# EFFECTS OF HIGH-INTENSITY INTERVAL EXERCISE ON VASODILATOR FUNCTION IN CHILDREN

by

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the degree of Master of Science .

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## Abstract

**Purpose:** Exercise training can improve vascular function through anti-atherogenic effects on the vascular endothelium, a response which can be discerned following individual bouts of exercise. Although well characterized in adults, the effect of exercise intensity on the acute recovery patterns of vasodilator function is unknown in children. **Study Design:** Nine children (age = 10.5 ± 1.5 y, 6 girls) completed 1) high-intensity interval exercise (HIIE, six 1-minute sprints at 90% peak power ($W_{max}$), with 1-minute recovery) and 2) moderate-intensity exercise (MIE, 15 minutes at 44% $W_{max}$, total external work-matched to HIIE). Superficial femoral artery (SFA) diameter, blood flow, shear rates, and flow-mediated dilation (FMD) were measured before (Pre), immediately following (Post), and 60 minutes following (Post60) the exercise trials using duplex ultrasound. **Results:** Baseline diameter increased similarly following both HIIE (Pre 4.25 ± 0.42 mm, Post 4.76 ± 0.42 mm) and MIE (Pre 4.29 ± 0.49 mm, Post 4.62 ± 0.49 mm), returning to pre-exercise values 60 minutes later. Blood flow and antegrade shear rate were increased following HIIE and MIE, but to a greater extent after HIIE ($P < 0.05$). Retrograde shear rate was attenuated following both exercise conditions, remaining lower 60 minutes after exercise ($P$'s $< 0.001$). FMD was attenuated Post compared to Pre following HIIE (Δ -2.8%) and MIE (Δ -2.5%) ($P$'s $< 0.05$) and recovered to pre-exercise values with no difference between Post60 and Pre FMD. When FMD was corrected to account for changes in baseline diameter, there was no longer a significant main effect of time ($P = 0.34$) making the post-exercise nadir in FMD negligible. **Conclusions:** Acute bouts of external work-matched HIIE or MIE exert a similar impact on shear-mediated conduit artery vasodilation and FMD in children and this is reversible 60 minutes post-exercise. This suggests the mechanisms that govern the acute FMD response in adolescents and adults may be dissimilar in children.

## Preface

Chapter 2 will be submitted to *Medicine & Science in Sports & Exercise* for publication. I planned the experiment alongside Dr. Alison McManus and data collection occurred with the assistance of Ryan Simair, Kevin Castilloux, Laura Morris, and Victoria Armstrong. I completed all data analysis and wrote the manuscript. Dr. Alison McManus and Dr. Daniel Green helped immensely by providing useful feedback and review for the final manuscript and Dr. Nia Lewis trained me in the measurement techniques and analysis used in this research. This study received ethical approval from The University of British Columbia Clinical Research Ethics Board (H16-00077).

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## List of Abbreviations

| Abbreviation | Definition |
|---|---|
| $^{31}$P-MRS | $^{31}$P-magnetic resonance spectroscopy |
| a-v $O_2$ diff | Arterio-venous $O_2$ difference |
| ADP | Adenosine diphosphate |
| AT | Anaerobic threshold |
| ATP | Adenosine triphosphate |
| CVD | Cardiovascular disease |
| EDRF | Endothelium-derived relaxing factor |
| eNOS | Endothelium nitric oxide synthase |
| EPOC | Excess post-exercise oxygen consumption |
| FMD | Flow-mediated dilation |
| GET | Gas exchange threshold |
| H+ | Hydrogen ion |
| HHb | Muscle de-oxyhemoglobin |
| HI | High-intensity exercise |
| HIIE | High-intensity interval exercise |
| HIIT | High-intensity interval training |
| $HR_{max}$ | Maximum heart rate |
| La | Lactate |
| LO | Low-intensity exercise |
| MAS | Maximum aerobic speed |
| MAV | Maximum aerobic velocity |
| MIE | Moderate-intensity exercise |
| MIT | Moderate-intensity training |
| mmol/L | Millimoles per litre |
| MVC | Maximum voluntary contraction |
| NO | Nitric oxide |
| $O_2$ | Oxygen |
| $O_{2^-}$ | Superoxide anion |

| | |
|---|---|
| ONOO- | Peroxynitrite |
| OSI | Oscillatory shear index |
| PCr | Phosphocreatine |
| PCr:Pi | Phosphocreatine:inorganic phosphate ratio |
| PFK | Phosphofructokinase |
| pH | Potential of hydrogen (i.e. acid-base balance) |
| Pi | Inorganic phosphate |
| $PO_2$ | cellular pressure of $O_2$ |
| Q | Cardiac output |
| RER | Respiratory exchange ratio |
| ROS | Reactive oxygen species |
| SFA | Superficial femoral artery |
| SNA | Sympathetic nervous activity |
| SOD | Superoxide dismutase |
| SV | Stroke volume |
| $t\dot{V}O_2$ | $\dot{V}O_2$ kinetics speed |
| $\dot{V}CO_2$ | Volume of carbon dioxide |
| $\dot{V}O_2$ kinetics | Oxygen uptake kinetics |
| $\dot{V}O_{2max}$ | Maximum volume of oxygen uptake, Maximum aerobic power |
| VT | Ventilatory threshold |
| $W_{max}$ | Maximum power output |

## Acknowledgements

Firstly, I would like to thank my supervisor Ali McManus for giving me this opportunity and providing continual support and mentorship over the past two years. Throughout this process you have facilitated my constant growth and improvement on an academic and personal level, helping me harness the skills I need to excel at any task I set my mind on. As well, I would not be in this position if it were not for my committee member Jonathan Little. You allowed me to work in your lab as an undergraduate research assistant, sparking my interest in pursuing research, and for that I am grateful.

To my team in the PERL lab, all of you have made this an exceptional experience which made coming in to work a pleasure. I could not ask for a better group of people to work with.

The guidance and support from my family and friends have shaped me into the person I am today. Through tribulation and success, you have always been there. I have gained indispensable personal skills from you which have helped me begin and finish my education.

