EFFECTS OF CARBOHYDRATE SUPPLEMENTATION ON
VARIABLE-INTENSITY EXERCISE RESPONSES IN BOYS AND MEN

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ABSTRACT

THESIS: Effects of Carbohydrate Supplementation on Variable-Intensity Exercise
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This study examined the physiological and perceptual effects of carbohydrate (CHO) on variable-intensity exercise (VIE) in boys and men. It was hypothesized that CHO would increase RER in boys and men and that this increase would be greater in boys. Additionally, it was hypothesized that RPE would be attenuated by CHO. Five boys (10-12 years) and seven men (18-30 years) consumed CHO or a placebo (PL) beverage before and throughout VIE. VIE included three 12-min sets of cycling; intensity varied every 20-30 seconds between 25, 50, 75, and 125% $\text{VO}_{2\text{max}}$. Boys' post-exercise glucose was higher in the CHO trial than the PL trial and RER was lower in boys than men, but was not affected by trial. RPE increased over time but was not different between groups or trials. Though VIE responses varied between boys and men, CHO ingestion before and during VIE did not provide physiological or perceptual benefits.

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CHAPTER I

Introduction

Energy Resources / Pathways

Adenosine triphosphate (ATP) storage in the muscle is limited and therefore must be continually replenished in order for muscle action to continue beyond a few seconds (48). The resources available to replenish ATP include adenosine diphosphate (ADP), phosphocreatine (PCr), carbohydrate (CHO), and fat. ATP is generally synthesized from these sources through a variety of biochemical pathways including the phosphate pathway, glycolysis, and oxidative phosphorylation. The phosphate pathway is the most rapid means to produce ATP. In this pathway, a phosphate from either PCr or ADP is combined with ADP to produce ATP. As exercise duration extends, there is an increased reliance on glycolytic ATP production. Glycolysis is a series of reactions that splits glucose into two molecules of pyruvate and generates two to three ATP (110). The fate of pyruvate is largely dictated by the glycolytic rate. If pyruvate is produced at a rate greater than it is being oxidized by the mitochondria, some pyruvate will be converted to lactate (51). If pyruvate enters the mitochondria, it will undergo oxidation in the citric acid cycle and result in ATP production via oxidative phosphorylation in the electron transport chain. Fat as an energy source also can be oxidized in a similar manner (110).

The extent to which a particular fuel source and energy pathway is utilized during exercise depends on exercise duration and intensity. While each pathway contributes to ATP

production during most types of exercise, the proportional contribution of each given pathway will vary. Short-duration (0-30 seconds), high-intensity exercise relies on the PCr system due to its high rate of ATP generation (47). Exercise bouts lasting 30 seconds to 2 minutes rely primarily on glycolysis, while oxidation predominates during exercise that extends beyond 2 minutes (47). The type and amount of substrate utilized also will vary with exercise intensity. The percentage of fat used tends to be greatest at low exercise intensities and decreases as intensity increases, while the percentage of CHO used increases as intensity increases (60). During prolonged exercise at a constant intensity, fat use will increase and CHO use will decrease over time (108). In contrast to prolonged constant intensity exercise, variable-intensity exercise includes frequent changes of intensity from low to supramaximal levels. These intensities, along with the relative duration of each level vary widely between different sports and protocols. As a result, variable-intensity exercise does not follow a well-characterized or consistent metabolic pattern. Instead, the metabolic processes during variable-intensity exercise likely reflect a fusion of aerobic and anaerobic pathways, the weight of each varying with the specific demands of the activity.

Carbohydrate Supplementation

Due to the dependency on CHO as a fuel source during exercise combined with the limited amount of glycogen stored in the muscle and the liver, exercise that leads to glycogen depletion results in fatigue (121). Thus, strategies to supplement endogenous CHO stores with exogenous CHO have been extensively studied. One of most common types of CHO supplementation is in the form of a CHO-rich beverage. CHO drinks are typically consumed to replace lost fluids or to provide nutrition in the form of CHO before, during, or immediately

following exercise. Many variations exist, but most share the same basic components: CHO, electrolytes, and water, while some formulas also contain protein. The CHO component of a sports drink can consist of one or more of four types of CHO: fructose, glucose, sucrose, and maltodextrins. The exact formulas differ, but most commercially available drinks contain approximately 6% total CHO (24). Electrolyte concentrations vary more widely between brands, but most drinks contain a mixture of sodium, potassium, and chloride and an overall osmolality close to 300 mOsm/kg H₂O.

Extensive research on the effects of CHO supplementation on exercise performance has shown CHO consumption before and during prolonged aerobic exercise can delay fatigue (27, 43, 134). This benefit is likely due to either glycogen sparing (27, 134) or improved plasma glucose maintenance (43). Improved sprint performance following prolonged aerobic exercise also has been observed (45, 56, 114). Some increases in performance after continuous exercise bouts shorter than one hour have been observed (8), but this effect is less certain (36).

More recently, the effects of CHO supplementation on intense intermittent and variable-intensity activity have been studied (9, 14, 30, 90, 111, 114, 128). CHO supplementation allowed subjects performing high-intensity intermittent cycling to perform additional sprints before exhaustion (30) or to produce more power on a Wingate test following the intermittent cycling (114). CHO supplementation during variable-intensity shuttle running protocols has elicited a similar response, allowing subjects to complete more repetitions of the protocol before becoming exhausted (90, 128) and faster sprinting during the last 15 minutes of exercise (128). Additionally, soccer players who consumed a high-CHO diet showed increased high-intensity activity during a game when compared to players who did not consume a high-CHO diet though this effect has not been observed with pre- or mid-game CHO supplementation

(9). These studies included exercise at least one hour in duration. In contrast, “repeated-sprint” protocols shorter than 30 minutes in duration have failed to show performance improvements with CHO supplementation (14, 111).

CHO supplementation also may influence the perceptual responses to exercise. Marathon runners were able to run at an increased intensity while maintaining the same RPE when they ingested CHO (126). One study found cyclists had a lower RPE during prolonged cycling at the same intensity when ingesting CHO (125) though another found no difference (116). The effect of CHO supplementation on RPE during exercise appears to be related to substrate availability, specifically blood glucose availability (69).

Metabolic Responses to Exercise in Children

The metabolic response to exercise is different in children compared to adults. Children store a similar amount of ATP as adults (38), but demonstrate lower power outputs during anaerobic exercise testing (19, 62). The decreased power output may be related to lower lean body mass and thus lesser muscle mass (19). However, Davies et al. (29) observed lower peak power values in children even after correcting for lean body mass, suggesting other factors, perhaps metabolic in nature also limit anaerobic exercise performance in children. PCr stores at rest are similar between children and adults (40, 41, 135), suggesting the PCr pathway does not limit the child’s anaerobic performance. However, aspects related to the glycolytic pathway may limit anaerobic exercise performance in children. Muscle (40, 41) and liver (19) glycogen levels are lower in children. Thus, the total amount of stored glycogen available to an exercising child is inferior to the glycogen available to an adult. As a result, children may become glycogen-depleted more quickly during an exercise bout due to lower glycogen levels at the onset of

activity (19). Glycolytic capacity also may be inferior in children compared to adults as levels of phosphofructokinase (PFK), a rate-limiting enzyme of glycolysis, are lower (39, 41). Eriksson et al. (39) observed 50% lower PFK activity in boys compared to men. Lower lactate accumulation and smaller decreases in pH in children during exercise further supports the argument that children have a reduced glycolytic capacity (40, 41, 135). In contrast to limited glycolytic capability, children exhibit enhanced oxidative capability. This is evidenced by a decreased ratio of glycolytic to oxidative enzyme activity (54) and a lower respiratory exchange ratio (RER) during exercise compared to adults (118). These metabolic characteristics are consistent with the observation that children typically do not fatigue to the same extent as adults during high-intensity exercise. For example, boys can generate a higher percentage of their initial peak power during repeated high-intensity bouts compared to men (58, 97, 99). Traditionally this has been attributed to factors such as decreased lactate and H^+ ion accumulation (72, 135) and improved PCr recovery (44), although the child's smaller mass and absolute level of force production may also play a role (99).

Knowledge of the effects of CHO supplementation on children is limited, but given the smaller amount of glycogen stored in the child's muscle and liver (19) and the availability and popularity of sports drinks, it is an important area of research. Riddell et al. (104, 105) found CHO intake prior to and during prolonged aerobic exercise to spare endogenous CHO and fat usage, delay exhaustion by up to 40%, and improve post-exercise performance in children. Although CHO supplementation improved sprint performance in adolescents (77), it does not attenuate fatigue during repeated sprints in children (85) or adolescents (77), suggesting other factors causing fatigue may mitigate any benefit of CHO consumption.

Purpose and Hypotheses

Activity patterns in children tend to be intermittent and variable in nature, particularly during free play and sport. Therefore, the purpose of this study was to examine the effects of CHO supplementation in children during this type of activity. To observe these effects, physiological and perceptual responses during variable-intensity exercise with and without CHO supplementation were measured in both boys and men. It was hypothesized that CHO supplementation would not affect VO_2 or heart rate (HR) during exercise, but that the perceptual responses to this type of exercise would be attenuated. Additionally, it was hypothesized that RER would increase in both boys and men, and that the increase in RER would be greater for boys than men. This hypothesis is based on the results of Timmons et al. (119) that demonstrated boys' ability to oxidize a greater proportion of exogenous glucose compared to men. It was hypothesized that blood glucose would be maintained at or slightly above baseline values with CHO supplementation and decrease over time without supplementation. Lastly, it was hypothesized that lactate would be higher in men than boys during exercise, but would not be affected by CHO ingestion.

Assumptions

1. The testing procedures and equipment were valid and reliable.
2. All subjects gave a maximal effort during the graded exercise test.
3. All subjects complied with the instructions regarding food and drink intake, exercise restrictions, and caffeine avoidance prior to each trial.
4. All child subjects/parents accurately reported their pubertal status.

5. There were no significant alterations in diet or physical activity level in any of the subjects over the time course of the study.
6. The volume and content of the CHO beverage given during the experimental trial was sufficient to provide an ergogenic effect.
7. The CHO content and time course from beverage consumption to exercise were sufficient to allow gastric emptying and intestinal absorption of glucose.

Delimitations

The subject population consisted of 10-12 year old boys whose pubertal age was 1-2 according to Tanner (115) and young adult men (18-30 years). All subjects were determined to be healthy, as indicated on the health history questionnaire completed by each subject or his parent(s). Subjects were of average fitness ($35\text{-}55\text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and not taking any medications known to alter the physiological response to exercise.

CHAPTER II REVIEW OF LITERATURE

This chapter will review the literature related to this study. In the first section, the metabolic processes responsible for fueling exercise and the methodologies currently used to assess exercise will be discussed. The chapter will then review a relatively new genre of exercise, variable-intensity exercise and the protocols that have been utilized to study this type of exercise. A review of the literature on CHO supplementation and its effect on exercise performance, physiological processes, and perception of effort will follow. Lastly, the metabolic differences between children and adults will be discussed, as will the effects of CHO supplementation in children during exercise. A summary will conclude the chapter.

Exercise Metabolism

Adenosine triphosphate (ATP) is considered the “energy currency” of the cell. Many critical processes rely on ATP availability including, but not limited to, ion gradient maintenance and muscular contraction. ATP is comprised of three parts: adenine, ribose, and three phosphates. The second and third phosphates are attached via high-energy phosphate bonds, which store much more energy than a typical chemical bond (20). This energy is released when a phosphate is split from the remainder of the molecule through hydrolysis, creating adenosine diphosphate (ADP) and inorganic phosphate (P_i) (61):

Approximately $20\text{--}25 \text{ mmol} \cdot \text{kg}^{-1} \text{ dm}$ of ATP is stored in the muscle (48). During intense exercise, ATP turnover can exceed $15 \text{ mmol} \cdot \text{kg}^{-1} \text{ dm} \cdot \text{sec}^{-1}$ (48). At this rate, the muscle's entire store of ATP would be depleted within two seconds; therefore, it is necessary to replenish ATP rapidly at the onset of exercise in order for muscle action to continue. Due to effective resynthesis, ATP stores rarely decrease more than 30-40%, even during maximal exercise (47). Three main pathways exist to replenish ATP: the phosphate pathways, glycolysis, and oxidative phosphorylation.

