation is impaired in conditions where IR is present, such as with obesity and puberty. This is indicative of impaired MetFlex because lower glucose disposal contributes to lower glucose availability and oxidation for energy.

1.3.3 *Assessment of metabolic flexibility*

MetFlex has most commonly been assessed in the past using a hyperinsulinemic-euglycemic clamp combined with indirect calorimetry (Apostolopoulou et al., 2016; Galgani, Heilbronn, et al., 2008). However, the assessment is not ideal for clinical care, especially for children, because it is invasive, time consuming and requires specialized medical training. Conducting a ^{13}C-enriched CHO breath test could provide an alternative method to non-invasively examine IR (Jetha, Nzekwu, Lewanczuk, & Ball, 2009; Lewanczuk, Paty, & Toth, 2004; Mizrahi, Lalazar, Adar, Raz, & Ilan, 2010). Breath tests are not uncommon in clinical care and have been used in patients for other diagnostic purposes. For example, the ^{13}C urea breath test is currently used in

clinical care for *Helicobacter pylori* (Lewanczuk et al., 2004). The ^{13}C urea breath test is similar to the MetFlex test that is proposed in this thesis. Main differences between the tests are the type of stable isotope used, (i.e., ^{13}C-enriched CHO vs. urea) and the environmental condition (i.e., conducting the protocol under an exercise condition vs. under a resting condition).

Researchers have recommended exercise as a more appropriate condition to assess MetFlex than at rest (Galgani, Moro, et al., 2008). Measuring MetFlex at rest is not as suitable as measuring MetFlex during exercise because of the lower demand for energy utilization at the level of the muscle. When exercise is used as a dynamic stimulus, it places a greater stress on the system to adequately supply fuel to the muscles and use oxidative machinery. We hypothesized that the clinical utility (i.e., validity) and assessment of MetFlex would be improved by studying ^{13}C-enriched CHO oxidation during exercise. The technique required for this innovative approach of measuring MetFlex during exercise has been used in our laboratory for exercise metabolism studies, in which the amount of exogenous CHO (CHO_{exo}) oxidized for energy was studied in children (Chu, Riddell, Takken, & Timmons, 2011; Timmons, Bar-Or, & Riddell, 2003, 2007a). With this technique, MetFlex is assessed by calculating the CHO_{exo} oxidative efficiency (total amount of CHO ingested before exercise/total amount of CHO_{exo} oxidized during exercise x 100). A higher CHO_{exo} oxidative efficiency indicates better MetFlex, because of the greater capacity to match CHO oxidation during exercise with CHO availability during exercise.

1.4 **Carbohydrate digestion, metabolism and storage**

1.4.1 *Metabolic fate of ingested carbohydrate*

The pathway of ingested CHO is reviewed here to provide a general overview and understanding of how ^{13}C-enriched CHO is used for metabolic research. The three major classes of CHO are monosaccharides, oligosaccharides, and polysaccharides. The most abundant, naturally occurring monosaccharide is 6-carbon glucose. Other monosaccharides include sucrose, lactose, maltose, and fructose. The focus here will be on the absorption, transport, and metabolism or storage of glucose, because the studies presented in this thesis use drinks containing universally labeled ^{13}C-enriched D-glucose.

Absorption of glucose occurs in the wall of the small intestine through the mucosal cells via active transport. The process requires energy and a receptor. The carrier for glucose and galactose is known as sodium-glucose transporter 1 (SGLT1), and is dependent on the sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) pump. The carrier requires Na^+ to be preloaded before glucose or galactose can attach. Using adenosine triphosphate (ATP), glucose is then transported through the mucosal cell (Gropper, Smith, & Groff, 2004). Glucose exits the mucosal cell at the basolateral surface by leaking back across the brush border into the intestinal lumen and diffuses through the basolateral membrane into the circulation or is transported into circulation through a carrier in the serosal membrane (Gropper et al., 2004).

When glucose enters the portal circulation, it is first directed to the liver. The liver breaks down some of the glucose, while the remaining glucose that is not metabolized by the liver enters systemic circulation and is transported into peripheral tissues. Glucose uptake into different organs and tissues occurs via facilitated diffusion, which is a passive transport system mediated by glucose transporters (Gropper et al., 2004). The process can be insulin dependent, as seen in skeletal muscle at rest and in adipose tissue, or insulin independent as seen in the liver or in skeletal muscle during and after exercise. Out of 6 identified glucose transporter protein isoforms (GLUT-1 to GLUT-6), glucose transporter protein isoform-4 (GLUT-4) is the only known isoform that is regulated by insulin (Gropper et al., 2004). GLUT-4 is predominantly found in striated muscle and adipose tissue. Different from other GLUT isoforms that are mostly localized on the cell membrane, GLUT-4 proteins are stored in specialized vesicles within the cell and translocated to the cell membrane with insulin stimulation or contraction-mediated stimulation by skeletal muscle (Molina, 2013).

Glucose is distributed according to the body's energy demands at the time of ingestion. It can be transported to the brain, heart, kidneys, adipocytes, and skeletal muscle. Hormones that are involved with the main regulating mechanisms of glucose transport include insulin, glucagon, epinephrine, and corticosteroids. Allosteric enzyme activation or suppression are also involved with regulating glucose transport. After glucose is transported into different tissues, it is oxidized or stored through several pathways via glycogenesis,

gluconeogenesis, glycogenolysis, glycolysis or the tricarboxylic acid (TCA) cycle (Gropper et al., 2004).

1.4.2 *Glucose storage and metabolism for exercise*

An overview of glucose control in the postprandial state compared to in the postabsorptive state is provided to review differences between glucose storage and metabolism. In the postprandial state (i.e., after a meal), increased insulin secretion from the pancreas leads to increased glucose uptake in skeletal muscle, fat and the hepatosplachnic bed, and glycogen synthesis is increased (Molina, 2013). Most of the insulin-stimulated glucose uptake is stored as glycogen in the liver and skeletal muscle. In the postabsorptive state (i.e., fasting), glucose is released mainly from the liver through glycogenolysis and gluconeogenesis to be used for energy. Glycogenolysis, involving the breakdown of hepatic glycogen stores to glucose, initially accounts for majority of the hepatic glucose output. After an overnight fast, glycogenolysis accounts for ~50% of hepatic glucose output, and if there is prolonged fasting (i.e., after ~60 hours), it becomes negligible. Gluconeogenesis then becomes the predominant source of hepatic glucose output (Molina, 2013). During exercise, plasma glucose from the liver or from the diet is taken up into the muscle for energy.

To understand the regulation of metabolism during exercise, an overview of the neuroendocrine response to exercise is also important. The neuroendocrine response to exercise includes activation of the sympathetic

nervous system, release of growth hormone, activation of the hypothalamic-pituitary-adrenal axis leading to the secretion of catecholamines and cortisol, suppression of insulin secretion and stimulation of glucagon secretion (Molina, 2013). This response stimulates lipolysis, increasing free fatty acids (FFA), and stimulates hepatic glucose output. The catecholamines that are released stimulate hepatic and skeletal muscle glycogenolysis (Molina, 2013). In skeletal muscle, glycogenolysis produces glucose-6-phosphate, but because muscle lacks the enzyme glucose-6-phosphatase to produce glucose, the glucose-6-phosphate is either oxidized in the muscle or released from the muscle as lactate instead of as glucose. Lactate is delivered to the liver, and can be used for liver gluconeogenesis (Molina, 2013). During prolonged moderate intensity exercise, energy substrate selection starts to switch from predominantly lipid oxidation to predominantly CHO oxidation and the liver relies more on gluconeogenic substrates to produce glucose (Molina, 2013). When CHO_{exo} is ingested, it provides an additional source of fuel during exercise that is taken up into the muscle. CHO_{exo} may compete with other substrates and suppress usual fat oxidation (Chu et al., 2011).

Plasma glucose transported into the muscle first undergoes glycolysis, which refers to the breakdown of glucose to pyruvate. This process is anaerobic. Glucose-6-phosphate via glycogenolysis also produces pyruvate. Pyruvate can be used as both an anaerobic or aerobic substrate for energy. The anaerobic pathway involves the conversion of pyruvate to lactate when energy is required

quickly, such as for short, intense exercise. The aerobic pathway involves the breakdown of pyruvate to acetyl CoA, which goes on to be synthesized as fat or used for producing ATP for energy via the TCA cycle and electron transport chain (Whitney & Rolfes, 2008). In these processes, carbon dioxide (CO_2) is produced as a metabolic byproduct. It enters the circulation and is eliminated in the lungs. With the ^{13}C-enriched CHO methodology used in this thesis, the $^{13}C/^{12}C$ ratio of expired CO_2 in breath is measured to estimate CHO_{exo} oxidation rate.

1.5 Use of ^{13}C-enriched carbohydrate in metabolic studies at rest and during exercise

1.5.1 *^{13}C-enriched carbohydrate breath test at rest in association with insulin resistance and diabetes*

To date, a few studies have examined the association of a ^{13}C-enriched CHO breath test under resting conditions with IR in adults (Banerjee et al., 2009; Ibarra-Pastrana, Candia Plata, Alvarez, & Valencia, 2012; Lewanczuk et al., 2004; Mizrahi et al., 2010) and in children (Jetha et al., 2009). Lewanczuk and colleagues (2004) found a strong correlation for ^{13}C-enriched CHO breath test results, measured as change in $^{13}CO_2$ after drink ingestion, with glucose disposal rate (r = 0.69, p<0.0001) and insulin sensitivity index (r = 0.69, p<0.0001) determined using a hyperinsulinemic-euglycemic clamp. There is also evidence for a significant inverse association between $^{13}CO_2$ and homeostasis model assessment of insulin resistance (HOMA-IR) (Banerjee et al., 2009; Ibarra-Pastrana et al., 2012; Jetha et al., 2009; Mizrahi et al., 2010). One study,

however, found that the association no longer existed when corrected for waist circumference and BMI (Banerjee et al., 2009), suggesting that it could be adiposity, rather than IR, *per se*, that affects CHO_{exo} oxidation at rest. No other studies to date have examined this relationship with adiposity compared to IR.

Only one study has examined ^{13}C-enriched breath test results at rest in relation to IR in children (Jetha et al., 2009). In pre-pubertal children with obesity, $^{13}CO_2$ measured in breath after CHO ingestion was correlated with indices of IR, including HOMA-IR, fasting insulin, and 2-h insulin during an OGTT (Jetha et al., 2009). More research in children studying the efficacy of the test for identifying IR would provide information about its clinical potential.

1.5.2 *Exogenous carbohydrate oxidation during exercise in adults and children with obesity and diabetes*

The association of ^{13}C-enriched CHO_{exo} oxidation during exercise or CHO_{exo} oxidative efficiency and IR have not been directly studied before. This is important to study because if impaired MetFlex during exercise is associated with IR, it would highlight and reveal an impairment in the oxidative machinery within skeletal muscle that could contribute to an increased risk for T2DM. Some knowledge can be translated from studies examining CHO_{exo} oxidation during exercise in adults with obesity and in adults and children with type 1 diabetes mellitus (T1DM). However, the number of available studies is scarce. Only one study has examined CHO_{exo} oxidation during exercise in adults with obesity (Ravussin, Pahud, Thelin-Doerner, Arnaud, & Jequier, 1980). The authors

reported lower total CHO (CHO_{tot}) oxidation at rest, but similar rates of CHO_{exo} oxidation during exercise in adults with obesity compared to controls (Ravussin et al., 1980). These findings suggest that MetFlex during exercise was not impaired by excess adiposity. However, IR was not considered. To our knowledge, no studies on CHO_{exo} oxidation during exercise have been completed in adults with T2DM, or in children with obesity compared to controls.

There are a few existing studies on CHO_{exo} oxidation during exercise in adults and children with T1DM. The studies provide some insight on the link between MetFlex under exercise conditions and dysglycemia, although, the research is sparse and inconclusive. In boys with T1DM, the contribution of CHO_{exo} to total energy expenditure (EE) during exercise was lower compared to healthy controls (9.1% vs. 12.4%, respectively) (Riddell, Bar-Or, Schwarcz, & Heigenhauser, 2000). This occurred despite elevated insulin concentrations, which were two-fold higher in the T1DM group compared to controls at all times points (Riddell, Bar-Or, Hollidge-Horvat, Schwarcz, & Heigenhauser, 2000). The reported CHO_{exo} oxidative efficiency was also lower in T1DM compared to controls (12.1% vs. 16.2%, respectively). Reduced CHO_{exo} oxidation in T1DM could be explained by a lower rate of gastrointestinal absorption of CHO, IR which may limit plasma glucose uptake and oxidation, a reduction in skeletal muscle GLUT-4 transporters, and an impairment in pyruvate dehydrogenase (PDH), a key regulatory enzyme of CHO oxidation, which would reduce CHO oxidation (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000). Support for reduced

GLUT-4 by Klip et al. (1990) was reported in rats with streptozotocin-induced diabetes compared to control rats. However, important to note that these mechanisms may not be directly translated and applied to individuals with T2DM. For example, there are data showing the amount of GLUT-4 is not reduced in T2DM (Zierath, Krook, & Wallberg-Henriksson, 2000). Although PDH is not measured directly in children for ethical reasons, elevated FFA concentrations are expected to inactivate the PDH complex according to the glucose-fatty acid cycle proposed by Randle and colleagues (1963). Unfortunately, FFA levels were not measured in the studies in this thesis, but severe obesity may be expected to contribute to elevated FFA at rest, resulting in substrate competition and a reduction in MetFlex during exercise.

Another topic for consideration is the effect of insulin administration on CHO_{exo} oxidation in adults with T1DM. CHO_{exo} oxidation was reduced in adults without insulin administration compared to individuals with insulin infusion and healthy controls (Krzentowski et al., 1981). The T1DM group without insulin infusion utilized 40-45% of the 100 g CHO load, and relied more on lipid stores than the other groups (Krzentowski et al., 1981). However, no differences were found for CHO_{exo} oxidation between T1DM with insulin infusion and the control group. These findings confirm that insulin secretion has a significant role on CHO_{exo} oxidation.

Robitaille and colleagues (2007) reported some conflicting data showing no difference in CHO_{exo} oxidation during exercise in adults with T1DM compared

to a control group. The participants with T1DM received their usual insulin dose before breakfast. Interestingly, plasma glucose oxidation was lower and muscle glycogen utilization was higher in T1DM compared to controls (Robitaille et al., 2007). To provide a clearer understanding of CHO availability versus oxidation, a few studies have examined the rate of plasma glucose disappearance during exercise in individuals with T1DM (Chokkalingam et al., 2007; Raguso et al., 1995; Shilo, Sotsky, & Shamoon, 1990; Zinman et al., 1977). Findings showed that the rate of plasma glucose disappearance is not different between T1DM and controls (Shilo et al., 1990; Zinman et al., 1977). However, Chokkaligam and colleagues (2007) reported plasma glucose oxidation was significantly lower than the rate of plasma glucose disappearance in individuals with T1DM, which suggests any impaired CHO oxidation could be related to oxidative machinery or substrate competition, but not due to impaired delivery of CHO to the muscle. Lastly, it is also important to consider exercise intensity. Raguso and colleagues (1995) reported no difference during exercise at 75% $\dot{V}O_{2max}$, but at 45% $\dot{V}O_{2max}$, the rate of plasma glucose disappearance was ~50% lower in T1DM compared to controls.

In summary, studies on T1DM suggest IR contributes to less efficient use of available CHO, demonstrating poor MetFlex. Based on these findings in individuals with T1DM, it was hypothesized that children with obesity and IR also have impaired MetFlex. **Table 1.2** provides a summary of CHO_{exo} oxidative efficiency in healthy and metabolically compromised conditions, including people

living with obesity, IR, prediabetes, or diabetes. Studies examining exercise training effects on CHO_{exo} oxidative efficiency were also included. Other than 2 studies (Chu, Morrison, Riddell, Raha, & Timmons, 2016; Chu et al., 2011), the table does not provide very much data on CHO_{exo} oxidative efficiency in children with obesity and T2DM, because few studies exist. For this reason, the research presented in this thesis is relevant and contributes new knowledge to the scientific community.

Table 1.2 Overview of exogenous carbohydrate oxidative efficiency in health and disease

Study Details	Age	CHO_{exo} oxidative efficiency during exercise
Riddell et al., 2000 Participants: Healthy, untrained boys	13 - 17 y	Reported: • 34.2 ± 2.2%
Riddell et al., 2001 Participants: Healthy, nonobese, habitually active boys	10 - 14 y	Calculated: • 33.1% in glucose trial • 30.3% in glucose + fructose trial
Timmons et al., 2003 Participants: Healthy, pre- and early-pubertal boys and men	9.8 y boys 22.1 y men	Reported: • 36.8 ± 2.0% in boys* • 26.0 ± 2.1% in men
Timmons et al., 2007 Participants: Healthy boys	12 y 14 y	Calculated: • 44% in 12 y boys • 36% in 14 y boys
Timmons et al., 2007 Participants: Healthy girls	12 y 14 y	Calculated: • 25.4% in 12 y girls • 24.3% in 14 y girls
Chu et al., 2011 Participants: Pre- and early-pubertal boys with obesity	9 - 12 y	Calculated: • 15.9%

Chu et al., 2016 (Chapter 3) Participants: children with obesity and NGT or IGT	8 - 17 y	Reported: • 17.0 ± 3.6% in children with IGT • 17.1 ± 4.4% in children with NGT
Bosch et al., 1994 Participants: Healthy, endurance-trained men	26 y	Calculated: • 85% or 93% at the end of exercise
Jeukendrup et al., 1997 Participants: Untrained and trained adults	20.9 ± 0.5 y untrained 25.1 ± 1.4 y trained	Calculated: • 31% in untrained adults • 35.7% in trained adults Calculations were based on the last hour of exercise and not the full 2 hours.
Burelle et al., 1999 Participants: Sedentary men and trained men	23 ± 1 y untrained 20 ± 1 y trained	Reported: • 33.6% in sedentary men • 44.4% in trained men Calculations did not include the first 30 min of exercise.
Krzentowski et al., 1984 (Exercise training study) Participants: Healthy men	25 y	Calculated: • 34.5% before exercise training • 40.3% after exercise training
Ravussin et al., 1980 Participants: Adults with obesity and controls	29 y	Calculated: • 33.6% in adults with obesity • 28.1% in controls
Riddell et al., 2000 Participants: Boys with T1DM and controls	13 - 19 y	Reported: • 12.1 ± 1.3% in T1DM* • 16.2 ± 0.8% in controls
Krzentowski et al., 1981 Participants: Adults with T1DM and controls	18 - 21 y	Calculated: • 84% in T1DM with insulin infusion • 92% in controls • 43% in T1DM without insulin infusion
Robitaille et al., 2007 Participants: Adults with T1DM and controls	26.5 ± 6.8 y	Reported: • 17% in T1DM • 21% in controls

*indicates that significant difference was identified between groups in the manuscript. Abbreviations: $\dot{V}O_{2max,}$ maximal oxygen uptake; BW, body weight; CHO_{exo}, exogenous carbohydrate; FFM, fat free mass; T1DM, type 1 diabetes mellitus. Note: For additional notes on study protocol and reported exogenous carbohydrate oxidation, refer to **Appendix J.**

1.5.3 *Clinical relevance of metabolic flexibility testing in children with obesity*

The progression from NGT to IGT to T2DM appears to be more rapid in children compared to adults (Weiss et al., 2005; Weiss & Caprio, 2005). Weiss and colleagues (2005) showed that insulin sensitivity in children with obesity rapidly deteriorated over 18 to 24 months. In their sample, 10% of subjects initially classified as NGT developed IGT and 24% of subjects initially classified as IGT developed T2DM. On a more positive note, some children who had IGT reverted back to NGT within 18 to 24 months, even with minimal reductions in BMI z-score (Weiss et al., 2005). This suggests that lifestyle interventions do not necessarily have to attain significant weight loss for health benefits. In children with obesity, maintaining a stable body weight during active growth may be an effective strategy to reduce or delay the risk of developing obesity-related co-morbidities. Exercise as therapy is one lifestyle modification that can improve health outcomes such as glycemia and IR independent of weight loss (Bell et al., 2007; Nassis et al., 2005; van der Heijden, Toffolo, Manesso, Sauer, & Sunehag, 2009). Since IR is improved with exercise interventions, MetFlex may also be improved. Research assessing MetFlex before and after exercise training would help elucidate the effectiveness of an intervention in children and evaluate components of clinical utility.

Unfortunately, because of a rapid deterioration in beta-cell function that occurs in children with obesity, which can be exacerbated during puberty, timing is critical and the opportune window for early and effective intervention is narrow.

MetFlex testing could be advantageous in providing an alternative screening method for risk factors associated with T2DM. The non-invasive nature of the test would eliminate the need for multiple blood samples, which create anxiety for some children. If MetFlex testing is deemed valid and reliable in children, the hope is to use the test to increase the number of children screened for risk factors associated with T2DM and to implement earlier intervention. Findings presented in this thesis will contribute additional scientific knowledge and stimulate discussions about the clinical utility of MetFlex testing.

1.6 **Effects of exercise on insulin signaling, insulin action and metabolic flexibility in humans**

1.6.1 *Effects of acute exercise on insulin resistance*

One of the main reasons exercise is beneficial for intervention and prevention of T2DM is that exercise has marked acute and chronic effects on IR and peripheral glucose uptake. To help explain how exercise improves IR, a brief overview of the insulin signaling pathway which regulates glucose transport is provided here and presented in **Figure 1.2**. At rest, insulin regulates glucose uptake into skeletal muscle by activation of a protein signaling cascade, which leads to translocation of GLUT-4 to the plasma membrane. Glucose uptake through GLUT-4 occurs via facilitative diffusion (Henriksen, 2002). Exercise also interacts with the insulin signaling pathway and increases GLUT-4 expression and translocation to the cell membrane. GLUT-4 translocation to the plasma membrane during or immediately after exercise is mediated by muscle

contraction instead of insulin. Insulin independent glucose uptake associated with exercise is beneficial, especially if insulin secretion is impaired due to impaired beta-cell function. Some of the key intracellular proteins involved with exercise mediated glucose transport are highlighted in **Figure 1.2**.

Acute endurance exercise has shown to lead to higher rates of tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1) and increased activity of phosphatidylinositol-3 kinase activity (PI3-kinase) in untrained healthy individuals and individuals with IR (Cusi et al., 2000; Kirwan et al., 2000; Röhling, Herder, Stemper, & Müssig, 2016). In contrast, other studies suggest that the link between exercise and GLUT-4 translocation likely occurs independent of proximal insulin signaling molecules (i.e., distal to the binding of insulin to the insulin receptor and activation of PI3-kinase) (Frøsig & Richter, 2009). Interactions with distal signaling molecules downstream of PI3-kinase that have been investigated more recently include atypical protein kinase C (aPKC) and akt substrate of 160 kDa (AS160) (Frøsig & Richter, 2009). However, the underlying pathways of these interactions are not fully understood. Another important signaling pathway affected by exercise involves adenosine monophosphate-activated protein kinase (AMPK). Increased adenosine monophosphate to ATP ratio (AMP:ATP) during exercise leads to AMPK activity and subsequent glucose uptake (Röhling et al., 2016). In addition, changes in calcium (Ca^{2+}) concentration during muscle contraction also influence GLUT-4 translocation. Increased cystolic Ca^{2+} concentration leads to increased

Ca^{2+}/calmodulin complexes, such as Ca^{2+}/calmodulin-dependent protein kinase 2 (CaMKII), which are important for glucose uptake (Lanner, Bruton, Katz, & Westerblad, 2008; Röhling et al., 2016). The Ca^{2+} signaling pathway appears to be impaired in individuals with T2DM (Nitert et al., 2012; Röhling et al., 2016).

In general, individuals with T2DM have impaired insulin signaling, despite having normal amounts of GLUT-4 transporters (Zierath et al., 2000). In adults with T2DM, a single bout of exercise at 60-70% of maximal oxygen uptake ($\dot{V}O_{2max}$) for 45-60 min increased plasma membrane GLUT-4 protein by 74 ± 20%, which was similar to adults without T2DM (Kennedy et al., 1999). The protein expression of GLUT-4 was not increased by the acute exercise (Kennedy et al., 1999). The duration of the acute effects of exercise on IR is unclear. A critical time-point identified by Frøsig and Richter (2009) suggested that exercise-induced molecular signaling and improvements in glucose uptake occurred 3-4 hours after exercise. At 24 hours after an acute bout of endurance exercise, Cusi et al. (2000) did not find improved whole body insulin-stimulated glucose disposal in adults with T2DM measured with a hyperinsulinemic-euglycemic clamp. These results suggest that improvements in IR influenced by exercise are transient and return to baseline after ~24 hours.

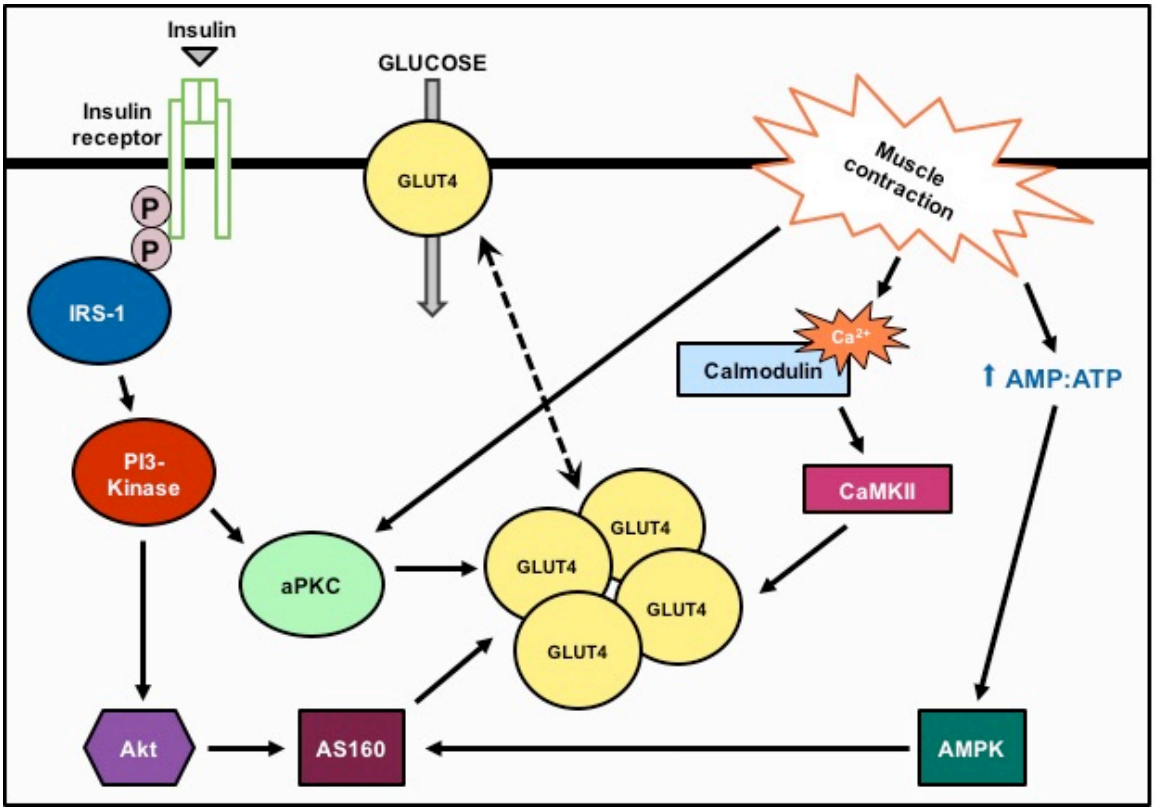

Figure 1.2 Simplified overview of intracellular proteins involved with glucose uptake in skeletal muscle. At rest, insulin binds to the insulin receptor and activates a protein signaling cascade, which leads to the translocation of GLUT-4 to the plasma membrane. Binding of insulin to the insulin receptor results in increased insulin receptor tyrosine phosphorylation and insulin receptor tyrosine kinase activity. This signaling sequence does not seem to be regulated by exercise. During or immediately after exercise, muscle contraction leads to GLUT-4 translocation via interactions with aPKC or AS160, as well as calmodulin activation by Ca^{2+}. Modified from Frøsig and Richter (2009) and Röhling et al (2016). Abbreviations: IRS-1, insulin receptor substrate-1; PI3-kinase, phosphatidylinositol-3 kinase activity; aPKC, atypical protein kinase C; Akt, Akt/protein kinase B; AS160, Akt substrate of 160 kDa; Ca^{2+}, calcium; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase 2; AMP, adenosine monophosphate; ATP, adenosine triphosphate; AMPK, adenosine monophosphate-activated protein kinase.