To the love of my life, Tanya Pullen, we have pursued this goal side-by-side since 2011. We have nearly completed this chapter, and I am looking forward to starting the next one with you when we get married in September. I could not have done it without you, and Lilo.

# Chapter 1 Introduction

Currently only 14% of 5- to 11-year-old Canadian children meet the daily recommendation of at least 60 minutes of moderate-to-vigorous physical activity (Statistics Canada, 2012-13). This raises concern since adequate amounts of physical activity are essential for promoting optimal cardiovascular health (Andersen et al. 2011). Physical inactivity in the young increases cardiovascular disease (CVD) risk factor susceptibility, such as obesity and type II diabetes (Andersen et al. 2006), which can lead to the onset of CVD later in life (Andersen et al. 2006, 2011). CVD is the leading cause of mortality worldwide and the number one cause of death of Canadian men and women (Public Health Agency of Canada, 2015). The process leading to CVD can begin in childhood (Newman, Wattigney, & Berenson, 1991), increasing children's risk for developing CVD and suffering a myocardial infarction or stroke (Juonala et al. 2010). Despite this epidemiological evidence we know little about the impact exercise has on cardiovascular function in children. Given this crisis of physical inactivity it is crucial to consider the impact different types of physical activity have on cardiovascular health in children and determine the dose-response to protect against cardiovascular impairment.

High-intensity interval exercise (HIIE) involves repeated short high-intensity bursts of exercise interspersed with recovery periods (Billat, 2001). Evidence suggests HIIE training may be equally effective (Sperlich et al. 2010; Baquet et al. 2010) or superior (McManus et al. 2005; Buchan et al. 2011; Bendiksen et al. 2014; Bond et al. 2015a) compared to continuous moderate-intensity exercise (MIE) in improving aerobic fitness and cardiovascular health in children, despite the large differences in training volume. The allure of promoting HIIE for children is based upon several observations that suggest this form of exercise may be a more practical and effective choice. The natural movement and activity patterns in children represents a pattern similar to interval-style exercise: children typically engage in intermittent activity for short durations with low intensity recovery periods in-between, suggesting this type of exercise may be more akin to their movement habits compared to continuous exercise (Bailey et al. 1995; Hoos et al. 2014). From a pragmatic perspective, interval-style exercise may help children achieve their physical activity recommendations in a range of environments such as classrooms (Ma, Mare, & Gurd, 2014a; Ma, Mare, & Gurd, 2014b; Weston et al. 2016), alleviating the common barrier of 'lack of time' by utilizing time efficient, low volume interval

exercise (Gibala, 2007). Furthermore, this style of exercise has been reported to be more enjoyable than continuous exercise in adults (Jung, Bourne, & Little, 2014; Martinez et al. 2015; Kong et al. 2016) and there is some empirical evidence suggesting children and adolescents also rate HIIE as highly enjoyable (Wisløff et al. 2007; Tjønna et al. 2009; Crisp et al. 2012; Bond et al. 2015b, 2015c). These findings suggest children may be more likely to participate in HIIE and obtain the cardiovascular health benefits associated with it.

Although the clinically apparent complications of CVD such as myocardial infarction, stroke, and peripheral vascular disease appear in middle to older age, the process leading to CVD can begin in early childhood. This presents itself as impaired arterial vasodilator function, a key precursor of later CVD in youth (Celemajer et al. 1992). Noninvasive assessment of the function of the vascular endothelium measured via flow-mediated dilation (FMD) has been used to measure childhood cardiovascular risk (Hopkins et al. 2015) and is used as a general marker of vascular health (Moens et al. 2005; Thijssen et al. 2011a). Recently, this technique has been utilized to assess acute (short-term) changes in vasodilator function in response to bouts of exercise (Dawson et al. 2013). It is understood that longer-term exercise training may augment vasodilator function in children (Watts et al. 2005; Seeger et al. 2011; Naylor et al. 2016; for a meta-analysis see Dias et al. 2015), however the pathways by which this occurs remain elusive. Investigating the recovery patterns of vasodilator function post-exercise provides insight into the adaptive response of blood vessel function which provide the stimulus for beneficial, long-term adaptation (for a review see Dawson et al. 2013).

The primary focus of this chapter is to review the extant literature relating to HIIE and vascular health in children. Firstly, HIIE will be reviewed with a focus on how children respond to HIIE. Following, a review of the effect of HIIE on cardiorespiratory fitness will provide evidence on the effectiveness of HIIE in children and indicate disparate adaptive responses compared to adults. Lastly, arterial vasodilator function will be reviewed, including the vascular system and measurement techniques used, with an emphasis on the recovery patterns post-exercise and possible physiological mechanisms which underlie these responses.

## 1.1 High-Intensity Interval Exercise (HIIE)

High-intensity interval exercise (HIIE) involves repeated short high-intensity bouts of

exercise separated with brief recovery periods (Billat, 2001). This intermittent style of exercise allows manipulation of the structure of the exercise (duration, intensity, and repetitions of interval and recovery bouts) which provides the stimulus for adaptation (Gibala et al. 2012).

Interval-style exercise has been used extensively by athletes in the context of their sport to provide them with superior athletic achievements. Variations of HIIE have been performed as early as the 1950's with Swedish cross-country skiers while measuring heart rate, maximal oxygen uptake, and blood lactate (see Billat, 2001). Shortly thereafter Olympic champion Emil Zatopek popularized interval training to enhance athletic performance. This ignited interest in HIIE leading to the first published scientific literature describing interval training by Reindell and Roskamm (1959) and investigations into the acute physiological responses to interval training by Astrand and colleagues in 1960 (Astrand et al. 1960a, 1960b). More recently research attention has shifted away from using interval-style exercise for athletic prowess, instead as a way of decreasing time and volume yet achieving similar or superior health benefits compared to longer duration low-to-moderate-intensity exercise. Indeed, in as little as 2-8 weeks HIIE training can improve muscle oxidative capacity (Burgomaster et al. 2005; Gibala et al. 2006; Little et al. 2010), physical fitness (McManus et al. 2005; Mucci et al. 2013; Barker et al. 2014; Forbes et al. 2017), and arterial function (Burgomaster et al. 2008; Bond et al. 2015a) in youth and adults, suggesting intensity provides a potent stimulus to produce fast adaptation.