Phosphate Pathways

The quickest method of replenishing ATP is the phosphocreatine (PCr) pathway. The muscle contains about $80 \text{ mmol} \cdot \text{kg}^{-1} \text{ dm}$ of PCr (four times the amount of stored ATP) (48). When the ATP concentration drops, ADP is phosphorylated with the phosphate from a molecule of PCr. This reaction is catalyzed by the enzyme creatine kinase:

The PCr pathway is critical at the onset of exercise, as it can reach a peak rate of ATP production within 1.3 seconds (47) and can replenish up to $9 \text{ mmol ATP} \cdot \text{kg dm}^{-1} \cdot \text{sec}^{-1}$ (48). Approximately 50% of the ATP produced during a short sprint (5-6 sec) comes from the PCr reaction. However, PCr stores are limited and are largely depleted within the first 10 seconds of exercise (48). Unlike ATP stores, it is possible for PCr stores to become completely depleted during intense exercise (18). During recovery, PCr is resynthesized when ATP donates P_i to Cr (reverse of CK reaction). The process of resynthesis has a half-time of approximately 30 seconds, and because it is dependent on aerobic ATP production, it is sensitive to oxidative metabolism (48).

A second phosphate pathway uses adenylate kinase to catalyze the combination of two molecules of ADP to form a molecule of ATP and one molecule of adenosine monophosphate (AMP).

The AMP byproduct of this reaction is deaminated by AMP deaminase to produce inosine monophosphate (IMP) and ammonia (48). This pathway is used to supplement ATP resynthesis when energy demands exceed the capacities of the other energy systems (48).

Glycolysis

The glycolytic pathway provides energy by breaking down CHO fuels (glucose or glycogen). Normal postabsorptive blood glucose is about 5.5 mM (20). Blood glucose typically rises with the onset of exercise due to a feed-forward mechanism that stimulates the liver to release glucose. The concentration may fall slightly over time, but will typically stay within 10% of the resting value (20). Glycogen is the storage form of glucose and is found primarily in the liver and muscles. In a normal 70-kg male, the liver stores about $250 \text{ mmol} \cdot \text{kg}^{-1}$ and about $100 \text{ mmol} \cdot \text{kg}^{-1}$ dm is stored in the muscle (120).

CHO in the form of blood glucose or muscle glycogen can be broken down through glycolysis. When glucose is the substrate, glycolysis results in the net gain of two ATP, while using glycogen as the substrate will result in the net gain of three ATP.

The glycolytic pathway consists of a series of reaction beginning with glucose-6-phosphate (G6P) and ending with pyruvate. G6P is formed by phosphorylating glucose (catalyzed by hexokinase)

or by breaking down glycogen into glucose-1-phosphate and converting it to G6P. Glucose phosphorylation requires one ATP, thus reducing the net gain of ATP through glycolysis compared to starting with glycogen. The first two steps provide the activation energy for the continuation of the glycolytic pathway (110). First, G6P is isomerized to form fructose-6-phosphate (F6P) which is then phosphorylated to form fructose-1,6-bisphosphate (F1,6BP). This reaction is catalyzed by phosphofructokinase (PFK) and is often referred to as the committed step of the glycolytic pathway because it is irreversible (61). F1,6BP is split into two three-carbon molecules (61). The remaining steps of glycolysis convert these molecules into pyruvate, producing ATP, NADH, and H^+ in the process (61). At this point, pyruvate has two possible fates. It can either enter the mitochondria to be oxidized or be reduced to lactate by the enzyme lactate dehydrogenase. The fate is dependent on the relative activities of glycolysis and the mitochondria. Pyruvate is consumed by the mitochondria for oxidation when possible (51); if pyruvate is being produced at a greater rate than the mitochondria can oxidize it then it will be reduced to lactate (110).

The two major points of glycolytic regulation are at the steps catalyzed by hexokinase and PFK. Hexokinase regulates glycolysis by controlling glucose uptake. If the concentration of G6P in the cell increases, hexokinase activity will decrease, thereby decreasing glucose uptake. The activity of PFK is controlled by the energy level of the cell. High levels of ATP or citrate (a citric acid cycle intermediate) will inhibit PFK, as these substances indicate sufficient energy levels. Hydrogen ions can also inhibit PFK. Conversely, high levels of ADP, AMP, or P_i will stimulate PFK and increase the glycolytic rate, as these substances indicate low energy levels (61).

Oxidative Phosphorylation

Oxidative metabolism occurs entirely in the mitochondria. Pyruvate from glycolysis is converted to acetyl-coenzyme A (acetyl-CoA). Each acetyl-CoA will turn the citric acid cycle one time. The main purpose of the citric acid cycle is to break down the acetyl-CoA and reduce the coenzymes NAD^+ and FADH^+ . Each acetyl-CoA produces three carbon dioxide (CO_2) molecules, one ATP, three NADH, three H^+ , and one FADH_2 . The CO_2 is exhaled, and the NADH and FADH_2 continue on to the electron-transport chain to be fully oxidized (110). The electron-transport chain reactions take place in the inner mitochondrial membrane. Free energy is released as electrons from NADH and FADH_2 travel through a series of electron acceptors, with oxygen being the final electron acceptor in the series. This energy is used to pump hydrogen ions across the inner mitochondrial matrix into the intermembrane space, creating an electrochemical gradient. When hydrogen ions return back down this gradient to the mitochondrial matrix, they release free energy that is used to synthesize ATP from ADP and P_i (61). Each NADH releases enough energy to form three ATP and each FADH_2 releases enough energy to form two ATP (110).

In addition to glucose and glycogen, fat can also be used for energy. The average adult male stores about 12,000 g of fat in adipose tissue, plus an additional 300 g of fat in the muscle. Thus, fat availability for energy production is virtually unlimited in humans (68). However, unlike glucose and glycogen, fat, primarily in the form of fatty acids can only be metabolized oxidatively. Fatty acids are broken down into acetyl-CoA through beta-oxidation. These molecules of acetyl-CoA produced from fat are oxidized by the citric acid cycle and electron transport chain in the same way as acetyl-CoA molecules from CHO (110).

Each of the three energy pathways contributes to ATP resynthesis during most types of exercise. However, the dominant pathway is mostly determined by the exercise intensity and duration. Short-duration, high-intensity exercise relies predominantly on the phosphagen systems and glycolysis, as these pathways replenish ATP more rapidly than oxidation. However, as exercise duration increases, oxidative metabolism becomes dominant. Both fat and CHO can be metabolized oxidatively (47). However, the proportions vary based on exercise intensity and duration. Fat use is highest at low intensities and decreases with increasing intensity whereas CHO use follows an opposite pattern (26). This shift occurs because high-intensity exercise requires ATP to be replenished at a rate that exceeds the maximal rate of fat oxidation (26). As exercise duration increases, intramuscular fuel stores (muscle glycogen and intramuscular triglyceride) decrease, and the reliance on plasma glucose and free fatty acids will increase (26). As CHO stores become depleted, exercise intensity will decrease to an intensity that can be maintained by fat oxidation (26).

Exercise Assessment

Needle Muscle Biopsy

Much of the research assessing muscle fuel stores and energy production has been obtained via the needle muscle biopsy. The muscle biopsy technique allows for direct biochemical analysis of most muscle metabolites and intermediates, including ATP, PCr, AMP, glycogen, fat, pyruvate, and lactate, among others. The technique offers the unique advantage of the possibility for enzyme activity assays, as well (16). Multiple biopsies can be used to measure concentration changes over time in any of these variables. However, muscle biopsies also have disadvantages. Only concentration in a small percentage of muscle fibers is measured,

meaning that the results must be interpreted with caution if extrapolating findings to whole muscle or whole body conclusions. Additionally, muscle biopsies are invasive and thus not conducive to research in certain populations or to examining acute changes over multiple time points during exercise. Therefore, other less invasive methods have also been developed to study exercise metabolism.

Blood

Blood measurements provide insight into metabolic processes occurring during exercise through a minimally invasive process. The blood compartment can be accessed through venapuncture or capillary sampling. These methods are less invasive than a muscle biopsy and thus more conducive to multiple samplings or use in special populations (i.e. children). However, blood metabolite concentrations are representative of the whole body and are affected by both active and non-active muscle groups as well as other organs. Substances commonly measured in blood samples include substrates such as glucose, lactate, free fatty acids, and glycerol as well as several hormones. However, the concentration of most substrates during exercise reflect a balance between the amount of substrate entering the blood and the amount of substrate taken up by various tissues. For example, blood glucose concentration (one measure of CHO availability) is affected by glucose release from the liver and glucose uptake by active muscle (120). Changes in blood lactate concentration imply changes in glycolytic flux during exercise of varying intensity (120). As with glucose, the blood lactate concentration reflects a balance between metabolic lactate production and lactate clearance, and its rate of production cannot be ascertained. Free fatty acids and glycerol are released into the blood from adipose tissue. The free fatty acids in the blood are rapidly turned over as they are taken

up and used by various tissues or reesterified (51). Glycerol is often used as a marker of lipolysis, as one molecule of glycerol is released for triglyceride that is broken down and is not cleared from the blood as quickly as fatty acids (61).

Isotope Tracers

Tracer methodology involves the use of a molecule (tracer) that is functionally identical to the molecule of interest, but contains a distinguishing feature allowing its metabolism to be measured. One commonly used tracer is carbon-13. Glucose or fatty acids labeled with carbon-13 can be ingested or infused and then levels of carbon-13 molecules in the blood or expired air can be measured. This technique allows for observation and quantification of fatty acid and glucose oxidation. When combined with whole-body indirect calorimetry measurements, glycogen and triglyceride oxidation rate can be approximated by multiplying the rate of uptake by the percent of the rate of uptake that is oxidized (132). The tracer method is advantageous as it can measure whole body fuel oxidation as well as changes in oxidation rates over time. However, isotope tracer methodology has several drawbacks. Tracer techniques cannot measure metabolite concentrations and tracer infusion may still be too invasive for routine use in some populations. Additionally, the equipment needed to measure tracer concentration is very expensive and requires substantial training.

Nuclear Magnetic Resonance Spectroscopy

A truly noninvasive technology is nuclear magnetic resonance spectroscopy (NMRS). NMRS has the unique ability to measure *in vivo* metabolite concentrations non-invasively and in real time. Atomic nuclei have magnetic properties, which cause them to spin randomly in the

earth's magnetic field. When a strong magnetic field is applied, the angle of their spin is altered and they absorb energy, which they release during the interval between excitation waves. This energy is detected by a receiver coil and displayed as frequencies and peak intensities. The frequency indicates which compound is being observed and the area under the peak intensity indicates the concentration of that compound (107). Several metabolites can be measured with NMRS by measuring different atoms. ^{31}P measurements can provide information about PCr, Pi, ATP, ADP, and intracellular pH. Lactate concentration is measured with ^1H nuclei and muscle and liver glycogen are measured with ^{13}C nuclei (70). However, this technique is very costly and the type of exercise that can be studied is very limited.

Indirect Calorimetry

Perhaps the most commonly used method of assessing exercise metabolism is indirect calorimetry. There is a direct relationship between oxidative ATP production and oxygen uptake. As glucose and fat are oxidized, oxygen is consumed and carbon dioxide is produced. The amount of each gas that is consumed or produced can be quantified by measuring the differences in oxygen and carbon dioxide concentrations between inspired air and expired air (130). However, this method cannot account for ATP produced anaerobically, so this technique does not measure ATP synthesized by the phosphagen or glycolytic systems. The specific type of fuel used during aerobic exercise can also be assessed with indirect calorimetry using the respiratory exchange ratio (RER). The RER is the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed. This ratio is equal to one when CHO is combusted because one molecule of CO_2 is produced for every molecule of O_2 that is consumed. Less CO_2 is produced per molecule of O_2 consumed because fats are more reduced. The whole-body RER

reflects the balance between these two types of substrate. Although RER is a useful tool to examine fuel use non-invasively, there are several assumptions inherent to the use of RER for this purpose. First, it must be assumed that the use of protein as an energy source is negligible. Protein is not completely oxidized because of the nitrogen it contains; therefore it is not impossible to calculate protein use from the RER. It must also be assumed that the process of gluconeogenesis in the liver is not affecting the RER. Lastly, it must be assumed that the RER is not affected by the CO_2 formed through buffering excess hydrogen ions released from lactic acid in the blood (130).