1.6.2 *Effects of short-term exercise training on insulin resistance*

The positive effects of exercise training on IR are well supported (Goodyear & Kahn, 1998). For the purpose of this thesis, discussion of exercise

training effects on IR will be limited to exercise training studies with a shorter intervention time (7 to 10 days in adults and 6 to 8 weeks in children). It is valuable for lifestyle intervention programs to determine how exercise can improve IR in individuals at risk for developing T2DM. Collating studies of shorter duration can help elucidate when health benefits are expected to occur independent of changes in weight, and how early potential improvements in MetFlex may be observed.

Current knowledge about the mechanisms underlying the effects of exercise training on IR in humans is limited. There is, however, evidence in animal models that suggests an increase in protein expression of some molecules, including insulin receptor tyrosine phosphorylation and IRS-1 (Henriksen, 2002). In humans, Houmard and colleagues (1999) showed that exercise training interacted with the insulin signaling pathway via upregulation of PI3-kinase activity to improve IR. The study measured PI3-kinase activity in the vastus lateralis muscle of healthy, young, sedentary men before and after 7 days of continuous exercise training (60 min/day; 75% $\dot{V}O_{2max}$) (Houmard et al., 1999). However, similar results on insulin-stimulated PI3-kinase activity were not replicated by Tanner et al. (2002) after 7 days of continuous exercise training (60 min/day; 70% $\dot{V}O_{2max}$) in middle-aged men with IR, despite improved IR measured after the intervention.

Several other studies have reported that exercise training consisting of 7 or 10 consecutive days improved IR in adults with IGT (Arciero, Vukovich,

Holloszy, Racette, & Kohrt, 1999) and T2DM (Kirwan, Solomon, Wojta, Staten, & Holloszy, 2009; Rogers et al., 1988). In men with mild T2DM, 7 days of exercise training (30 min/day on a treadmill and 20-30 min/day on a cycle ergometer; 68% $\dot{V}O_{2max}$) improved glucose tolerance (Rogers et al., 1988). Glucose disposal measured using clamp procedures is also improved after 7 days of exercise training in adults with T2DM (Kirwan et al., 2009; O'Gorman et al., 2006; Winnick et al., 2008). Kirwan et al. (2009) prescribed 7 days of exercise training (30 min/day of cycling and 30 min/day of treadmill walking; 70% $\dot{V}O_{2max}$) in adults with T2DM and showed improved insulin responsiveness of peripheral glucose uptake and enhanced insulin suppression of hepatic glucose production measured with a hyperinsulinemic-euglycemic clamp. Results were found independent of any change in weight in the participants. In contrast to Kirwan et al.'s results, Winnick and colleagues (2008) did not find a significant effect of 7 days of exercise training (50 min/day of treadmill walking; 70% $\dot{V}O_{2max}$) on hepatic insulin sensitivity in adults with T2DM. However, exercise training improved whole body and peripheral insulin sensitivity as expected (Winnick et al., 2008). Interestingly, O'Gorman et al. (2006) reported increased insulin-mediated glucose disposal in adults with obesity and T2DM but not in adults with obesity and without T2DM after 7 consecutive days of exercise training (60 min/day of cycling; 75% $\dot{V}O_{2max}$). One possible explanation is that there is an inverse relationship between degree of IR and magnitude of change in glucose

disposal after exercise training. This means adults with the highest IR had the highest relative response (O'Gorman et al., 2006).

Exercise training studies as short as 7 or 10 days of intervention have not yet been conducted in children. To date, exercise training studies examining IR in children have included at least 6 weeks of intervention before measuring the effects of exercise training on IR and other health outcomes (Fedewa, Gist, Evans, & Dishman, 2014; Kim & Park, 2013; Lee & Kim, 2013). In children with obesity, Kim et al. (2007) and Kelishadi et al. (2008) showed decreased HOMA-IR after 6 weeks of an aerobic-type exercise intervention, which included jump rope (40 min/day; 5 days/week) (Kim et al., 2007) or fitness-oriented activities, games and running (60 min/day; 3 days/week) (Kelishadi, Hashemi, Mohammadifard, Asgary, & Khavarian, 2008). Both studies also reported decreased weight and percent body fat after the exercise training (Kelishadi et al., 2008; Kim et al., 2007). Ben Ounis and colleagues (2008) reported decreased HOMA-IR in children who followed a 2 month exercise only intervention (90 min/day; 4 days/week; at predetermined exercise intensity that elicited maximal fat oxidation) and children who followed a dietary program plus the 2 month exercise intervention, but not in children who followed the diet only intervention. Weight loss was reported in the diet only and diet and exercise intervention groups, but not in the exercise only intervention group (Ben Ounis et al., 2008). Similarly, Bell et al. (2007) reported improved IR independent of weight loss after 8 weeks of a combined aerobic and resistance exercise training in children with

obesity. In summary, evidence suggests exercise alone is an effective intervention strategy for reducing IR in children with obesity (Kim & Park, 2013). A shorter intervention in children would advance our knowledge about initial metabolic responses to exercise training, and MetFlex testing would help determine if we can use a CHO challenge test with exercise to quantify these improvements.

One final consideration for using exercise interventions to improve IR is exercise modality. The positive effects of aerobic training on IR are well accepted, but there are much less data on the effects of resistance training (Marson, Delevatti, Prado, Netto, & Kruel, 2016) and high intensity interval training (Racil et al., 2016). A recent meta-analysis on the effects of aerobic, resistance and combined aerobic and resistance exercise training on IR markers in children reported that fasting insulin was only improved with aerobic training, and not in studies that implemented resistance training or combined aerobic and resistance training (Marson et al., 2016). Aerobic training also improved HOMA-IR. However, there was an insufficient number of studies available to assess the effect of resistance training and combined aerobic and resistance training on HOMA-IR (Marson et al., 2016). Not many studies have investigated the effect of high intensity interval training on IR in children with obesity, but one study showed improved HOMA-IR after 12 weeks of exercise intervention (Racil et al., 2016). Due to a limited number of studies, the best modality or combinations of

exercises for optimal intervention in children with obesity cannot be clearly delineated at this time.

1.6.3 *Effects of exercise training on metabolic flexibility*

Improvements in MetFlex have been reported in a few studies examining the effects of exercise training in adults with obesity (Battaglia, Zheng, Hickner, & Houmard, 2012; Malin et al., 2013; Meex et al., 2010). Malin et al. (2013) studied MetFlex in adults with IFG, IGT or IFG+IGT after 12 weeks of exercise (60 min/day; 5 days/week; 85% maximal heart rate (% HR_{max})), and found improved IR was correlated with enhanced MetFlex after exercise training. However, improved MetFlex was only found in the IFG and IGT groups, and not the IFG+IGT group (Malin et al., 2013). MetFlex was measured under resting conditions using a hyperinsulinemic-euglycemic clamp combined with indirect calorimetry. Using similar methods, but a different 12 week exercise training program (45 min/day; 3x/week; 55% of predetermined maximal workload), Meex et al. (2010) showed that MetFlex was restored after exercise training in men with T2DM when compared to a control group matched for weight, BMI and age. In a study using a different method of assessing MetFlex (MetFlex in response to a high-fat diet), Battaglia et al. (2012) reported increased fat oxidation in response to a high-fat diet in lean adults, but not in adults with obesity. Exercise training for 10 consecutive days (60 min/day; 70% $\dot{V}O_{2max}$) improved fat oxidation in both

adults with obesity and without obesity (Battaglia et al., 2012). The studies mentioned above all assessed MetFlex under resting conditions.

There has not been extensive research on MetFlex conducted under exercise conditions. An overview of studies that have investigated the effects of exercise training on fat and CHO metabolism provide some insight. Aerobic exercise training is beneficial for improving fat oxidation (Brandou, Dumortier, Garandeau, Mercier, & Brun, 2003; Johnson et al., 2010; Nordby et al., 2015), which in theory would improve MetFlex by maintaining or restoring the ability to switch to a greater reliance of fat oxidation under fasting conditions and prolonged exercise conditions (DiPietro, 2010). In post-menopausal women, who have a lower overall capacity for fat oxidation during exercise compared to young women, 12 weeks of aerobic exercise training (60 min/day; 5 days/week; 65% $\dot{V}O_{2max}$) led to increased FFA mobilization and fat oxidation during exercise (Johnson et al., 2010). Similarly, fat oxidation measured during exercise was improved in children with obesity after 2 months of exercise intervention (45 min/day; 1 day/week; at predetermined exercise intensity that elicited maximal fat oxidation; plus 1 day/week of additional aerobic exercise encouraged with a phone call) (Brandou et al., 2003).

Measuring CHO_{exo} oxidation can be used to achieve a better understanding of how substrate oxidation changes after exercise therapy. There appears to be one existing study that has examined this. In healthy men, aerobic exercise training (60 min/day; 5 days/week; 30-40% $\dot{V}O_{2max}$) over 6 weeks led to

a 17% increase in CHO_{exo} oxidation during exercise (Krzentowski et al., 1984). No significant effect of exercise training was found on CHO_{exo} oxidation at rest (Krzentowski et al., 1983). The results suggest that MetFlex in response to CHO_{exo} improves with exercise training and that it is best examined under exercise conditions.

In contrast to the study mentioned above, Jeukendrup et al. (1997) reported similar CHO_{exo} and endogenous CHO (CHO_{endo}) oxidation during exercise in endurance-trained adults compared to untrained adults, but fat oxidation rates were higher in the endurance-trained adults. This suggests that exercise training or lower IR contributes to improved fat oxidation during exercise. However, the study did not involve an exercise intervention, but selected participants based on training status. Additional research is required to determine how exercise training influences substrate selection and oxidation during exercise and how responses in CHO_{exo} oxidation or fat oxidation are related to IR and risk for T2DM.

The strength of assessing MetFlex under exercise conditions is the ability to examine a dynamic, physiological response, where metabolic machinery must respond to a greater demand for energy compared to at rest (DiPietro, 2010; Galgani, Moro, et al., 2008). The research in this area is in its early stages, and we do not have adequate information to infer if measuring MetFlex to increased fat availability or MetFlex to increased CHO availability is most appropriate, or if alternative methodologies would provide a better measurement of MetFlex under

exercise conditions. Therefore, exercise training-related effects on MetFlex measured during exercise warrant further examination.

Knowledge about MetFlex in response to exercise training in children is more limited compared to adults. In children with obesity, we know that exercise training improves components of metabolic health, such as lipid levels, IR, and fat oxidation during aerobic exercise (Ben Ounis et al., 2008; Brandou et al., 2003). Studies have not yet examined the effect of exercise training on CHO_{exo} oxidative efficiency in children. Thus, one of the specific objectives of this thesis was to investigate the effect of 7 consecutive days of exercise on IR and MetFlex during exercise in children with obesity (Chapter 5).

1.7 **Summary**

The increasing incidence of T2DM in children is a public health concern that highlights health complications are occurring earlier in life. Currently, available screening tools and diagnostic cut-points for diabetes for the clinical care of children are not well supported by evidence-based studies. The progression of NGT to IGT to T2DM also can occur silently over time. Thus, the development of alternative screening tools that are not very invasive may be beneficial for children. Based on research on MetFlex in adults and the association between reduced MetFlex and IR (Apostolopoulou et al., 2016; Galgani, Moro, et al., 2008), a greater understanding of MetFlex in children could help identify risk factors associated with T2DM, such as IGT, dysglycemia and IR. To our knowledge, no studies in adults or children have examined these

associations with MetFlex (defined as CHO_{exo} oxidative efficiency) during exercise or if MetFlex can be improved with exercise therapy in children.

Further understanding the effects of exercise therapy on MetFlex in children may help determine who would benefit most from traditional interventions for T2DM that encourage lifestyle changes, such as increasing physical activity, and who might benefit most from other types of interventions, such as pharmacological therapy. One of the strengths of the MetFlex test presented in this thesis, which was modified from exercise metabolism studies, is the non-invasive nature of the test because it does not require blood work or insulin infusion. Therefore, the clinical utility of the test should be examined further. The studies presented in Chapters 3, 4, and 5 of this thesis aim to supplement the gap in scientific knowledge on MetFlex during exercise in children and to increase our general knowledge of MetFlex testing to help determine if it can be used to identify metabolic complications associated with T2DM in the future.

1.8 **Thesis objectives and hypotheses**

1.8.1 *General objectives*

The general objective of this thesis was to advance our knowledge of MetFlex under exercise conditions in children with obesity.

1.8.2 *Specific objectives*

The specific objectives of the studies in this thesis were to:

1) examine MetFlex during exercise in children with IGT compared to children with NGT (Chapter 3);
2) determine the validity and reliability of using MetFlex during exercise as a screening tool for identifying dysglycemia and IR in children with obesity (Chapter 4);
3) determine the effect of 7 consecutive days of exercise training on MetFlex and IR in children with obesity (Chapter 5).

1.8.3 *Specific hypotheses*

The specific hypotheses of the studies in this thesis were:

1) MetFlex during exercise would be lower in children with IGT compared to children with NGT (Chapter 3);
2) MetFlex during exercise would be negatively associated with fasting glucose and insulin resistance (Chapter 4);
3) 7 consecutive days of exercise training would increase MetFlex during exercise and decrease IR in children with obesity (Chapter 5).

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CHAPTER 2

2 PROJECT DESIGN, STUDY PARTICIPANTS, AND METHODOLOGICAL CONSIDERATIONS

Pediatric research presents with several challenges to consider that are unique from research in adults. Among these are biological maturation and physiological changes during growth. In addition, obesity creates a milieu of factors that may influence puberty and metabolism. Discussion of these concerns are contained in this chapter. Special considerations and rationale related to study design and participant selection are also included to support the general methodology presented in this thesis. However, more specific methodological details of each study are described in the subsequent chapters.

2.1 Study Design

The subsequent chapters in this thesis were written based on two studies. Study 1 (Chapter 3 and 4) followed a cross-sectional design. Study 2 applied an interventional study design (Chapter 5). Both studies included the same experimental methodology to measure MetFlex during exercise. The exercise intensity of the MetFlex test was set at 45% $\dot{V}O_{2max}$. Although past studies in healthy children and children with T1DM completed in our laboratory have used intensities between ~60-70% $\dot{V}O_{2max}$ (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000; Timmons et al., 2003, 2007a, 2007a), we anticipated that this would be a

challenge for children who were more sedentary. Hence, the exercise intensity was lowered to suit the clinical population studied. Furthermore, 45% $\dot{V}O_{2max}$ is an intensity of exercise that elicits predominantly fat oxidation (Brooks & Mercier, 1994) unless CHO_{exo} is provided before exercise, in which case, CHO_{exo} oxidation would be increased and fat oxidation suppressed during exercise (Chu et al., 2011). Evidence that CHO_{exo} oxidation is correlated with intensity of exercise up to about 50% $\dot{V}O_{2max}$ (Pirnay et al., 1982) also supports the chosen exercise intensity. If greater individual variability in CHO_{exo} oxidation is thought to occur at higher exercise intensities, results from MetFlex testing above 50% $\dot{V}O_{2max}$ would be more difficult to interpret.

In the exercise intervention study, the MetFlex test was conducted at the same absolute workload after training as before training. Since $\dot{V}O_{2max}$ was not expected to change after 7 days of exercise, we predicted that using the same pre-training workload would not affect the results of the study. Friedlander and colleagues reported no differences in CHO_{tot} oxidation after 10 and 12 weeks of exercise training in men (Friedlander, Casazza, Horning, Huie, & Brooks, 1997) and women (Friedlander et al., 1998), respectively, when cycling at the same absolute workload as pre-training, which further supports the study design (Chapter 5). If CHO_{tot} oxidation measured during exercise is not expected to change after exercise training, we can further examine how CHO_{exo} and CHO_{endo} oxidation changes and if CHO_{exo} oxidative efficiency improves in response to exercise training. Notably, Friedlander et al. (1997) reported lower post-training

CHO_{tot} oxidation in the men when tested at the same relative workload. However, this was not considered a concern for the study because the absolute and relative post-training workloads were expected to be very similar.

One of the benefits of measuring MetFlex is that it is less invasive than blood work and insulin infusion. It was expected that the children in the study would prefer the MetFlex test when compared to an OGTT. To assess acceptability of the MetFlex test, we used a modified Likert questionnaire (**Appendix A, Figure A.1**). The questionnaire was based on a 5 point Likert scale (Streiner, Norman, & Cairney, 2014) and included select statements, including 'Overall I enjoyed the test' and 'the test was boring', from the Physical Activity Enjoyment Scale (Motl et al., 2001). Additional statements were added to the questionnaire to ensure applicability to both the OGTT and MetFlex test. A total score for acceptability for each test was calculated out of 18 by adding the scores for 'I liked the taste of the drink', 'the test did not take too long', 'I would not mind doing the test a second time' and 'overall I enjoyed the test', and subtracting the scores for 'the test was boring' and 'the test was painful'. In participants who completed the questionnaire for both tests (n=30), the acceptability scores for the OGTT test and MetFlex test were 8.1 ± 3.3 and 8.1 ± 3.9, respectively (p=0.96) (**Appendix A, Table A.2**). When asked which test the child liked better (n=28), 12 (43%) of the children indicated the OGTT test and 16 (57%) of the children indicated the MetFlex test. Based on these data,

acceptability of MetFlex was similar to an OGTT, and there was a slight preference for completing MetFlex testing over an OGTT.

2.2 Study Participants

Participants recruited for the studies included boys and girls ages 8 to 17 years old. Because of the wide age range, the sample of children consisted of different stages of pubertal development. At the time of recruitment, there was a paucity of research on MetFlex under exercise conditions and no evidence to indicate MetFlex would be affected by sex or puberty. Similarly, there are no age or sex specific guidelines when completing an OGTT. Since one of the study aims was to examine the validity of MetFlex for identifying risk factors associated with T2DM, and an OGTT is currently used to diagnose prediabetes and diabetes, we also did not make adaptations to the MetFlex test according to age or sex. Much like the OGTT, the amount of CHO provided before exercise was based on body weight (1.75 g/kg of body weight) up to a maximum of 75 g. All participants in the studies consumed the maximum amount of CHO (75 g) because of their weight.

Participants for all of the studies were recruited from the Children's Exercise and Nutrition Centre at the McMaster Children's Hospital. One of the nurses and an exercise physiologist in the clinic asked eligible patients if they could be contacted about the study and completed consent-to-contact forms. In total, 133 consent-to-contact forms were received. Of these, 46 (35%) of the

families decided to participate, 59 (44%) decided not to participate, and 28 (21%) did not respond to phone calls or emails about the study. In study 1, there were 7 participants who dropped out of the study after enrolment, resulting in 34 children who participated. See **Appendix B** for a summary of recruitment details.

For the intervention study, participants who provided consent-to-contact for future recruitment after the first study were contacted. The initial study design of this pilot study set out to include only boys with IGT to examine if 7 days of exercise could be a quick strategy to improve MetFlex and reverse increased glycemia and IR. However, due to challenges with recruitment, parameters were modified to include boys and girls and any children with obesity who were referred to have an OGTT by their physician. At the time of recruitment, there were no data to indicate that the effect of exercise on MetFlex and IR is different in boys and girls or in children with NGT and IGT. Therefore, recruitment continued from the Children's Exercise and Nutrition Centre until the study was completed. No children dropped out of the second study because of the intervention. Only one child enrolled, completed a baseline visit, but did not complete the entire study due to personal time constraints.

2.3 **Methodological considerations**

2.3.1 *Timing of puberty in children with obesity*

The association between increased adiposity and the timing of puberty has been discussed for several decades (Frisch, 1972). In girls, there is substantial

evidence suggesting a positive association between greater adiposity and early maturation (Dai et al., 2014; Frisch, 1972; Wang, 2002). In boys, there are more conflicting views (Tinggaard et al., 2012). Some studies have reported that greater adiposity is associated with early maturation (Dai et al., 2014), while others have reported the opposite (Wang, 2002). Possible explanations for these differences could be related to the poor reliability of identifying the onset of puberty (Rasmussen et al., 2015; Villamor & Jansen, 2016), variable definitions of early maturation and obesity duration in different studies (Villamor & Jansen, 2016), and a potential non-linear relationship between obesity and maturation, where overweight could be associated with early maturation but severe obesity is associated with late puberty (Tinggaard et al., 2012; Villamor & Jansen, 2016).

Understanding obesity and the timing of puberty is relevant for research related to childhood obesity because of physiological changes that occur in this period of development. Adiposity could influence puberty through mechanisms related to leptin or other signaling pathways involving insulin-like growth factor-1 (IGF-1), insulin, adiponectin, and c-reactive protein, which can affect the expression of sex hormone binding globulin to trigger the onset of puberty (Pinkney et al., 2014; Villamor & Jansen, 2016). In the context of this thesis, we know very little about how any of these factors may interact with substrate metabolism and MetFlex. The focus of the subsequent chapters include discussion of insulin, IR and MetFlex. An in-depth assessment of MetFlex and changes during puberty, which involve changes in adipokines and hormonal

signaling, were beyond the scope of this thesis. While we cannot dismiss these potential factors, we were limited in our ability to assess whether they may or may not influence MetFlex in children with obesity. Future examination is warranted, specifically to observe how the timing of puberty and physiological changes with growth and development are associated with MetFlex.

2.3.2 *Estimating biological age using years from peak height velocity*

A method used to estimate biological age used in our laboratory, developed by Mirwald et al. (2002), is estimating years from peak height velocity (PHV). In the development of the equation, years from PHV was determined by using age of PHV as the maturational benchmark and subtracting chronological age. The difference in years indicates the maturity offset (Mirwald et al., 2002). The ratio of leg length to sitting height was also used to predict maturational status. Leg length to sitting height ratio increases as a child approaches age of PHV and decreases at and after age of PHV. When measured on two separate occasions about 1 year apart, maturity can be roughly identified. Use of the ratio requires sequential measurements of leg length to sitting height taken at least 1 year apart, which is not practical for cross-sectional studies. For this reason, Mirwald et al. (2002) examined and developed a multiple regression equation that incorporated a single measurement of leg length to sitting height ratio to estimate maturity offset. In the sex-specific equations suggested, years from PHV can be predicted by including measurements of height, sitting height, weight, and

chronological age (Mirwald et al., 2002). The degree of accuracy was deemed reasonable by the authors with the ability to predict maturity offset within an error of ± 1 year 95% of the time (Mirwald et al., 2002).

Some caveats to consider for estimating years from PHV in the studies include accuracy and application to different clinical populations. The majority of children included in the development of the multiple regression equation were healthy, typically developing children. The data were from the Saskatchewan Pediatric Bone Mineral Accrual Study, which included a cohort of children of which 2% were underweight, 77% normal weight and 22% overweight or obese (Bailey, 1997), according to Centers for Disease Control and Prevention (CDC) guidelines. In addition, the measurement of sitting height to leg length could be affected by inaccuracy if there is greater adiposity around a child's gluteal muscles, hips and legs, which could overestimate their sitting height. In summary, the application of the equations to children with obesity has yet to be confirmed. There currently is no other easily accessible method to estimate maturation, specific for children with obesity. To provide another estimate of pubertal development, we asked participants to identify their stage of development based on the Tanner method (Tanner, 1962). Other than using a physician's exam, the absence of an easy, reliable estimate of biological maturation is a limitation faced when attempting to estimate puberty in childhood obesity research.

2.3.3 *Indices used to determine insulin sensitivity and beta-cell function*

The gold standard methods for measuring insulin sensitivity and beta-cell function in humans include performing a hyperinsulinemic-euglycemic clamp and frequently sampled intravenous glucose tolerance test, respectively. Due to the invasive nature of these techniques, other indices derived from glucose and insulin concentrations measured after an overnight fast or during an OGTT have been adapted for use. The assay protocols for glucose and insulin measurements performed in this thesis are presented in **Appendix C.** Surrogate indices of insulin sensitivity and beta-cell function that are reported in the following chapters include HOMA-IR, whole body insulin sensitivity index (WBISI), and insulin secretion-sensitivity index-2 (ISSI-2). An overview of each of these indices and how they may be related to the pathophysiology of T2DM and glucose availability during exercise is described below.