Intensity is a primary factor influencing on the adaptive response to exercise training in children (Armstrong & Barker, 2011), but how the intensity is administered during an exercise bout must be considered. Utilizing a higher intensity with intermittent rest periods has become popular due to its practicality, time-saving quality, and safety compared to continuous high-intensity exercise or other variations of maximal sprint exercise (Gibala et al. 2012). The intensity used and the duration of interval to recovery ratio (work:rest) dictates the primary energy systems utilized to produce adenosine triphosphate (ATP), thereby providing different metabolic stressors during exercise and recovery which determine the adaptive response to exercise. Work intervals longer than 45 seconds require large (~40%) contribution from the aerobic system (Gastin, 2001) and shorter rest intervals creates a greater reliance on aerobic metabolism during consecutive intervals (Laursen & Jenkins, 2002). On the other hand, longer

recovery periods (>1.5 minutes) lead to restoration of the aerobic system due to return to near resting levels of acid-base balance (pH) and phosphocreatine (PCr). This allows removal/oxidation of lactate (La) prior to subsequent sprints, lowering intramuscular hydrogen ions (H+) which are known to inhibit glycolysis and PCr recovery (Gaitanos et al. 1993; Putman et al. 1995). The following intervals can then be performed at higher power outputs via greater anaerobic glycolytic contribution with less factors limiting anaerobic glycolysis (Buchheit & Laursen, 2013b). This is observed with HIIE consisting of short all-out sprints which produces the highest blood La accumulation and therefore greater glycolytic contribution to work (Buchheit & Laursen, 2013b).

The most popular and effective protocols used in the literature (annotated as the work to rest ratio in seconds [s]), which will be referred to in the following sections are repeated Wingate tests (30:240 s), the Tabatta protocol (20:10 s), 4 x 4 (240:240 s), repeated sprints (6:24 s), one on:off (60:60 s), and running sprints (10-30:10-30 s). Depending on the study addressed, the duration of the work and rest intervals and the total repetitions completed may vary. Research using cycling, running, and upper-body ergometers/handgrip exercise are discussed but there are limitations and benefits associated with each. Intensity and load is better controlled in cycle ergometry compared to running exercise because cycle ergometers allow control over exercise power output (as a relative % maximum power output [$W_{max}$] or % maximum aerobic power [$\%\dot{V}O_{2max}$]) compared to running which is commonly prescribed relative to maximum aerobic speed (MAS) or velocity (MAV). Considering the difficulties in comparing exercise completed at different intensities, comprehensive comparison between exercise intensities requires constants and variations of the exercise protocol. Thus, control over power output in cycling allows sounder comparison of intensities and durations by matching a constant total exercise workload with variations in intensities.

## 1.2 HIIE and Children

Higher intensity exercise is well tolerated in children (Hebestreit, Mimura, & Bar-Or, 1993); however, maturational and developmental disparities exist between children and adults which may influence the acute and chronic response to this type of exercise. A brief review of these follows.

### 1.2.1 Maturational Differences

#### Muscle Morphology and Oxidative Potential

Large changes occur in muscle fibre type composition from birth through to old age (see Figure 1.1). Following birth muscle fibres are non-differentiated (Son'kin & Tambovtseva, 2012) and type I fibres increase during the first 2 years following birth at a fast rate making them more predominant and much larger than type II fibres in children (Oertel, 1988). The relative proportion of these fibres decreases from age 10- to 19-years-old (Armstrong, Barker, & McManus, 2015) while the amount of type II fibres increases ~35 to 50% likely due to transformation of type I to type II fibres during pubertal development (Tambovtseva & Kornienko, 1986, 1987; Lexell et al. 1992).

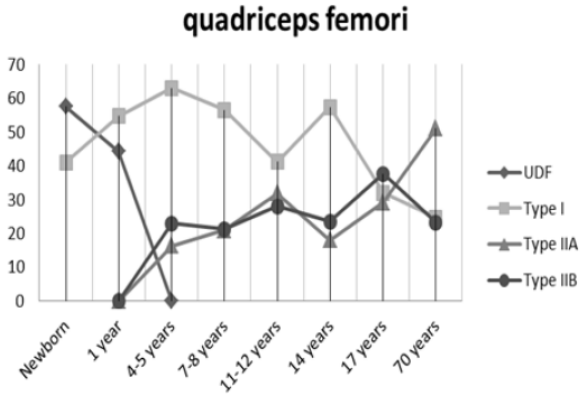
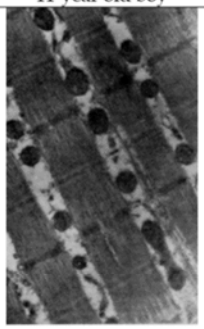
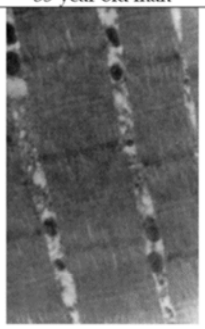



**Figure 1.1 Age-related changes of muscle fibre composition in men.** Vast changes occur in muscle morphology from 4 months intrauterine development until 70 years of age (represented by relative % of each fibre type, Y axis). Through pubertal development, type II fibres gradually increase as type I fibres decrease, possibly due to transformation of the latter to the former. UDF, undifferentiated fibres. Reproduced from 2012 Son'kin Valentin, Tambovtseva R. Published in Biochemistry, Genetics and Molecular Biology under CC BY 3.0 license. Available from: http://dx.doi.org/10.5772/31457, ©permission not required.