Perception

The detection and interpretation of sensations experienced during exercise is termed perceived exertion. Perceived exertion integrates external stimuli from physical work with internal stimuli reflecting physiological functions. No single physiological variable fully explains perceived exertion, but factors most commonly linked to perceive exertion are HR, VO_2 , ventilation, blood lactate and pH (53). Some of these variables (such as ventilation) are consciously sensed while others are not. The concept of measuring perceived exertion was originally introduced in 1961 by Gunnar Borg. Borg created the first rating of perceived exertion (RPE) scale and a modified form of this scale is still commonly used today in research and clinical settings (92). RPE can be reported as an overall rating or as a series of differentiated ratings (i.e. central vs. local, upper vs. lower body, etc.).

A new scale, designed to be more developmentally appropriate for use in children, was introduced in 1994. This scale, the children's effort rating table (CERT) used the more familiar 1-10 number range and child-chosen descriptions of effort (129). This scale was shown to have

greater validity (73, 78) and reliability (78) in children than the Borg scale when comparing RPE to VO_2 , HR, and power output. A pictorial version of the CERT (P-CERT) depicting a child running up a set of steps has since been validated and shown to be more reliable than the Borg scale when considering the relationships between RPE and VO_2 , HR, and minute ventilation (74). The Cart and Load Effort Rating (CALER) scale is another pictorial scale depicting cycling while pulling various loads of bricks. The researchers validating this scale found that practice with the scale increased reliability in young children (42). The OMNI RPE scale was designed to be applicable to children varying in race, gender, and health status. The OMNI scale is very similar to the P-CERT, though these scales were independently developed, with P-CERT designed in the UK and the OMNI scale developed in the US; these scales are equally valid (74). Several exercise mode-specific variations of the OMNI scale have been generated, including scales for walking, running, cycling, and stepping. The creators of the OMNI scale were the first to correlate differentiated (chest, leg, and overall) RPE values to VO_2 in children (106).

Variable-Intensity Exercise

Traditional exercise testing often falls into one of three categories: graded exercise, submaximal continuous exercise, or anaerobic exercise. Graded exercise is used to measure peak exercise responses or determine physiological thresholds (i.e. lactate threshold) while submaximal continuous exercise protocols allow researchers to make measurements during steady-state conditions and study how responses change over time. Anaerobic exercise testing methods are used to assess power and anaerobic energy production. However, few daily life and sport activities are entirely aerobic or anaerobic in nature, thus an additional type of exercise, variable-intensity exercise has been studied more recently. A direct method of

analyzing sport activity is motion analysis, first utilized by Reilly & Thomas (102). Match analysis can quantify distance covered, type of motion (sprinting, jogging, walking, jogging backwards) and other skills such as passing and scoring (28). This method is valid, but because game-play is not controlled, it is difficult to empirically test the effects of an intervention. Repetitive sprints are a basic method of quantifying intermittent activity in the laboratory (112). However, sport activity is more complex than maximal sprints interspersed with rest. New protocols have been designed to better simulate sport activity; these protocols are primarily based on match analysis. Shuttle running, treadmill, and recently, cycling protocols have been used to assess performance and the effects of interventions.

Shuttle Running

Nicholas et al. (89) developed the Loughborough Intermittent Shuttle Test (LIST), a two-part shuttle-running protocol designed to mimic the varied nature of activity in sports such as soccer or rugby. Part A consisted of five 15-minute cycles of walking, sprinting, recovery, jogging, and running. Part B, the performance test component, consisted of alternating bouts of jogging and running without recovery to exhaustion. The researchers found that their protocol elicited similar physiological responses (HR, blood lactate, fluid loss) to those observed during a game of soccer in men. Another shuttle-running protocol, the Yo-Yo Intermittent Recovery Test, consists of bouts of shuttle runs interspersed with active rest periods. The shuttle runs progressively increase in speed throughout the test until the subject fails to complete a bout. The test in its entirety is typically 6-20 minutes in duration (71). Unlike the LIST, this test does not involve varying intensities, thus it is less similar to game play.

Treadmill

Drust et al. (37) developed a 46-minute treadmill protocol utilizing a randomized pattern of four speeds (6 km/hr, 12 km/hr, 15 km/hr, and 21 km/hr). A group of seven university soccer players performed the protocol and the observed physiological responses (VO_2 , HR) were similar to those seen in a typical soccer game. Grieg et al. (49) also developed a treadmill protocol based on average speed and duration of different activity types during a soccer game. The speeds and duration were extrapolated from video match analysis. Using this protocol, they found physiological (HR, blood lactate, salivary cortisol concentration, and perceptual responses) in ten male semi-professional soccer players during intermittent exercise were greater than during steady state exercise at the same average speed. However, the responses recorded during intermittent exercise were attenuated compared to the same measurements recorded during an actual game.

Cycling

An unpublished study from this laboratory utilized a novel protocol on the cycle ergometer to mimic intermittent, variable-intensity activity. The exercise consisted of three 12-min sets of four three-minute cycles. Each three-minute cycle was as follows: 60 seconds at 150 W, a 10-second sprint against 5% of the subject's body mass, 50 seconds at 100 W, and 60 seconds of active recovery with no resistance. Sprint mean power tended to decline over time ($p = .055$). The absolute work rates in this protocol were equivalent for all subjects with the exception of the sprint resistance, which was based on body mass. Thus the exercise was likely not equally fatiguing for all subjects (50). The novel variable-intensity exercise (VIE) protocol

used in the present study aims to better control workload by individualizing intensity to each subject's fitness level.

Carbohydrate Supplementation

Carbohydrates are one of three types of macronutrients and exist as monosaccharides, disaccharides, and polysaccharides. Polysaccharides are primarily utilized for energy, but are integrated into some structural elements, as well. These include glycoproteins, glycolipids, and proteoglycans. Glycoproteins, which make up most of the cell membrane's integral proteins, include a short, hydrophilic CHO chain on the externally exposed region of the protein (13). These CHO chains often act as enzymes or as receptors for binding hormones that activate the attached integral protein and consequently the intracellular enzyme cascade (51). Glycolipids serve as recognition sites on the plasma membrane surface (13); approximately a tenth of membrane lipid molecules are glycolipids (51). Proteoglycans are CHO-protein complexes that make up the extracellular matrix of most animal cells (13).

CHO is a crucial energy source, as it is the only macronutrient that can be utilized by the brain (51). As prolonged exercise depletes muscle CHO stores, active muscles begin to utilize blood glucose as an energy source, which in turn depletes liver glycogen. If exercise continues beyond the point of muscle and liver glycogen depletion, glucose homeostasis cannot be maintained, leading to hypoglycemia and ultimately cerebral dysfunction (75). To prevent this, CHO supplements, often in the form of a beverage, are commonly consumed before, during, or immediately following exercise. These drinks serve to replace lost fluids and provide nutrition in the form of CHO. Drinks consumed before and during exercise are primarily for maintenance of fuel sources, while post-exercise drinks aid in the process of glycogen resynthesis. Many drink

variations exist, but most share the same basic components: CHO, electrolytes, and water; some formulas also contain protein. Electrolyte concentration varies widely between brands, but it is common for drinks to contain a mixture of sodium, potassium, and chloride. Most commercially available drinks are designed to be isotonic with plasma (24).

The CHO component of a sports drink can consist of one or more of four following types of CHO: glucose, fructose, sucrose, and maltodextrins (glucose polymers). Some types of CHO are more easily oxidized compared to others; CHO types are divided into two categories based on their rate of oxidation. Rapidly oxidized CHO can be oxidized at rates up to 60 g per hour and include glucose, sucrose, maltose, and maltodextrins. Slowly oxidized CHO are oxidized at no more than 30 g per hour and include fructose and galactose, among others (66). The exact formulas vary with respect to type of CHO, but most commercially available drinks contain 6-8% total CHO (24). Concentrations beyond this amount may negatively affect the rate of gastric emptying (91).

Exogenous CHO utilization is affected by the rate of ingestion as well as the intensity of the exercise being performed. The highest rates of exogenous glucose oxidation and the greatest CHO sparing have been observed during an ingestion rate of 60 g/hr (127). Ingestion of more than 60-70 g of CHO per hour does not increase oxidation and excess CHO likely accumulates in the intestine, which may cause gastrointestinal distress (65). Ingestion of multiple types of CHO can improve the peak exogenous CHO oxidation rate beyond the maximal oxidation rate that can be achieved with ingestion of a single CHO type. A peak oxidation rate of 1.26 g/min was observed after ingestion of 1.2 g glucose/min and 0.6 g fructose/min compared to a peak oxidation rate of 0.83 g/min after ingesting 1.8 g glucose/min (63). A similar effect

was found when 1.2 g glucose/min and 0.6 g sucrose/min were ingested but was not apparent when 0.6 g maltose/min were substituted for the sucrose (64).

The utilization of exogenous CHO increases as exercise intensity increases, but only up to a point. Pirnay et al. (96) found greater exogenous CHO oxidation when exercise intensity was increased from 22 to 39% of VO_{2max} . Exogenous CHO oxidation continued to increase as the exercise intensity was increased to 51% of VO_{2max} , but no additional change in exogenous oxidation was observed when the exercise intensity was increased to 64% VO_{2max} . Timing of ingestion does not appear to affect exogenous oxidation rates. Studies giving a single CHO load at the onset of exercise found similar oxidation rates as studies giving similar total amounts of CHO as repetitive feedings during exercise (66).

Performance

Most researchers agree CHO supplementation during prolonged exercise can improve exercise performance. Support for the positive effects of CHO supplementation appeared in the early 1920s when Levine et al. (80) studied 11 men running the 1924 Boston Marathon. They found that runners who consumed CHO during the race did not exhibit the negative effects (i.e. weakness, exhaustion) of some of the other runners who did not consume CHO. They also found a strong correlation between post-race blood glucose and physical condition. This was one of the first studies to suggest that the ingestion of glucose during exercise of long duration might prevent hypoglycemia and fatigue. These proposed ergogenic effects of CHO supplementation on endurance performance have been confirmed by other researchers. Coyle et al. (27) found ingestion of exogenous CHO prevented a drop in glucose in highly fit adult men cycling at 71% of VO_{2max} to exhaustion when compared to water ingestion. Additionally,

subjects maintained the exercise intensity for four hours when fed CHO compared to three hours in the water trial. In a follow-up study, Coggan and Coyle (23) concluded that fatigue is partially caused by a drop in plasma glucose concentration and can be reversed by ingestion or intravenous administration of CHO.

Though the beneficial effects of CHO supplementation on performance during prolonged exercise are well accepted, the effects of CHO supplementation on shorter-duration (one hour or less) exercise are less clear. Anantaraman et al. (2) found moderately fit men and women who consumed CHO before cycling were able to maintain more power during the final 20 minutes of one hour of high-intensity exercise compared to subjects who consumed a placebo (PL). Subjects initially pedaled at a cadence of 80 revolutions per minute against a resistance corresponding to 90% of their VO_{2max} ; resistance was kept constant and power output based on pedal cadence was continuously recorded. The researchers suggest that the glucose from the CHO drink may have served as a substrate as muscle glycogen may have been somewhat depleted after 40 minutes.

CHO supplementation has elicited performance benefits during some types of intermittent and VIE as well. Leatt and Jacobs (76) observed muscle glycogen sparing in ten male soccer players who consumed a CHO drink before and during a soccer match versus a PL. CHO supplementation has been repeatedly shown to benefit intermittent exercise in the laboratory setting as well. Davis et al. (30) used a protocol of intermittent cycling alternating between one minute at 120-130% of VO_{2max} and 3 minutes of rest until exhaustion. Time to exhaustion increased significantly (on average 27 minutes, or seven additional 1-minute bouts) when a CHO drink was ingested compared to a PL. The average increase in time to fatigue was 27 minutes, which corresponds to seven additional cycles of the exercise protocol. The

researchers suggested the delay in fatigue might be due to selective glycogen sparing in Type II muscle fibers, or an increase in muscle glycogen resynthesis during the three-minute rest periods. There is also evidence the body can oxidize a large percentage of exogenous CHO, providing 16-20% of the energy required for the activity (95).