Homeostasis model assessment, developed by Matthews and colleagues (1985), uses fasting measurements of glucose and insulin. In the fasted state, glucose is homeostatically maintained, insulin levels are not significantly changing and hepatic glucose production is constant (Muniyappa, Madan, & Quon, 2000). The model is based on interactions between glucose and insulin dynamics, in which glucose concentrations depend on insulin secretion by the beta-cells to suppress hepatic glucose production and insulin concentrations depend on the beta-cell response to glucose concentrations (Matthews et al., 1985; Muniyappa et al., 2000). HOMA-IR is determined with the following

equation: (fasting glucose x fasting insulin)/22.5. The units for fasting glucose and fasting insulin are mmol/L and μU/mL, respectively. HOMA-IR reflects primarily hepatic IR determined by the product of fasting glucose and fasting insulin. In an individual with hyperglycemia (fasting glucose ≥ 7.0 mmol/L) (Goldenberg & Punthakee, 2013), fasting insulin levels are insufficient to suppress hepatic glucose production and maintain normal glucose concentrations. There exists a good correlation between HOMA-IR and whole body insulin sensitivity determined with a clamp (r=0.70, p<0.0001), however, the potential for hepatic and peripheral IR to differ in an individual raises a concern (Matsuda & DeFronzo, 1999). Although there may be a reasonable correlation between hepatic and peripheral IR, HOMA-IR is more likely to indicate hepatic IR and is limited in providing information about peripheral glucose uptake (Matsuda & DeFronzo, 1999). Thus, in the context of exercise physiology research, HOMA-IR is not the best index to reflect IR of skeletal muscle and glucose availability during exercise.

WBISI, also known as the Matsuda index, was developed by Matsuda and DeFronzo (1999) and is derived from glucose and insulin concentrations measured during an OGTT. Using a dynamic test with an oral glucose load more closely resembles the normal physiological response to a meal compared to conducting a hyperinsulinemic-euglycemic clamp. However, some complexities to consider with an OGTT include possible differences in gastric emptying, incretin hormone secretion and action, and splanchnic glucose uptake, which may

contribute to additional variability (Brown & Yanovski, 2014). Nevertheless, Matsuda and DeFronzo (1999) reported a good correlation between WBISI derived from an OGTT and whole body insulin sensitivity determined with a clamp (r=0.73, p<0.0001). This correlation has also been confirmed in pediatric studies (George et al., 2011; Yeckel et al., 2004). WBISI is calculated with the following equation: 10,000/√ (fasting glucose x fasting insulin)(mean glucose x mean insulin). The units for glucose and insulin are mg/dL and μU/mL, respectively. From a physiological perspective, WBISI provides different information than HOMA-IR and represents a combination of hepatic and peripheral insulin sensitivity. Therefore, the index contributes additional information about skeletal muscle glucose uptake after an oral glucose load at rest. A higher WBISI could suggest good availability of glucose or glycogen in skeletal muscle to be utilized during exercise.

To derive indices for beta-cell function, confirmation of the hyperbolic relationship between components of insulin secretion and insulin sensitivity is first required (Retnakaran, Qi, Goran, & Hamilton, 2009). Two indices, derived from measurements during an OGTT, that have mathematically demonstrated the hyperbolic relationship are ISSI-2 (Retnakaran et al., 2008) and the insulinogenic index/fasting insulin (Utzschneider et al., 2009). Both indices were correlated with the disposition index measured with a frequently sampled intravenous glucose tolerance test, but ISSI-2 showed a modestly stronger correlation (ISSI-2: r=0.24, p=0.0003; insulinogenic index/fasting insulin: r=0.17, p=0.0022) (Retnakaran et

al., 2009). Based on these results, ISSI-2 was used in this thesis to provide a surrogate measurement of beta-cell function. ISSI-2 is determined by multiplying the ratio of area under the curve insulin (AUCins) to area under the curve glucose (AUCgluc) by WBISI. The units for glucose and insulin are mg/dL and μU/mL, respectively. ISSI-2 provides information about the insulin response of the beta-cells to a glucose load in relation to insulin sensitivity. A low ISSI-2 value would indicate impaired insulin secretion to compensate for reduced insulin sensitivity. After a glucose load before exercise, reduced ISSI-2 could suggest decreased insulin secretion in response to the glucose load, which may result in reduced skeletal muscle glucose uptake. However, the index does not provide a direct measure of skeletal muscle glucose uptake nor does it imply that glucose uptake would be impaired during exercise because contraction-mediated glucose uptake could remain unaffected.

2.3.4 *General technique and considerations for ^{13}C-enriched carbohydrate use in exercise metabolism studies*

In general, ^{13}C-enriched CHO methodology was developed using concepts presented by Péronnet and colleagues (1990). The studies conducted in children herein were based on the pioneering work of Riddell and colleagues in the late 1990s and 2000s (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000; Riddell, Bar-Or, Schwarcz, et al., 2000). They were the first to apply the ^{13}C-enriched CHO methodology to exercising children. Our laboratory has continued to apply this novel method over the past 10 years. The methodology consists of a ^{13}C-

enriched CHO beverage that is given orally followed by breath samples that are measured periodically during exercise to determine the $^{13}CO_2/^{12}CO_2$ ratio using isotopic ratio mass spectrometry. For more details on the timing of breath sample collection during the MetFlex protocol, refer to **Appendix D**. The breath samples are collected directly from a hose that attaches the mouthpiece to the metabolic cart, and stored in Exetainer tubes for later analysis, as described by Mahon & Timmons (2014). A description of the protocol used for the breath analysis is provided in **Appendix E.**

The ^{13}C enrichment of the drink was estimated using prior experiments conducted by Péronnet and colleagues confirmed by gas chromatography mass spectrometry. ^{13}C-glucose isotopic composition of the CHO drink is expressed as a change per 1000 difference versus the $^{13}C/^{12}C$ ratio from the international standard ^{13}C Pee Dee Belemnitella-1 ([$\delta^{13}C$]PDB-1). The drink enrichment used in this thesis was approximately +100‰ [$\delta^{13}C$]PDB-1, which provided a strong measurement signal to minimize background shift (Massicotte, Péronnet, Adopo, Brisson, & Hillaire-Marcel, 1992). Background shift refers to a small, natural shift in the isotopic composition of CO_2 that is related to the oxidation of muscle glycogen containing ^{13}C from the diet. Therefore, CHO_{exo} oxidation can also be overestimated because of the isotopic composition of CO_2 provided form endogenous substrates, which is often not taken into account (Péronnet et al., 1990). In the studies in this thesis, participants were asked to avoid corn and

corn-based products for at least 5 days before the experimental visit to minimize any error that could be associated with this background shift.

^{13}C methodology has limitations to consider, one of which includes a delay in measuring $^{13}CO_2$ production by tissues at the mouth due to the bicarbonate pool in the body (Pallikarakis, Sphiris, & Lefebvre, 1991). This could lead to a slight underestimate of CHO_{exo} oxidation rate. Although, this discrepancy is much less of a concern with moderate intensity exercise compared to at rest, because the CO_2 production rate is a lot higher (Pirnay et al., 1982). Another possible criticism of the methodology is the recycling of ^{13}C in the form of lipid (via lipogenesis) or glucose (via the Cori cycle). Since the metabolic pathways share common pools, crossing-over of labeling could occur. However, Ravussin et al. (1980) discussed that even after a large intake of CHO_{exo}, the conversion of CHO into fat seems to be of minor importance. Pirnay et al. (1982) reported that the approximate error due to glucose recycling is less than 10%.

2.3.5 *Specific considerations for measuring the isotopic composition of breath samples*

Due to unforeseen maintenance delays with the IDMicro system (IDMicro Breath Version 2.0, Compact Science Systems, Staffordshire, UK) used for measuring the isotopic composition of breath samples in one of the studies (Chapter 5), CHO_{exo} oxidation rates were calculated using breath results measured on two systems. Isotope ratio mass spectrometry was completed using the IDMicro system for 9 out of 12 participants, and the Europa Scientific 20/20

gas isotope ratio mass spectrometer (Europa Scientific, Cincinnati, OH) for the remaining 3 participants. **Appendix F** shows a Bland-Altman plot for $^{13}CO_2$ versus [δ^{13}C]PDB-1 values. There is good agreement between the two systems. However, there is a small bias for higher measurements using the Europa Scientific system when the values are lower for $^{13}C/^{12}C$ ratio, such as breath collected at rest before and after ^{13}C-enriched CHO drink consumption. When translated to calculating CHO_{exo} oxidative efficiency, there may be a potential difference of ± 0.8%. This would not have affected the study results in a meaningful way (Chapter 5).

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CHAPTER 3

3 NO DIFFERENCE IN EXOGENOUS CARBOHYDRATE OXIDATION DURING EXERCISE IN CHILDREN WITH AND WITHOUT IMPAIRED GLUCOSE TOLERANCE

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3.1 ABSTRACT

The capacity to match carbohydrate (CHO) utilization with availability is impaired in insulin-resistant, obese adults at rest. Understanding exogenous carbohydrate (CHO_{exo}) oxidation during exercise and its association to insulin resistance (IR) is important, especially in children at risk for type 2 diabetes. Our objective was to examine the oxidative efficiency of CHO_{exo} during exercise in obese children with normal glucose tolerance (NGT) or impaired glucose tolerance (IGT). Children attended 2 visits, and were identified as NGT (n=22) or IGT (n=12) based on 2-h oral glucose tolerance test (OGTT) glucose levels of <7.8 mmol/l or ≥7.8 mmol/l, respectively. Anthropometry, body composition and aerobic fitness ($\dot{V}O_{2max}$) were assessed. Insulin and glucose at baseline, 30, 60,

90 and 120 min during the OGTT were used to calculate measures of insulin sensitivity. On a separate day, a ^{13}C-enriched CHO drink was ingested before exercise (3 × 20 min bouts) at 45% $\dot{V}O_{2max}$. Breath measurements were collected to calculate CHO_{exo} oxidative efficiency. CHO_{exo} oxidative efficiency during exercise was similar in IGT (17.0 ± 3.6%) compared to NGT (17.1± 4.4%) (p=0.90) despite lower whole body insulin sensitivity in IGT at rest (p=0.02). Area under the curve for insulin (AUCins) measured at rest during the OGTT was greater in IGT compared to NGT (p=0.04). The ability of skeletal muscle to utilize CHO_{exo} was not impaired during exercise in children with IGT.

3.2 INTRODUCTION

The rise in childhood obesity has led to an increased number of type 2 diabetes diagnoses. The presence of type 2 diabetes in childhood suggests that the progression of impaired glucose tolerance (IGT) to type 2 diabetes is accelerated in children, compared with adults, in whom the progression is gradual and may occur over a decade (Weiss & Caprio, 2005). Based on adult data, this progression could involve impaired metabolic flexibility (MetFlex) associated with insulin resistance (IR), resulting in a loss of physiologic reserve and adaptability to nutrient provision (Kelley et al., 1999). MetFlex refers to the capacity to adapt substrate utilization with substrate availability, and is an integral component of skeletal muscle health and plasticity. In insulin-resistant obese adults, impaired MetFlex at rest was characterized by impaired fat oxidation rate in the fasted

state and reduced switching to glucose oxidation with the provision of carbohydrate (CHO), when compared to lean adults (Kelley et al., 1999). A poor dependence on fat oxidation in the fasted state by skeletal muscle significantly predicted the severity of insulin-resistant CHO oxidation (Kelley et al., 1999).

Currently, there is a lack of knowledge about skeletal muscle metabolism, particularly on exogenous CHO (CHO_{exo}) oxidation, in relation to IR and type 2 diabetes in the pediatric population (Aucouturier, Duché, & Timmons, 2011). Weiss et al. (2003) reported lower peripheral CHO disposal and CHO utilization during a hyperinsulinemic-euglycemic clamp in obese children with IGT compared with obese children with normal glucose tolerance (NGT). CHO disposal was also lower during insulin stimulation under resting conditions in obese children compared to lean children (Robinson et al., 1998). These data suggest that, at least under resting conditions, CHO availability in obese children with IGT may be altered due to impaired CHO disposal to skeletal muscle.

A better understanding of CHO_{exo} oxidative efficiency in overweight and obese children under dynamic conditions, such as exercise, may help elucidate the role of skeletal muscle and substrate metabolism on the pathogenesis of IR, IGT and risk for type 2 diabetes. Over the past two decades, CHO_{exo} oxidation rate and CHO_{exo} oxidative efficiency, defined as the CHO_{exo} oxidized divided by the total CHO_{exo} ingested, have been explored by utilizing a ^{13}C-glucose stable isotope technique that quantifies the amount of orally-ingested CHO oxidized for energy (Chu et al., 2011; Riddell, Bar-Or, Hollidge-Horvat, et al., 2000; Timmons

et al., 2003, 2007a; Timmons, Bar-Or, & Riddell, 2007b). However, no studies have investigated CHO_{exo} oxidative efficiency during exercise in children with IGT or type 2 diabetes. Studies looking at CHO_{exo} oxidation in individuals with type 1 diabetes provide some insight and evidence for impaired CHO_{exo} oxidative efficiency (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000). Despite elevated insulin levels, the contribution of CHO_{exo} oxidation to total energy expenditure during 60 min of exercise was lower in boys with type 1 diabetes than boys without diabetes (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000). While IR appeared to affect CHO_{exo} oxidative efficiency in boys with type 1 diabetes (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000), findings reported in adults with type 1 diabetes compared to healthy controls showed no difference in CHO_{exo} oxidation during exercise (Krzentowski et al., 1981; Robitaille et al., 2007).

The primary aim of our study was to examine CHO_{exo} oxidative efficiency during exercise in obese children with IGT and NGT. We hypothesized that children with IGT would have reduced CHO_{exo} oxidative efficiency during exercise (CHO_{exo} utilization divided by total CHO_{exo} availability) compared to children with NGT. Secondary objectives included investigating the contribution of CHO_{exo}, endogenous CHO and total fat to total energy expenditure during exercise and comparing measures of insulin sensitivity and beta-cell function between groups.

3.3 METHODS

Participants. Children ages 8-17 years old were invited to participate in the study, which was approved by the Hamilton integrated Research Ethics Board. Participants were recruited from the Children's Exercise and Nutrition Centre at the McMaster Children's Hospital among children who were referred to have an oral glucose tolerance test (OGTT) or who had recently completed an OGTT. Children were excluded if they had a known motor delay or physical disability that would interfere with completing the exercise sessions, or if they were taking medications that were thought to influence insulin sensitivity, such as Metformin. Overweight was defined as ≥85th body mass index (BMI) for age percentile according to Centers for Disease Control and Prevention (CDC) cut-points. Based on the Canadian Diabetes Association guidelines, children were identified as NGT or IGT based on their OGTT results if 2-h glucose concentrations were <7.8 mmol/L or 7.8-11.0 mmol/L and/or fasting glucose concentrations were <6.1 mmol/L or 6.1-6.9 mmol/L, respectively (Goldenberg & Punthakee, 2013). Written informed parental consent and child assent were obtained from all families.

Preliminary Visit. Participants were asked to attend 2 study visits. During the preliminary visit, the OGTT was performed first. Participants were asked to fast overnight for at least 10 h. The OGTT was followed by anthropometric and maturity assessments, including weight, standing height, and sitting height, and a maximal aerobic fitness ($\dot{V}O_{2max}$) test. For participants who had a recent OGTT within the past 3 mo, the preliminary visit started with anthropometric

assessments. Body composition was assessed using bioelectrical impedance analysis (Inbody 520, Biospace Co., Seoul, Korea). BMI percentile for age was calculated and years from peak height velocity were estimated using equations provided by Mirwald et al. (2002). Self-assessment of sexual maturation was completed using Tanner staging, where the child and parent (if needed) looked at pictures for pubic hair development in boys or breast development in girls and identified which stage resembled the child's development. $\dot{V}O_{2max}$ was determined on a cycle ergometer (Corival Lode, Groningen, The Netherlands) using a progressive, continuous exercise protocol to maximal exertion. $\dot{V}O_{2max}$ was reached when 2 of the following criteria were met: i) heart rate (HR) ≥ 185 beats; ii) respiratory exchange ratio ≥ 1.1; and/or iii) an inability to maintain a cadence of 60 rpm despite verbal encouragement. The highest 30-s average for oxygen uptake ($\dot{V}O_2$) was considered the $\dot{V}O_{2max}$. HR was monitored continuously during the test (Polar Electro Oy, Kempele, Finland). $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) were measured using a metabolic cart (Vmax29, SensorMedics, Palm Springs, CA, USA). The preliminary visit was completed ~5 days before the experimental visit.

Experimental Visit. During the second visit, CHO_{exo} oxidative efficiency during exercise was assessed. Participants were asked to avoid corn or corn-derived products during the week leading up to the experimental visit to reduce background enrichment of expired CO_2 from naturally derived ^{13}C (Schoeller, Klein, Watkins, Heim, & MacLean, 1980). On the day of the visit, participants did

not consume any food at least 3 hours before the session. Breath was sampled for 3 min using a mouthpiece and hose system connected to the metabolic cart. For later analysis of $^{13}C/^{12}C$ ratio, duplicate samples of expired air (10 mL) were stored in Exetainer tubes (Labco Exetainer, Lampeter, Ceredigion, UK). An initial resting breath sample was taken before participants ingested the ^{13}C-enriched CHO drink. After a 30 min rest period, another resting breath sample was collected before the start of 60 min of exercise (3 × 20 min bouts with 5 min breaks) at 45% $\dot{V}O_{2max}$. Breath samples were collected at 10 min and 20 min of each exercise bout.

Experimental Beverage and Substrate Utilization. Participants ingested 1.75 g of glucose per kg of body mass up to a maximum of 75 g of glucose in 300 mL of water. The experimental beverage was enriched with uniformly labelled ^{13}C-glucose (D-glucose, [U-13]C6, 99%, Cambridge Isotope Laboratories, Inc., Tewksbury, MA) to a composition of +100‰ [$\delta^{13}C$]PDB-1, where [$\delta^{13}C$]PDB-1 is the Pee Dee Belemnitella-1 international standard. Breath samples were collected to calculate CHO_{exo} oxidation and percent contribution of CHO_{exo}, endogenous CHO (CHO_{endo}), and total fat to total energy expenditure during exercise.

Total CHO oxidation rate and total fat oxidation rate were calculated using the following equations (Péronnet & Massicotte, 1991)

$$\text{total CHO oxidation rate (g/min)} = 4.59 \cdot \dot{V}CO_2 \text{ (l/min)} - 3.23 \cdot \dot{V}O_2 \text{ (l/min)}$$

$$\text{total fat oxidation rate (g/min)} = -1.70 \cdot \dot{V}CO_2 \text{ (l/min)} + 1.69 \cdot \dot{V}O_2 \text{ (l/min)}$$

The ratio of $^{13}C/^{12}C$ in expired CO_2 was determined by Metabolic Solutions, Inc (Nashua, NH) using a Europa Scientific 20/20 gas isotope ratio mass spectrometer (Europa Scientific, Cincinnati, OH).

CHO_{exo} oxidation rate was calculated using the following equation modified from Mosora et al. (1976)

$$CHO_{exo} \text{ oxidation rate (g/min)} = \dot{V}CO_2 [(R_{exp} - R_{ref})/(R_{exo} - R_{ref})] (1/k)$$

where $\dot{V}CO_2$ is in liters per minute STPD, R_{exp} is the ^{13}C-enrichment of expired CO_2 during exercise, R_{ref} is the ^{13}C-enrichment of expired CO_2 at rest before CHO_{exo} ingestion, R_{exo} is the ^{13}C-enrichment of CHO_{exo} in the drink, and k (0.7426 l/g) is the volume of CO_2 produced by complete oxidation of 1 g of glucose (Péronnet & Massicotte, 1991). CHO_{endo} was determined as the difference between total CHO oxidation rate and CHO_{exo}.

To calculate CHO_{exo} oxidative efficiency, we determined area under the curve (AUC) for CHO_{exo} using the trapezoidal method. All participants received 75 g of CHO before exercise. Therefore,

$$CHO_{exo} \text{ oxidative efficiency (\%)} = [\text{AUC } CHO_{exo} \text{ (g)}/75 \text{ g } CHO_{exo}] * 100$$

In order to normalize CHO_{exo} for fat free mass (FFM), AUC CHO_{exo} (g) was divided by FFM and multiplied by 1,000 to calculate AUC CHO_{exo} relative to FFM

in mg/kg FFM. Rest breaks between exercise bouts were omitted when calculating AUC CHO_{exo}.

Blood Analysis. During the OGTT, blood was drawn at baseline and at 30, 60, 90, and 120 min after consumption of a 75 g CHO drink (Glucodex, Rougier Pharma, Mirabel, Quebec, Canada), according to standard clinical procedures. Serum samples were analyzed for glucose using a colourimetric assay kit (cat. no. 10009582, Cayman Chemical, Ann Arbor, Michigan, USA) and insulin using a high sensitivity ELISA kit (KAQ1251, Invitrogen, Burlington, Ontario, Canada). The calculated intra-assay coefficient of variation was 3.2 and 6.8 for glucose and insulin, respectively. AUC glucose (AUCgluc) and AUC insulin (AUCins) were determined from the OGTT using the trapezoidal method. To maintain sample size, fasting glucose and insulin measured on the same day by the McMaster Core Facility (McMaster Children's Hospital) were used for 4 participants. Results were unchanged when these participants were removed from the analysis. Homeostasis model assessment of insulin resistance (HOMA-IR) and whole body insulin sensitivity index (WBISI) using the Matsuda index (Matsuda & DeFronzo, 1999) were also derived from glucose and insulin concentrations. An oral disposition index was calculated using the insulin secretion-sensitivity index-2 (ISSI-2), which is defined as the ratio of AUCins to AUCgluc multiplied by WBISI to provide a measurement of beta-cell function (Retnakaran et al., 2009).

*Statistical Analysis***.** All values are expressed as means ± SD. Variables were checked for normality using the Shapiro-Wilk test (IBM SPSS Statistics,

version 20). Non-parametric data were log-transformed and normality was re-assessed. Independent t-tests were used to compare CHO_{exo} oxidative efficiency and insulin sensitivity measurements between groups. If data did not achieve normality after log-transformation, the Mann-Whitney *U*-test was used to compare variables between groups. Two-way ANOVA was used to test for group, time, and group x time interaction effects (PRISM 5, GraphPad Software). Statistical significance was set at p value ≤ 0.05.

3.4 **RESULTS**

Twelve children with IGT and twenty-two children with NGT participated. Two hour glucose concentrations during the OGTT were 8.8 ± 0.8 mmol/L in the IGT group and 5.7 ± 1.3 mmol/L in the NGT group. Participant characteristics are shown in **Table 3.1**. No differences between groups were found for percent body fat (p=0.42) or $\dot{V}O_{2max}$ (p=0.32), which was 20.7 ± 3.6 ml/kg/min and 22.2 ± 4.4 ml/kg/min in IGT and NGT, respectively. In addition, no significant differences were found for participant characteristics between boys and girls, except for height which was greater in boys compared to girls (p=0.01). To evaluate insulin sensitivity, we calculated HOMA-IR, WBISI and AUCins derived from insulin and glucose concentrations determined from an OGTT. In the NGT group, 2 children were excluded due to missing time points during the OGTT. No children had to be excluded in the IGT group. HOMA-IR tended (p=0.06) to be higher in the IGT group (8.8 ± 3.7) compared to the NGT group (6.5 ± 2.7). WBISI and AUCins

values are shown in **Figure 3.1**. To test statistical difference between groups both WBISI and AUCins were log-transformed. The oral disposition index assessed as ISSI-2 was lower in children with IGT (508 ± 141) compared to children with NGT (759 ± 304) (p=0.004; Mann-Whitney U-test).

Table 3.1 Participant characteristics

	NGT (n=22)	IGT (n=12)
Boys/Girls	11/11	3/9
Tanner I/II/III/IV/V	0/7/5/2/8	0/2/3/5/2
Age (years)	14.4 ± 2.12	15.05 ± 2.34
Height (cm)	165.7 ± 10.2	161.3 ± 11.8
Weight (kg)	95.1 ± 27.1	94.8 ± 26.4
YPHV (years)	1.74 ± 1.82	2.30 ± 1.43
BMI percentile	98.6 ± 1.4	99.1 ± 0.6
Percent body fat (%)	42.8 ± 6.3	44.4 ± 4.1
Fat free mass (kg)	53.3 ± 11.3	52.7 ± 15.7

Data are presented as means ± SD. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; BMI, body mass index; YPHV, years from peak height velocity. No significant differences were identified between groups using independent t-tests.

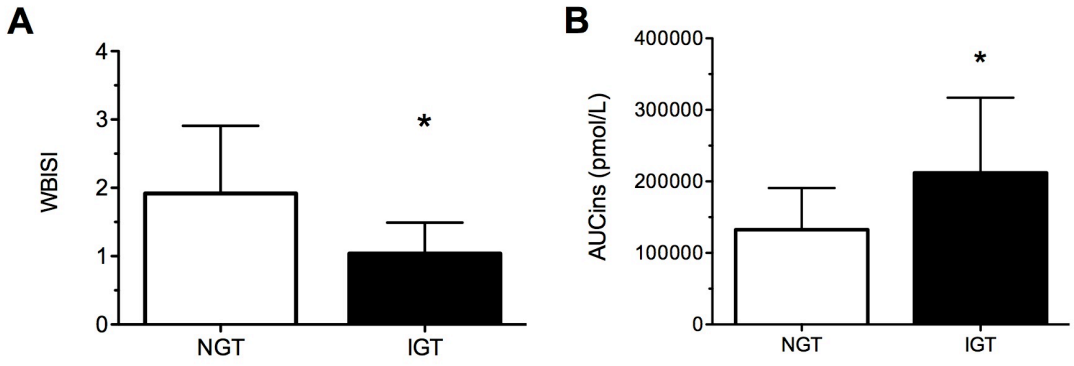

Figure 3.1 A) whole body insulin sensitivity index (WBISI) in children with normal glucose tolerance (NGT) or impaired glucose tolerance (IGT). **B)** area under the curve insulin concentrations measured at rest (AUCins) in children with NGT or IGT. Data are presented as means ± SD. *Significantly different than NGT, $p<0.05$.

To assess CHO_{exo} oxidative efficiency at visit 2, the exercise intensity was set at 45% $\dot{V}O_{2max}$, which corresponded to a workload of 45 ± 17 Watts and 47 ± 14 Watts in the IGT and NGT group, respectively ($p=0.64$). There were no differences in CHO_{exo} oxidative efficiency or AUC CHO_{exo} relative to FFM in children with NGT compared to children with IGT (**Figure 3.2**). Secondary analyses assessing the energy contribution of CHO_{exo}, CHO_{endo} and total fat to total energy expenditure also showed no differences between groups (**Figure 3.3**).