Due to the differences in metabolic properties of these fibre types, children have higher activity of oxidative enzymes compared to pubertal adolescents (Berg, Kim, & Keul, 1986; Eriksson, Gollnick, & Saltin, 1974). Eriksson, Gollnick, & Saltin (1974) found that succinate dehydrogenase, an important enzyme complex for mitochondrial respiration, is 25% more

abundant in children than formerly observed in adults (Gollnick et al. 1972). Furthermore, resting oxidative enzymes are higher in adolescents compared to young adults (Haralambie, 1982) revealing a progressive decline in resting oxidative enzyme expression through maturation. Age-related differences in oxidative metabolism are further supported by higher adenosine diphosphate (ADP) concentrations (Taylor et al. 1997) and a lower resting phosphocreatine:inorganic phosphate ratio (PCr:Pi) suggesting increased mitochondrial respiration in children (Chance et al. 1986). Moreover, children possess a lower potential for anaerobic metabolism, with glycogen content (Eriksson, 1972) and glycolytic enzymes (Kaczor et al. 2005) lower in children compared to adults. For example, resting levels of phosphofructokinase (PFK), the glycolytic rate-limiting enzyme, are ~66% lower than observed in adults (Gollnick et al. 1972; Eriksson, Gollnick, & Saltin, 1974). These data demonstrate a progressive decline in oxidative enzyme activity with age and maturation and concomitant incline in glycolytic enzymes.

**Table 1.1 Skeletal muscle mitochondria in boy and man.** Muscle biopsy in an 11-year-old boy reveals a larger mitochondria diameter and greater numbers of mitochondria in relation to myofibril area compared to a 35-year-old man. Reproduced from 2012 Son'kin Valentin, Tambovtseva R. Published in Biochemistry, Genetics and Molecular Biology under CC BY 3.0 license. After Kornienko, 1979; modified. Available from: http://dx.doi.org/10.5772/31457, ©permission not required.

| Index | 11-year old boy | 35-year old man | Difference, % |
|---|---|---|---|
| Electron micro photos of somatic muscle lengthwise cuts (m. Quadriceps Femori) |  |  | |
| Mean diameter of mitochondria, micron | 236 | 175 | -35 |
| Mean thickness of myofibrils, micron | 505 | 590 | +14 |
| Ratio of mitochondria area to myofibril area | 0,034 | 0,016 | -113 |
| Ratio of mitochondria total area to myofibril total area | 0,153 | 0,097 | -58 |

**Metabolic Response to Exercise**

The metabolic response to exercise can be measured using noninvasive techniques such as oxygen uptake kinetics ($\dot{V}O_2$ kinetics) and $^{31}$P-magnetic resonance spectroscopy ($^{31}$P-MRS). In the transition to exercise, measures of pulmonary $\dot{V}O_2$ kinetics reveal a window into identifying the mechanisms involved in controlling the dynamic adjustment of the oxidative capacity of the body (Armstrong & Barker, 2009). Compared to adults, children possess a faster pulmonary $\dot{V}O_2$ kinetic response time or tau ($t\dot{V}O_2$) during moderate-intensity exercise (Fawkner et al. 2002; Leclaire et al. 2013) and a faster $t\dot{V}O_2$ with an attenuated slow-component during high-intensity exercise (Fawkner et al. 2004). Sex differences in $\dot{V}O_2$ kinetics are apparent at an early age as boys have a faster $t\dot{V}O_2$ during high-intensity exercise compared to girls of the same age (~10- to 11-years-old; Fawkner & Armstrong, 2004). Additionally, longitudinal research comparing children at 11-years-old and 13-years-old reveals age-dependent slowing of pulmonary $\dot{V}O_2$ kinetics response time during high-intensity exercise (Fawkner & Armstrong, 2004a; see Figure 1.2). Age-related differences may be due to a higher arterio-venous oxygen difference (a-v $O_2$ diff) during identical work-rates compared to adults (Prado, Dias, & Trometta, 2006), indicating a better balance of oxygen delivery and oxygen utilization. This is supported with evidence demonstrating that children have faster muscle de-oxyhemoglobin (HHb) kinetics compared to adults, reflecting a faster local fractional oxygen extraction (Leclaire et al. 2013). Furthermore, children have faster local blood flow adjustment during exercise (Koch & Eriksson, 1973; Leclaire et al. 2013) making it possible that enhanced oxygen delivery in children lead to higher levels of cellular pressure of oxygen ($PO_2$) during moderate and intense exercise (Koch, 1984), thereby increasing blood-myocyte oxygen flux and oxidative phosphorylation (McDonough et al. 2005). Therefore, faster $t\dot{V}O_2$ observed in children are likely due to enhanced oxygen extraction and local oxygen delivery (Leclaire et al. 2013), the result of a larger type I fibre composition (Pringle et al. 2003), enhanced oxidative enzymes (Poole & Jones, 2012), and improved local oxygen delivery (Koch, 1984; Murias, Spencer, & Paterson, 2014).

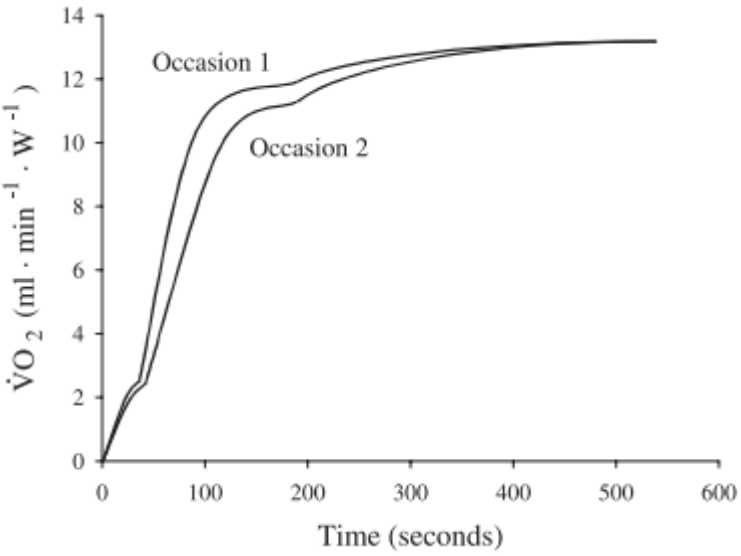



**Figure 1.2 Longitudinal changes in the pulmonary $\dot{V}O_2$ kinetic response in children at 11-years-old (occasion 1) and at 13-years-old (occasion 2).** The pulmonary $\dot{V}O_2$ kinetic response slows with age and undergoes changes during early pubertal development. Over 2 years time, 11-year-old children have a slowing of pulmonary $\dot{V}O_2$ kinetics and an increase in the slow component (upward rise in $\dot{V}O_2$ towards an elevated steady state) during high-intensity cycling exercise. Reproduced from Fawkner & Armstrong, 2004a, ©permission not required.