Nicholas et al. (90) examined the effects of CHO ingestion on nine adult male athletes during shuttle running. The protocol consisted of repeating 15-minute cycles; each cycle started with walking, then a sprint, followed by a jog at 55% of VO_{2max} , followed by faster running at 95% of VO_{2max} . This protocol was repeated five times for a total of 75 minutes, and then subjects alternated jogging and fast running until exhaustion. Subjects consumed either a CHO drink or PL immediately before and every 15 minutes during exercise. The subjects who consumed the CHO were able to continue the final run to fatigue for 2.2 additional minutes (33% longer), which was significant. Using similar protocols, other researchers have also observed increased time to exhaustion (43, 82) in CHO-loaded men when ingesting CHO during exercise compared to a PL. A study by Welsh et al. (128) also found improvements in ten male and female athletes during shuttle running with CHO ingestion. In this study, four 15-minute quarters separated by a 20-minute half-time (to simulate game conditions) included varying intensities of shuttle running, followed by a run to fatigue. Increased time to fatigue was seen following CHO ingestion. Additionally, CHO ingestion was correlated with faster sprint times in the fourth quarter. Subjects also performed better on a motor skill test and reported significantly less fatigue during the CHO trial compared to the PL trial. A similar study found improved maintenance of sprint speed with CHO ingestion compared to a PL (82).

To date, no performance benefits have been observed with CHO supplementation before or during short-duration (less than one hour) variable-intensity exercise. No effect of

CHO on peak anaerobic performance was observed in a study of seven men by Wouassi et al. (133). Likewise, CHO consumption did not improve performance in ten trained male cyclists during a series of four intermittent 1.6 kilometer cycling time trials compared to a PL trial (111).

Physiological responses

CHO supplementation during exercise does not affect VO_2 (55, 86, 88, 124) or HR (30, 55, 86, 90, 124), regardless of the type of exercise (continuous or intermittent). DeBock et al. (31) observed a consistently higher RER in young men during two hours of exercise with CHO compared to a fasted state. (0.93 vs 0.88). Utter et al. (124) also found higher RER values in experienced adult runners at the end of a three-hour treadmill run at 70% of $\text{VO}_{2\text{max}}$ with CHO compared to a PL. However, this effect has not been consistently observed, as Marmy-Conus et al. (86) did not observe any RER differences between CHO and PL in trained men during 60 minutes of cycling at 71% of $\text{VO}_{2\text{max}}$. The lack of a difference in this study may have been related to the shorter exercise duration. Many studies have found no differences in glycogen utilization with CHO ingestion (43, 55, 88). However, some researchers suggest a reduced rate of glycogen depletion in type II fibers only, termed selective glycogen sparing (30, 31). Evidence supporting this selective glycogen sparing was reported by De Bock et al. (31) after 2 hours of cycling at 75% $\text{VO}_{2\text{max}}$. Glycogen content in type II fibers, as quantified by fluorescence microscopy, decreased from 8.7 to 7.1 $\text{OD}/\mu\text{m}^2$ when CHO was ingested before and during exercise, while it decreased from 9.6 to 4.5 $\text{OD}/\mu\text{m}^2$ in the fasted condition. CHO did not affect the exercise-induced glycogen depletion in type I fibers. Some researchers also suggest an increased rate of muscle glycogen resynthesis during the rest intervals of intermittent exercise protocols (30), though this theory has not been directly tested.

Blood glucose concentration is affected by CHO ingestion before and during exercise. Blood glucose concentration is generally maintained at or above resting levels when CHO is consumed during exercise, while blood glucose concentration tends to decrease when water or a PL is ingested during prolonged exercise (21, 88). Davis et al. (30) found higher blood glucose concentration throughout 60-90 minutes of intermittent cycling (1-minute bouts of 120-130% $\text{VO}_{2\text{peak}}$ separated by 3-minute rest periods), while Foskett et al. (43) observed a difference only at fatigue during an intermittent shuttle running protocol lasting 131 – 158 minutes. Depending on the drink and exercise protocol, blood glucose concentration sometimes decreases at the onset of exercise. This is due to elevated muscle glucose uptake (stimulated by both high insulin concentrations and contraction-mediated glucose uptake) that cannot be matched by the glucose rate of appearance (86). When CHO is ingested during exercise, liver glucose output is drastically reduced (86). Blood lactate concentration is typically not affected by CHO supplementation, regardless of exercise mode or intensity (21, 43, 90).

Perceptual responses

Several studies have revealed an effect of CHO on RPE in adults during prolonged exercise in that CHO attenuates perceived exertion during the later stages of prolonged exercise (7, 21, 69, 124). Each of these studies employed a continuous protocol of either running (124) or cycling (7, 21, 69) at 70% of $\text{VO}_{2\text{max}}$ performed for 2-3 hours. CHO also attenuated RPE compared to PL in men during two hours of intermittent cycling at 73% of $\text{VO}_{2\text{max}}$ (123). CHO supplementation led to an attenuated increase in RPE compared to PL in all of these studies. However, this effect was not observed until the latter half of the exercise bout. Leg and overall RPE were affected, but chest RPE was not (21, 123). The effect of CHO on RPE during VIE has

been examined using the LIST shuttle-running protocol. Two studies using versions of the LIST protocol found RPE was lower in CHO than a PL after 40-90 minutes (6, 82). These results mimic the continuous exercise results, as RPE is not affected until the later stages of the exercise bout.

Research on the effects of CHO on RPE during shorter-duration (one hour or less) exercise is limited. There was no effect of CHO in either boys or men during 60 minutes of cycling at 70% of VO_{2peak} (116). These results align with the previous studies employing cycling at approximately 70% of VO_{2max} where RPE was not affected by CHO until after 90 minutes in duration. A study examining the effect of CHO on RPE during intermittent exercise used a running protocol of 12 x 800 meter intervals at 3-km race pace with 90 seconds of recovery between intervals (a total of 28 minutes in duration). No effect of CHO on RPE was observed, as subjects reported a mean RPE of 18 (Borg 6-20 scale) during both treatments (32). When differentiated RPE was examined, Davis et al. (30) found leg RPE was greater in the PL trial than the CHO trial in adult men and women after 40 minutes of intermittent supramaximal cycling, but found no differences in chest or overall RPE.

Children

It is well accepted that exercise responses vary between children and adults. Children are not physiologically equal to small adults. However, due ethical constraints associated with research in children, some of these differences are not well characterized. A limited number of studies utilizing blood collection and the muscle biopsy method have been performed, but most of what is known about exercise in children has been obtained via non-invasive methods such as gas exchange, HR monitoring, and perceptual measurements.

Exercise Metabolism

Children and adults store similar concentrations of ATP (38) in skeletal muscle. Children and adults also store similar amounts of PCr (40, 41, 135) and creatine kinase activity per total protein content does not differ between children and adults (67). Blood glucose concentrations are similar at rest and during moderate exercise regardless of age (19), but age-related discrepancies exist in glycogen storage. Both muscle (40, 41) and liver (19) glycogen concentrations are lower in children compared to adults. PFK activity is lower in children (39, 41); Eriksson et al. (39) observed a 50% lower activity in boys compared to men, suggesting a decreased glycolytic capacity. Though resting pH is not different between children and adults (135), children experience smaller decreases in pH during exercise as well as lower lactate accumulation; these findings further support the notion that children have a reduced glycolytic capacity (40, 41, 135). However, children have a concomitantly enhanced oxidative capability. This is evidenced by a decreased ratio of glycolytic to oxidative enzyme activity; the ratio of PFK to isocitrate dehydrogenase is two-fold higher in adults (0.884 vs 1.633) (54). Increased activity of other oxidative enzymes, such as succinate dehydrogenase (41) and fumarase (15) activities also has been observed in children.

Children also use fuel differently than adults. This knowledge is based on studies examining RER differences between children and adults. Children consistently exhibit lower RER values compared to adults at the same absolute (109) or relative intensity (113, 119). Stephens et al. (113) examined RER during exercise in three groups of boys (10, 12, and 15 years) and one group of young adult men (22 years). Each subject cycled for 5-6 minutes at five submaximal exercise intensities (30, 40, 50, 60, and 70% of $\text{VO}_{2\text{peak}}$). Lower RER was observed in the younger groups compared to the older groups. The lower RER values observed in the younger children

confirm a greater reliance on the oxidation of fat for fuel in younger/less mature children. The contribution of blood glucose to exercise energy expenditure is 37% greater in children compared to adults (119), though total CHO use is lower in children. These findings suggest children use less CHO during exercise because glycogen is limited (119). There is some evidence for higher FFA turnover in boys (35), but this is not fully supported by other research (131).

There are also performance differences when comparing children and adults. Adults can produce more force, even when force is considered relative to body mass, but children typically do not fatigue to the same extent as adults during high-intensity exercise. For example, boys can generate a higher percentage of their initial peak power during repeated high-intensity bouts compared to men (58, 97, 99). This is often attributed to children's lower initial peak power, likely caused by their smaller muscle mass, and therefore inferior ability to produce force (99) but may also be related to children's lower lactate accumulation (41), better acid-base regulation (98), and faster PCr resynthesis (17, 18, 44, 52).

Perception

In addition to the physiological and performance differences between adults and children during exercise, perceptual responses appear to differ between children and adults. Bar-Or (10) observed that children rated their exercise intensity lower than adults for any given HR and that this relationship held true when the exercise intensity was defined as a percentage of maximal HR. Ratel et al. (99) also found adults reported significantly higher RPE values than children during high-intensity 10 x 10-second intermittent running and cycling tests. However, the effect of age on RPE is not clear. Mahon et al. (83) observed a trend for RPE to be higher in children ($p = 0.07$) and to increase more over time in children ($p = 0.06$) than adults during 16

minutes of cycling at a work rate corresponding to ventilatory threshold. Other researchers have corroborated the higher RPE (116) and greater increase in RPE over time in boys compared to men using varying continuous exercise protocols (22, 116). Both the higher RPE and greater rate of change in RPE over time may be related to low glycogen within the child's muscle and the proposed glycostat mechanism, which provides feedback from the muscle to the CNS to regulate exercise intensity (93, 101). Children have lower glycogen levels at a fixed level of exercise, and would experience greater sensory signaling from the muscle if this mechanism is valid.

Carbohydrate Supplementation

The first study to assess the effect of CHO supplementation on performance in children was performed by Hendelman et al. (59) in 1997. High school boys performed timed performance rides (2500 meters against a resistance equal to 5% body mass) following 75 minutes of cycling at 60% of VO_{2max} after three different pre-exercise feedings: 1) candy bar, 2) fat-free fig bar, 3) PL drink. No differences were observed in substrate utilization (assessed by RER) or performance time. The authors speculated the absence of a statistical difference may have been due to the relatively low CHO content of the pre-exercise snacks. In contrast, Riddell et al. (105) found the ingestion of a glucose plus fructose drink delayed exhaustion in 10-14 year old boys by 40% during a ride at 90% of VO_{2max} following 90 minutes of cycling at 55% VO_{2max} compared to water, but glucose alone did not significantly delay exhaustion.

A novel study by Riddell et al. (104) characterized the responses of adolescents to ingested exogenous CHO. They measured the amount of exogenous CHO that was oxidized during exercise using carbon-13 stable isotope tracer methodology in adolescent (13-17 yr) boys performing 2 hours of exercise at 60% of VO_{2max} (104). Exogenous glucose ingestion increased

blood glucose and spared endogenous CHO and fat during exercise. The shift toward an increased percentage of fat use with increasing duration was prevented and the percentage of CHO utilized was maintained by the CHO ingestion. The researchers found that a significant portion (40%) of the total energy demands after two hours of this type of exercise were met by oxidizing exogenous glucose. They also found exogenous glucose oxidation to account for 50% of the total CHO oxidation, thus sparing muscle glycogen. In a similar study, Riddell et al. (105) assessed the influence of glucose versus glucose plus fructose or a PL during 90 minutes of cycling at 55% of VO_{2max} . In this study, exogenous CHO oxidation contributed approximately 16% to total energy regardless of the type of CHO (glucose or glucose plus fructose). Endogenous fat and CHO were spared by glucose ingestion as 17% less fat and 14% less endogenous CHO were oxidized during exercise with CHO ingestion compared to water.