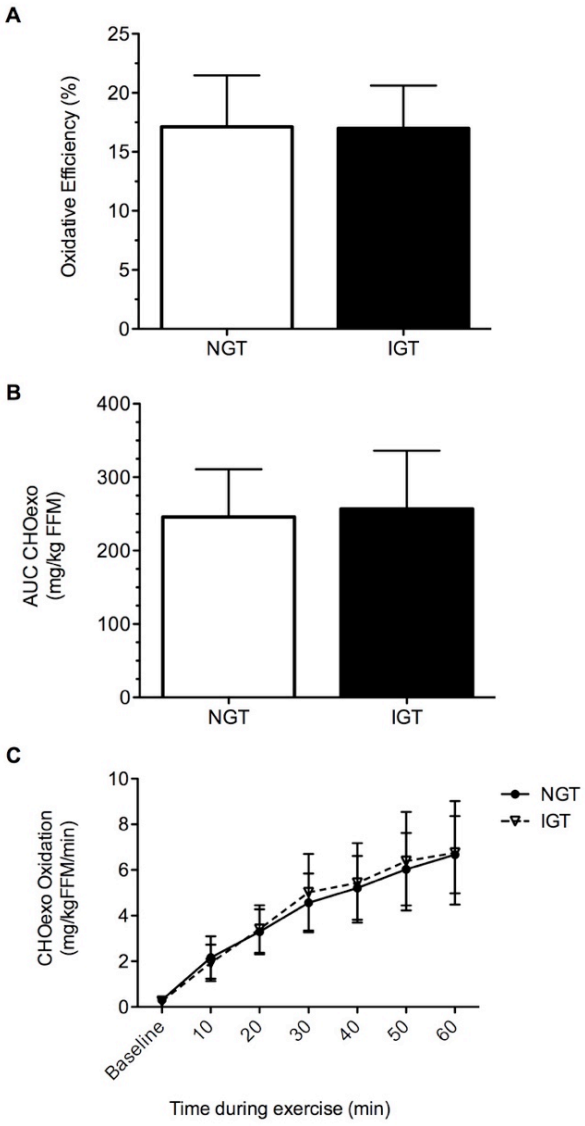

Figure 3.2 A) oxidative efficiency of exogenous carbohydrate (CHO_{exo}) of children with normal glucose tolerance (NGT) or impaired glucose tolerance (IGT). **B)** area under the curve CHO_{exo} utilization (AUC CHO_{exo}) normalized to kg of fat free mass (FFM) in children with NGT and IGT. **C)** CHO_{exo} oxidation over 60 min of exercise in children with NGT (closed circles) and IGT (open triangles). Data are presented as means ± SD. Independent t-tests showed no significant difference between groups in *A* (p=0.93) or *B* (p=0.66). Two-way ANOVA showed a significant time effect (p<0.01) and no significant group effect (p=0.46) or group x time interaction effect (p=0.96) in *C*.

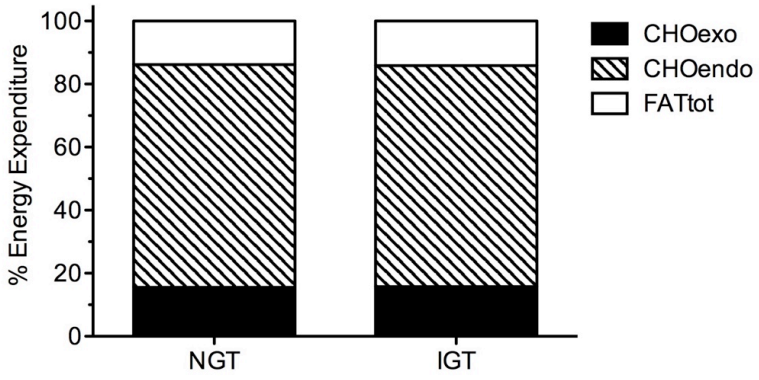

Figure 3.3 Contribution of exogenous carbohydrate (CHO_{exo}), endogenous carbohydrate (CHO_{endo}) and total fat (FAT_{tot}) oxidation to total energy expenditure during 60 min of exercise in children with NGT or IGT. Independent t-tests showed no significant differences between groups.

3.5 DISCUSSION

The main finding of this study was that CHO_{exo} oxidative efficiency during exercise was not significantly different in obese children with IGT compared to obese children with NGT, despite a 39% lower WBISI and 33% lower ISSI-2 in the IGT group. This finding suggests that either exercise overrides impairments in CHO_{exo} uptake and oxidation due to IR in children or that CHO_{exo} oxidation is not yet impaired in these children compared to children without IR. Elevated insulin secretion may have helped compensate for reduced insulin sensitivity and reduced beta-cell function. In support of this, AUCins at rest was higher in the IGT group compared to the NGT group. Greater insulin secretion in response to CHO_{exo} could have sustained CHO uptake into skeletal muscle and helped maintain the availability of CHO_{exo} for oxidation in the IGT group.

This is the first study to measure CHO_{exo} oxidative efficiency during exercise in obese children with IGT. We believe it is an important area of research, especially in light of reduced CHO_{exo} oxidative efficiency in children with type 1 diabetes compared with children without type 1 diabetes (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000). However, studies in adults with type 1 diabetes did not report similar findings (Krzentowski et al., 1981; Robitaille et al., 2007). In our study population, CHO_{exo} oxidative efficiency [17.0 ± 3.6% (IGT) and 17.1 ± 4.4% (NGT)] is comparable to values in healthy boys (16.2 ± 0.8%) and higher than values in boys with type 1 diabetes (12.1 ± 1.3%) reported by Riddell and colleagues (2000). These authors also showed the contribution of CHO_{exo} to total energy expenditure was lower in boys with type 1 diabetes (9.1 ± 1.0%) compared to the control group (12.4 ± 0.5%) (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000). Comparing these data to our results (**Figures 3.2 and 3.3**), it is possible that obese children utilize at least similar amounts of CHO_{exo} as non-obese children under exercise conditions due to endocrine-related compensatory mechanisms at rest before exercise, which increase CHO_{exo} availability in skeletal muscle. This was supported by no difference in CHO_{exo} oxidation at baseline between groups, which was measured 30 min after drink consumption before exercise (**Figure 3.2C**). Another explanation is that non-insulin mediated mechanism(s) during exercise, such as contraction mediated CHO uptake, is not impaired during exercise in obese children with or without IGT. We suggest that CHO_{exo} oxidative efficiency during exercise only becomes impaired when insulin

secretion can no longer maintain CHO disposal in the presence of declining insulin sensitivity (beta-cell dysfunction) and/or non-insulin mediated CHO uptake during exercise is disrupted. Furthermore, the underlying mechanism(s) that impairs CHO_{exo} oxidation may be different in children with type 1 diabetes than in children at risk for type 2 diabetes.

Although we cannot entirely disregard the possible effect of adiposity on CHO_{exo} oxidation at rest (Rueda-Maza, Maffeis, Zaffanello, & Schutz, 1996) or during exercise (Ravussin et al., 1980), evidence suggests a stronger influence of glucose tolerance and insulin sensitivity on substrate selection and utilization (Braun, Sharoff, Chipkin, & Beaudoin, 2004; Prior, Ryan, Stevenson, & Goldberg, 2014). Prior et al. (2014) reported lower CHO oxidation during submaximal exercise in obese, older adults with IGT compared to obese, older adults with NGT. Similarly, CHO oxidation during exercise at 45% $\dot{V}O_{2max}$, was lower in overweight women with low insulin sensitivity (WBISI < 4.0) compared to women with high insulin sensitivity (WBISI > 7) (Braun et al., 2004). In contrast to these studies involving adults, we did not find a significant difference in total CHO oxidation in obese children with IGT compared to obese children with NGT; however, we provided CHO_{exo} before exercise which can alter substrate selection (Chu et al., 2011). Clearly, more work is needed to assess CHO_{exo} oxidation during exercise in obese children and the role of IR.

Impaired MetFlex is strongly linked to the pathogenesis of IR and type 2 diabetes in adults (Galgani, Moro, et al., 2008). We propose that our study design

provides a novel, non-invasive way to assess MetFlex in children. Galgani and colleagues (2008) proposed that MetFlex should be examined under exercise conditions, because of the greater demand for energy during exercise compared to at rest. Past studies have demonstrated MetFlex at rest by observing the ability of skeletal muscle to switch between predominantly fat oxidation during fasting conditions to suppressed fat oxidation and predominantly CHO oxidation under insulin-stimulated conditions, such as after a meal (Kelley & Mandarino, 2000). Based on this method, MetFlex is assessed according to three components: a stressor, a regulator and an effector. In our study protocol, the stressor was CHO_{exo} ingestion (i.e., substrate availability) before exercise and the effector was CHO_{exo} oxidation (i.e., substrate utilization) during 60 min of submaximal exercise. However, we do not have the complete MetFlex profile because a regulator (i.e., insulin) was not directly measured before or during the exercise test. Based on insulin measured at rest, we speculate that CHO_{exo} oxidative efficiency during exercise was sustained by endocrine compensatory responses before exercise or by non-insulin mediated mechanisms associated with exercise in children with IGT. However, future research is necessary to confirm the role of insulin with MetFlex under exercise conditions.

Prior studies assessing MetFlex in children showed decreased CHO disposal at rest during insulin stimulation in obese children with IGT compared to obese children with NGT (Weiss et al., 2003), as well as in obese children compared to lean children (Robinson et al., 1998). CHO oxidation with insulin-

stimulation was also decreased in IGT versus NGT under resting conditions (Weiss et al., 2003). The major differences in these studies compared to the current study include the manipulation of insulin concentrations via a hyperinsulinimic-euglycemic clamp and the absence of exercise-induced insulin sensitivity. In theory, poor CHO disposal at rest may result in decreased muscle glycogen availability and use for exercise and a greater dependence on plasma glucose (Colberg et al., 1996) or CHO_{exo} when available. Our data showed no difference in CHO_{exo} oxidation during exercise between children with NGT and IGT, therefore suggesting children with IGT continue to rely on CHO_{exo} as an energy source, which is likely maintained by non-insulin mediated mechanisms during exercise. If there was an inability to utilize available CHO_{exo}, there could be impaired MetFlex and a switch in fuel selection to utilize and rely on more lipids instead of CHO for energy. We did not find inter-group differences in fat oxidation in the current study. A longitudinal study is warranted to observe changes in CHO_{exo} oxidative efficiency during exercise in normal weight, overweight and obese children with varying degrees of IR and glucose tolerance.

Our study findings should be considered in light of a few limitations. Sex, puberty and menstrual cycle phase may affect endogenous substrate oxidation during exercise (Devries, Hamadeh, Phillips, & Tarnopolsky, 2006; Michael C. Riddell, 2008; Timmons et al., 2003, 2007a), but very little is known about how these factors influence CHO_{exo} oxidative efficiency. It was not a primary objective of this study to compare boys and girls, and final numbers of boys and girls in the

IGT and NGT groups; however, we found similar results for IGT compared to NGT when the analysis was completed in the girls only. Unfortunately, because of the small number of boys with IGT, we could not look at this in the boys. Information on puberty and menstrual cycle phase was recorded when available. However, it was a challenge to control for these factors since most of the girls in this study experienced irregular cycles, which was not surprising given their stage of maturation. Nonetheless, a key strength of our study is using a non-invasive, feasible, and dynamic method to assess CHO_{exo} oxidative efficiency in obese children with IGT. No studies in either adults or children have examined CHO_{exo} oxidation during exercise have also measured insulin sensitivity and beta-cell function.

In conclusion, obese children with IGT maintained CHO_{exo} oxidative efficiency during exercise as well as obese children with NGT, despite lower WBISI and ISSI-2. To confirm the possible role of a compensatory insulin response in obese children with IGT, future work should compare CHO_{exo} oxidative efficiency during exercise in children with IGT and a reduced insulin response and secretion (beta-cell dysfunction) compared to a control group with normal or elevated insulin response and secretion (beta-cell compensation). This would help elucidate when impaired CHO_{exo} oxidative efficiency may have a critical influence and contribution to the pathogenesis of IR and type 2 diabetes. An alternative explanation for the results is that exercise overrides impaired CHO_{exo} oxidative efficiency, because of both the insulin dependent and non-

insulin dependent mechanisms of CHO disposal which occur during exercise. However, we cannot identify the proportion of CHO uptake that is attributed to insulin dependent mechanisms compared to non-insulin dependent mechanisms with our methodology. Observing CHO_{exo} oxidative efficiency longitudinally at rest and during exercise to identify when CHO_{exo} oxidation is disrupted has clinical implications for screening and developing diabetes intervention strategies.

3.6 ACKNOWLEDGEMENTS

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CHAPTER 4

4 VALIDITY AND RELIABILITY OF A NOVEL METABOLIC FLEXIBILITY TEST IN CHILDREN WITH OBESITY

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Chu L, Morrison KM, Riddell MC, Raha S, Timmons BW. Validity and reliability of a novel metabolic flexibility test in children with obesity. *J Appl Physiol* submitted on January 31, 2017.

4.1 ABSTRACT

Purpose: Existing methods for diagnosing diabetes and for identifying risk of diabetes development are completed under resting conditions and based on adult data. Studying additional methods to identify metabolic risk in children is warranted. Our objective was to examine the validity and reliability of a metabolic flexibility (MetFlex) test for screening glycemia and insulin resistance (IR) in children. We hypothesized higher MetFlex during exercise would be correlated with lower fasting glucose and homeostasis model assessment of insulin resistance (HOMA-IR), and higher whole body insulin sensitivity index (WBISI) and beta-cell function (ISSI-2). **Methods:** Thirty-four children with obesity (14 boys, 20 girls) attended 2 visits. At visit 1, an OGTT was followed by anthropometric and aerobic fitness ($\dot{V}O_{2max}$) assessments. Insulin and glucose during the OGTT were used to calculate HOMA-IR, WBISI and ISSI-2. At visit 2,

a ^{13}C-enriched carbohydrate drink was ingested before 60 min of exercise at 45% $\dot{V}O_{2max}$. Breath measurements were collected to calculate area under the curve exogenous carbohydrate (AUC CHO_{exo}) to measure MetFlex. **Results:** Pearson's *r* correlation showed no significant association between MetFlex during exercise with fasting glucose (*r*=-0.288, *p*=0.110). MetFlex was associated with log-HOMA-IR (*r*=-0.597, *p*=0.024), log-WBISI (*r*=0.575, *p*=0.051) and log-ISSI-2 (*r*=0.605, *p*=0.037) in boys but not girls. When repeated (n=18), MetFlex was deemed a reliable test (intraclass correlation coefficient=0.692). **Conclusion:** MetFlex during exercise was negatively associated with IR and positively associated with beta-cell function in boys. Further research is required to explore clinical utility of the MetFlex test and explain the lack of association in girls.

4.2 INTRODUCTION

The high prevalence of childhood obesity has led to a concerning rise in childhood type 2 diabetes (T2DM), a condition once predominantly diagnosed in adults. Current clinical tests used to screen and diagnose T2DM include fasting glucose, 2-h glucose during an oral glucose tolerance test (OGTT) and/or glycated hemoglobin levels (Goldenberg & Punthakee, 2013), all of which are measured at rest. While cut-offs used for T2DM diagnosis have been developed in adults and predict risk for retinopathy (Engelgau et al., 1997; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003; McCance et al., 1994), similar studies have not yet been conducted in children, and this lack of

evidence-based correlation between biomarkers and risk may lead to inaccurate diagnosis. Additional limitations of a routine OGTT test are its poor reproducibility (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003; Libman et al., 2008) and invasive nature (blood sampling).

The development and validation of alternative screening tools to identify early metabolic risk factors associated with T2DM in children, such as hyperglycemia and IR, would be beneficial. Metabolic flexibility (MetFlex) refers to the ability of a system to adapt substrate oxidation with substrate availability (Galgani, Moro, et al., 2008). Impaired MetFlex and adaptation to substrate could contribute to or indicate increased ectopic fat accumulation and be associated with lipotoxic mechanisms (Apostolopoulou et al., 2016). Insulin plays an important role in the body's response to nutrients, and adults with obesity and IR have impaired MetFlex at rest compared to healthy adults (Galgani, Moro, et al., 2008). Prior studies have combined a hyperinsulinemic-euglycemic clamp with indirect calorimetry in order to study MetFlex under resting conditions (Apostolopoulou et al., 2016; Galgani, Moro, et al., 2008; Kelley et al., 1999). This method is more invasive than currently available tests and not ideal for clinical care. To date, a few studies have investigated MetFlex using ^{13}C-glucose breath tests under resting conditions (Ibarra-Pastrana et al., 2012; Jetha et al., 2009; Lewanczuk et al., 2004). Consequently, MetFlex can be studied using a technique that does not require blood work.

The biggest limitation of previous work assessing IR via MetFlex, as noted by Galgani and colleagues (2008), is that testing MetFlex under resting conditions requires a low demand for energy. Therefore, testing under exercise conditions may be more valuable. The concept of measuring MetFlex during exercise in children has been explored by utilizing a ^{13}C-glucose technique, which quantifies the amount of orally-ingested carbohydrate (CHO) oxidized for energy (Chu et al., 2016, 2011; Riddell, Bar-Or, Hollidge-Horvat, et al., 2000; Timmons et al., 2003, 2007a, 2007b). To our knowledge, no studies in adults or children have examined the association between MetFlex under exercise conditions with fasting glucose, which is one criterion used for diagnosing T2DM.

The primary objective of this study was to examine the validity and reliability of the MetFlex test during exercise for glycemia and IR screening in children at risk of developing T2DM. Validity was assessed by measuring exogenous CHO (CHO_{exo}) oxidative efficiency during exercise, as a novel and feasible measure of MetFlex in children with obesity, and determining the association between MetFlex during exercise and fasting glucose and measurements related to IR, including homeostasis model assessment of insulin resistance (HOMA-IR), whole body insulin sensitivity index (WBISI) and a surrogate measure of beta-cell function, insulin secretion-sensitivity index-2 (ISSI-2). Examination of the association between MetFlex and 2-h glucose during an OGTT was not included because of the poor reproducibility of 2-h glucose previously reported in children with obesity (Libman et al., 2008). The reliability of

the MetFlex test during exercise was evaluated by repeat testing on a separate day in a subgroup of participants.

We hypothesized that MetFlex during exercise would be inversely associated with fasting glucose and HOMA-IR, and directly associated with WBISI and ISSI-2. We also hypothesized that the reliability of the MetFlex test would be deemed good based on an intraclass correlation coefficient (ICC) of at least 0.60 (Cicchetti, 1994).

4.3 **METHODS**

Participants: Children ages 8-17 years old were recruited from the Children's Exercise and Nutrition Centre at McMaster Children's Hospital. As previously described (Chu et al., 2016), these children were referred to have an OGTT or recently completed an OGTT (within the last 3 months). Children were eligible to participate if they were ≥85th body mass index (BMI) for age percentile according to Centers for Disease Control and Prevention (CDC) cut-points. Exclusion criteria included a known motor delay or physical disability that would interfere with completing the exercise session, or if the child was taking medications thought to influence insulin sensitivity, such as Metformin. The study was approved by the Hamilton integrated Research Ethics Board and written informed parental consent and child assent were obtained from all families. With the same group of children, we previously reported no differences in MetFlex when children were separated by glucose tolerance status (Chu et al., 2016). By

investigating the association between MetFlex and fasting glucose and IR, the aim was to provide a clearer understanding of MetFlex during exercise because IR usually occurs before impaired glucose tolerance is detected.

Preliminary Visit: At the preliminary visit, an OGTT was performed in those participants who had not undergone a recent assessment for glucose tolerance. For this, participants were asked to fast overnight for at least 10 hours. Blood was drawn at baseline and at 30, 60, 90, and 120 min after consumption of a CHO drink (Glucodex, Rougier Pharma, Mirabel, Quebec, Canada) (1.75 g/kg up to a maximum of 75 g), according to standard clinical procedures. This procedure was followed by anthropometric (weight, standing height, sitting height) and maturity assessments. Weight was measured using a digital weight scale (BWB-800; Tanita Corporation, Tokyo, Japan), and standing height and sitting height were measured using a calibrated stadiometer (Harpenden Stadiometer 602-VR; Holtan Limited, Crymych, United Kingdom). An average of two measurements was used, and if the difference was greater than 0.4 kg or 0.4 cm, the measurement was repeated. Percent body fat was assessed using bioelectrical impedance analysis (Inbody 520, Biospace Co., Seoul, Korea). BMI percentile for age was calculated using the 2000 CDC growth charts (Ogden et al., 2002) and years from peak height velocity was estimated using equations provided by Mirwald et al. (2002). Self-assessment of sexual maturation was completed using Tanner staging (Tanner, 1962), where the child and parent (if needed) looked at pictures for pubic hair development in boys or breast development in girls and

identified which stage resembled the child's development . Maximal aerobic fitness ($\dot{V}O_{2max}$) was then determined on a cycle ergometer (Corival Lode, Groningen, The Netherlands) using a progressive, continuous exercise protocol to maximal exertion (Bar-Or O & Rowland, 2004). $\dot{V}O_{2max}$ was reached when 2 of the following criteria were met: i) heart rate (HR) ≥ 185 beats; ii) respiratory exchange ratio ≥ 1.1; and/or iii) an inability to maintain a cadence of 60 rpm despite verbal encouragement. The highest 30-sec average for oxygen uptake ($\dot{V}O_2$) was considered the $\dot{V}O_{2max}$. HR was monitored continuously during the test (Polar Electro Oy, Kempele, Finland). $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) were measured using a calibrated metabolic cart (Vmax29, SensorMedics, Palm Springs, CA, USA). The preliminary visit was completed approximately 5 days before the experimental visit.

Experimental Visit: At the second visit, MetFlex during exercise was assessed. Participants were asked to complete a 24-h food and physical activity diary, so that we could ensure strenuous physical activity on the day before and day of the visit was avoided, and food was not consumed at least 3 hours before the session. Corn and corn-derived products were avoided during the week leading up to the experimental visit to reduce background enrichment of expired CO_2 from naturally derived ^{13}C (Schoeller et al., 1980). Breath was sampled for 3 min using a mouthpiece and hose system connected to the metabolic cart. For later analysis of $^{13}C/^{12}C$ ratio, duplicate samples of expired air (10 mL) were stored in Exetainer tubes (Labco Exetainer, Lampeter, Ceredigion, UK). An initial

resting breath sample was taken before participants ingested the ^{13}C-enriched CHO drink. After a 30 min rest period, another resting breath sample was collected before the start of 60 min of exercise (3 × 20 min bouts with 5 min breaks) at 45% $\dot{V}O_{2max}$. Breath samples were collected at 10 min and 20 min of each exercise bout.

Reliability of MetFlex: In a subgroup of participants, the experimental visit was repeated at least 5 days later to assess the reliability of measuring MetFlex during exercise.

Experimental Beverage and Substrate Utilization: Participants ingested 1.75 g of glucose per kg of body mass up to a maximum of 75 g of glucose in 300 mL of water. The experimental beverage was enriched with uniformly labelled ^{13}C-glucose (D-glucose, U-13C6, 99%, Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA) to a composition of +100‰ [$\delta^{13}C$]PDB-1, where [^{13}C]PDB-1 is the Pee Dee Belemnitella-1 international standard. Breath samples were collected to calculate CHO_{exo} oxidation rate.

The ratio of $^{13}C/^{12}C$ in expired CO_2 was determined by Metabolic Solutions, Inc (Nashua, NH, USA) using a Europa Scientific 20/20 gas isotope ratio mass spectrometer (Europa Scientific, Cincinnati, OH). CHO_{exo} oxidation rate was calculated using the following equation modified from Mosora et al. (1976)

$$CHO_{exo} \text{ oxidation rate (g/min)} = \dot{V}CO_2 \left[(R_{exp} - R_{ref})/(R_{exo} - R_{ref})\right] (1/k)$$

Where $\dot{V}CO_2$ is in liters per minute STPD, R_{exp} is the ^{13}C-enrichment of expired CO_2 during exercise, R_{ref} is the ^{13}C-enrichment of expired CO_2 at rest before CHO_{exo} ingestion, R_{exo} is the ^{13}C-enrichment of CHO_{exo} in the drink, and k (0.7426 l/g) is the volume of CO_2 produced by complete oxidation of 1 g of glucose (Péronnet & Massicotte, 1991).

MetFlex: To calculate CHO_{exo} oxidative efficiency, we determined area under the curve (AUC) for CHO_{exo} using the trapezoidal method. All participants received 75 g of CHO before exercise. In order to normalize CHO_{exo} for fat free mass (FFM), AUC CHO_{exo} (g) was divided by FFM and multiplied by 1000 to calculate AUC CHO_{exo} relative to FFM in mg/kg of FFM. Rest breaks between exercise bouts were omitted when calculating AUC CHO_{exo}.

Blood Analysis: Serum samples collected during the OGTT were analyzed for glucose using a colourimetric assay kit (Cat. No. 10009582, Cayman Chemical, Ann Arbor, Michigan, USA) and insulin using a high sensitivity enzyme-linked immunosorbent assay (ELISA) kit (KAQ1251, Invitrogen, Burlington, Ontario, Canada). The calculated intra-assay coefficient of variation was 3.2 and 6.8 for glucose and insulin, respectively. To maintain sample size, fasting glucose and insulin measured on the same day via ARCHITECT Glucose assay (Ref. 3L82-21 and 3L82-41, Abbott Laboratories, Limited, Saint-Laurent, Québec, Canada) and ARCHITECT Insulin Reagent Kit (Ref. 8K41, Abbott Laboratories, Limited, Saint-Laurent, Québec, Canada) by the McMaster Core Facility (McMaster Children's Hospital) were used for 4 participants. HOMA-IR

and WBISI using the Matsuda index (Matsuda & DeFronzo, 1999) were also derived from glucose and insulin concentrations. An oral disposition index was calculated using ISSI-2, which is defined as the ratio of AUCins to AUCgluc multiplied by WBISI to provide a measurement of beta-cell function (R. Retnakaran et al., 2009).

Statistical Analysis: All values are expressed as means ± SD. Variables were checked for normality using the Shapiro-Wilk test (IBM SPSS Statistics, version 20). Non-parametric data were log-transformed and normality was re-assessed. Pearson's *r* correlations were used to test the associations between MetFlex and fasting glucose and IR measurements. ICC (model: two-way mixed; absolute agreement; single measures) (Hallgren, 2012) was used to assess reliability of the MetFlex test during exercise. Confidence intervals were reported where applicable. Statistical significance was set at p value ≤ 0.05.

4.4 RESULTS

Fourteen boys and twenty girls participated. Although we planned to recruit children identified as overweight or obese, the majority of the participants were higher than the 95th BMI-for-age percentile, and only one child was slightly below (93.7th BMI-for-age percentile). Participant characteristics are shown in **Table 4.1**. There were no significant differences between boys and girls except for height (p=0.01). Fasting blood glucose concentrations and 2-h glucose concentrations during the OGTT were 4.8 ± 0.6 mmol/L and 6.9 ± 1.9 mmol/L,

respectively. In the group, 22 children had normal glucose tolerance and 12 children had impaired glucose tolerance according to Canadian Diabetes Association guidelines (Goldenberg & Punthakee, 2013). $\dot{V}O_{2max}$ was similar between boys and girls (22.4 ± 5.3 ml/kg/min and 21.4 ± 3.3 ml/kg/min, respectively, p=0.5).