Pulmonary $\dot{V}O_2$ kinetics are tightly coupled to the metabolic state of skeletal muscle (Barker et al. 2008). Specifically, the kinetics of muscle PCr breakdown and regeneration mirrors $\dot{V}O_2$ during step transitions (Rossiter et al. 1999; Barker et al. 2008; see Figure 1.3). Modeling equations and $^{31}$P-MRS allows measurement of high-energy phosphates (i.e. PCr, Pi) and provides a measure of muscle metabolism and mitochondrial function in-vivo (McCormack et al. 2011). Although not agreed upon by all (Willcocks et al. 2010), PCr kinetics differ between children and adults: PCr breakdown during exercise is attenuated in children compared to adolescents (Eriksson and Saltin, 1974) and adults (Ratel et al. 2008; Barker et al. 2010; Kappenstein et al. 2013). During plantar flexion HIIE involving 30 repetitions of 20:10 seconds, children (n = 16, age = 9.4 ± 0.5 y) had an attenuated decline in muscle PCr end exercise and during interval recoveries (Kappenstein et al. 2013). Barker and colleagues (2010) utilized exercise workloads specific to intracellular pH threshold and found that 9- to 12-year-

old children have a smaller decline in PCr above the threshold, signifying children have a smaller contribution for ATP resynthesis from anaerobic means compared to adults. Furthermore, PCr recovery is faster in children (Ratel et al. 2008; Fleischman et al. 2010) and is negatively correlated to the previous year's growth velocity (McCormack et al. 2011).

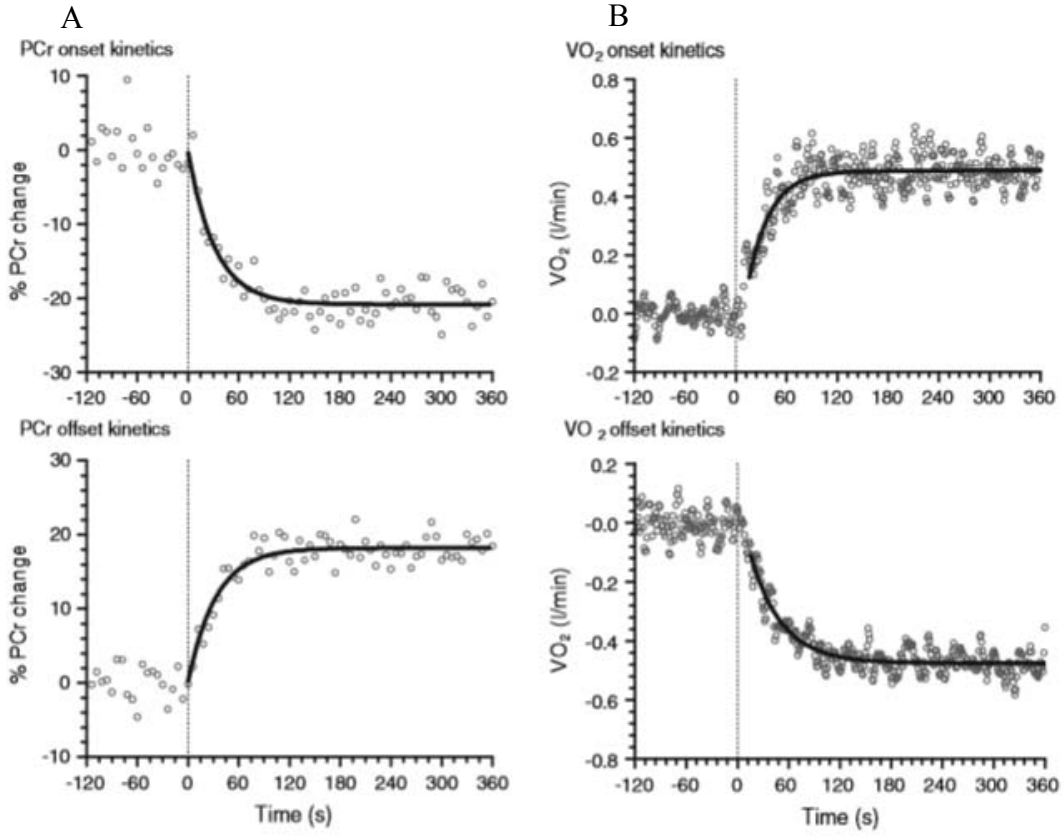



**Figure 1.3 Temporal response of PCr and $\dot{V}O_2$ in children.** At the initiation of knee-extensor exercise muscle PCr falls while $\dot{V}O_2$ rises (*column A*) while at exercise cessation PCr recovers as $\dot{V}O_2$ declines (*column B*). This response is similar in adults, exposing the link between PCr kinetics at the muscle and $\dot{V}O_2$ measured at the mouth. Reproduced from Barker et al. 2008, ©permission received.

Children can exercise at intensities close to $\dot{V}O_{2max}$ without producing high levels of La (Tolfrey & Armstrong, 1995) making their peak blood La smaller than adults (Eriksson & Saltin, 1974; Beneke, Hutler, & Leithauser, 2007). Although this is not agreed upon by all (Willcocks et al. 2010), during HIIE (10 x 10:30 s cycling sprints) children have faster blood H+ removal (Ratel et al. 2002) and faster cellular La clearance (Ratel et al. 2003) compared to

adults which allows for blood pH to be maintained at higher levels during exercise. This may be due to the preferential recruitment of type I fibres leading to an attenuated increase and decrease in Pi/PCr and pH, respectfully (Mizuno, Secher, & Quistorff, 1994), and enhanced oxidation of La to reconstruct ATP (Pilegaard et al. 1999; Ratel et al. 2003). Therefore, children may not produce less lactate *per se,* they may be more efficient at utilizing it as an energy source or regulating it through higher rates of ventilation, effectively regulating acid-base balance through $PaCO_2$ regulation (Ratel et al. 2002).