Timmons et al. (119) went on to characterize the difference responses to exogenous CHO between boys (10 yrs) and men during 60 minutes of cycling exercise at 70% of VO_{2max} . During the exercise bout, the boys utilized more fat and less CHO than the men in both the CHO and PL trials. Exogenous CHO was oxidized at a 37% higher rate relative to body mass and contributed 50% more to total energy expenditure in boys compared to men. This group of boys was the youngest of the groups studied in the previous three studies and the contribution of exogenous CHO to total energy was higher in this group (22% contribution) compared to 10-14-year-old boys (16% contribution) (105), 13-17-year-old boys (18% contribution) (104), and men (15% contribution) (119). Recently, Timmons et al. (118) corroborated the findings that younger and less mature boys oxidize a greater percentage of exogenous glucose than older boys and thus conserve relatively more glycogen when fed 1.68 g/kg body mass CHO prior to exercise at 70% of VO_{2max} . The researchers suggest this, along with the increased percentage of

fat oxidation, is a compensatory mechanism to preserve the low levels glycogen in the muscle. This study provides additional support for the theory that decreased CHO oxidation in younger boys is substrate-related and not due to a reduced glycolytic capacity. A similar study showed the decrease in endogenous CHO and fat oxidation with CHO supplementation was also true for girls (117), but the unlike the boys, reliance on exogenous CHO was not different between age groups.

Timmons et al. (116) found no effect of CHO on RPE in 9-10 year-old boys cycling at 70% of VO_{2peak} for 60 minutes. Comparable results were obtained by Meyer et al. (87) with boys cycling in the heat at 50% VO_{2peak} for 50 minutes (one 20-min bout and two 15-min bouts) prior to a ride to exhaustion at 90% VO_{2peak} . Riddell et al. (105) did not observe any effect of glucose or glucose plus fructose ingestion in 10-14 year old boys during 90 minutes of cycling at 55% VO_{2peak} . One study by Riddell et al. (103) did find lower average RPE in 13-17 year old boys during 60 minutes of cycling at 60% VO_{2peak} when CHO was ingested before and during exercise compared to a water trial. However, the glucose trial was always performed after the water trial and the subjects were not blinded to the drink composition in this study. Therefore, the differences in RPE may have been due to a trial order effect or to psychological effects of CHO consumption.

The first study examining the effects of CHO supplementation on repeated anaerobic exercise in children was conducted by Marjerrison et al. (85). Pre- and early-pubertal (8-12 yr, pubertal stage 1-2) boys consumed either 1.0 g/kg body mass of 22.2% CHO or an equal volume of PL drink 30 minutes prior to the exercise. An elevation in blood glucose was observed after CHO ingestion, indicating that increased glucose was potentially available to the muscle. The subjects then performed four Wingate Anaerobic Tests (WAnT) with two minutes of rest

between each WAnT. Mean power tended to decline over time but this effect was not significant ($p = 0.053$), no performance enhancements were observed during the CHO trial as both peak and mean power were similar between trials. The results from this study suggest an enzymatic limitation to performance as opposed to a substrate deficiency. As a follow-up to this study, Lee et al. (77) studied repeated anaerobic exercise in adolescent (13-16 yr, pubertal stage ≥ 3) boys. Prior to the exercise protocol, subjects consumed either 1.5 g/kg body mass of 22.2% CHO or an equal volume of PL. The exercise protocol consisted of a WAnT followed by ten 10-second sprints and a second WAnT. Resistance was set at 0.075 kiloponds/kilogram body mass for both the WAnTs and the sprints. A 30-second recovery period consisting of unloaded pedaling at 60 rpm was allowed between each exercise bout. WAnT peak and mean power decreased over time during both trials ($p < 0.05$). Power was higher in the CHO trial compared to the PL trial during the first WAnT when expressed as either peak (442.9 versus 396.3 W) or mean (330.5 versus 229.1 W) power. Peak and mean power values were similar between trials during the second WAnT. There were no statistically significant interaction effects. This study suggests a beneficial effect of CHO on sprint performance, although this benefit manifested as improved initial power instead of attenuated fatigue over time, as was hypothesized by the authors. No effects of CHO on RPE were observed in either the study by Marjerrison et al. (85) or Lee et al. (77).

Summary

Each of three ATP replenishment processes (the phosphate pathways, glycolysis, and oxidative phosphorylation) contributes during most types of exercise, though the dominant pathway depends on exercise intensity and duration. Likewise, fuel sources vary based on

exercise intensity and duration, with fat use highest at low intensities and decreasing with increasing intensity and CHO use following an opposite pattern. Several methods are used to study exercise metabolism, including the needle muscle biopsy, blood measurements, isotope tracers, NMRS, indirect calorimetry, and perceptual measurements. Each of these methods has advantages and disadvantages related to their validity, usefulness, and feasibility. Indirect calorimetry and blood and perception measurements are the most frequently used methods, as well as the methods utilized in the present study due to their relatively non-invasive nature.

VIE, a type of exercise mimicking sport or play, consists of short, repetitive bouts of varying intensity and duration. Shuttle running, treadmill, and recently, cycling protocols have been used to assess VIE performance and the effects of interventions.

When prolonged exercise depletes muscle CHO stores, active muscles begin to utilize blood glucose as an energy source eventually leading hypoglycemia. To prevent this, CHO supplements are commonly consumed before, during, or immediately following exercise to replace CHO. CHO supplementation during continuous or intermittent prolonged exercise can improve exercise performance, but the potential benefits of CHO supplementation on shorter-duration (one hour or less) exercise are not well supported. CHO supplementation does not affect VO_2 , HR, or blood lactate concentration. Blood glucose concentration is generally maintained at or above resting levels when CHO is consumed during exercise, but the effects on RER and glycogen depletion are disputed. CHO attenuates perceived exertion during the later stages of prolonged exercise, but does not appear to affect RPE during exercise shorter than one hour in duration. Leg RPE appears to be more sensitive to the effects of CHO than chest or overall RPE.

Exercise responses vary between children and adults. Children and adults have similar levels of ATP, PCr, blood glucose, plasma free fatty acids but lower muscle and liver glycogen. Children also have lower glycolytic enzyme activity, greater oxidative enzyme activity, and use more fat as fuel during exercise. Perceptual responses also differ between children and adults; some researchers have observed lower RPE in children while others show higher RPE in children. CHO supplementation enhances exercise performance during prolonged exercise in children and adolescents. In addition, children are able to oxidize more exogenous CHO compared to adults, suggesting the decreased CHO oxidation in children is substrate-related and not due to a reduced glycolytic capacity. CHO does not appear to affect RPE during aerobic exercise in children when the subjects are blinded to the drink composition. The fatigue response in children and adolescents during repeated anaerobic bouts is not attenuated by CHO supplementation. However, one study shows a beneficial effect of CHO on adolescents' sprint performance in the form of improved initial power. RPE is not significantly affected by CHO during this type of exercise.

Children's activity during play inherently follows an intermittent pattern and consists of varied intensities; several sports performed by both children and adults also follow this pattern. CHO is commonly ingested during this type of exercise by both children and adults, though the effects of this practice are not well studied. The available literature suggests that performance, perception, and some physiological processes during varied-intensity exercise are affected by CHO supplementation, but whether these effects would be measurable during exercise shorter than one hour in duration was not known. Based on the current knowledge, no effect of CHO on VO_2 , HR, or blood lactate was expected, but CHO was expected to increase or maintain blood glucose and increase CHO oxidation and thus RER during VIE in both boys and men. The results

of Timmons et al. (119), indicating greater exogenous CHO oxidation in boys compared to men, led to the hypothesis that the increase in RER would be greater in boys. CHO has not been previously shown to affect perceptual responses during short-duration exercise in either boys or men; however, it was hypothesized that the effect of CHO of RPE attenuation would present earlier during VIE due to the increased metabolic demand this type of activity compared to steady-state exercise.

CHAPTER III METHODS

Subjects

12 boys ages 10 – 12 years, pubertal stages 1 & 2, according to Tanner (115) and 11 men ages 18-30 years were recruited for this study. Boys self-selected their pubertal stage using the Tanner pubic hair maturity criteria. All subjects had a VO_{2peak} between 35 and 55 $ml \cdot kg^{-1} \cdot min^{-1}$, indicating average fitness for these groups (4). Subjects were healthy and not taking any medications that might affect physiological responses to exercise. Subjects consumed a normal diet and did not ingest caffeine or participate in strenuous exercise for 24 hours prior to each trial. This study was approved by Ball State University's Institutional Review Board and subject permission or parental permission and child assent were obtained prior to testing.

Study Design

Each subject reported to the laboratory on three separate days over a 6-week period. Visit 1 served to familiarize subjects to the laboratory and testing procedures. Additionally, a graded exercise test and practice VIE session were performed. Visits 2 and 3 were the experimental trials, during which either CHO or PL beverages were given. Drink administration was counterbalanced, double-blinded, and utilized a crossover design.

Procedures

Visit 1 (Familiarization/Graded Exercise Test)

Upon arrival, the study procedures were explained and subject consent or parental consent and child assent were obtained. Child subjects and/or their parents completed the maturation form. All subjects or their parents completed a health history questionnaire and height and weight were measured. Subjects read written instructions regarding the use of the OMNI 0-10 rating of perceived exertion (RPE) scale and then answered a set of standardized questions to ascertain their understanding of the scale. All subjects then practiced cycling at two different intensities (25 and 50 Watts (W)) for 2.5 minutes at each workload while using a mouthpiece breathing valve and nose clip. This served to familiarize the subjects with the breathing valve and cycle ergometer. A 5-minute rest period was allowed and then a graded exercise test was performed on the cycle ergometer to determine peak values for VO_2 and work rate. For children, the protocol commenced at 25-50 W and increased by 25 W every two minutes until a near maximal effort; thereafter work rate increased by 12-13 W per minute until volitional exhaustion. For adults, the protocol commenced at 50-100 W and increased by 50 W every two minutes until a near maximal effort; thereafter work rate increased by 25 W per minute until volitional exhaustion. HR and whole-body rating of perceived exertion (RPE_{body}) were recorded at the end of each stage and a mouthpiece breathing valve and nose clip were used to collect expired air samples. A peak effort was defined as the attainment of at least two of the following criteria: 1) failure to maintain a pedal rate > 50 rpm, 2) respiratory exchange ratio ≥ 1.00 (children) or ≥ 1.10 (adults), 3) maximal HR $\geq 95\%$ of age-predicted maximum ($208 - 0.7 \times \text{age}$), and 4) RPE ≥ 8 .

Following the graded exercise test a 15-minute rest period was allowed, after which the subjects practiced the VIE protocol on the cycle ergometer. The protocol consisted of repeating 2-minute cycles consisting of exercise at percentages of the peak work rate: 20 seconds at 25%; 30 seconds at 50%, 20 seconds at 125%, 30 seconds at 25%, and 20 seconds at 75%. This 2-minute cycle was completed six times for a total of 12 minutes. HR and whole-body RPE were recorded throughout each set.

Visits 2 & 3 (Experimental Trials)

Subjects recorded and replicated their diet the night prior to visits 2 and 3 and consumed a controlled breakfast (Carnation® No Sugar Added Instant Breakfast Drink) one hour before arriving to the laboratory. Child subjects consumed one serving of the breakfast drink while adult subjects consumed two servings of the breakfast drink. This helped to minimize any effect of diet on the physiological response to exercise. Upon arrival to the laboratory, subjects rested in a seated manner for 30 minutes. After the rest, subjects warmed up for 5 minutes at 25% of peak work rate on the cycle ergometer and then began the VIE. The VIE protocol consisted of three 12-minute sets of 6 x 2-minute cycles. Each 2-minute cycle consisted of exercise at percentages of peak work rate measured during the graded exercise test: 20 seconds at 25%, 30 seconds at 50%, 20 seconds at 125%, 30 seconds at 25%, and 20 seconds at 75%. A 3-minute rest was given after the first and second sets of exercise. HR and RPE_{body}, RPE_{chest}, and RPE_{legs} were recorded every six minutes throughout the exercise.

CHO or PL drinks were administered upon arrival to the laboratory, after 15 minutes of the seated rest, at the end of the seated rest, and during the 3-minute rests between 12-minute sets of exercise. All drinks for a given visit were equal in volume (2.5 ml/kg body weight) and

content (6% CHO or PL). This drink regimen (volume and timing) was modeled after Riddell et al. (105). Finger-stick blood samples for the assessment of glucose and lactate concentration were taken upon arrival to the laboratory, after the 30-minute rest, during each 3-minute rest period, and immediately following the last exercise bout. Expired air was collected and analyzed for VO_2 , RER, and V_E during the second 12-minute set of exercise only. A schematic of the protocol for visits 2 and 3 is shown below:

Figure 3-1. Experimental Visit Protocol.