Table 4.1 Participant characteristics

	Boys (n=14)	Girls (n=20)
Tanner I/II/III/IV/V	0/4/4/2/3	0/4/4/5/7
Age (years)	14.5 ± 2.2	14.8 ± 2.2
Height (cm)*	169.6 ± 11.9	160.4 ± 8.3
Weight (kg)	102.4 ± 31.6	89.8 ± 21.6
BMI-for-age percentile	98.7 ± 1.6	98.9 ± 0.9
YPHV	1.33 ± 1.94	2.37± 1.38
Percent body fat	42.1 ± 6.9	44.3 ± 4.5
Fat free mass (kg)	58.0 ± 14.2	49.6 ± 10.8
HOMA-IR	6.4 ± 3.8	7.2 ± 2.8
WBISI**	1.9 ± 1.0	1.5 ± 0.9
ISSI-2**	649.48 ± 280.85	677.68 ± 294.67

Data are presented as mean ± SD. BMI, body mass index; YPHV, years from peak height velocity; HOMA-IR, homeostasis model assessment of insulin resistance; WBISI, whole body insulin sensitivity index; ISSI-2, insulin secretion-sensitivity index-2. *Significant difference between groups using independent t-tests (p≤0.05). **mean ± SD reported in 12 boys and 19 girls.

At visit 2, the intensity of exercise was set at 45% $\dot{V}O_{2max}$ for the MetFlex test, which corresponded to a workload of 46 ± 15 Watts. In contrast to our hypothesis, MetFlex during exercise, measured by AUC CHO_{exo} (mg/kg FFM), was not significantly associated with fasting glucose (r=-0.288, p=0.110).

However, CHO_{exo} oxidation (mg/kg FFM/min) measured at rest before exercise was negatively associated with fasting glucose (r=-0.45, p= 0.010). Data for CHO_{exo} oxidation at rest was log-transformed for all correlations.

In the entire group, MetFlex was not significantly correlated with log-HOMA-IR (r=-0.265, p=0.131), log-WBISI (r=0.137, p=0.454) or log-ISSI-2 (r=0.345, p= 0.053). At rest, CHO_{exo} oxidation (mg/kg FFM/min) was negatively correlated with log-HOMA-IR (r=-0.425, p=0.013) and positively correlated with log-ISSI-2 (r=0.444, p=0.011), but was not related to log-WBISI (r=0.161, p=0.454). Secondary analyses examining correlations for MetFlex during exercise and log-HOMA-IR, log-WBISI and log-ISSI-2 in boys and girls separately are shown in **Figure 4.1**.

In a subgroup of participants (**Table 4.2**), MetFlex during exercise was repeated to assess individual reliability of the measurement. Independent t-tests showed no differences in standard anthropometric measurements between the children who were included in the reliability analysis compared to those who were not (data not shown). The reliability of MetFlex during exercise was deemed good, as shown in **Table 4.3**. CHO_{exo} oxidation rates at rest and at 30 min and 60 min of exercise collected on two separate days (**Figure 4.2**) were also reliable, as shown in **Table 4.3**.

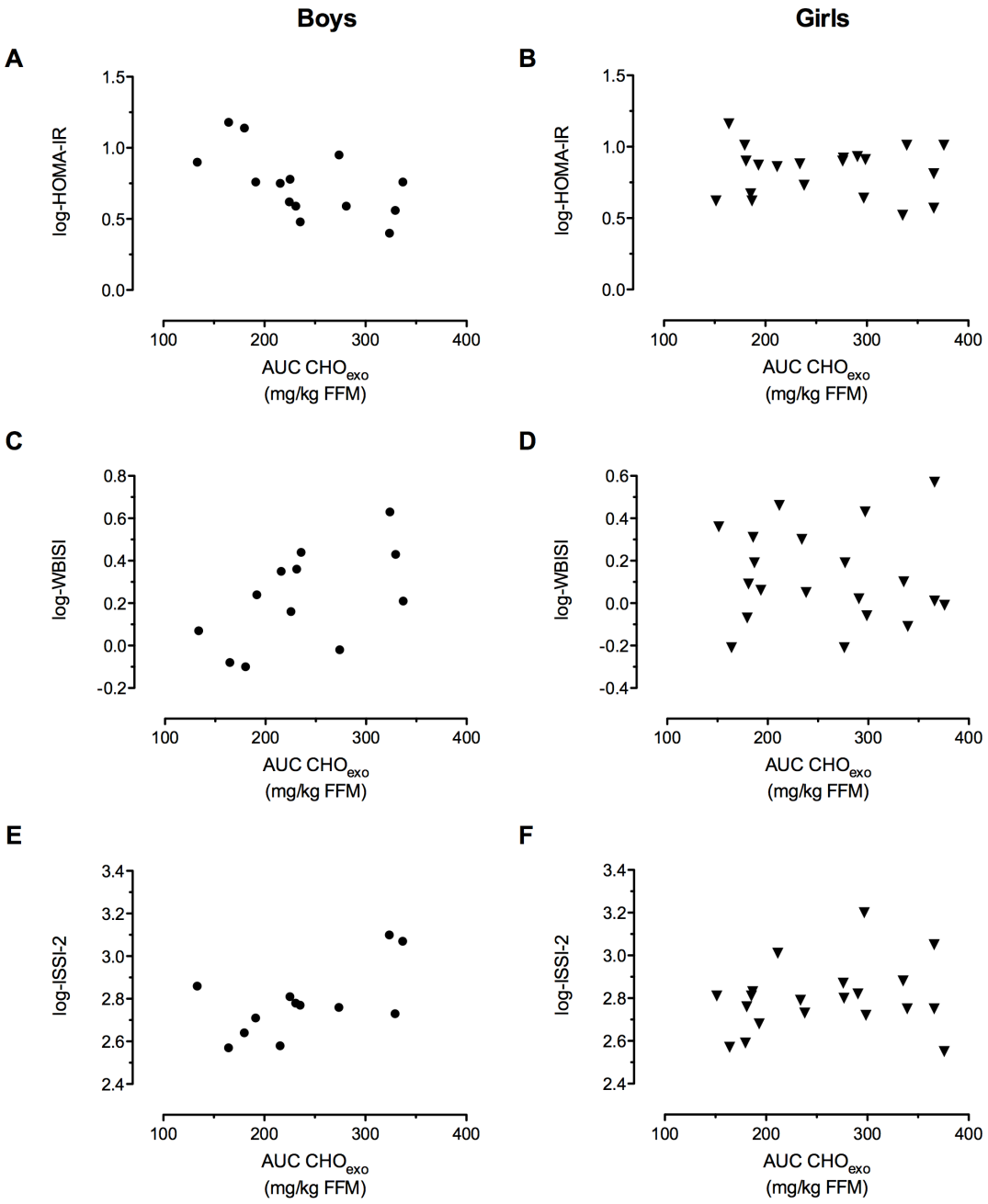

Figure 4.1 Correlations for area under the curve exogenous carbohydrate oxidation (AUC CHO_{exo}) and log-HOMA-IR in boys (A: r=-0.597, p=0.024) and girls (B: r=-0.082, p=0.731), log-WBISI in boys (C: r=0.575, p=0.051) and girls (D: r=-0.042, p=0.862), and log-ISSI-2 in boys (E: r=0.605, p=0.037) and girls (F: r=0.197, p=0.404).

Table 4.2 Participant characteristics in subgroup of children in reliability analysis

	Boys (n=7)	Girls (n=11)
Tanner I/II/III/IV/V	0/2/3/2/0	0/2/3/4/2
Age (years)	14.2 ± 3.1	14.7 ± 2.0
Height (cm)	166.6 ± 10.8	159.4 ± 8.2
Weight (kg)	96.0 ± 25.1	82.7 ± 17.0
BMI-for-age percentile	98.5 ± 2.2	98.5 ± 1.1
YPHV	1.33 ± 1.94	2.37± 1.38
Percent body fat	40.2 ± 7.9	43.2 ± 4.7
Fat free mass (kg)	56.6 ± 13.7	46.6 ± 8.0
$\dot{V}O_{2max}$ (mg/kg/min)**	23.3 ± 5.3	22.6 ± 3.1
HOMA-IR	6.8 ± 5.3	6.4 ± 2.4
WBISI**	2.3 ± 1.3	1.7± 0.9
ISSI-2**	593.9 ± 333.0	679.8 ± 221.7

Data are presented as mean ± SD. BMI, body mass index; YPHV, years from peak height velocity; HOMA-IR, homeostasis model assessment of insulin resistance; WBISI, whole body insulin sensitivity index; ISSI-2, insulin secretion-sensitivity index-2. ** mean ± SD reported in 6 boys and 11 girls.

Table 4.3 Reliability of the metabolic flexibility test and exogenous carbohydrate oxidation at rest, and at 30 min and 60 min of exercise (n=18)

	Pearson's *r* coefficient	Intraclass correlation coefficient (95% CI)	Mean absolute difference
MetFlex reported as AUC CHO_{exo} (mg/kg FFM)	0.707 (p=0.001)	0.692 (0.35, 0.81)	8.2 mg/kg FFM
CHO_{exo} oxidation rate at rest (mg/kg FFM/min)	0.835 (p<0.001)	0.822 (0.59, 0.93)	0.016 mg/kg FFM/min
CHO_{exo} oxidation rate at 30 min of exercise (mg/kg FFM/min)	0.659 (p=0.003)	0.662 (0.30, 0.86)	0.162 mg/kg FFM/min
CHO_{exo} oxidation rate at 60 min of exercise (mg/kg FFM/min)	0.674 (p=0.002)	0.617 (0.24, 0.84)	0.397 mg/kg FFM/min

Intraclass correlation coefficient (ICC) using two-way mixed model with absolute agreement and single measures results. MetFlex, metabolic flexibility; AUC CHO_{exo}, area under the curve exogenous carbohydrate oxidation; CHO_{exo}, exogenous carbohydrate; FFM, fat free mass.

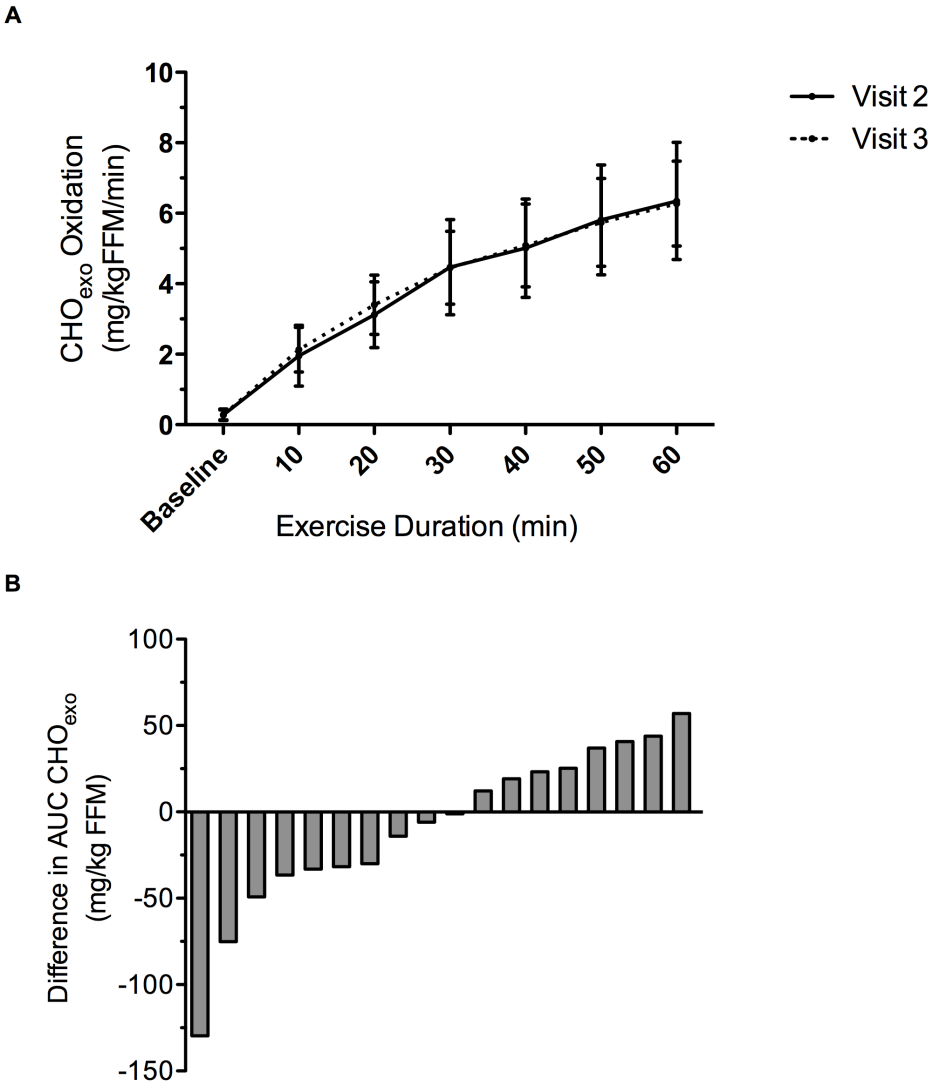

Figure 4.2 A) Exogenous carbohydrate (CHO_{exo}) oxidation rate normalized to fat free mass (FFM) over 60 min of exercise on visit 2 (solid line) and visit 3 (dotted line). Data are presented as mean ± SD. **B)** Difference in area under the curve CHO_{exo} (AUC CHO_{exo}) normalized to FFM measured on 2 separate days in the same participants. Bars represent each individual participant (range: -130 mg/kg FFM/min, 57 mg/kg FFM/min; n=18).

4.5 DISCUSSION

Our primary objective was to examine the validity and reliability of a MetFlex test during exercise for glycemia and IR screening in children at risk of

developing T2DM by virtue of excess adiposity. Although no significant associations between MetFlex during exercise and fasting glucose or IR were found in the entire group, secondary analyses revealed associations with IR and beta-cell function when the group was separated by sex. MetFlex during exercise was inversely associated with HOMA-IR and directly associated with WBISI and ISSI-2 in boys but not in girls. When CHO_{exo} oxidation at rest was examined, there was an inverse association with HOMA-IR and a direct association with ISSI-2 in all participants. The association between CHO_{exo} oxidation at rest and WBISI was not significant. The reliability of the MetFlex test on separate days showed good agreement (ICC=0.692) (Cicchetti, 1994), which suggests very good potential for clinical application, pending improved validity in both boys and girls with future work.

Currently, children who receive clinical care, and are identified as overweight or obese with at least two risk factors for diabetes, are referred to have an OGTT (American Diabetes Association, 2016b). An advantage of the OGTT is the ability to identify impaired glucose tolerance and diagnose T2DM even when fasting glycemia is within the normal range (Bartoli, Fra, & Carnevale Schianca, 2011). However, a few limitations of performing OGTTs in children should be considered. The criteria for diagnosing T2DM (Goldenberg & Punthakee, 2013) were based on cut-offs predicting the risk of retinopathy in adults (Engelgau et al., 1997; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003; McCance et al., 1994), and not in

children. For this reason, developing non-invasive measures that help screen for metabolic compromise preceding the development of dysglycemia would be beneficial for T2DM prevention (Levy-Marchal et al., 2010). Another caveat of the OGTT to consider is the poor reproducibility of 2-h glucose during an OGTT (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003; Libman et al., 2008). In an asymptomatic child, a positive OGTT test indicating T2DM requires a repeat test to confirm the diagnosis (American Diabetes Association, 2016b). Libman and colleagues (2008) reported poor reproducibility of 2-h glucose (ICC=0.34, CI=0.14,0.57) compared to fasting glucose (ICC=0.73, CI=0.58, 0.82) in children identified as overweight. Compared to the results for 2-h glucose shown by Libman et al. (2008), the reliability of the MetFlex test reported in this study was considerably higher (2-h glucose, ICC=0.34 vs. MetFlex, ICC=0.69).

In contrast to our hypothesis, no significant correlations were found for MetFlex during exercise and fasting glucose. Compared to controls, individuals with T2DM have lower insulin-stimulated skeletal muscle glucose transporters (GLUT-4) at the level of the plasma membrane in the presence of hyperglycemia (Zierath et al., 1996). It is likely that in our study population with obesity, but not T2DM, CHO_{exo} transport to skeletal muscle (i.e., CHO_{exo} availability) was not impaired. Furthermore, no child in our study had impaired fasting glucose according to CDA guidelines (Goldenberg & Punthakee, 2013). Another possible explanation is related to non-insulin mediated mechanisms during exercise,

which could promote normal levels of CHO uptake into peripheral tissues, primarily skeletal muscle (Hayashi, Wojtaszewski, & Goodyear, 1997). Altogether, the data suggest that underlying mechanism(s) that affect CHO oxidation during exercise are independent of resting glycemia in the absence of T2DM.

We originally hypothesized an inverse association between MetFlex during exercise and elevated glycemia, because boys with type 1 diabetes showed lower CHO_{exo} oxidation and percent CHO_{exo} oxidative efficiency during exercise compared to controls, despite higher insulin concentrations (Riddell, Bar-Or, Hollidge-Horvat, et al., 2000). Two potential mechanisms suggested by the authors were the possibility of reduced GLUT-4 density, which was associated with IR in type 1 diabetes (Klip et al., 1990; Riddell, Bar-Or, Hollidge-Horvat, et al., 2000) or an impairment in a key regulatory enzyme of glucose oxidation, pyruvate dehydrogenase (PDH), which could be inactivated by elevated levels of FFA, as explained by the glucose-fatty acid cycle proposed by Randle and colleagues (1963). FFA levels were not measured in this study, so we can only speculate that severe obesity, contributing to elevated FFA at rest, would result in substrate competition and reduce MetFlex during exercise. Future steps towards identifying when MetFlex during exercise is impaired in children with obesity would help refine and improve the adaptability of the MetFlex test.

Interestingly, we showed significant associations between MetFlex during exercise with surrogate measures of IR. One of the earliest risk factors for T2DM

is IR, but there is no clear biochemical measure of IR that is clinically recommended (Levy-Marchal et al., 2010). Physicians may infer IR from clinical signs, such as acanthosis nigricans or the presence of components of metabolic syndrome (Borai, Livingstone, & Ferns, 2007). In a research setting, the gold standard for determining IR is the hyperinsulinemic-euglycemic clamp, which is invasive and not ideal for clinical use. Past studies have aimed to find alternative ways to identify IR using ^{13}C-glucose breath tests under resting conditions (Ibarra-Pastrana et al., 2012; Jetha et al., 2009; Lewanczuk et al., 2004). Results were promising, showing a positive relationship with expired $^{13}CO_2$ after ingestion of a ^{13}C-glucose drink with IR (Ibarra-Pastrana et al., 2012; Jetha et al., 2009; Lewanczuk et al., 2004). Greater $^{13}CO_2$ measured in the breath indicated greater utilization of the available substrate (i.e. greater MetFlex). Similar to previous studies (Ibarra-Pastrana et al., 2012; Jetha et al., 2009; Lewanczuk et al., 2004), our resting measure of MetFlex (CHO_{exo} oxidation at rest) was negatively associated with IR. We also found a positive association between CHO_{exo} oxidation at rest and beta-cell function, which, to the best of our knowledge, has not been reported before.

By testing MetFlex under exercise conditions, a greater demand to utilize available substrates was placed on the system (Galgani, Moro, et al., 2008). Indeed, the finding that MetFlex during exercise is negatively associated with HOMA-IR and positively associated with WBISI and beta-cell function indicates the test could prospectively complement clinical screening procedures; however,

our data currently supports this only in boys. Additionally, potential of the MetFlex test to be modified and used as a field-based test should be investigated in the near future. This would allow for more children to be screened for T2DM risk factors, outside of clinics and in locations where children spend a majority of their time, such as in schools. However, our intention is not to apply MetFlex during exercise as a replacement of current diagnostic tests for T2DM, but to investigate its potential as a reliable, non-invasive screening tool to help predict risk for T2DM.

The results presented in this study should be considered in the context of a few limitations. First, substantial evidence suggests that menstrual cycle phase affects substrate oxidation (Devries et al., 2006; Michael C. Riddell, 2008; Timmons et al., 2003, 2007a). We recorded information on pubertal development and menstrual cycle phase when available. It was a challenge to control for these factors in the study, especially because it is not uncommon for younger girls, particularly those with obesity, to experience irregular menstrual cycles. Out of 14 girls that had reached menarche before the initial study visit, both irregular (n=4) and regular menstrual cycles (n=7) were reported, with the remaining girls reporting use of birth control (n=1), only one period since menarche (n=1) and no additional information (n=1). A second limitation was that MetFlex was not measured under resting conditions during an additional visit to compare with MetFlex measured under exercise conditions. One of the key strengths of the study was the ability to assess the association of variables with MetFlex using a

less invasive method than a hyperinsulinemic-euglycemic clamp, and apply the concept of MetFlex during exercise in children with obesity. Even though there is evidence showing a link between MetFlex at rest and IR (Banerjee et al., 2009; Ibarra-Pastrana et al., 2012; Jetha et al., 2009; Lewanczuk et al., 2004; Mizrahi et al., 2010), the examination of MetFlex during exercise provides valuable information, because lifestyle intervention often includes increasing habitual physical activity and/or participating in a personalized exercise program. The present study investigated the correlation of MetFlex during exercise with fasting glycemia, but also IR and beta-cell function, which is different from what was reported previously on glucose tolerance status (Chu et al., 2016). This contributed novel findings about MetFlex in children with obesity.

In summary, MetFlex has promise as a non-invasive measure of obesity related dysmetabolism in the pediatric population, especially if future research aims to attain a better understanding of possible sex differences. In addition, the reliability of the MetFlex test during exercise was good, which highlights good potential for clinical use. Clinical application could include MetFlex testing in a multidisciplinary weight management clinic with an exercise physiologist amongst other clinicians to help identify T2DM risk in children who have not been referred for an OGTT. However, factors related to clinical utility such as assessment of necessary space, equipment, personnel and costs would require future evaluation. Future research directions may include evaluating the relationship of MetFlex and other metabolic outcomes associated with obesity, such as hepatic

steatosis and dyslipidemia. Longitudinal studies would also be beneficial for investigating the possibility of using MetFlex results to predict risk of developing T2DM.

4.6 ACKNOWLEDGEMENTS

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CHAPTER 5

5 EFFECT OF 7 DAYS OF EXERCISE ON METABOLIC FLEXIBILITY AND INSULIN RESISTANCE IN CHILDREN WITH OBESITY

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5.1 ABSTRACT

Purpose: The capacity to match carbohydrate (CHO) oxidation with CHO availability (deemed metabolic flexibility (MetFlex)) may be important for type 2 diabetes prevention. In adults, impaired MetFlex is associated with insulin resistance (IR), which can be improved with as little as 7 days of exercise. Whether this occurs similarly in children is unknown. We hypothesized 7 consecutive days of exercise would improve MetFlex and IR in children with obesity. **Methods:** Twelve children (8 boys, 4 girls) completed 2 study visits before (PRE) and after (POST) exercise training. At visit 1, fasting blood was collected, and anthropometry and aerobic fitness ($\dot{V}O_{2max}$) assessed. At visit 2, a ^{13}C-enriched CHO drink was ingested before exercise (3 x 20 min) at 45% $\dot{V}O_{2max}$. Exogenous CHO oxidative efficiency (MetFlex) was calculated from breath samples. During training, participants alternated between continuous and

high intensity interval cycling sessions at home under supervision. **Results:** In spite of good training adherence, there was no improvement in MetFlex (PRE: 20.7 ± 1.8%, POST: 18.9 ± 4.9%, p=0.22) or HOMA-IR (PRE: 8.7 ± 4.6, POST: 8.1 ± 6.0, p=0.51). **Conclusion:** Future research should investigate exercise volume, sex, and pubertal effects on the early responsiveness of MetFlex to exercise therapy.

5.2 INTRODUCTION

The rise in prevalence of type 2 diabetes mellitus (T2DM) in children over the past few decades (Dabelea et al., 2014; Hamman et al., 2014) stresses a critical need for effective prevention and treatment strategies. Insulin resistance (IR), a common risk factor for the development of T2DM, is associated with reduced metabolic flexibility (MetFlex) (Apostolopoulou et al., 2016; Galgani, Moro, et al., 2008). MetFlex refers to the ability to adapt substrate oxidation to substrate availability (Galgani, Moro, et al., 2008). It is impaired under resting conditions in adults with obesity compared to healthy adults (Galgani, Moro, et al., 2008). Past studies combined a hyperinsulinemic-euglycemic clamp with indirect calorimetry to study MetFlex under resting conditions (Apostolopoulou et al., 2016; Galgani, Heilbronn, et al., 2008; Kelley, Goodpaster, Wing, & Simoneau, 1999; Mandarino, Consoli, Jain, & Kelley, 1996). A newer approach, using the ^{13}C-enriched carbohydrate (CHO) technique for exercise studies involving children in our laboratory (Chu et al., 2011; Riddell, Bar-Or, Hollidge-

Horvat, et al., 2000; Timmons et al., 2007a, 2007b) and recently implemented to assess MetFlex in children with obesity (Chu et al., 2016), is less invasive and more appropriate for use in children. Studying MetFlex in children is valuable because impaired MetFlex could be one of the initial problems disrupting substrate oxidation, possibly contributing to hyperglycemia.

There is evidence that exercise training, for as little as 7 days, improves the metabolic profile of adults (Kirwan et al., 2009; Mikus, Oberlin, Libla, Boyle, & Thyfault, 2012; Winnick et al., 2008). In children, exercise training also improves IR, but past studies included programs lasting at least 6 weeks (Fedewa et al., 2014; Kim & Park, 2013; Lee & Kim, 2013). To our knowledge, no study has reported on the effect of a shorter exercise intervention on IR in children. We tested the hypothesis that MetFlex and IR would improve in response to a brief period of exercise therapy (i.e., 7 days), independent of changes in weight, body composition or $\dot{V}O_{2max}$.

The primary objective of this study was to examine the effect of 7 days of exercise training on MetFlex during exercise and IR in children who by virtue of their obesity are at greater risk of T2DM. Secondary objectives included: 1) assessing changes in body weight, body composition and aerobic fitness after 7 days of exercise training, and 2) determining the association between exercise training intensity and change in MetFlex.

5.3 METHODS

Study Design: Children with obesity, who were scheduled for an oral glucose tolerance test (OGTT), ages 8-17 years old were recruited from the Children's Exercise and Nutrition Centre at the McMaster Children's Hospital. Because the OGTT is the clinical test of choice to identify impaired glucose tolerance and T2DM, it was used as the criterion. Children who provided consent-to-contact from a previous study in our laboratory (Chu et al., 2016) were also recruited. Exclusion criteria included known motor delays or physical disability that would interfere with completing the study, or medications that could influence IR, such as Metformin. The study was approved by the Hamilton Integrated Research Ethics Board, and written informed parental consent and child assent were obtained from all families. Participants were asked to attend 2 study visits before (PRE) and after (POST) a 7 day home-delivered exercise training program.