The enhanced oxidative capabilities during higher intensity exercise discussed provide evidence of maturational- and age-dependent changes in metabolic responses to HIIE. These differences influence the recovery process during and following HIIE, ultimately impacting the adaptive response children experience.

**Exercise Recovery**

The recovery process between HIIE intervals and following exercise cessation is an important feature of the purported health benefits. Even after the cessation of exercise $\dot{V}O_2$ remains elevated above resting values. This was first observed by Hill and colleagues in the early 1920's (Hill & Lupton, 1923) and reflects an increased metabolic 'repayment' of oxygen deficit the occurred with exercise (McArdle, Katch, & Katch, 1991). This excess post-exercise oxygen consumption or EPOC (Gaesser & Brooks, 1984) occurs within 1 hour post-exercise and the mechanisms involved are comprised of replenishment of oxygen stores in blood and muscle, resynthesis of ATP and PCr, La removal, maintenance of body temperature, and return to baseline circulation and ventilation levels (for a review see Børsheim & Bahr, 2003). HIIE largely influences the mechanisms of EPOC due to the strong relationship between EPOC and exercise intensity (Laforgia et al. 1997; Laforgia, Withers, & Gore, 2006; Hazell et al. 2012) as well as the intermittent style of HIIE (Cunha et al. 2016).

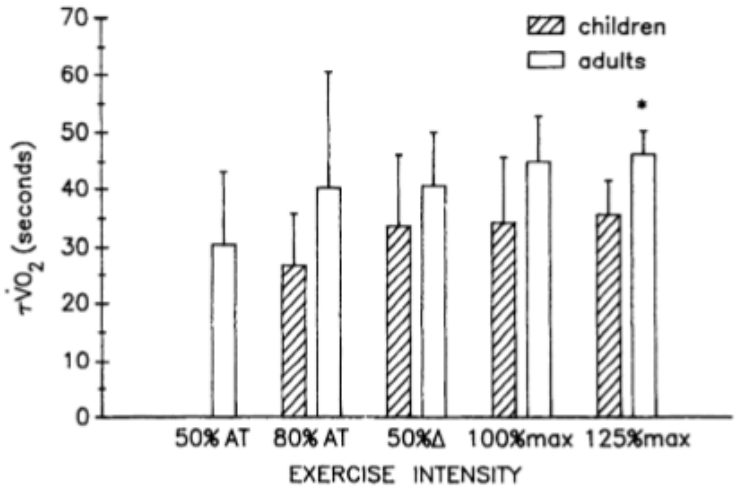



**Figure 1.4 $\dot{V}O_2$ recovery in children and adults.** The recovery of $\dot{V}O_2$ is faster in children at the highest intensity of exercise compared to adults (*$P < 0.001$) and although the recovery of $\dot{V}O_2$ slows in adults as intensity progresses, there is no effect of work intensity on recovery $\dot{V}O_2$ in children. Reproduced from Zanconato, Cooper, & Armon, 1991, ©permission not required upon request.

Children recover faster than adults following high-intensity exercise (Zanconato, Cooper, & Armon, 1991; Hebestreit, Mimura, & Bar-Or, 1993; Ratel et al. 2006; see Figure 1.4). Following one 30 second Wingate pre-pubertal boys (age = 9-12 y) have faster recovery of ventilation, heart rate, and $\dot{V}O_2$ compared to men (Hebestreit, Mimura, & Bar-Or, 1993). During exercise intervals children have faster $\dot{V}O_2$ kinetics and less oxygen debt compared to adults due to greater oxidative contribution towards ATP production, maintaining higher PCr levels and less accumulation of H+ and La (Kappenstein et al. 2013). Conversely, adults must recover from lower end-interval PCr levels leading to a larger decrease in pH (Kappenstein et al. 2013) which can impair calcium release and decrease force generation (Nakamura & Schwartz, 1970), limiting exercise performance and prolonging the rate of recovery (Allen, Lamb, & Westerblad, 2008). Furthermore, children have a lower RER during a relative workload, signifying preferential oxidation of lipids over carbohydrate utilization (Boisseau & Delamarche, 2000; Mahon & Timmons, 2014) leading to less reliance on carbohydrates and therefore less glycolytic byproducts which can extend interval and post-exercise recovery. Albeit, a relationship exists between maturation, maximal anaerobic power, and fatigue resistance which confounds these observations (Falgairette et al. 1991; Falk & Dotan, 2006).

Ratel and colleagues (2006) found children have a lesser decrease in mean power output, mean force output, and running velocity in treadmill interval sprints compared to adults. The faster recovery of power and force output in pre-pubertal boys than in young men is possibly explained by lower total mechanical work, higher aerobic turnover, and lower level of acidosis (Hebestreit, Mimura, & Bar-Or, 1993). The ability of children to recover from high-intensity exercise may also be a result of limited capacity for power output due to inability to recruit type II muscle fibres (Falk & Dotan, 2006). Peak power increases from age 7 to 17 years in both boys and girls (375% and 295%, respectfully) due to increases in muscle size and changes in muscle fibre type composition leading to better utilization of type II fibres and higher rates of glycolytic enzymes (Dotan et al. 2012). Therefore, large differences in attained power outputs may be a primary reason for faster recovery since children have less to recover from (Falk & Dotan, 2006).

While the aim of the preceding review was to inform readers on children's enhanced oxidative metabolism during exercise, the subsequent sections will review cardiorespiratory and vascular adaptations to HIIE and continue to build on the notion that these characteristics may very well shape the adaptive response to exercise. Each section's focus will be on the response to acute bouts of exercise while longer-term training studies will be discussed to observe how the acute response lead to training-induced adaptations.