VO_2 : oxygen consumption; RER: respiratory exchange ratio; WU: 5-minute warm-up; RPE: rating of perceived exertion (OMNI 0-10 scale); HR: heart rate

Instrumentation

Height and mass were measured using a wall-mounted stadiometer (Seca 222, Hanover, MD) and a calibrated digital scale (Toledo 1D1 Multirange Scale, Worthington, OH), respectively.

The mass recorded during visit 1 served as the subject's mass for drink administration for both experimental trials. HR was recorded with a Polar Monitor® (Polar USA, Inc., Stamford, CT). The OMNI 0-10 RPE scale was used to assess perceived exertion (106). All exercise was performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Gas exchange measurements were obtained using standard open-circuit spirometry techniques. Subjects breathed through a mouthpiece connected to a two-way breathing valve (Model 2600, Hans Rudolph, Kansas City, MO) while wearing a nose clip. The volume of inspired air was determined by a Parkinson-Cowan dry gas meter (Rayfield Equipment, Waitsfield, VT). Expired air was sampled from a plexiglass mixing chamber and dehydrated through drying line (Perma Pure, INC., Toms River, NJ) before being analyzed for oxygen and carbon dioxide concentrations. Gas concentration analyses were performed with an S-3A/I oxygen analyzer and a CD 3A carbon dioxide analyzer (Applied Electrochemistry, Inc., Pittsburgh, PA). Analyzers were calibrated before each testing session, first with ambient room air, and then with a gas containing known concentrations of oxygen and carbon dioxide. The analyzers and dry gas meter were connected to a computer where a metabolic software program calculated VO_2 and RER. Statistical analyses were performed using SPSS 14.0 software (Chicago, IL).

Blood Analyses

Capillary blood samples were obtained via a sterile fingerstick procedure. Glucose was analyzed directly from the fingerstick using an automated blood glucose monitor and reagent strips (BD Logic, Waltham, MA). After analyzing blood glucose, two 30- μl samples were collected in capillary tubes. Each 30- μl sample was immediately lysed in 100 μl of 8% perchloric

acid and frozen at -20°C for later analysis. After all samples were collected, lactate assays were performed in duplicate as follows. Samples were thawed and then centrifuged for 5 minutes at 15,000 rpm in an IEC Micromax RF ultracentrifuge (Needham Heights, MA). One ml of a reagent solution containing distilled water, glycine hydrazine buffer, lactate dehydrogenase, and NAD⁺ was pipetted into a series of culture tubes. This solution allowed measurement of lactate through NADH formation. 25µl of supernatant from each sample was added to the reagent. After vortexing each tube to mix thoroughly, tubes were incubated for 45 minutes in a 37° water bath. NADH was analyzed by measuring sample absorbance at 340 nm with a calibrated Beckman Coulter Inc. Spectrophotometer (DU 530 Life Science UV/Vis, Fullerton, CA). Lactate concentration was determined based on the NADH concentration using a conversion factor (94).

Data Processing

Peak WR values were calculated as the sum of the last WR completed, plus a portion of the final stage in progress. Subjects received credit for a pro-rated amount of the final increase based on the percentage of the stage they completed before volitional exhaustion; a percentage of the final WR increase (equal to the percentage of the stage that was finished) was added to the WR of the last fully completed stage. Values obtained during the graded exercise were recorded as follows: the highest 1-minute average was used for RER and VO_{2peak} ; the highest 30-second average HR was recorded as the peak HR. During the second 12-minute set of each VIE protocol, VO_2 and RER values were averaged for each 2-minute cycle. The gas exchange data from the first 2-minute cycle was excluded from analysis.

Statistical Analyses

Data are reported as means $\pm$ SD. Anthropometric and graded exercise test measurements were analyzed by an independent two-tailed t-test (boys vs. men). Physiological and perceptual responses to VIE were analyzed with a 3-way mixed repeated measures ANOVA (time x trial x group). Time and trial were within-group factors while group was the between-group factor. Bonferroni *post-hoc* testing was performed to determine specific differences where significant time or interaction effects were observed. The dependent variables for the ANOVAs were HR, % HR_{max}, RPE_{body}, RPE_{leg}, RPE_{chest}, VO₂, % VO_{2peak}, RER, blood glucose concentration, and blood lactate concentration. Statistical significance was set at $p < 0.05$.

CHAPTER IV RESULTS

Out of 23 subjects (12 boys and 11 men) recruited to participate in the study, 12 subjects (5 boys and 7 men) successfully completed all trials. Four boys and two men did not fall within the $\text{VO}_{2\text{max}}$ range that was specified for inclusion in this study (between 35 and 55 ml/kg/min); all boys and one man had $\text{VO}_{2\text{max}}$ values that fell below the range while one man had a $\text{VO}_{2\text{max}}$ value that exceeded the range. In addition, three boys and two men did not successfully complete the experimental trials: one boy and one man terminated the trials due to gastric distress, one boy and one man were unable to complete the VIE, and one boy withdrew from the study for unknown reasons. Subject characteristics for the subjects that successfully completed all trials and thus were included in the statistical analyses were as follows. The age, height, and mass of the men ($n = 7$) were 23.8 ± 2.6 years, 172.6 ± 9.1 cm, and 76.6 ± 13.0 kg, respectively. For the boys ($n = 5$) these measures were 11.3 ± 1.1 years, 146.3 ± 5.1 cm, and 43.3 ± 8.9 kg. Four of the boys were pubertal stage 1, and one boy was pubertal stage 2, according to Tanner (115).

Maximal Exercise Responses

All subjects successfully completed the maximal exercise test; each subject achieved at least two of the four pre-determined criteria for a successful test. One subject (man) met two criteria, six subjects (two men and four boys) achieved three criteria, and five subjects (four men

and one boy) achieved all four criteria. The maximal exercise responses for men and boys are presented in Table 4-1. $\text{VO}_{2\text{max}}$ in $\text{L}\cdot\text{min}^{-1}$ was greater in men ($p < 0.05$), but there were no differences between groups when $\text{VO}_{2\text{max}}$ was expressed relative to mass. Men achieved a higher RER at maximal exercise ($p < 0.05$). A trend toward a higher maximal HR was observed in the boys ($p < 0.07$). RPE at maximal exercise was similar between groups. Peak WR was statistically higher in the men (302 ± 50 W) compared to the boys (147 ± 11 W).

Table 4-1. Maximal exercise responses for men and boys.

	Men	Boys
VO_2 ($\text{L}\cdot\text{min}^{-1}$)	3.54 ± 0.60	1.88 ± 0.13 *
VO_2 ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	46.3 ± 3.7	44.5 ± 6.5
RER	1.24 ± 0.05	1.14 ± 0.03 *
HR	188 ± 12	200 ± 6
RPE	9.7 ± 0.7	9.0 ± 1.2

VO_2 : oxygen consumption; RER: respiratory exchange ratio; HR: heart rate; RPE: rating of perceived exertion (OMNI 0-10 scale) *Different from men ($p < 0.05$).

Variable Intensity Exercise

Physiological Responses

Figure 4-1 shows the blood glucose responses to VIE. There was a time by trial by group interaction effect; because this is the most meaningful effect and these interactions led to the other interaction and main effects, the other effects will not be discussed in detail. In CHO, men had higher pre-exercise blood glucose than boys (124 ± 17 vs. 96 ± 10 $\text{mg}\cdot\text{dL}^{-1}$, $p < 0.05$). In PL, men had higher post-exercise blood glucose than boys (94 ± 8 vs. 82 ± 5 $\text{mg}\cdot\text{dL}^{-1}$, $p < 0.05$). In men during the CHO trial compared to the PL trial, pre-drink and pre-exercise blood glucose were higher ($p < 0.05$), and blood glucose was lower at 12 minutes ($p < 0.05$). In boys, blood glucose was lower during the PL trial compared to the CHO trial after exercise (96 vs. 82 $\text{mg}\cdot\text{dL}^{-1}$, $p < 0.05$). In men during the CHO trial, blood glucose increased from pre-drink to pre-exercise (98 to 124 $\text{mg}\cdot\text{dL}^{-1}$, $p < 0.05$), decreased from 124 $\text{mg}\cdot\text{dL}^{-1}$ pre-exercise to 75 $\text{mg}\cdot\text{dL}^{-1}$ at 12

minutes ($p < 0.05$), and remained significantly lower than pre-exercise at 24 minutes ($91 \text{ mg}\cdot\text{dL}^{-1}$). Blood glucose significantly increased from 12 minutes to 24 and 36 minutes (74 to 91 and $100 \text{ mg}\cdot\text{dL}^{-1}$, respectively) in men. There were no significant effects of time in men during the PL trial or in boys during either trial.

Figure 4-1. Blood glucose concentration response to VIE for men (■, □) and boys (●, ○) in CHO (■, ●) and PL (□, ○) trials. Group x trial x time interaction ($p < 0.05$)

VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo ^aIn CHO, group effect ($p < 0.05$) ^bIn PL, group effect ($p < 0.05$) ^cIn men, trial effect ($p < 0.05$) ^dIn boys, trial effect ($p < 0.05$). Effect of time in men ($p < 0.05$) not shown.

VO_2 and $\%\text{VO}_{2\text{max}}$ responses are presented in Table 4-2. There was a time main effect for VO_2 ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Specifically, VO_2 at 16 minutes was lower versus 20, 22, and 24 minutes ($p < 0.05$) and the VO_2 at 18 minutes was lower versus 20 and 24 minutes ($p < 0.05$). There was an identical time effect when VO_2 was expressed as a percentage of $\text{VO}_{2\text{max}}$; VO_2 at 16 minutes was lower versus 20, 22, and 24 minutes ($p < 0.05$) and the VO_2 at 18 minutes was lower versus 20 and 24 minutes ($p < 0.05$). There were no other main effects or any interaction effects on VO_2 when expressed in either $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ or as a percentage of $\text{VO}_{2\text{max}}$.

Table 4-2. Respiratory-metabolic responses to VIE in CHO and PL trials in men and boys.

Time (min)	CHO					PL				
	16	18	20	22	24	16	18	20	22	24
VO₂ (ml·kg⁻¹·min⁻¹)										
Men	31.8	32.2	33.2	33.0	33.2	31.9	32.3	33.0	33.3	33.5
	± 2.4	± 2.4	± 2.6	± 2.6	± 2.7	± 2.1	± 1.8	± 1.8	± 1.9	± 2.1
Boys	32.5	32.9	33.6	33.2	33.3	32.1	32.3	32.3	32.5	32.6
	± 1.9	± 2.4	± 2.0	± 1.6	± 2.3	± 3.7	± 3.7	± 3.8	± 3.6	± 3.4
*										
%VO_{2max}										
Men	68.6	69.4	71.6	71.1	71.6	68.8	69.9	71.5	71.9	72.5
	± 4.9	± 5.0	± 4.7	± 5.3	± 5.5	± 3.7	± 4.0	± 4.5	± 4.5	± 4.3
Boys	72.3	73.1	74.6	73.9	73.9	72.2	72.6	72.9	72.6	73.6
	± 3.9	± 3.2	± 3.8	± 4.4	± 3.3	± 3.1	± 2.2	± 2.2	± 4.1	± 3.4
*										

Presented values are 2-minute averages from each cycle of the second 12-min bout of VIE. The first two minutes of the cycle have been excluded as a wash-out period. VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo; VO₂: oxygen consumption; %VO_{2max}: percentage of maximal oxygen consumption *Main effect of time ($p < 0.05$)

Figure 4-2 shows the RER response to VIE. RER was lower in boys (0.96) than men (0.99) (main effect of group, $p < 0.05$). There was a main effect of time on RER ($p < 0.05$). Specifically, RER increased from 0.95 at 16 minutes to 0.98 at 18 and 20 minutes ($p < 0.05$). There were no significant trial or interaction effects.

Figure 4-2. RER response to VIE for men (■,□) and boys (●,○) in CHO (■,●) and PL (□,○) trials.

VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo; RER: respiratory exchange ratio *Group main effect ($p < 0.05$) **Main effect of time ^aDifferent from 16 minutes ($p < 0.05$)

Lactate values are presented in Table 4-3. There were as a time by group interaction effect. Men had significantly higher lactate than boys at all exercise time points (minutes 12, 24, and 36). In men, lactate was higher at all exercise time points compared to pre-drink or pre-VIE ($p < 0.05$). Pre-drink and pre-VIE lactate values were not different ($p > 0.05$). In boys, only the 12-minute value was significantly different from the pre-drink or pre-VIE value. The lactate values at minutes 24 and 36 were not significantly different from any other time points, nor one another. There were significant main effects for time and group, but no other interactions.