Visit 1: Children arrived after a minimum 10-h overnight fast for blood work to calculate homeostasis model assessment of insulin resistance (HOMA-IR), derived from glucose and insulin concentrations (Matthews et al., 1985). Blood sampling was followed by anthropometric and maturity assessments. Weight was measured using a digital weight scale (BWB-800; Tanita Corporation, Tokyo, Japan). Standing height and sitting height were measured using a calibrated stadiometer (Harpenden Stadiometer 602-VR; Holtan Limited, Crymych, United Kingdom). An average of two measurements was used, and repeated if the

difference was greater than 0.4 kg or 0.4 cm. Body composition was assessed using bioelectrical impedance analysis (Inbody 520, Biospace Co., Seoul, Korea), and BMI percentile for age was calculated. Years from peak height velocity were estimated using equations provided by Mirwald et al. (2002). Based on previously described criteria (Chu et al., 2016), aerobic fitness ($\dot{V}O_{2max}$) was determined on a cycle ergometer (Corival Lode, Groningen, The Netherlands). Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured using the mixing chamber setting (30 sec average) on a calibrated metabolic cart (Vmax29, SensorMedics, Palm Springs, CA, USA). HR was monitored continuously during the test (Polar Electro Oy, Kempele, Finland).

Visit 2: Before arriving, 24-h food and physical activity diaries were completed. Participants were asked to avoid corn or corn-derived products during the week to reduce background enrichment of expired CO_2 from naturally derived ^{13}C (Schoeller et al., 1980). Strenuous physical activity on the day before and day of the visit was avoided, and no food was consumed at least 3-h before. MetFlex during exercise (3 x 20 min) at 45% $\dot{V}O_{2max}$ was assessed, as previously described (Chu et al., 2016). During the test, expired gas from the mouth was sampled for 3 min periods using the metabolic cart. Duplicate samples of expired air (10 mL) were stored in Exetainer tubes (Labco Exetainer, Lampeter, Ceredigion, UK) for later analysis of breath enrichment ($^{13}C/^{12}C$ ratio). After this visit, participants started their home-exercise training program. At least 48-h after

the last training session, participants attended a 3rd and 4th visit to repeat procedures from visit 1 and 2.

Experimental Beverage and Substrate Utilization: Participants ingested a 75 g CHO drink enriched with uniformly labelled ^{13}C-glucose (Cambridge Isotope Laboratories, Inc., Tewksbury, MA) and breath samples were collected to calculate CHO_{exo} oxidation rate before and during exercise. Additional details are described in Chu et al. (2016). Breath enrichment was determined by isotope ratio mass spectrometry using the IDMicro Breath Version 2.0 (Compact Science Systems, Staffordshire, UK) for 9 participants and the Europa Scientific 20/20 gas isotope ratio mass spectrometer (Europa Scientific, Cincinnati, OH) for 3 participants due to unforeseen maintenance delays with the IDMicro system. Comparison of breath samples analyzed by the two systems showed an average difference of ~4% in $^{13}CO_2$ values versus the $^{13}C/^{12}C$ ratio of the ^{13}C Pee Dee Belemnitella-1 ([δ^{13}C]PDB-1). This means a 5% difference in [δ^{13}C]PDB-1 values measured on the IDMicro system compared to Europa Scientific 20/20 system could affect CHO_{exo} oxidative efficiency calculations by ± 0.8%. Because pre- and post-training breath samples from the same participant were analyzed using the same system, we do not expect this small difference to affect our results in any meaningful way.

MetFlex: By calculating the percent of exogenous CHO (CHO_{exo}) oxidized divided by the amount of CHO_{exo} orally-ingested, we have a surrogate measure of MetFlex that quantifies substrate oxidation versus availability. To calculate

CHO_{exo} oxidative efficiency, we determined area under the curve (AUC) for CHO_{exo} using the trapezoidal method. Rest breaks between exercise bouts were omitted. All participants received 75 g of CHO. Therefore, CHO_{exo} oxidative efficiency = AUC CHO_{exo} (g)/75g x 100. AUC CHO_{exo} was also normalized to fat free mass (FFM). MetFlex was reported in 2 ways: 1) CHO_{exo} oxidative efficiency (%), and 2) AUC CHO_{exo} (mg/kg FFM).

Blood Analysis: Serum samples were analyzed for glucose using a colourimetric assay kit (Cat. No. 10009582, Cayman Chemical, Ann Arbor, Michigan, USA) and insulin using a high sensitivity enzyme-linked immunosorbent assay (ELISA) kit (KAQ1251, Invitrogen, Burlington, Ontario, Canada). The calculated intra-assay coefficient of variation was 3.4% and 8.2% for glucose and insulin, respectively.

Exercise Training Program: Participants completed 7 consecutive days of exercise training supervised by researchers involved with the study. A stationary cycle ergometer (Monark Cardio-Care 827 E Monark Exercise AB, Varberg, Sweden) was transported to the child's home. HR was monitored during the training. On the 1st, 3^{rd}, 5^{th} and 7^{th} days of training, children completed continuous exercise consisting of 3 repetitions of 15-min bouts of exercise (5-10 min rest periods) at an exercise intensity eliciting 80% of maximal HR (% HR_{max}). On the 2^{nd}, 4^{th}, and 6^{th} days of exercise training, children completed interval exercise consisting of 6 sets of 4 repetitions of 15-s (1 min rest periods between sprints; 5-10 min rest periods between sets). Children pedaled as fast as they

could against the same resistance as the prior continuous exercise session The duration of daily exercise was 45 min during the continuous sessions and 6 min during the high intensity sessions for a total of 198 min of deliberate moderate-to-intense exercise over the course of training. By using two different types of exercise, we hoped to increase enjoyment and reduce boredom for children, while ensuring a significant physiological stimulus.

Sample Size: Pilot data in boys with obesity reported a CHO_{exo} oxidative efficiency of ~22% during 60 min of exercise (data not shown), while a study in healthy boys without obesity reported a CHO_{exo} oxidative efficiency of ~30% (Timmons et al., 2003). To improve from 22% to 30% with $\propto$=0.05 and β=0.1 (90% power), 12 participants were needed. Healthy girls have a CHO_{exo} oxidative efficiency of ~25% (Timmons et al., 2007a), but the ideal sample size for including girls could not be determined because of the lack of data in girls with obesity.

Statistical Analysis: Values were expressed as means ± SD. Variables were checked for normality using the Shapiro-Wilk test (IBM SPSS Statistics, version 20). Non-parametric data were log-transformed. Paired t-tests were used to compare pre- and post-training values. Pearson's *r* correlation was used to examine associations between exercise training intensity and changes in MetFlex. Statistical significance was set at $p \leq 0.05$.

5.4 **RESULTS**

Participant Characteristics

Participant characteristics for 12 children (8 boys, 4 girls) who completed the study are shown in **Table 5.1**. No differences in PRE and POST anthropometric and $\dot{V}O_{2max}$ measurements were found.

Recruitment and Retention

Recruitment occurred from approximately July 2014 to June 2016. Over this time, 43 consent-to-contact forms for the study were completed and received from the Children's Exercise and Nutrition Centre. Out of the families who agreed to be contacted, 11 (26%) agreed to participate. Reasons for declining were distance (n=6), too busy (n=10), child did not want to (n=4) and not enough space in home (n=1). Twelve families were not contacted because the phone number on file was out of service (n=2), the child was too old (n=2), or the family did not respond to phone messages or emails (n=8). In children who completed a previous study in our laboratory (Chu et al., 2016) and provided consent-to-contact (n=21), 10 were contacted and 2 agreed to participate. Other children declined because of distance and/or time (n=6), the child did not like cycling (n=1) or an unspecified reason (n=1). Altogether, 13 children enrolled in the study, but one girl withdrew after visit 1 for reasons unrelated to the study.

Training Adherence

In 12 children who completed the study, 2 children (1 boy, 1 girl) missed one session each and completed an extra day at the end of the training program. Another girl completed all training sessions except 2 out of 3 sets of one continuous exercise session due to equipment malfunction, and 4 out of 6 sets of one interval exercise session because of fatigue. The other participants completed 7 days of training without major issues.

Exercise intensity during training sessions

During the continuous exercise session, average HR recorded over the 4 days was 76.9 ± 3.0% HR_{max}, which was slightly lower than the target HR set at 80% HR_{max}. Participants met the target HR, within ± 5 beats/min, 70.4 ± 20.2% of the time. HR measured after each set of intervals and averaged for the 3 high intensity interval sessions was 86.4 ± 5.5% HR_{max}.

Effect of exercise training on MetFlex and IR

No significant improvements in MetFlex during exercise or HOMA-IR were identified after training. Individual data for change in MetFlex and HOMA-IR are shown in **Figure 5.1**. MetFlex measured as CHO_{exo} oxidative efficiency was improved by ~2.3% in 5 children, did not change in 2 children, and declined by ~6.4% in 5 children. AUC CHO_{exo} in mg/kg FFM and HOMA-IR were log-transformed for the statistical analysis.

Table 5.1 Participant characteristics

	PRE	POST
Tanner (I/II/III/IV/V)	0/7/1/2/2	---
Boys/Girls	8/4	---
Age (years)	14.2 ± 1.9	---
YPHV	1.30 ± 1.37	---
Height (cm)	163.0 ± 10.8	163.3 ± 11.4
Weight (kg)	93.0 ± 24.0	94.0 ± 24.5
BMI-for-age percentile	98.4 ± 2.7	97.8 ± 3.4
Percent body fat	45.2 ± 4.5	45.1 ± 4.7
Fat free mass (kg)	50.4 ± 11.2	51.1 ± 11.9
$\dot{V}O_{2max}$ (mg/kg BW/min)*	21.5 ± 4.8	21.5 ± 3.9
Fasting glucose (mmol/L)	4.8 ± 0.3	4.7 ± 0.5
Fasting insulin (uU/mL)	40.0 ± 19.6 (16.3, 91.0)	37.8 ± 22.0 (17.0, 91.8)
HOMA-IR	8.7 ± 4.6 (3.0, 20.6)	8.1 ± 5.7 (3.4, 23.6)
MetFlex: CHO_{exo} oxidative efficiency (%)	20.7 ± 1.8	18.9 ± 4.9
MetFlex: AUC CHO_{exo} (mg/kg FFM)	319.2 ± 61.1 (212.7, 397.9)	289.8 ± 97.5 (190.4, 442.8)

Data are presented as mean ± SD. Ranges are reported for variables that were non-normally distributed. YPHV, years from peak height velocity; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; FFM, fat free mass; MetFlex, metabolic flexibility; CHO_{exo}, exogenous carbohydrate; AUC, area under the curve; PRE, before exercise training; POST, after exercise training. No significant differences were found PRE and POST using paired samples t-tests ($p \leq 0.05$). *one boy was excluded due to a technical error on the metabolic cart during his $\dot{V}O_{2max}$ after the exercise training.

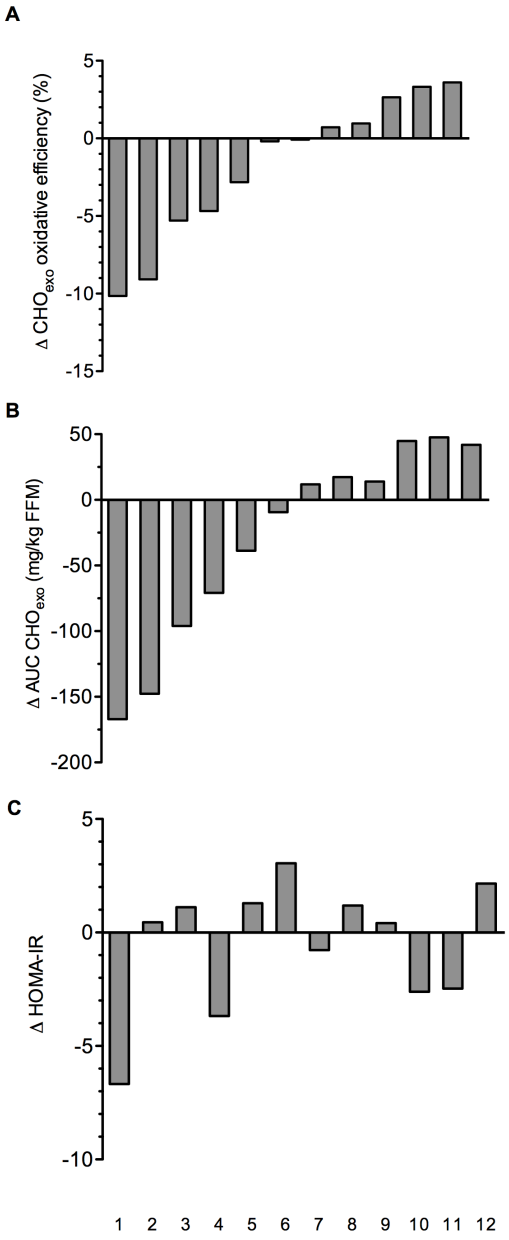

Figure 5.1 A) Individual change in exogenous carbohydrate (CHO_{exo}) oxidative efficiency after 7 days of exercise training. Using a paired t-test, no significant difference in oxidative efficiency was found after exercise training (PRE: 20.7 ± 1.8%, POST: 18.9 ± 4.9%, p=0.22). **B)** Individual change in area under the curve exogenous carbohydrate (AUC CHO_{exo}) relative to fat free mass (FFM) after 7 days of exercise training. Participants are numbered on the x-axis and ordered according to change in CHO_{exo} oxidative efficiency. Using a paired t-test, no significant difference in AUC CHO_{exo} relative to FFM was found after exercise training (PRE: 319.2 ± 61.1 mg/kg FFM, POST: 289.8 ± 97.5 mg/kg FFM, p=0.20). **C)** Individual change in HOMA-IR after 7 days of exercise training. Participants are numbered on the x-axis and ordered according to change in CHO_{exo} oxidative efficiency. Using a paired t-test, no significant difference in HOMA-IR was found after exercise training (PRE: 8.7 ± 4.6, POST: 8.1 ± 6.0, p=0.51).

Effect of exercise training intensity on change in MetFlex

Although no effect of training on MetFlex was observed, there was a strong trend for the relationship between increased CHO_{exo} oxidative efficiency and the time exercise training within the prescribed target HR range (r=0.56, p=0.06) **(Figure 5.2)**. Since a few children were unable to maintain the target HR range for the entire continuous exercise sessions, we re-assessed outcomes for only those who met the target HR range at least 75% of the time (n=8). Similar to results in the group as a whole, no significant differences in MetFlex or HOMA-IR after training were found (**Table 5.2**).

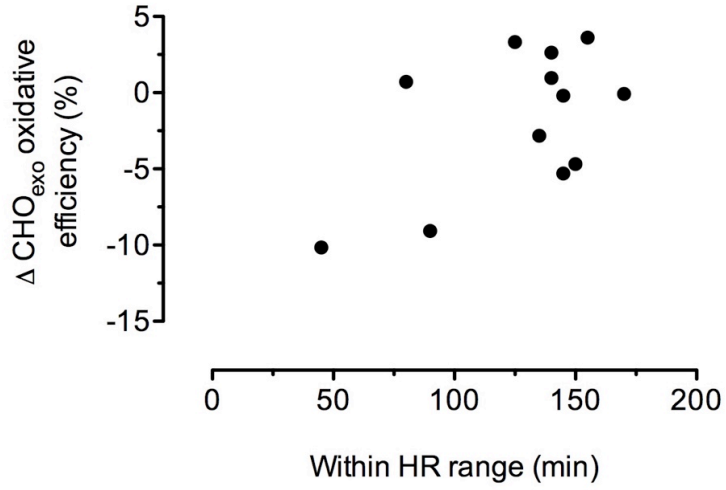

Figure 5.2 Correlation between time spent cycling within heart rate range at 80% of maximal heart rate (% HR_{max}) in minutes (min) and change in exogenous carbohydrate (CHO_{exo}) oxidative efficiency after 7 days of exercise training. Pearson's r correlation showed a positive trend (r=0.56, p=0.06).

Table 5.2 Participant characteristics and results in children who were at least 75% adherent to the prescribed target heart rate range during exercise training sessions

	PRE	POST
Tanner (I/II/III/IV/V)	0/4/1/2/1	---
Boys/Girls	4/4	---
Age (years)	14.3 ± 2.1	---
YPHV	1.6 ± 1.4	---
Height (cm)	161.5 ± 10.7	162.2 ± 11.4
Weight (kg)	93.6 ± 29.7	95.0 ± 30.4
BMI-for-age percentile	98.1 ± 3.2	97.4 ± 4.1
Percent body fat	44.8 ± 5.4	44.6 ± 5.7
Fat free mass (kg)	50.9 ± 13.3	51.8 ± 14.3
$\dot{V}O_{2max}$ (mg/kg BW/min)*	21.8 ± 5.2	21.7 ± 4.5
Fasting glucose (mmol/L)	4.8 ± 0.4	4.8 ± 0.4
Fasting insulin (uU/mL)	40.4 ± 22.8	40.3 ± 25.1
HOMA-IR	8.8 ± 5.3 (3.0, 20.6)	8.9 ± 6.5 (3.4, 23.6)
MetFlex: CHO_{exo} oxidative efficiency (%)	19.9 ± 1.6	19.2 ± 3.7
MetFlex: AUC CHO_{exo} (mg/kg FFM)	309.5 ± 71.4 (212.7, 397.9)	296.7 ± 100.1 (200.5, 442.8)

Data are presented as mean ± SD. Ranges are reported for variables that were non-normally distributed. YPHV, years from peak height velocity; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; FFM, fat free mass; MetFlex, metabolic flexibility; CHO_{exo}, exogenous carbohydrate; AUC, area under the curve; PRE, before exercise training; POST, after exercise training. No significant differences were found PRE and POST using paired samples t-tests ($p \leq 0.05$). *one boy was excluded due to a technical error on the metabolic cart during his $\dot{V}O_{2max}$ after the exercise training.

5.5 DISCUSSION

The primary objective of this study was to examine the effect of 7 days of exercise training on MetFlex during exercise and IR in children with obesity. Results showed no improvements in MetFlex or HOMA-IR, and large inter-individual variability in response. No changes in body weight, body composition or $\dot{V}O_{2max}$ were found after training.

To date, only a few studies in adults (Malin et al., 2013; Meex et al., 2010) and none in children have assessed the effect of exercise training on MetFlex measured using a hyperinsulinemic-euglycemic clamp and indirect calorimetry. MetFlex was improved after a 12 week exercise intervention in adults with impaired fasting glycemia or impaired glucose tolerance (Malin et al., 2013) and in adults with T2DM (Meex et al., 2010). To our knowledge, prior studies have not examined the responsiveness of MetFlex measured under exercise conditions to exercise training in children.

Several studies provide evidence of improved IR after exercise training in adults with T2DM (Kirwan et al., 2009; O'Gorman et al., 2006; Winnick et al., 2008). These studies showed better insulin-mediated glucose disposal after 7 days of exercise training (Kirwan et al., 2009; O'Gorman et al., 2006; Winnick et al., 2008). However, O'Gorman reported that similar improvements were not found in adults without T2DM (O'Gorman et al., 2006), suggesting that the greatest benefits from exercise training might occur in individuals with the greatest deterioration in metabolic profile.

How IR was measured is important to consider. Although studies show good correlation between HOMA-IR and peripheral insulin sensitivity measured using a hyperinsulinemic-euglycemic clamp (Emoto et al., 1999; Sarafidis et al., 2007), HOMA-IR is limited in providing information about what is occurring at the muscle level. In theory, HOMA-IR calculated using fasting insulin and fasting glucose is a better indicator of hepatic insulin sensitivity than peripheral insulin sensitivity. Kirwan et al. (2009) reported that 7 days of exercise led to improvements in peripheral insulin sensitivity and enhanced suppression of hepatic glucose output in adults with T2DM. In contrast, Winnick et al. (2008) found that improvements were mainly attributed to peripheral and not hepatic insulin sensitivity. For ethical and practical reasons, we did not conduct a hyperinsulinemic-euglycemic clamp before and after training, but it would be interesting to investigate this in future studies.

In previous adult studies, the exercise training consisted of continuous, aerobic exercise for 40-60 min at ~70-75% $\dot{V}O_{2max}$ (Kirwan et al., 2009; O'Gorman et al., 2006; Winnick et al., 2008) compared to our training alternating between continuous and interval sessions. Nevertheless, substantial evidence suggests high intensity interval training provides similar improvements in IR and metabolic profile when compared to moderate intensity continuous exercise training (Fisher et al., 2015; Jelleyman et al., 2015).

The trend for a relationship between prescribed exercise time within ± 5 beats/min of 80% HR_{max} and MetFlex measured as change in CHO_{exo} oxidative

efficiency suggests that the exercise stimulus could be an important factor for improving MetFlex in children with obesity. It may be that a certain exercise threshold is required to improve MetFlex over such a short period of time. However, when we excluded children with less than 75% adherence to the target HR range, individual variability in change in MetFlex remained. Thus, the necessary exercise threshold may not have been reached within 7 days even in the children with good training adherence.

The novelty of the study design highlights factors that require careful consideration when studying MetFlex. To build on our initial work, future studies involving children should investigate short-term exercise training on peripheral insulin sensitivity and/or compare a larger volume of exercise training on MetFlex and IR. A strength of the study is that we demonstrated the feasibility of measuring MetFlex during exercise. Past studies have been conducted at rest, which arguably does not sufficiently stress the metabolic system because of the low energy demand. Furthermore, findings show large inter-individual variability in response to exercise therapy in children. In a previous study, the reliability of MetFlex measured on two separate days was deemed good with an intraclass correlation coefficient of ~0.7 (Chu et al., *in review*). In 10 out of 12 participants in this study, the change in MetFlex was greater than the mean difference reported in the reliability study, which was 0.5% for CHO_{exo} oxidative efficiency (unpublished data) and 8.2 mg/kg FFM for AUC CHO_{exo} (Chu et al., *in review*).

Therefore, a large proportion of the difference in MetFlex could be attributed to individual variability and not the test itself.

The study results should be considered in light of a few limitations. First, the number of families that were asked by the clinic team if they would like to be contacted for the study and declined was not recorded. Due to the nature of clinic-based recruitment and patient confidentiality, a clinician made initial contact and this was difficult to track. Thus, the true feasibility of recruiting is not entirely known. Second, our sample consisted of boys and girls at different stages of puberty, but there were not enough participants to tease out possible sex and/or pubertal effects. When estimating sample size, we were limited to previous studies that were heterogeneous in exercise intensity. On the issue of sex, there is an absence of studies related to MetFlex and CHO_{exo} oxidation in girls with obesity, so the possible influence of sex on MetFlex is unclear. Similarly, very little is known about pubertal effects on MetFlex during exercise conditions. Some evidence from non-obese children suggests that pubertal stage influences CHO_{exo} oxidation in boys (Timmons et al., 2003), but not in girls (Timmons et al., 2007a). It is possible that some individuals were more sensitive to changes in MetFlex with exercise therapy based on pubertal stage. Results of the current study can be used to design future trials investigating MetFlex in response to training with consideration of potential sex and pubertal effects.

In summary, 7 days of exercise training did not improve MetFlex during exercise or HOMA-IR in children with obesity. The data support large inter-

individual variability in the early response to short-term exercise training in children, and this requires further examination. The shortest amount of exercise training necessary to improve MetFlex or IR in children with obesity remains to be determined. Future research is warranted to investigate the best mode, volume and intensity of exercise to improve risk factors associated with developing T2DM in children.

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CHAPTER 6

6 SUMMARY OF FINDINGS AND GENERAL DISCUSSION

The general objective of this thesis was to advance our knowledge about MetFlex under exercise conditions in children with obesity. With a better understanding of MetFlex, the specific aim was to determine if MetFlex could be used as a screening tool to identify risk factors, such as IGT, dysglycemia and IR, associated with the development of T2DM. In this thesis we examined if children with IGT have impaired MetFlex compared to children with NGT (Chapter 3). This was followed by investigating the validity and reliability of the MetFlex test to screen for dysglycemia and IR in children with obesity (Chapter 4). Since exercise therapy is often included as a component of intervention programs for children at risk for T2DM, the effect of 7 days of exercise on MetFlex and IR was also investigated (Chapter 5). The combined studies have helped advance scientific knowledge and have produced novel results related to MetFlex during exercise in children with obesity.

The main research findings presented in this thesis included that the diagnosis of IGT in children does not indicate impaired MetFlex during exercise compared to NGT in children (Chapter 3), even with greater IR in the IGT group. There was, however, an association between MetFlex and IR (measured as HOMA-IR and WBISI), as well as beta-cell function (measured as ISSI-2) in boys with obesity, but not in girls with obesity (Chapter 4). No association was found

between MetFlex and fasting glucose (Chapter 4) or 2-h glucose during an OGTT (**Appendix G**), which were measured under resting conditions. Reliability of the MetFlex test was good (ICC=0.692) (Chapter 4). Lastly, MetFlex and HOMA-IR did not improve after 7 consecutive days of exercise in children with obesity (Chapter 5). Our data suggested large inter-individual variability in these responses, at least in the early stages of exercise therapy. The contribution of CHO_{exo}, CHO_{endo}, and FAT_{tot} to total EE also did not change after the exercise intervention (**Appendix H**). Therefore, indicating substrate selection during exercise was similar after 7 days of exercise in children with obesity. In the following sections, the potential clinical utility of the MetFlex test will be discussed. The significance of the main research findings will also be explained further in context of the scientific literature.

6.1 Evaluation of the clinical utility of metabolic flexibility testing

The term clinical utility is typically used to describe the assessment of clinical effectiveness and/or economic evaluations (Smart, 2006). In Smart's multi-dimensional model of clinical utility, additional factors to consider related to clinical utility were identified. These factors based on clinicians' judgments on clinical utility include appropriateness, accessibility, practicability, and acceptability (Smart, 2006). A summary of Smart's dimensions of clinical utility is provided in **Appendix I**. In this section of the thesis, Smart's multi-dimensional model will be used to guide the discussion on clinical utility of MetFlex testing for

children at risk for developing T2DM. Aspects of appropriateness and acceptability are discussed in more detail below, and will be the focus of the discussion. At this time, accessibility and practicability cannot be fully evaluated because of the paucity of research on MetFlex and these components of clinical utility, which may be areas to study in future research.