### 1.2.2 Cardiorespiratory Fitness

Maximal oxygen uptake ($\dot{V}O_{2max}$ or maximal aerobic power) provides a measure of an individual's capacity for aerobic ATP resynthesis to produce energy for work (McArdle, Katch, & Katch, 1991). $\dot{V}O_{2max}$ has implications for performance and health as it represents the functional state of the oxygen transport system (Baquet, Praagh, & Berthoin, 2003). Although more characteristic in adults, children do not typically attain steady state $\dot{V}O_2$ values during maximal exercise testing and secondary characteristics to define $\dot{V}O_{2max}$ can lead to under-predictions of true $\dot{V}O_{2max}$ in children, therefore the extensive breadth of research regarding training-induced improvements in aerobic power are reported as $\dot{V}O_{2peak}$. Studies reviewed had to meet several criteria: 1) contain a high-intensity interval group and a control

and/or a moderate-intensity exercise group, and 2) study participants who were healthy and normal weight.

High-intensity interval training (HIIT) causes a significant increase in $\dot{V}O_{2peak}$ in children (Rotstein et al. 1986; McManus, Armstrong, & Williams, 1997; Baquet et al. 2002; McManus et al. 2005; Nourry et al. 2005; Gamelin et al. 2009; Obert et al. 2009; Baquet et al. 2010; Rosenkranz et al. 2012; Mucci et al. 2013; Barker et al. 2014; see Table 1.2). McManus et al. (2005) employed Wingate-type HIIT (7 x 30:165 s 'all-out' cycling @ 100% $W_{max}$) 3x/week for 8 weeks and observed a larger improvement in $\dot{V}O_{2peak}$ (+11.4%) compared to the moderate-intensity condition (+7.9%), regardless of large difference in exercise volumes (~3.5 min in the interval training group compared to ~20 min with moderate cycling). Other comparisons of exercise intensities support these findings (McManus, Armstrong, & Williams, 1997; Baquet et al. 2010) and it is the intense nature and interval-style of HIIE that may lead to similar improvements. Specifically, improvements in cardiorespiratory fitness may be mediated by adaptations in stroke volume (SV) and cardiac output (Q), thereby increasing oxygen delivery (Eriksson & Koch, 1973; Koch & Eriksson, 1973). Eriksson and Koch (1973) endurance trained 9 boys (age = ~11.7 y) 3x/week for 16 weeks and found increased $\dot{V}O_{2peak}$ was due to improvements in SV (+19%) with no change in a-$vO_2$ difference (i.e. peripheral oxygen extraction), later supported by observations of increased SV following 2 months of HIIT in pre-pubertal children (Obert et al. 2009). Utilizing a surrogate measure of SV several authors have noted improvements in $O_2$pulse (ml of oxygen ejected per heart beat) following 8 weeks of HIIT in pre-pubertal children (~1 $mlO_2 \cdot$beat increase) (McManus et al. 2005; Mucci et al. 2013). These findings are in line with work by Obert and colleagues (2003) who found training-induced improvements in $\dot{V}O_{2peak}$ are due to increased pre-load, decreased afterload, and cardiac enlargement. Although higher intensities cause near attainment of maximum heart rate which can limit preload, ejection fraction, and SV, peak SV can be reached and maintained during interval recovery periods, allowing cardiac adaptations to take place during interval rest periods (Buchheit & Laursen, 2013a).

Compared to the work in children, research in adults suggest the majority of HIIT induced adaptations take place peripherally through improved oxygen extraction and utilization at the muscle. Jacobs et al. (2013) completed cycling HIIT (6 sessions of 8-12 x

60:75 s @ 100% $W_{max}$) and found improvements in $\dot{V}O_{2peak}$ (+8%) were due to expansion of muscle mitochondria (+20%, assessed via cytochrome c oxidase activity) and greater leg deoxygenation with no changes in Q or total hemoglobin. This agrees with others who have reported a 38% increase in citrate synthase (a marker of muscle oxidative potential) (Burgomaster et al. 2005), an increase in pyruvate dehydrogenase (Burgomaster, Heigenhauser, & Gibala, 2006), and an increased capacity for lipid oxidation and glycogen sparing (Burgomaster et al. 2008) following the same training protocol. Furthermore, following leg extensor HIIT (15 x 60:180 s) the number of capillaries in contact with muscle fibres were increased (+19% type 1 fibres and +21% type II fibres) possibly leading to a larger microcirculatory volume and more peripheral oxygen delivery at the active muscle fibres (Krustrup, Hellsten, & Bangsbo, 2004). Interestingly, just one session of HIIE stimulates the transcriptional process of mitochondrial biogenesis 3 hours into exercise recovery, coinciding with increased mRNA expression of mitochondrial genes which lead to increased mitochondrial protein content and enzyme activity 24 hours post-exercise (Little et al. 2011).

Unlike work in adults, short-term adaptations following HIIE in children are scarce. Most studies reviewed in Table 1.2 were 7-8 weeks in duration, however Barker and colleagues (2014) observed improvements in $\dot{V}O_{2peak}$ in adolescents following 6 sessions of HIIE (~5%) and although no mechanisms were directly measured, they suggest a possibility of peripheral adaptations in line with previous adult research utilizing similar exercise protocols (Burgomaster et al. 2008; Jacobs et al. 2013). Higher work intensities necessitate recruitment of fast-twitch muscle fibres (Gollnick, Piehl, & Saltin, 1974; Altenburg et al. 2007) and the oxidative capacities in children's less developed type II fibres may increase with exercise training, although this remains unclear. More work is needed in this area to identify how individual bouts of exercise stimulates adaptation in the cardiorespiratory network, a time-course of the response, and where this occurs. Albeit, it is likely that children's high potential for oxidative phosphorylation and aerobic properties at the muscle alongside a smaller SV and Q may lead to enhanced central oxygen delivery with limited peripheral adaptations following HIIT (Erikkson & Koch, 1973; Lussier & Buskirk, 1977; Mandigout et al. 2001; Obert et al. 2003; McManus et al. 2005). This theory, although deduced from mechanisms involved in cardiorespiratory improvements, may provide insight into child-adult differences in peripheral vascular adaptation in the forthcoming chapters.