Table 4-3. Blood lactate response to VIE in CHO and PL trials in men and boys.

Time Point	CHO					PL				
	Pre-drink	Pre-VIE	12 min	24 min	36 min	Pre-drink	Pre-VIE	12 min	24 min	36 min
Lactate (mmol·L⁻¹)										
Men	2.7 ± 0.8	3.3 ± 0.8	8.9* ^a ± 2.0	9.4* ^a ± 2.7	9.4* ^a ± 3.0	2.7 ± 1.1	2.5 ± 1.6	9.1* ^a ± 2.3	9.7* ^a ± 2.9	10.6* ^a ± 3.2
Boys	2.4 ± 0.5	3.1 ± 0.8	5.8 ^a ± 0.8	5.2 ± 0.5	5.3 ± 0.3	2.4 ± 0.5	2.4 ± 0.7	5.6 ^a ± 0.3	4.8 ± 0.5	4.6 ± 0.9

VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo *Different between groups ($p < 0.05$) ^aDifferent from pre-drink and pre-VIE ($p < 0.05$)

The HR response to VIE is presented in Figure 4-3a. HR was not different between trials, nor were there any interaction effects. HR increased over time (main effect of time, $p < 0.05$). Specifically, HR increased significantly from 151 bpm at minute 6 to all other time points and from 162 bpm at minute 12 to all subsequent time points. HR also increased significantly between minutes 18 and 24 (168 to 172 bpm) and between minutes 30 and 36 (171 to 174 bpm). There was a main effect of group; HR was higher in boys than men (173 vs. 160 bpm). When HR during was set relative to HR_{max} (shown in Figure 4-3b), only the main effect of time was significant. Percent of HR_{max} increased significantly from 78% at minute 6 to all other time points and from 83% at minute 12 to all subsequent time points. Percent of HR also increased significantly from minute 18 to minutes 24 and 30 (87 vs. 89 and 88%, respectively) and from minutes 30 to 36 (88 to 90%).

Figure 4-3a. HR response to VIE for men (■,□) and boys (●,○) in CHO (■,●) and PL (□,○) trials.

HR: heart rate; VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo *Group main effect ($p < 0.05$) **Time main effect ($p < 0.05$) ^aHigher than 6 minutes ($p < 0.05$) ^bHigher than 12 minutes ($p < 0.05$) ^cHigher than 18 minutes ($p < 0.05$) ^dHigher than 30 minutes ($p < 0.05$)

Figure 4-3b. HR (relative to HR_{max}) response to VIE for men (■, □) and boys (●, ○) in CHO (■, ●) and PL (□, ○) trials.

HR: heart rate; HR_{max} : maximal heart rate; VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo*Main effect of time ^aHigher than 6 minutes ($p < 0.05$) ^bHigher than 12 minutes ($p < 0.05$) ^cHigher than 18 minutes ($p < 0.05$) ^dHigher than 30 minutes ($p < 0.05$)

Perceptual Responses

Perceptual responses to VIE are shown in Figures 4-4a, b, and c; for all RPE measures there was a main effect of time ($p < 0.05$). There were no main effects for group or trial and no interaction effects for any of the RPE measures. Specifically, RPE_{body} increased significantly from minute 6 to all other time points, from 12 minutes to 24 and 36 minutes, from 18 minutes to 30 and 36 minutes, and from 30 to 36 minutes. RPE_{chest} increased significantly between minute 6 and all other time points, from 12 minutes to 24 and 36 minutes, from 18 minutes to 24 and 36 minutes, and from 30 minutes to 36 minutes. RPE_{legs} increased significantly between minute 6 and all other time points, from 12 minutes to 24 and 36 minutes, from 18 minutes to 24, 30,

and 36 minutes, from 24 to 36 minutes, and from 30 to 36 minutes. There were no main effects of group or trial, and no interaction effects.

Figure 4-4a. RPE_{body} response to VIE for men (■, □) and boys (●, ○) in CHO (■, ●) and PL (□, ○) trials.

RPE_{body} : whole-body rating of perceived exertion (OMNI 0-10 scale); VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo *Time main effect ($p < 0.05$) ^aHigher than 6 minutes ($p < 0.05$) ^bHigher than 12 minutes ($p < 0.05$) ^cHigher than 18 minutes ($p < 0.05$) ^dHigher than 30 minutes ($p < 0.05$)

Figure 4-4b. RPE_{chest} response to VIE for men (■, □) and boys (●, ○) in CHO (■, ●) and PL (□, ○) trials.

RPE_{chest} : chest-specific rating of perceived exertion (OMNI 0-10 scale); VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo *Time main effect ($p < 0.05$) ^ahigher than 6 minutes ($p < 0.05$) ^bhigher than 12 minutes ($p < 0.05$) ^chigher than 18 minutes ($p < 0.05$) ^dhigher than 30 minutes ($p < 0.05$)

Figure 4-4c. RPE_{legs} response to VIE for men (■, □) and boys (●, ○) in CHO (■, ●) and PL (□, ○) trials.

RPE_{legs} : leg-specific rating of perceived exertion (OMNI 0-10 scale); VIE: variable-intensity exercise; CHO: carbohydrate; PL: placebo *Time main effect ($p < 0.05$) ^ahigher than 6 minutes ($p < 0.05$) ^bhigher than 12 minutes ($p < 0.05$) ^chigher than 18 minutes ($p < 0.05$) ^dhigher than 24 minutes ($p < 0.05$) ^ehigher than 30 minutes ($p < 0.05$)

CHAPTER V DISCUSSION

This study investigated the physiological and perceptual responses to variable-intensity exercise with and without CHO supplementation in boys and men. CHO supplementation has been shown to enhance performance and decrease RPE during prolonged exercise (21, 69, 123, 124); short-duration and variable-intensity exercise responses are not well characterized. This lack of information is particularly relevant to children, as their typical activity pattern and sports participation is composed mostly of short exercise bouts of varying intensities. In addition, it has been shown that children are more reliant on exogenous CHO during prolonged aerobic exercise than adults (119). This enhanced use of this energy source has been attributed to the child's reduced capacity to store muscle glycogen (41). It was hypothesized that CHO supplementation would increase RER in both groups, but that the increase in RER would be greater for boys than men. Additionally, it was hypothesized that CHO supplementation would not affect VO_2 or HR during exercise, but that the perceptual responses to this type of exercise would be attenuated. Although RER was lower in boys, as hypothesized, CHO supplementation did not affect RER in either group. HR and VO_2 were not affected by CHO, as was hypothesized. Blood glucose was not different between trials with the exception of the post-exercise value in boys only, where blood glucose concentration was lower, in partial support of the hypothesis that blood glucose would decrease over time without supplementation. The hypothesis that blood glucose would be maintained at or slightly above baseline values with CHO

supplementation was not supported, as men's blood glucose dropped significantly at the onset of exercise. Lastly, it was hypothesized that lactate would be higher in men than boys during exercise, but would not be affected by CHO ingestion.. Lactate concentration was higher in men than boys during exercise and did not vary between the CHO and PL trials, in agreement with the hypothesis. As hypothesized, $\%HR_{max}$ and VO_2 (% of VO_{2max} and mass-relative) were not affected by CHO. RPE increased over time, as expected, and was similar in boys and men; however CHO did not attenuate RPE, contrary to the hypothesis.

Physiological Responses

Glucose

It was hypothesized that blood glucose would be maintained at or slightly above baseline values with CHO supplementation and decrease over time without supplementation. This hypothesis was partially supported in that boys had lower blood glucose post-exercise in PL versus CHO, but was not supported over time in men, possibly due to large variations in blood glucose during the CHO trial. The blood glucose response to the beverages varied between the men and the boys. Men had higher blood glucose concentrations prior to exercise in the CHO trial and immediately post-exercise in the PL trial compared to the boys. In addition, blood glucose was more variable over time in men; although this variation was limited to the CHO trial. Specifically, pre-drink blood glucose concentration was already elevated in the CHO compared to the PL trial ($\sim 10 \text{ mg}\cdot\text{dL}^{-1}$). With the consumption of CHO, blood glucose increased further in the men (pre-exercise) before dropping at the end of the first exercise bout and then gradually increasing to the end of exercise. It is uncertain why the pre-drink blood glucose values varied within this group, as all subjects indicated adherence to the dietary restrictions and

consumption of the provided breakfast as directed. This discrepancy in blood glucose concentration prior to drink consumption is not ideal, but the pre-drink glucose concentrations were in the normoglycemic range so any confounding effect this may have had on the results could be minimal, but this is uncertain. In contrast, boys experienced little variation in blood glucose over time, regardless of trial, although blood glucose was significantly greater post-exercise in the CHO versus PL trial.

The higher pre-exercise blood glucose in men compared to boys in the CHO trial indicates a greater responsiveness to the CHO ingestion in men. Potential reasons for this difference in responsiveness could include variations in gastric emptying or insulin sensitivity. Although glucose concentration in the CHO drink was constant between groups, the men consumed a greater absolute amount of glucose. As a result, it is possible that the intestinal glucose diffusion gradient was greater in the men and resulted in a more rapid entry of glucose into circulation. Insulin sensitivity is higher in pre-pubertal children compared to adolescents, and may be higher than in adults, though these groups have not been directly compared (5). If this is the case, the higher insulin sensitivity would result in a lower glucose concentration in the blood. Although blood glucose was not measured after CHO drink ingestion, Timmons et al. (119) observed higher blood glucose in men compared to boys (120 vs. 108 mg·dL⁻¹, respectively) 20 minutes after ingestion of a CHO-containing breakfast.

The 49 mg·dL⁻¹ decrease in blood glucose in men after the first set of exercise was likely due to the effects of insulin and muscle contraction synergistically increasing the uptake of glucose from the blood (33). Insulin stimulates translocation of GLUT4 glucose transporters to the sarcolemma, allowing movement of glucose into the muscle. GLUT4 translocation is also stimulated by dynamic exercise through an independent mechanism (81). However, when

GLUT4 translocation is stimulated simultaneously by insulin and muscle contraction, the amount of glucose taken up exceeds the amount that can be taken up by insulin or exercise alone (33). Similar decreases have been observed with CHO ingestion 45 minutes prior to exercise (25, 57, 79); twice the amount of CHO was ingested as a bolus in these studies, but the insulin response to the CHO was likely similar, explaining the drop in blood glucose in men after the first set of exercise.

Boys in the present study did not exhibit much variation in blood glucose concentration in either trial. Children may experience a transient decrease in blood glucose concentration over the first 10-15 minutes of exercise without any form of CHO supplementation (34, 35, 105), though this does not always occur (104). If such a decrease occurs, blood glucose concentration will gradually increase back towards a normal value and is then maintained (34, 35, 105). Alternatively, if pre-exercise blood glucose is elevated glucose will drop over time towards a normoglycemic value (116, 118, 119) if no additional CHO is provided. Transient decreases in blood glucose during exercise with CHO supplementation have also been observed (105), though this is not always the case (104). Lastly, while glucose ingestion may significantly increase blood glucose over time (104, 105), the combination of glucose and fructose results in the maintenance of baseline blood glucose concentration (105). These findings are in contrast to the observations in the present study; boys did not exhibit a decrease in blood glucose early in exercise during either trial, and blood glucose was maintained near a normoglycemic value throughout exercise in both trials. The lack of a decrease in blood glucose in boys early in exercise is probably because boys did not become hyperglycemic in either trial, and thus did not have a large amount of circulating insulin at the beginning of exercise.

In the boys, post-exercise blood glucose concentration differed between trials in the present study, but neither value varied significantly from the initial blood glucose concentration, so it is difficult to ascertain which condition is more favorable. Fatigue and increased RPE are associated with hypoglycemia, but at $82 \text{ mg}\cdot\text{dL}^{-1}$, hypoglycemia was not yet an issue. It is possible that blood glucose would have continued to decrease if the exercise duration was extended, potentially creating a hypoglycemic state. Timmons et al. (119) observed decreased blood glucose in boys and men after 60 minutes of cycling without supplementation; when the same exercise was performed with CHO supplementation, decreased post-exercise blood glucose was observed only in men. No pre-drink to post-exercise differences were observed in the present study, but the CHO trial responses in Timmons et al. are consistent with the variation in blood glucose over time in men, but not boys during the CHO trial in the present study. .