6.1.1 *Appropriateness of the metabolic flexibility test based on effectiveness*

Aspects of appropriateness of a test to consider are its effectiveness and relevance. In this thesis, we aimed to provide evidence-based support for the effectiveness of the MetFlex test (Chapter 4). Because there was no difference in MetFlex in children with IGT compared to NGT (Chapter 3), and no association with fasting glucose with MetFlex (Chapter 4), the MetFlex test would be ineffective for diagnosing prediabetes or diabetes. Instead, the test could be most useful for identifying when muscle glucose disposal is impaired, indicating increased IR, and identifying the presence of early impairments in substrate utilization. In boys with obesity, we found that MetFlex was negatively associated with HOMA-IR and positively associated with WBISI and beta-cell function measured as ISSI-2 (Chapter 4). Due to the lack of association in girls with obesity (Chapter 4), more research is required before the test can be implemented for clinical care. In an ideal research setting, subsequent research would include a longitudinal study to determine cut-points for MetFlex to predict T2DM and other health complications. It is however, important to note that

longitudinal studies have yet to be completed in children for the current glycemic cut-points for the clinical diagnosis of prediabetes and diabetes. Therefore, further investigation on the appropriateness of clinical screening and diagnostic tests, in general, for children at risk for developing T2DM is recommended.

A strength of the MetFlex test that stands out from the study results reported in this thesis is the good reliability of the test (Chapter 4). Poor reproducibility of 2-h glucose during an OGTT (Libman et al., 2008; Santaguida et al., 2005), which is currently used to diagnose prediabetes and diabetes, suggests supplementary clinical tests would be valuable. Nevertheless, based on the available scientific literature and results from our study, there is insufficient evidence to replace the OGTT with any other test at this time. Despite poor reliability for 2-h glucose during an OGTT, the ability to diagnose IGT from 2-h glucose measurements is important because IGT remains a strong predictor of the development of T2DM (Santaguida et al., 2005).

In summary, the clinical utility of MetFlex testing in its current state would be ineffective. Primary reasons include the lack of association with risk factors in girls with obesity and the absence of cut-points for MetFlex that would indicate an increased risk for T2DM. More work investigating changes in MetFlex in the same participants over time would help elucidate if MetFlex could be used as a tool to show improvements at clinical follow-up appointments. The research area in children is still in its early stages, therefore, MetFlex testing should not be considered a solely valuable research tool without further examination of its

potential as a clinical tool. There remains copious research potential to build on the novel findings presented in this thesis.

6.1.2 *Appropriateness of the metabolic flexibility test based on relevance*

To evaluate the relevance of MetFlex testing, the variables that best predict the development of prediabetes, T2DM and other health outcomes need to first be highlighted. This helps identify what information is obtained from current tests that are used, and what added information would enhance clinical care for children. Weiss and colleagues (2005) reported the best predictors of developing T2DM in children were severe obesity, IGT and ethnicity. Many studies, mainly in adults, support both IFG and IGT as strong risk factors for T2DM, while combined IFG+IGT was the strongest risk factor (Santaguida et al., 2005). A review by Santaguida et al. (2005) indicated that majority of the risk for progressing to T2DM is associated with identified IFG or IGT rather than other factors. Treating IFG and/or IGT would be beneficial for preventing T2DM and other health outcomes such as CVD, stroke and microvascular disease. Alas, information on the prognosis of T2DM based on worsening glycemia after IGT diagnosis could not be found in the pediatric literature.

Important questions to ask are how can we predict which children are most at risk for IFG or IGT, and where does impaired MetFlex fall along the spectrum of NGT to IGT to T2DM development? To address the first question, identifying beta-cell failure would be valuable. As described earlier, glucose homeostasis

depends on both insulin secretion and insulin action (Arslanian, 2000). With the current available tests, impairments in either of these are difficult to identify without blood work. Beta-cell failure (i.e., declining insulin secretion) relative to insulin sensitivity is thought to be the primary mechanism that contributes to the development of prediabetes (Hannon & Arslanian, 2015). Bacha and colleagues (2010) reported adolescents with obesity who showed signs of glucose dysregulation were more likely to have impaired insulin secretion rather than reduced insulin sensitivity. Consequently, although IR is one of the earliest abnormalities in children with obesity (Arslanian, 2000), it is beta-cell failure that significantly affects glycemia, which could lead to glucose intolerance and progression to T2DM (Hannon & Arslanian, 2015). In our study, we showed that MetFlex was associated with ISSI-2 in boys (Chapter 4). This suggests that MetFlex could provide a surrogate measure of beta-cell function. However, similar to the association with HOMA-IR and WBISI found only in boys described above, it is unlikely for MetFlex testing to be implemented for clinical use if it is valid in boys but not in girls, at least without further study or explanation. In addition, more research is necessary to determine if it is possible to differentiate MetFlex values or determine cut-points for identifying elevated IR versus reduced beta-cell function.

To address where impaired MetFlex occurs in the progression to T2DM, the interaction between dysglycemia and glucose transport activated via insulin-stimulation or muscle contraction requires more understanding. It is unclear

whether impaired glucose oxidation during exercise is a result of a defect in insulin-stimulated glucose uptake (i.e., decreased muscle glycogen stores available for oxidation), contraction-mediated glucose uptake (i.e., decreased plasma glucose oxidation), or both. Insulin-stimulated glucose transport can be reduced by elevated FFA, glucose toxicity and inflammatory markers (Shepherd & Kahn, 1999). One hypothesis is that elevated FFA in the blood, due to diet or excess adiposity, competes with glucose uptake via substrate competition. Evidence suggests that contraction-mediated stimulation of GLUT-4 translocation occurs normally in individuals with IR (Shepherd & Kahn, 1999). However, Nitert et al. (2012) reported that the Ca^{2+} signaling pathway that contributes to glucose uptake during exercise is impaired in individuals with T2DM. When IR and other metabolic health complications start to affect substrate selection, availability and oxidation during exercise remains uncertain.

Another important question related to clinical relevance of a test is how the test will impact clinical decision-making. In certain situations, additional information about a patient does not always impact their prescribed treatment plan. For example, clinicians who work with children with obesity do not usually use HOMA-IR as a biochemical measure when choosing the best treatment and intervention plan for an individual. One possible reason is that fasting glucose or other biochemical measures, such as lipid levels, provide adequate information to determine the intervention or treatment strategy. Clinicians should be able to use test results as a guide to decide on the best intervention program for patients.

The advantage of using an exercise condition compared to a resting condition for MetFlex testing is the ability to determine if IR is limited to the insulin signaling pathway or due to a general resistance in mechanisms involved with GLUT-4 translocation (Zierath et al., 2000). Hypothetically, if dysglycemia were a result of a defect in insulin-stimulated glucose transport, but not contraction-mediated glucose transport, exercise therapy would be an effective intervention to improve IR. However, if there was also a defect in contraction-mediated glucose transport, changes to usual substrate oxidation and impaired MetFlex during exercise may occur. In this case, exercise therapy may need to be supplemented with other treatment strategies, such as pharmacological therapy. This represents one way that the MetFlex testing could impact existing treatment strategies and clinical-decision making.

When the responsiveness of the MetFlex test to 7 days of exercise training was examined, results showed large inter-individual variability (Chapter 5). If MetFlex had improved after 7 days of exercise training, the test could be recommended as a motivational tool by helping track improvements in MetFlex independent of factors that may take longer to change during an intervention, such as weight or percent body fat. Since MetFlex did not improve after 7 days of exercise training (Chapter 5), a clearer understanding of MetFlex and its responsiveness to exercise training is necessary before clinical application can be evaluated further. Additional research is required to determine if a longer

exercise training program would lead to more consistent improvements in MetFlex and result in less individual variability.

In summary, the clinical utility of MetFlex testing is a pertinent research pursuit because of the current inability to measure some physiological outcomes (i.e., beta-cell dysfunction) within a clinical setting. Furthermore, MetFlex testing could supplement clinical measurements (i.e., glycemia), with information about substrate oxidation when a patient is challenged with an exercise stimulus. This could provide insight about whether exercise therapy would be beneficial for that particular patient living with obesity. On the contrary, the individual variability in improvements in MetFlex between children (Chapter 5) cautions its clinical utility until there is better evidence to show that MetFlex improves in parallel with improved IR after exercise training. Hence, there is currently not enough support to recommend using MetFlex test results for clinical-decision making.

6.1.3 *Acceptability of the metabolic flexibility test based on participant ratings*

We did not address the acceptability of the test according to clinicians, but acceptability scores filled out by children who completed both the MetFlex test and an OGTT are summarized in **Appendix A, Table A.2**. Total acceptability scores were not different for the MetFlex test compared to the OGTT (Chapter 2). This suggests children tolerated the MetFlex test well relative to the standard clinical test used to diagnose diabetes. We expected there would be a greater score for enjoyment for the MetFlex test compared to the OGTT because of the

elimination of blood work. However, this was not the case. Possible reasons for this could be that many children recruited in the study have completed clinical blood work regularly for their physician and are accustomed to it and/or many children found the bike seat during the MetFlex test to be very uncomfortable, especially if cycling was not a regular activity the child enjoyed.

6.2 **Novelty of findings**

The general objective of this thesis was to advance our knowledge about MetFlex under exercise conditions in children with obesity. Indeed, the results presented in this thesis provoke interesting discussion and areas for future research. Building on this initial groundwork related to MetFlex under exercise conditions will provide a better understanding of the role of MetFlex in health and disease. **Table 6.1** summarizes the novel findings from the studies in this thesis and their scientific contribution to the literature.

Table 6.1 Novelty of findings

Chapter	Novel Findings	Scientific Contributions and Summary
3	Glucose tolerance status did not impact MetFlex during exercise in children with obesity	First study to measure MetFlex under exercise conditions in children with obesity; Children with IGT did not have reduced MetFlex during exercise compared to children with NGT even when IR was greater in the children with IGT; Increased compensatory insulin secretion after a CHO load is one possible explanation for not finding a difference in MetFlex during exercise between groups. Higher insulin concentrations maintain CHO_{exo} availability and oxidation, and therefore could help maintain MetFlex during exercise
4	Fasting blood glucose (measured at rest) was not associated with MetFlex during exercise in children with obesity	First study to investigate the association between MetFlex during exercise and glycemia; Pearson's *r* correlation did not show a significant association between MetFlex during exercise with fasting glucose
	Surrogate measurements of IR (measured at rest) were not associated with MetFlex during exercise in the whole group. When separated by sex, IR was associated with MetFlex during exercise in boys, but not in girls	First study to investigate the validity of a novel, non-invasive MetFlex test for identifying IR, a primary risk factor associated with T2DM; MetFlex during exercise was negatively associated with HOMA-IR and positively associated with WBISI and ISSI-2 in boys with obesity, but not in girls with obesity; Future work is necessary to determine validity of the MetFlex test to be used in girls
	The proposed test used to measure MetFlex during exercise is a reliable test	First study to investigate the reliability of a novel, non-invasive MetFlex test; MetFlex is a reliable test when repeated in the same individuals on a separate day; The ICC was 0.692 for MetFlex reported as AUC CHO_{exo} in milligrams per kg of FFM
5	No change in MetFlex during exercise and HOMA-IR was reported in children with obesity after 7 days of exercise	First study to investigate the effect of 7 consecutive days of cycling exercise on MetFlex during exercise and HOMA-IR in children with obesity; No significant differences in MetFlex during exercise and HOMA-IR were found after short term exercise training; There was large inter-individual variability in the response of MetFlex during exercise and HOMA-IR to 7 days of exercise
	No change in substrate selection during exercise was reported after 7 days of exercise	The contribution of CHO_{exo}, CHO_{endo} and FAT_{tot} to total energy expenditure during 60 min of exercise did not change after 7 consecutive days of cycling exercise in children with obesity (**Appendix H**).

6.3 Limitations and Challenges

To guide future work related to MetFlex under exercise conditions, it is important to acknowledge the limitations and challenges encountered during the completion of this thesis. The novelty of the MetFlex protocol is a strength of the research presented, but because of this, there are also limited studies to corroborate the findings and confirm that the outcome provides a suitable measurement of MetFlex. For this reason, a more detailed discussion of the different components of MetFlex and how the MetFlex protocol presented supports or fails to reflect these components can be found below.

Kelley and Mandarino (2000) indicated that a primary aspect of MetFlex in skeletal muscle is the capacity to switch between different substrates, however, this capacity can be lost in IR. According to Kelley and Mandarino's definition, impaired MetFlex is characterized by a loss of physiologic reserve, specifically a disrupted adaptation to fasting conditions shown by a failure to increase lipid oxidation and a disrupted response to insulin shown by a failure to suppress lipid oxidation (Kelley & Mandarino, 2000). Instead of measuring lipid metabolism, the MetFlex protocol presented in this thesis focused on MetFlex to CHO_{exo} and to some extent CHO_{tot} metabolism. Hence, knowledge on MetFlex to increased lipid availability and lipid oxidation during exercise is still lacking. In support of studying MetFlex of CHO, Kelley and Mandarino (2000) reported that impaired skeletal muscle lipid oxidation under fasting conditions predicted the severity of impaired CHO oxidation associated with IR.

Other authors have discussed MetFlex in broader terms, defining MetFlex as the capacity of a system to adapt substrate oxidation to substrate availability (Galgani, Moro, et al., 2008). In the MetFlex protocol presented in this thesis, substrate oxidation was measured at baseline before exercise and during 60 min of exercise. The changing substrate availability was the CHO_{exo} provided and the changing energy demand was created by using exercise as a stimulus. A limitation of the MetFlex protocol was that insulin was not measured at the onset of exercise to confirm if insulin concentrations indeed increased after CHO_{exo} ingestion and decreased at the onset of exercise as expected. The insulin response that occurred after CHO ingestion, but before the start of exercise, was assumed to be similar to the insulin response measured during the OGTT at rest. Measuring insulin is important because of its effect on CHO disposal. Unknown insulin levels before the onset of exercise or during exercise was a limitation, because the proportion of CHO_{exo} uptake and oxidation that was attributed to insulin dependent mechanisms compared to insulin independent mechanisms related to exercise remained unclear (as discussed in Chapter 3). Therefore, the MetFlex protocol used in this thesis represented MetFlex to increased CHO_{exo} availability at the level of the skeletal muscle, but did not show if a maintenance or impairment of MetFlex was related to insulin action on CHO uptake, contraction-mediated CHO uptake or both. In retrospect, data on insulin response would have improved our understanding of the mechanisms related to CHO_{exo} uptake at rest and during exercise in children. However, additional blood work

could have also deterred children from participating. A few of the challenges with conducting research studies in children that regularly attend a tertiary care clinic include competing appointments (both clinical and other research appointments), time constraints, and families often being overwhelmed with their child's health. For these reasons, recruitment and scheduling was difficult, especially when attempting to recruit a more homogenous group of children such as recruiting only boys with IGT (previously discussed in Chapter 2).

6.4 Future research directions

It is anticipated that more research on MetFlex under exercise conditions will be conducted in healthy populations, and in adults and children with chronic diseases. Studies in adults could provide opportunities to investigate MetFlex under more controlled conditions to investigate hormonal effects. Particularly in women, more regular menstrual cycles or the use of oral contraceptives could provide information on whether or not sex hormones influence MetFlex. Research in adults may also provide opportunities to complete additional bloodwork or use more invasive methodologies, such as muscle biopsies. This could help clarify the role of plasma FFA and proteins on glucose uptake in skeletal muscle and MetFlex during exercise. Studies to confirm the association between MetFlex under exercise conditions with IR and beta-cell function are needed to elucidate the clinical potential of MetFlex testing. Identifying if reduced MetFlex is more likely to occur in individuals with certain chronic conditions, such as cystic fibrosis

or diabetes, will provide further understanding about MetFlex in health and disease.

There is currently no research on the link between MetFlex measured under resting conditions and MetFlex measured under exercise conditions, and its association with metabolic complications. Someone with impaired MetFlex at rest is expected to also have impaired MetFlex during exercise, however it is unclear if this would occur simultaneously or if reduced MetFlex at rest occurs before reduced MetFlex during exercise or vice versa. In order to investigate this, participants would need to complete a MetFlex assessment at rest using indirect calorimetry with a hyperinsulinemic-euglycemic clamp or a CHO challenge, and then complete MetFlex assessment with CHO intake and an exercise challenge. Alternatively, MetFlex in response to a high-fat diet could also be completed to examine fat availability and oxidation at rest compared to during exercise. No studies have previously examined MetFlex to increased fat availability under exercise conditions.

In the future, the MetFlex test could potentially influence clinical decision-making through the development of a field test for children using the knowledge obtained in this thesis. By designing a test that is accessible in schools or community centres, more children could be screened for risk factors associated with T2DM. This would allow for children with an increased risk for T2DM to be identified and treated with earlier intervention. To develop a field test, ideal modifications would include condensing MetFlex testing into one visit and

developing equations to estimate $\dot{V}O_2$ and $\dot{V}CO_2$ based on other measurements, such as HR. Previously developed equations for estimating $\dot{V}O_{2max}$ can be used to determine the workload for the exercise during the MetFlex test. Lastly, breath measurements would be collected for later analysis to determine the $^{13}CO_2/^{12}CO_2$ ratio and calculate CHO_{exo} oxidative efficiency. The concept is promising in theory, nevertheless, a lot of work remains to be completed in order to appropriately evaluate the utility of such a field test for children.

Before efficacy research on a new field test is conducted, there is a need to better understand MetFlex under exercise conditions. The validity of MetFlex to identify risk factors associated with T2DM in children requires further study and confirmation. As highlighted previously (Chapters 3,4,5), future research observing potential sex and pubertal effects on MetFlex is necessary. In order to do this, a more homogenous sample of children would be needed to study MetFlex, such as recruiting mainly pre-pubertal girls or pre-pubertal boys. Furthermore, longitudinal studies observing changes in MetFlex in both healthy, typically developing children and children with varying degrees of obesity and IR are warranted. This could help determine MetFlex cut-points to predict the development of T2DM and other health complications. Identifying possible factors that could influence the responsiveness of MetFlex to exercise training would also be beneficial for the prevention of health complications. If MetFlex testing shows better potential to be used as a screening tool in the future, components of clinical utility, including accessibility and practicability, will require evaluation. As it

is often the case with research, we now have a slightly more advanced understanding of MetFlex under exercise conditions in children, but are left with many more research questions to answer. The pursuit of these questions and later advancements in scientific knowledge will hopefully contribute to improved screening and treatment for T2DM in children with obesity.

6.5 References

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APPENDICES

APPENDIX A – Acceptability questionnaire

Please rate how you feel about the test you just did by placing an X on the circle with the appropriate number.

I liked the taste of the drink					
	①	②	③	④	⑤
	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
The test was boring					
	①	②	③	④	⑤
	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
The test was painful					
	①	②	③	④	⑤
	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
The test did not take too long					
	①	②	③	④	⑤
	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
I would not mind doing the test a second time					
	①	②	③	④	⑤
	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
Overall, I enjoyed the test					
	①	②	③	④	⑤
	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree

Figure A.1 Modified Likert questionnaire used to assess acceptability.

	MetFlex Test average rating /5	OGTT average rating /5
I liked the taste of the drink (+)	3.1 ± 1.2	2.8 ± 1.2
The test was boring (-)	2.3 ± 0.8	2.5 ± 1.0
The test was painful (-)	2.8 ± 1.3	2.2 ± 1.3
The test did not take too long (+)	3.0 ± 0.9	2.9 ± 1.0
I would not mind doing the test a second time (+)	3.4 ± 0.9	3.4 ± 1.0
Overall, I enjoyed the test (+)	3.7 ± 0.9	3.8 ± 0.8
Total acceptability score /18	**8.1 ± 3.9**	**8.1 ± 3.3**

Table A.2 Acceptability scores for the metabolic flexibility test and oral glucose tolerance test. The ratings with a "+" were added and "-" were subtracted to calculate a total acceptability score. MetFlex, metabolic flexibility; OGTT, oral glucose tolerance test.

APPENDIX B – Summary of recruitment details

	# of consent-to-contact forms	# of participants not enrolled	# of participants who did not respond	# of participants who declined	Reasons for declining participation
2012	34	24	12	12	• Distance (n=1) • Too busy (n=4) • Child did not want to (n=4) • Not interested (n=1) • No show at OGTT (n=1) • Medical reasons (n=1)
2013	28	18	6	12	• Distance (n=3) • Too busy (n=4) • Child did not want to (n=2) • Not interested (n=2) • No show at OGTT (n=1)
2014	35	19	3	16	• Distance (n=1) • Too busy (n=5) • Child did not want to (n=1) • No show at OGTT (n=2) • Enrolled in other studies (n=1) • Unknown reason (n=1) • Still cannot contact (n=2) • Waiting to hear back (n=3)
2015	24	18	5	13	• Distance (n=3) • Too busy (n=4) • Child did not want to (n=2) • Space was not appropriate (n=1) • Phone number out of service (n=1) • Child recently turned 18 years old (n=2)
2016	12	8	2	6	• Distance (n=2) • Too busy (n=3) • Child did not want to (n=1)
Total	**133**	**87**	**28**	**59**	

Table B.1 Number of families that declined participation in Study 1 or Study 2 in this thesis (Chapters 3, 4 or 5) and reasons for declining.

	# of participants enrolled	# completed study	# dropped out	Reasons for not completing study
2012	10	7	3	• No longer wanted to participate. Didn't like the exercise (n=2). • Medical reasons unrelated to the study (n=1).
2013	10	9	1	• Medical reasons unrelated to the study (n=1).
2014	16	14	2	• Could not complete exercise (due to medical reasons) (n=1). • Participant did not want to continue with the exercise sessions (n=1).
2015	6	6	0	• N/A
2016	4	3	1	• Too busy (n=1)
Total	**46**	**39**	**7**	

Table B.2 Number of participants who enrolled and completed one or both studies in this thesis or dropped out. Participants were also recruited from Study 1 to participate in Study 2 (n=2).

APPENDIX C – Assay protocols for measuring glucose and insulin

Glucose

Kit: Glucose Colorimetric Assay Kit (Cat. No. 1009582, Cayman Chemical)
Assay Range: 0 - 25 mg/dL

Preparation:

1. Thaw serum samples, which were stored at -80°C.
2. Dilute SODIUM PHOSPHATE ASSAY BUFFER with 40 mL HPLC-grade water.
3. Dilute serum samples by 10 or another factor with diluted assay buffer.
    - Ex. 40 μL: 360 μL
4. Reconstitute GLUCOSE COLORIMETRIC ENZYME MIXTURE with 6 mL of diluted assay buffer.
5. Make 100 mg/dL GLUCOSE STOCK.
    - Dilute 50 μL of 1000 mg/dL glucose standard with 450 μL of diluted assay buffer
6. Prepare standards according to manual instructions.

Performing the Assay:

1. Add 85 μL of DILUTED ASSAY BUFFER to all wells.
2. Add 15 μL of each standard or sample in duplicates.
3. Add 100 μL of ENZYME MIXTURE to all wells.
4. Cover and incubate at 37°C for 10 min.
5. Remove plate cover and read absorbance at 500-520 nm.

Insulin

Kit: Human Insulin ELISA Kit (Cat. No. KAQ1251, Invitrogen)
Assay Range: 0.17 - 250 μU/mL

Preparation:

1. Reconstitute standards and controls with distilled water.
    - 2 mL for zero standard
    - 1 mL for other standards and controls
2. For wash solution, add 199 volumes of distilled water to 1 volume of wash solution (200x).
    - Ex. 2 mL of wash solution + 400 mL of distilled water
3. Thaw serum samples, which were stored at -80°C.
4. Dilute serum samples if necessary with zero standard. If there is not enough of the zero standard to dilute samples, PBS at physiological pH can be added to the zero standard.
    - Dilution used for studies in this thesis was 1:3

Performing the Assay:

1. Add 50 μL of standard, control or samples in wells in duplicates.
2. Add 50 μL of ANTI-INSULIN HRP CONJUGATE into all wells.
3. Cover plate and incubate for 30 min at room temperature.
4. Aspirate solution from wells. Wash wells 3x. After washing invert plate and tap dry.
5. Add 100 μL of CHROMOGEN SOLUTION into all wells within 15 min of washing.
6. Incubate for 15 min in the dark at room temperature.
7. Add 100 μL of STOP SOLUTION into each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
8. Blank plate against a chromogen blank composed of 100 μL each of chromogen solution and stop solution. Read the absorbance of each well at 450 nm within 1 hour of adding the stop solution.

APPENDIX D – Metabolic flexibility protocol

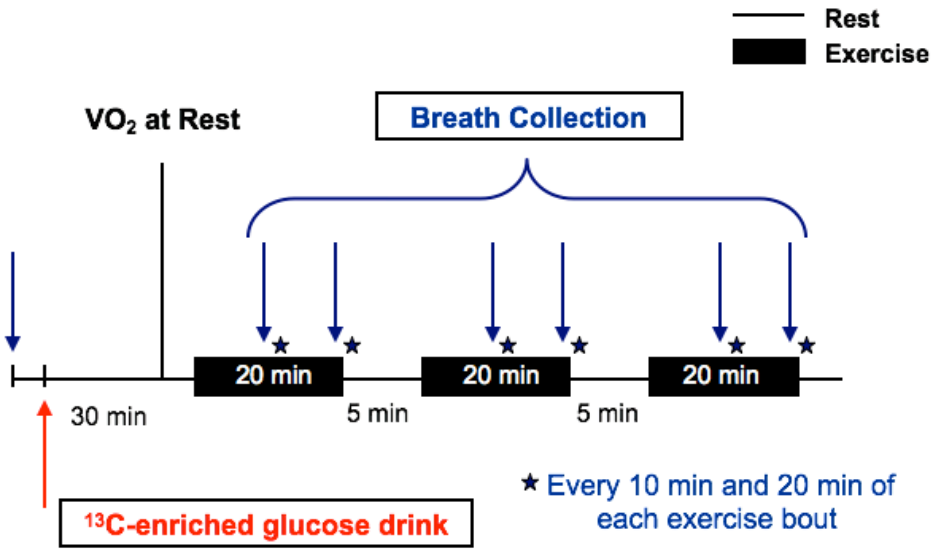

Figure D The metabolic flexibility protocol included 8 time points for breath collection (blue arrows): at rest before drink consumption, at rest 30 min after drink consumption, and every 10 and 20 min of each exercise bout. A ^{13}C-enriched glucose drink was ingested 30 min before exercise. Participants cycled at 45% $\dot{V}O_{2max}$ for 60 min (3 x 20 min bouts) with approximately 5 to 10 min rest periods in between exercise bouts.