Cardiorespiratory-Metabolic

CHO supplementation had no effect on any of the cardiorespiratory-metabolic variables examined in this study, but some group differences and time effects exist. As hypothesized, average RER was lower in boys compared to men, regardless of trial. RER is an indicator of fuel use, thus the lower value in boys suggests they utilized a greater amount of fat during the exercise protocol regardless of trial (CHO or PL). This was expected, as children typically display lower RER values during exercise (113, 119), regardless of whether CHO is consumed or not (119). The lower RER values in children are typically attributed to a decreased ability to utilize carbohydrate along with an enhanced fat oxidation capacity (19). Differences in oxidative (15,

41) and glycolytic (39, 41) enzyme activities, and/or children's decreased capacity to store muscle glycogen (41), contribute to this unique fuel use pattern.

Contrary to the hypothesis that CHO would increase RER more in the boys than in the men, there was no difference between groups in the manner in which RER changed as function of trial. This is in contrast to what has been shown by others. For example, studies by Timmons et al. (119) and Riddell et al. (104, 105) reported that CHO consumption in boys before and during prolonged aerobic exercise significantly increased RER values. Moreover, they showed that boys were able to oxidize more CHO when exogenous CHO was ingested and that they were able to oxidize a larger percentage of the exogenous CHO compared to men (119). The combination of small sample size and variability in measurements limited the statistical power necessary to detect a difference in RER between trials in boys compared to men in the present study. Moreover, the differences between boys' and men's RER in the study by Timmons et al. (119) were not significant until 50 minutes of exercise, so it is possible that the exercise protocol in this study was too short for an effect of CHO on RER to be realized.

Another point of consideration is the specific metabolic demand imposed by the VIE in this study. In contrast to the studies by Riddell et al. (104, 105) and Timmons et al. (117, 119), which included continuous, moderate-intensity (55-70% $\text{VO}_{2\text{max}}$) cycling performed for 60 to 90 minutes, the current study utilized an exercise protocol with large variations in intensity and shorter duration. Glycolytic flux should be relatively constant during continuous exercise, while the fluctuations in metabolic demand during VIE prevented a steady-state condition from being established. This type of exercise may alter the ability to utilize exogenous glucose, though benefits from exogenous CHO have been observed during a variable-intensity shuttle running protocol (1, 43). However, the high intensity of this exercise may have challenged the upper

limits of glycolytic flux. Although children are able to increase their CHO utilization when exogenous CHO is provided (104, 105, 117, 119), there is undoubtedly an upper limit to glycolytic flux, and the demands during VIE may have been too high, or the overall duration too brief, for the potential effects of the CHO supplementation to be realized.

Resting blood lactate was higher in both groups than what is commonly reported (51). Blood lactate increased with exercise in both boys and men, though the increase was larger in men, as hypothesized. Higher blood lactate values in men compared to boys are well documented in the literature (113). Ratel et al. found that ten 10-second sprints resulted in a ~ 6 mmol·L⁻¹ greater lactate accumulation in men compared to boys of a similar age to the boys in this study (99). Blood lactate concentration reflects the overall balance between lactate production and lactate clearance from the blood. Lactate is produced as a byproduct of glycolysis; the decreased glycolytic and increased oxidative capacity observed in children may lead to lower lactate production. Lactate clearance by skeletal muscle is affected by blood flow and the ability to metabolize lactate oxidatively. In that muscle blood flow is increased in children and children have greater oxidative enzyme activities (54), the child's skeletal muscle may be better equipped than the adult muscle to clear lactate. The lack of a trial effect on blood lactate agrees with the hypothesis and suggests that the CHO supplementation did not affect the muscle's overall metabolic activity. This is in agreement with studies examining the effects of CHO in adults during shuttle running (43, 90). In contrast, studies examining the effects of CHO in children have found higher lactate values during the CHO trials at the end of exercise (105, 116, 118). It is important to consider, however, that the exercise protocol in those studies was continuous at a moderate intensity, and that the observed differences did not occur until 60 minutes of exercise or later.

CHO supplementation did not affect HR or VO_2 . In contrast, Riddell et al. (105) and Timmons et al. (116, 119) observed a slightly higher HR (~ 4 bpm) in subjects when glucose was ingested, while another study by Riddell et al. (104) saw no effect. In men, CHO did not significantly affect HR during a variable-intensity shuttle running protocol, either (43, 90). The increase in HR over time in the present study is consistent with the cardiovascular drift phenomenon associated with prolonged or high-intensity exercise. Cardiovascular drift is caused by a combination of increased blood flow to the skin for thermoregulation, decreasing stroke volume, and changes in sympathetic control mechanisms (20). HR was higher in boys than in men, as would be expected (122). When HR was expressed as a percentage of HR_{max} , no differences between groups remained.

VO_2 relative to body weight and $\%\text{VO}_{2\text{max}}$ were similar between boys and men during exercise, indicating that the child and adult protocols were well matched for relative intensity. There was a slight increase in the mean VO_2 during the second set of VIE. This is likely due to a combination of the slow component of oxygen uptake kinetics (46), an accumulation of an oxygen debt from the supramaximal bouts, and the VO_2 drift phenomenon (130). The precise mechanisms for VO_2 drift are not known, but factors thought to contribute to VO_2 drift include increased ventilation, increased recruitment of type II muscle fibers and increases in circulating catecholamines (130).

Perceptual Responses

Both overall (RPE_{body}) and differentiated ($\text{RPE}_{\text{chest}}$, RPE_{legs}) measures of perceived exertion were utilized in this study. Differentiated RPE measures are valuable for assessing the perceptual responses to CHO during exercise because often some, but not all, RPE values are

affected (30, 123). Furthermore, information about the contribution of local sensations to the overall RPE can be helpful in isolating various factors influencing perception of effort during exercise. As expected, all RPE measures increased over time in both boys and men, but there were no differences between boys and men with respect to RPE. Although the differentiated measures of RPE were not statistically compared to one another, the relationship pattern between the differentiated measures was identical to that reported in the literature (106); the grand mean for RPE_{legs} (5.6) was higher than for RPE_{body} (5.0), which was higher than for RPE_{chest} (4.2). No effect of CHO was observed on any of the RPE measures in this study; this is contrary to the hypothesis that RPE would be attenuated in the CHO trial. A variety of outcomes relative to RPE differences between boys and men have been reported in the literature. Bar-Or and Ward (12) observed lower RPE values in children compared to men during exercise at the same %HR_{max}. Ratel et al. (99, 100) also found lower RPE values in boys compared to men during intermittent, high-intensity exercise. However, Mahon et al. (83) found RPE was higher in children at ventilatory threshold during a graded exercise test. When RPE responses are examined over time during exercise, boys tend to experience faster increases in RPE than men (84, 116).

In men, CHO supplementation has been shown to decrease body and leg RPE, but not chest RPE, during the later stages of prolonged, constant intensity exercise (21, 123). During prolonged (~60-90 minutes) intermittent exercise, leg RPE may be attenuated by CHO supplementation, but this effect may not be observed until the later stages of the exercise protocol (30). It is possible that the protocol utilized in this study was too short for an RPE benefit to be realized. However, many subjects indicated they were completely exhausted at the end of 36 minutes, and would have been unable to complete additional 12-minute bouts.

RPE during intermittent exercise of a shorter duration is not affected by CHO (32, 111). CHO did not affect RPE during a varied-intensity shuttle running protocol (1, 43), though sprint (43) and skill (1) were improved. This outcome suggests that even if there is no perceptual benefit to CHO supplementation, subsequent performance may still be improved. This notion is crucial to real-life applications, such as game play.

An effect of CHO supplementation on boys has only been observed in one study by Riddell et al. (103). Unfortunately, drink administration was not blinded in this study and the trial order was fixed. Other studies examining the effects of CHO on continuous exercise in boys have found no effect of CHO (105, 116). Additionally, no effects of CHO on RPE were observed during repeated WAnT exercise in boys (85) or adolescents (77). These observations in children align with the findings in the present study that CHO does not attenuate the perceptual responses to VIE.

Responses to Maximal Exercise

All subjects achieved two or more of the pre-established criteria used to indicate a successful maximal exercise test. The physiological responses were consistent with reported values for recreationally active boys and men of similar ages. The VO_{2max} values observed in boys in both absolute and mass relative terms were within the range of typical values for VO_{2max} (3), though as expected, VO_2 in absolute terms was higher in the men. There were no significant differences between groups for VO_{2max} when expressed relative to body mass ($ml \cdot kg^{-1} \cdot min^{-1}$), indicating that the groups were of similar aerobic fitness. RER was higher in men than boys, which, based on the greater capacity for glycolytic metabolism in men (3, 11), was expected. HR_{max} tended to be greater in boys, though the effect was not significant ($p < 0.07$); this

difference was expected due to the age-related variation in maximal HR. RPE at maximal exercise was comparable between groups. That boys and men rated the effort sensation of maximal exercise equally with respect to the RPE provides evidence of a consistent anchor for effort perception during VIE.

Summary

The intent of this study was to test the hypotheses that CHO supplementation would increase RER in both groups (more so in the boys), and attenuate the perceptual responses to VIE, but not affect VO_2 or HR. It was also hypothesized that blood glucose would be maintained at or above baseline during exercise with CHO and decrease during exercise with PL and that blood lactate would be higher in men than boys during exercise but not vary between trials. Boys and men had similar and expected responses to maximal exercise, indicating the groups were matched for fitness. Men, but not boys, experienced blood glucose variations in response to CHO; this suggest a greater responsiveness to CHO ingestion in men. For boys only, blood glucose concentration differed between trials post-exercise, but neither condition appeared preferable as both values were within the normoglycemic range. CHO supplementation did not alter cardiorespiratory-metabolic responses during VIE. RER was lower in boys, indicating greater fat oxidation, but did not vary between trials, as was hypothesized. As hypothesized, blood lactate accumulation was higher in men, but did not vary between trials. $\%\text{HR}_{\text{max}}$ and VO_2 (% of $\text{VO}_{2\text{max}}$ and mass-relative) were similar between groups and trials, indicating the protocols were matched for relative intensity. RPE increased over time, as expected, and was similar in boys and men. CHO did not attenuate RPE, as hypothesized; this may have been due to the short duration of the protocol, as previous studies have not observed a benefit of CHO until the latter

stages of prolonged exercise. However, evidence from the literature suggests performance may be improved regardless of any perceptual benefit of CHO.

Limitations

There are several limitations with the present study. First, due to the intense nature of the exercise, some of the assumptions inherent to the use of RER to determine fuel utilization may have been violated. The intention of the VIE protocol was to elicit a continually changing metabolic environment, thus all measurements were taken under non-steady state conditions. In addition, hyperventilation could have elevated RER independently of exercise metabolism. Indeed, the mean RER values during exercise were close to 1.00 in the men, and even exceeded 1.00 in some subjects, indicating some degree of non-metabolic CO₂ production. A second limitation of this study was the small sample size. As noted, approximately half of the subjects who volunteered for the study completed all of the experimental trials. Thirdly, unequal error variances (significant Levene's tests) were observed for some dependent variables (HR, lactate, glucose, VO₂, and all RPE measures) at various time points; this was likely due to the unequal sample sizes. Since these inequalities should only affect group main effects approaching significance, only the main effect of group on HR is concerning. However, the adult-child difference in HR at the same relative-intensity exercise is well documented, and thus not a cause for concern. Lastly, no performance measurement was employed in this study. CHO supplementation may benefit performance during this type of activity, even when no perceptual benefits are realized (1); a performance test would have enhanced the overall meaningfulness of the study.

Future work

It is not clear whether the time effects observed in the present study would change further if the exercise duration were extended. For example, a typical soccer game consists of two 45-minute halves. However, the intensity of the current protocol is not likely sustainable over an additional 45-minute period and it may not be feasible for children to exercise for such a long duration in the laboratory. Undoubtedly, other factors, such as boredom, would affect responses during longer-duration efforts. If longer-duration examination is desired, the VIE protocol should be adjusted to more closely mimic the metabolic demands of a “real-life” sport activity, such as soccer. Future work should also examine the potential performance benefits associated with CHO supplementation in addition to physiological and perceptual changes. The application of the CHO strategy utilized in the present study to the field may be an appropriate and informative approach to studying the effects of CHO supplementation in children.

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