APPENDIX E – Analysis of Breath $^{13}CO_2$ protocol

System: Europa Scientific 20/20 gas isotope ratio mass spectrometer (Europa Scientific)

Materials and Equipment:

1. Breath samples collected in Exetainer tubes (Labco, Ltd., 10 mL evacuated, no additive)
2. Reference gas standard (REF/NEW) (Northeast Airgas 5% CO2, in Helium)
3. Control gas (CG) standards (CG1, CG2, CG3) (Northeast Airgas, Custom Production or equivalent)
4. PDZ Europa ABCA (Crewe, United Kingdom) equipped with a bar code reader

Procedure:

1. Verify that the control gas (CG1, CG2, CG3) and reference gas samples (REF/NEW) are less than 7 days old according to preparation date.
    - If the control gases are more than 7 days old, discard the tubes, prepare fresh tubes.
2. Log onto instrument computer.
3. Load the Autosampler.
    - If not using the bar code reader, unique sample names/numbers are entered in the "Enter Samples" list found via the "Prepare System" icon. This should be done while loading the autosampler. If using the bar code reader load samples as follows but do not enter names in "Enter Samples".
    - Sample tubes must be a 10 cc Exetainer tube.
    - The autosampler is loaded in the following order:
        i. The sample run begins with one NEW sample (same as "REF/NEW") tube and the second sample being designated as REF/NEW in the "Sample Data Entry" list.
        ii. Three controls (CG1, CG2, CG3) in random order.
        iii. One more NEW sample.
        iv. Up to 15 samples to be analyzed.
        v. Two more NEW samples, with the second being designated as REF/NEW in the "Sample Data Entry" list.
        vi. Up to 15 samples to be analyzed.
        vii. One more NEW sample.
        viii. Up to 15 samples to be analyzed.
        ix. Two more NEW samples, with the second being designated as REF/NEW in the "Sample Data Entry" list.
        x. Continue with this pattern until all samples are loaded.
        xi. Then end the run with three controls (CG1, CG2, CG3) in random order and then 2 more NEW samples, with the second being designated as REF/NEW in the "Sample Data Entry" list.
4. Press Save and OK.

System: IDMicro Breath Version 2.0 (Compact Science Systems)

Log in from the computer
Log in to the Breath user: User #2, password: xxxxx

System warm-up:

- In standard test: press Start up, then press "start test". Takes 30 min for filament to be ready, the current, trap and limits will be green too. If off for a long time, let it go for longer). Wait 2-3 hours for things to flow nicely.

Prior to running samples:

- Always run the calibration before you start and run between sample batches.

Run calibration in this order:

1. **Auto Ratio Centre 45 (Peak centre)** - Press start test.
2. **Stability 45Delta test** - Press start test. This is to stabilize with time. Takes about 10-20 min. The ratios are 0.1%, 0.1% and 0.2%. Repeat if not within range.
3. **Reproducibility delta sigma -** Stability with pulses on and off. Takes 10-20 minutes. The ratios are 0.1%, 0.1% and 0.2%. Repeat if not within range.
4. **Linearity 45 auto test -** Linearize the 45/44 and 46/44 with samples. Watch out sample peak is not lower than 1.E-09. This would be in the statistical noise.

Note: If any of the above are out of range, repeat the test before going to the next test. This can take 1-3 hours.

Now the Breath Analyzer is ready to run samples.

Start running batch of samples:

1. Press **New.** Set up samples (1-5 blocks/1-4 column/1-11 row).
2. Set the samples up in batch. Select **Save**. (Make sure **Helicobactor Pylori test** is selected). This is where to select duplicates. Note the date or file name.
3. Press Batches: Press prepared and select the set that was just saved.
4. Select queue the samples first by highlight the batch and press **QUEUE. Start Test.**

Additional Notes:

- Always have blanks in the first 2 rows and run them 2x before and after the batch.
- It will take 1.5 min per sample.

APPENDIX F – Bland-Altman plot of $^{13}CO_2$ versus [δ^{13}C]PDB-1 measured with the IDMicro system and the Europa Scientific system

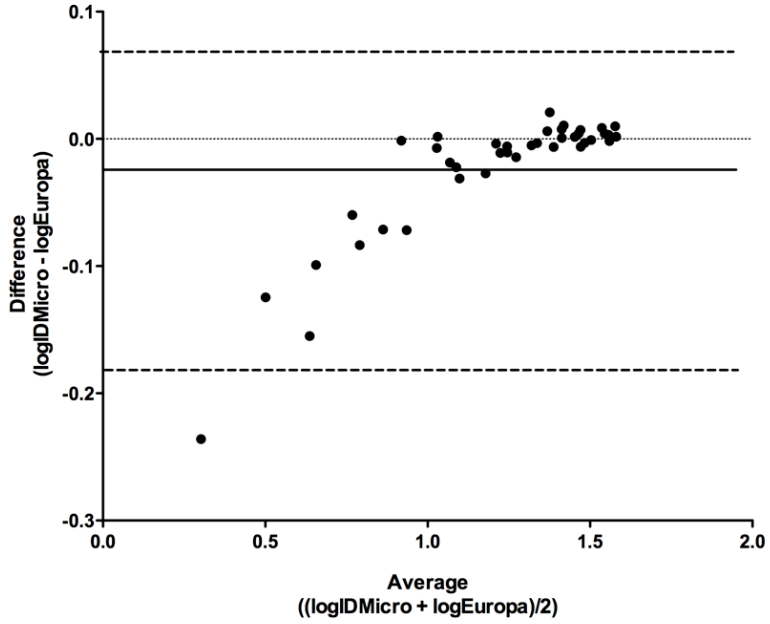

Figure F Bland-Altman plot shows good agreement between $^{13}CO_2$ versus delta 13-carbon Pee Dee Belemnitella-1 ([δ^{13}C]PDB-1) values measured with the IDMicro system and Europa Scientific system. There is a small bias for higher values measured with the Europa Scientific system compared to the IDMicro system when there is a lower isotopic enrichment in the breath samples. However, we do not expect this small difference to affect study results in any meaningful way (Chapter 5).

APPENDIX G – Association between metabolic flexibility and 2-h glucose during an oral glucose tolerance test

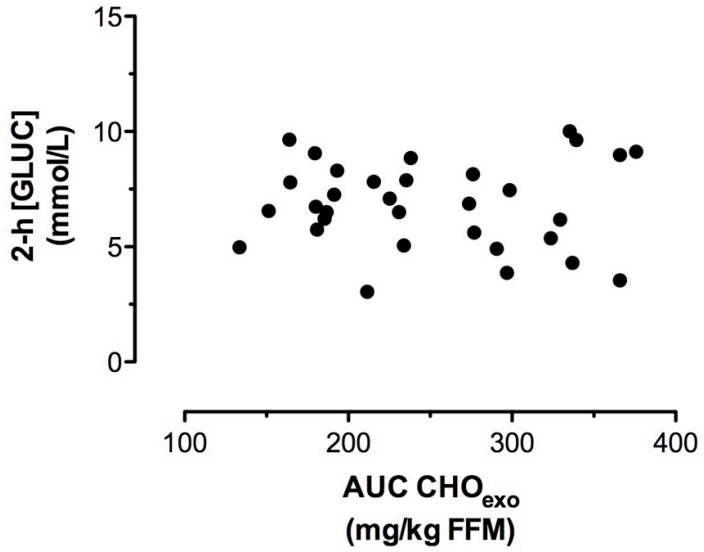

Figure G Pearson's *r* correlation showed no significant association between metabolic flexibility measured as area under curve exogenous carbohydrate oxidation during exercise with 2-h glucose during an oral glucose tolerance test (r=0.003, p=0.989). Abbreviations: 2-h [GLUC], 2-h glucose concentration; AUC CHO_{exo}, area under the curve exogenous carbohydrate oxidation; FFM, fat free mass.

APPENDIX H – Change in exogenous and endogenous carbohydrate and total fat contribution to total energy expenditure after 7 days of exercise training

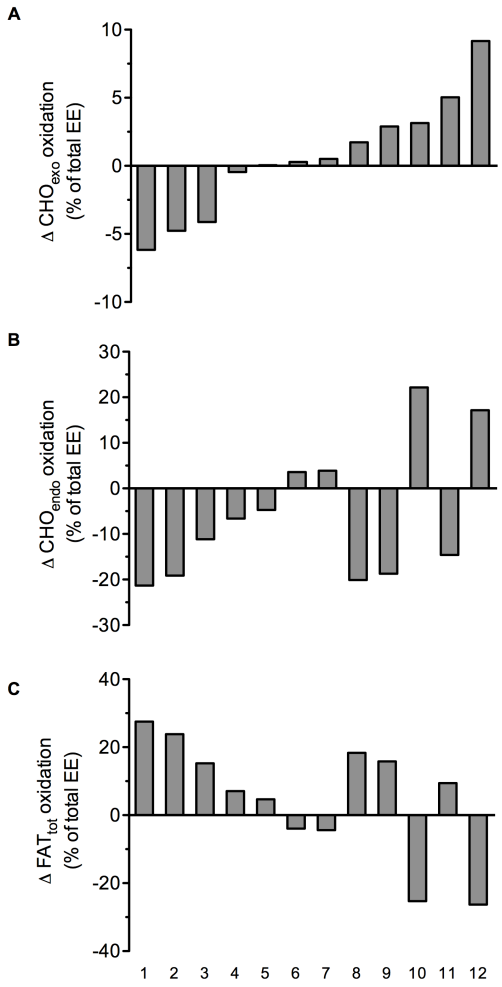

Figure H A) Individual change in exogenous carbohydrate (CHO_{exo}) contribution to percent of total energy expenditure (EE) after 7 days of exercise training. Using a paired t-test, no significant difference in CHO_{exo} contribution to total EE was found after exercise training (PRE: 16.8 ± 2.6%, POST: 17.5 ± 4.3%, p=0.63). **B)** Individual change in endogenous carbohydrate (CHO_{endo}) contribution to percent of total EE after 7 days of exercise training. Participants are numbered on the x-axis and ordered according to change in CHO_{exo} contribution to total EE. Using a paired t-test, no significant difference in CHO_{endo} contribution to total EE was found after exercise training (PRE: 69.0 ± 13.5%, POST: 63.2 ± 12.4%, p=0.20). **C)** Individual change in total fat (FAT_{tot}) contribution to percent of total EE after 7 days of exercise training. Participants are numbered on the x-axis and ordered according to change in CHO_{exo} contribution to total EE. Using a paired t-test, no significant difference in FAT_{tot} contribution to total EE was found after exercise training (PRE: 14.2 ± 12.3%, POST: 19.4 ± 13.6%, p=0.32).

APPENDIX I – Summary of Smart's dimensions of clinical utility

Component	Aspects	Examples of issues to consider
Appropriate	• Effective • Relevant	• Existence of evidence • Impact on existing treatment • Importance for clinical decision-making
Accessible	• Resources • Procurement	• Costs and cost-effectiveness • Availability, supply, and quality • Financing
Practicable	• Functional • Suitable • Necessary training or knowledge	• Do the material, methods, or instructions perform their tasks *in situ*? • Adequacy of current levels and potential future needs • Constraints on training
Acceptable	• To clinicians • To clients • To society (public or stakeholders)	• Ethical, legal, social, or psychological concerns that may affect practice or treatment • Ethical, legal, social, or psychological concerns that may affect acceptance

Table I Adapted from Smart (2006). Main components of clinical utility that were discussed in the thesis included appropriateness and acceptability of the metabolic flexibility test. Accessibility and practicability could not be evaluated at this time because of limited research, but may be components to consider for future research. Note: reference is found in Section 6.5.

APPENDIX J – Overview of exogenous carbohydrate oxidative efficiency in health and disease

Study Details	Age	Notes on study protocol	CHO_{exo} oxidation reported in study	CHO_{exo} oxidative efficiency
Riddell et al., 2000 Participants: Healthy, untrained boys	13 - 17 y	• 60% $\dot{V}O_{2max}$ • 4 x 30 min → 120 min • 3 g CHO/kg BW	• 57.8 g/120 min CHO_{exo} oxidation	Reported: • 34.2 ± 2.2%
Riddell et al., 2001 Participants: Healthy, nonobese, habitually active boys	10 - 14 y	• 55% $\dot{V}O_{2max}$ • 3 x 30 min → 90 min • +47.7‰ [δ^{13}C]PDB-1 glucose • +48.0‰ PDB fructose • volume 25 ml/kg BW • 6% glucose drink vs. 3% glucose + 3% fructose drink • mean BW: 44.9 kg	• 0.36 g/min glucose (bout 3) • 0.31 g/min fructose (bout 3) • calculated for 90 min	Calculated: • 33.1% in glucose trial • 30.3% in glucose + fructose trial
Timmons et al., 2003 Participants: Healthy, pre- and early-pubertal boys and men	9.8 y boys 22.1 y men	• 70% $\dot{V}O_{2max}$ • 2 x 30 min → 60 min • 6% CHO drink was ingested • +20.9‰ [δ^{13}C]PDB-1 • volume 24 ml/kg BW • mean BW: 35.1 kg in boys; 82.6 kg in men	Boys vs. men • 30 min: 6.4 ± 0.5 vs. 3.9 ± 0.3 mg/kg/min • 45 min: 9.4 ± 0.7 vs. 6.4 ± 0.5 mg/kg/min • 60 min: 10.7 ± 0.4 vs. 8.4 ± 0.7 mg/kg/min	Reported: • 36.8 ± 2.0% in boys* • 26.0 ± 2.1% in men

Timmons et al., 2007 Participants: Healthy boys	12 y 14 y	• 70% $\dot{V}O_{2max}$ • 60 min cycling • 6% CHO drink (4% sucrose, 2% glucose) • +10‰ [δ^{13}C]PDB-1 • 12 ml/kg BW + 4 ml/kg BW every 15 min during exercise → 28 ml/kg BW • mean BW: 44.0 kg in 12 y boys; 60.2 kg in 14 y boys	Last 15 min of exercise: • 12.4 ± 0.7 mg/kg/min in 12 y boys • 10.2 ± 0.9 mg/kg/min in 14 y boys	Calculated: • 44% in 12 y boys • 36% in 14 y boys
Timmons et al., 2007 Participants: Healthy girls	12 y 14 y	• 67% $\dot{V}O_{2max}$ • 60 min cycling • 6% CHO drink (4% sucrose, 2% glucose) • +10‰ [δ^{13}C]PDB-1 • 12ml/kg BW + 4 ml/kg BW every 15 min → 28 ml/kg BW • mean BW: 46.6 kg in 12 y girls; 58.2 kg in 14 y girls	Last 15 min of exercise: • 7.1 ± 0.5 mg/kg/min in 12 y girls • 6.8 ± 0.4 mg/kg/min in 14 y	Calculated: • 25.4% in 12 y girls • 24.3% in 14 y girls
Chu et al., 2011 Participants: Pre- and early-pubertal boys with obesity	9 - 12 y mean age: 11.4 y	• exercise intensity that elicited maximal fat oxidation • 3 x 20 min → 60 min • 6% CHO drink • provided 16 ml/kg FFM/min + 4 x 4 ml/kg FFM → 32 ml/kg FFM • mean BW: 67.4 kg • mean FFM: 24.33 kg	Last 20 min of exercise: • 5.1 ± 1.5 mg/kg FFM/min	Calculated: • 15.9%

Chu et al., 2016 (Chapter 3) Participants: children with obesity and NGT or IGT	8 - 17 y	• 45% $\dot{V}O_{2max}$ • 3 x 20 min → 60 min • +100‰ [δ^{13}C]PDB-1 • 75 g CHO drink		Reported: • 17.0 ± 3.6% in children with IGT • 17.1 ± 4.4% in children with NGT
Bosch et al., 1994 Participants: Healthy, endurance-trained men	26 y	• 70% $\dot{V}O_{2max}$ • 180 min • 10% CHO drink • 500 ml/h → 1500 ml	End of exercise: • 0.83 g CHO ingestion • 0.77 g CHO oxidation • CHO_{exo} oxidation rate was 63 (8) µmol/min/kg FFM • Mean FFM: 62.7 kg	Calculated: • 85% or 93% at the end of exercise
Jeukendrup et al., 1997 Participants: Untrained and trained adults	20.9 ± 0.5 y untrained 25.1 ± 1.4 y trained	• 60% $\dot{V}O_{2max}$ • 120 min cycling • 8% CHO drink • -11.2‰ [δ^{13}C]PDB-1 • 8 ml/kg + 2 ml/kg every 15 min → 24 ml/kg • mean BW: 75.4 ± 3.8 kg in untrained; 72.3 ± 1.2 kg in trained	CHO_{exo} oxidation from 60 to 120 min: • 44.8 ± 2.6 g in untrained adults • 49.5 ± 1.5 g CHO in trained adults peak CHO_{exo} oxidation rates: • 0.96 ± 0.03 g/min in untrained adults • 0.95 ± 0.07 g/min in trained adults	Calculated: • 31% in untrained adults • 35.7% in trained adults Calculations were based on the last hour of exercise and not the full 2 hours.

Burelle et al., 1999 Participants: Sedentary men and trained men	23 ± 1 y untrained 20 ± 1 y trained	• 68% $\dot{V}O_{2max}$ • 90 min • 100 g CHO drink (split into 4 equal volumes) • +25‰ [δ^{13}C]PDB-1	• 33.6 ± 1.2 g/h in sedentary men* • 44.4 ± 1.8 g/h in trained men	Reported: • 33.6% in sedentary men • 44.4% in trained men Calculations did not include the first 30 min of exercise.
Krzentowski et al., 1984 (Exercise training study) Participants: Healthy men	25 y	• 6 weeks of training (60 min cycling; 5 days/week; 30-50% $\dot{V}O_{2max}$) • protocol: • 40% $\dot{V}O_{2max}$ • 105 min • +18.4‰ [δ^{13}C]PDB-1 • received 100 g CHO_{exo} and continued exercising for 90 min, then rested for 90 min • protocol after exercise training was at 32.1% $\dot{V}O_{2max}$ (same absolute intensity as before training)	• 34.5 ± 2.9 g/3 h before exercise training* • 40.3 ± 2.7 g/3 h after exercise training • Training increased CHO_{exo} oxidation by 17%	Calculated: • 34.5% before exercise training • 40.3% after exercise training
Ravussin et al., 1980 Participants: Adults with obesity and controls	29 y	• 20% $\dot{V}O_{2max}$ • 120 min cycling • Received 100 g CHO_{exo} 1 h before exercise	• 33.6 ± 2.5 g in adults with obesity • 28.1 ± 2.3 g in controls	Calculated: • 33.6% in adults with obesity • 28.1% in controls

Riddell et al., 2000 Participants: Boys with T1DM and controls	13 - 19 y mean age: 15.1 ± 0.7 y	• 58.8% $\dot{V}O_{2max}$ • 60 min • +16.3‰ [δ^{13}C]PDB-1 • usual insulin dose in T1DM	• 10.4 g/60 min IDDM • 14.8 g/60 min healthy	Reported: • 12.1 ± 1.3% in T1DM* • 16.2 ± 0.8% in controls
Krzentowski et al., 1981 Participants: Adults with T1DM and controls	18 - 21 y	• 45% $\dot{V}O_{2max}$ • 4 h on treadmill • received 100 g of glucose	• 84 ± 8 g/4 h in T1DM with insulin • 92 ± 3 g/4 h in controls • 43 ± 11 g/4 h in T1DM without insulin	Calculated: • 84% in T1DM with insulin infusion • 92% in controls • 43% in T1DM without insulin infusion
Robitaille et al., 2007 Participants: Adults with T1DM and controls	26.5 ± 6.8 y	• 50.8% $\dot{V}O_{2max}$ • 60 min • +400‰ [δ^{13}C]PDB-1 • usual insulin dose in T1DM • received 30 g of glucose, 15 min before exercise	Exercise at 30 to 60 min: • 6.3 g/30 min in T1DM • 5.2 g/30 min in controls muscle glycogen: • higher in T1DM* plasma glucose oxidation: • lower in T1DM*	Reported: • 17% in T1DM • 21% in controls

*indicates that a significant difference was identified between groups in the manuscript. Abbreviations: $\dot{V}O_{2max,}$ maximal oxygen uptake; BW, body weight; CHO_{exo}, exogenous carbohydrate; ‰ [δ^{13}C]PDB-1, per mille versus delta 13-Carbon Pee Dee Belemnitella-1; FFM, fat free mass; T1DM, type 1 diabetes mellitus. Note: references are found in Section 1.9.

APPENDIX K – Copyright acknowledgements

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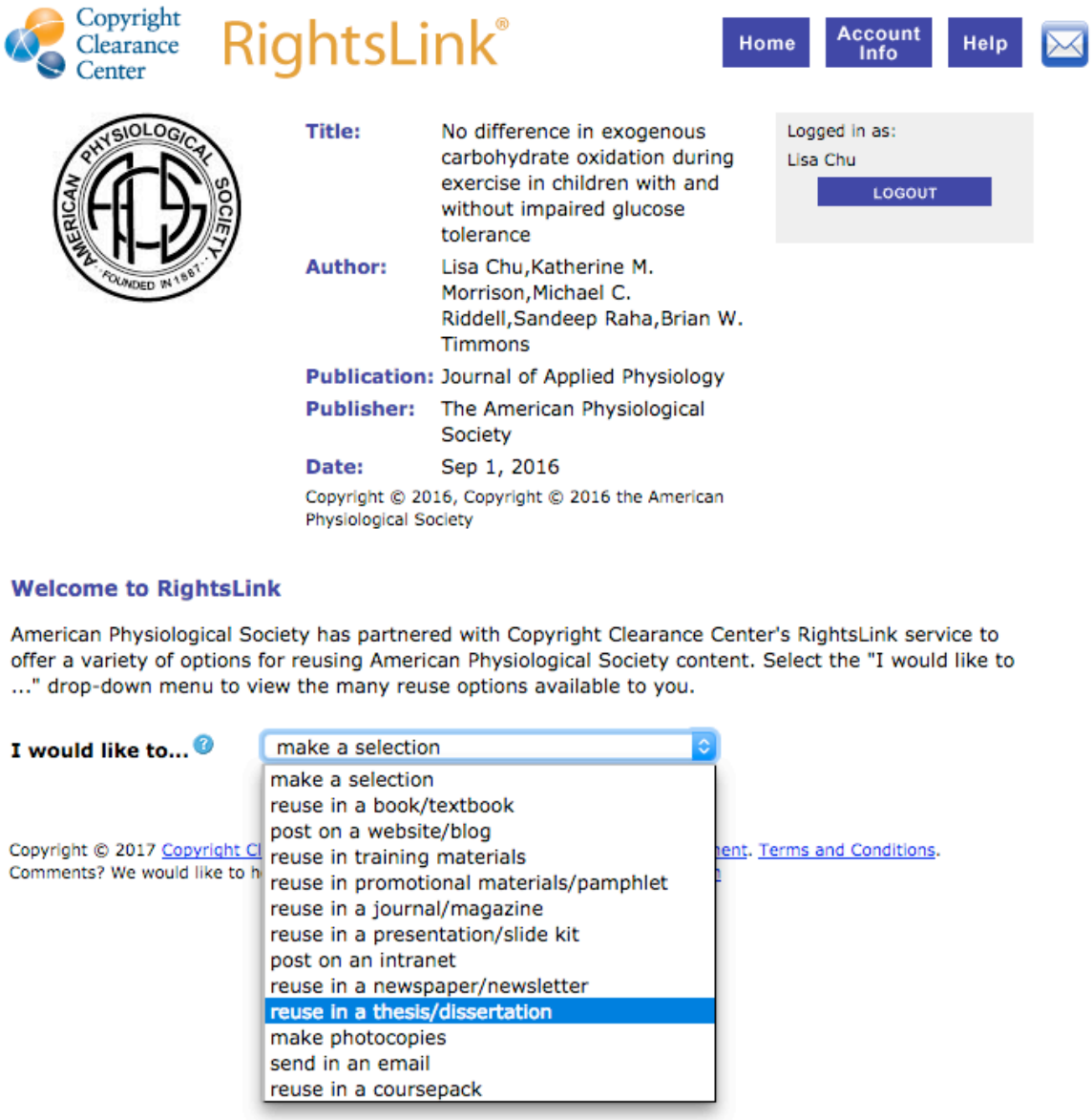

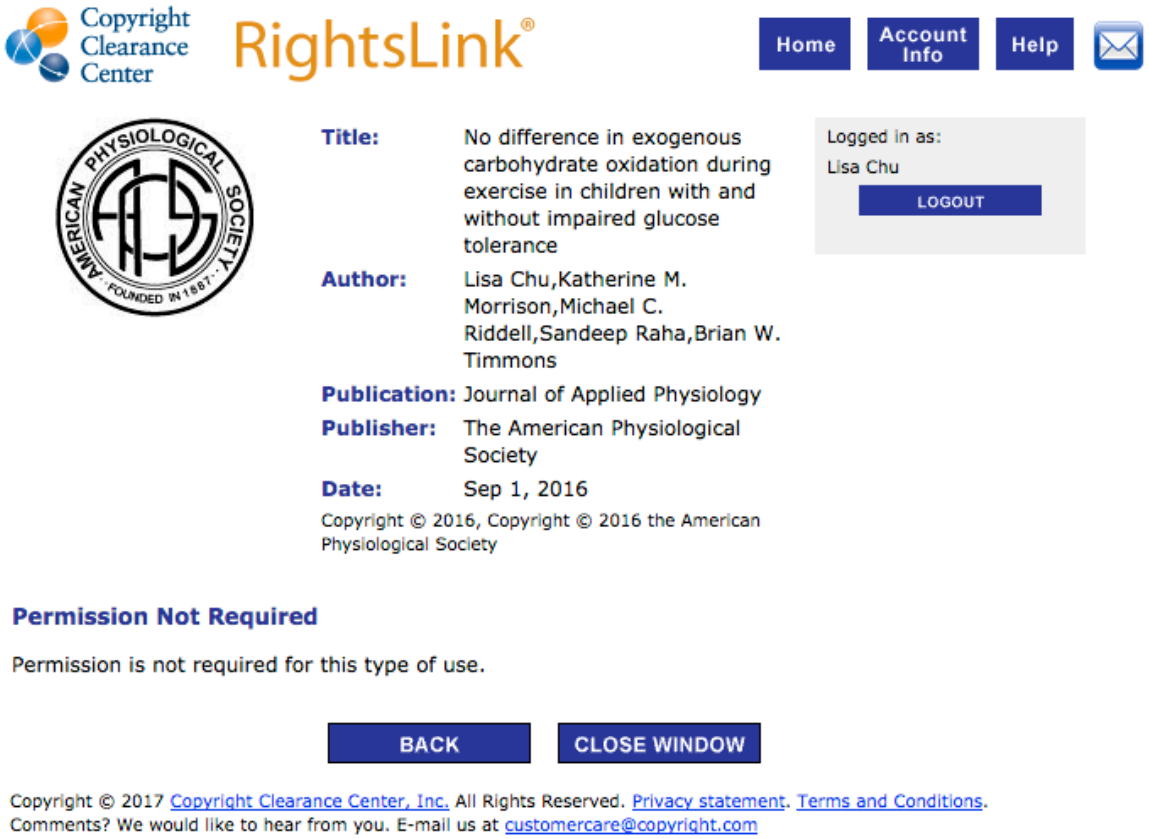

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