**Table 1.2** Summary of cardiorespiratory outcomes following HIIT.

| Citation | Participant Characteristics[a] | Study Design | Exercise Intervention[b] | Time commitment (min)[c] | $\dot{V}O_{2peak}$ (Δ%)[d] |
|---|---|---|---|---|---|
| Baquet et al. 2010 | 77 NW & OW children (34 girls, age = 8-11 y) | 7 wk, 3x/wk, running | HIIT: (n = 22, 11 girls; 30 min intermittent running, 5 sets x 5-10 reps of 10-20 s sprints @ 110-130% MAS w/ 10-20 s rest, 3 min rest between sets)<br>MIT: (n = 22, 10 girls; 1-4 sets x 6-20 min @ 80-100% MAV, 5 min rest between sets)<br>CON: (n = 19, 10 girls) | HIIT: ~25-35 min<br>MIT: ~18-39 min | HIIT: ↑ 4.8%*<br>MIT: ↑ 7%*<br>CON: ↓ 1.8% |
| Baquet et al. 2002 | 53 NW & OB children (30 girls, age = 8-11 y) | 7 wk, 2x/wk, running | HIIT: (n = 33, 20 girls; 30 min intermittent running, 4 sets x 5-10 reps of 10-20 s sprints @ 110-130% MAS w/ 10-20 s rest, 3 min rest between sets)<br>CON: (n = 20, 10 girls) | HIIT: ~25 min | HIIT: ↑ 8.2%*<br>CON: ↓ 2% |
| Barker et al. 2014 | 10 NW adolescents (0 girls, age = 14-16 y) | 2 wk, 3x/wk, cycling | HIIT: (n = 10; 1 set x 4-7 reps of 30 s 'all-out' cycling @ 7.5% body mass w/ 4 min rest between reps) | HIIT: ~14-27.5 min | HIIT: ↑ 5%* |
| Gamelin et al. 2009 | 52 NW children (23 girls, age = 9-10 y) | 7 wk, 3x/wk, running | HIIT: (n = 22, 10 girls; 30 min intermittent running, 4-5 sets x 5-20 reps of 5-30 s sprints @ 100-190% MAV w/ 10-30 s rest between reps)<br>CON: (n = 16, 9 girls) | HIIT: ~30 min | HIIT: ↑ 4.9%*<br>CON: ↓ 2.5% |
| McManus, Armstrong, & Williams, 1997 | 30 NW children (30 girls, age = 9-10 y) | 8 wk, 3x/wk, cycling and running | HIIT: (n = 12; 3-6 reps of 10 s running w/ 30 s rest, 3-6 reps of 30 s running w/ 90 s rest)<br>MIT: (n = 11; 20 min cycling @ 160-170 bpm [~75-85% $\dot{V}O_{2peak}$])<br>CON: (n = 7) | HIIT: ~20 min<br>MIT: ~20 min | HIIT: ↑ 8.4%*<br>MIT: ↑ 10%*<br>CON: ↓ 2% |

| Citation | Participant Characteristics[a] | Study Design | Exercise Intervention[b] | Time commitment (min)[c] | $\dot{V}O_{2peak}$ (Δ%)[d] |
|---|---|---|---|---|---|
| McManus et al. 2005 | 45 NW children (0 girls, age = 9-11 y) | 8 wk, 3x/wk, cycling | HIIT: (n = 10; 20 min cycling, 1 set x 7 reps of 30 s 'all-out' cycling @ 100% $W_{max}$ w/ 2 min 45 s rest between reps)<br>MIT: (n = 10; 20 min cycling @ 160-170 bpm [~75-85% $\dot{V}O_{2peak}$])<br>CON: (n = 15) | HIIT: ~20 min<br>MIT: ~20 min | HIIT: ↑ 11.4%*<br>MIT: ↑ 7.9%*<br>CON: ↑ 1.6% |
| Obert et al. 2005 | 24 NW children (9 girls, age= 9-11 y) | 8 wk, 2x/wk, running | HIIT: (n=9, 3 girls; 30 min intermittent running, 4 sets x 5-10 reps of 10-20 s sprints @ 100-130% MAS w/ 10-20 s rest, 3 min rest between sets)<br>CON: (n=9, 4 girls) | HIIT: ~30 min | HIIT: ↑ 15.5%*<br>CON: ↓ 0.6% |
| Rosenkranz et al. 2012 | 18 NW & OW children (16 girls, age = 7-12 y) | 8 wk, 2x/wk, running | HIIT: (n = 8; 30 min intermittent running, 4 sets x 5-10 reps of 10-20 s sprints @ 100-130% MAS w/ 10-20 s rest, 3 min rest between sets)<br>CON: (n = 8) | HIIT: ~30 min | HIIT: ↑ 24.5%*<br>CON: ↓ 8.9% |
| Rotstein et al. 1986 | 28 NW children (0 girls, age = 10-12 y) | 9 wk, 3x/wk, running and games | HIIT: (n = 16; 1-2 sets of 3 x 600 m w/ 2.5 min rest between sets, 5 x 400 m runs w/ 2 min rest between sets, 6 x 150 m runs w/ 1.5 min rest between sets)<br>CON: (n = 12) | HIIT: unknown | HIIT: ↑ 8%*<br>CON: ↑ 2% |

a NW – normal weight, OW – overweight, OB – obese, starting sample size

b HIIT – high-intensity interval training, MIT – moderate-intensity training, CON – control group, MAS – maximal aerobic speed, MAV – maximal aerobic velocity, $\dot{V}O_{2peak}$ – peak oxygen uptake, $HR_{max}$ – maximal heart rate, W – watts, final sample size for analysis

c Does not include warm-up or cool-down, time commitment per session including interval rest periods

d Percent change for groups

* Significant difference compared to pre-intervention

## 1.3 Vasodilator Function

In asymptomatic adults and those with disease, exercise is a well-known strategy to reduce the risk of CVD and related events (Lee et al. 2003; Mora et al. 2007; Lee et al. 2012). Exercise has been shown to decrease CVD risk in diseased (Alkarmi et al. 2010) and healthy (Green et al. 2011b) populations irrespective of traditional CVD risk factors (Green et al. 2003). Although it is understood that exercise can reduce primary and secondary cardiovascular events (Blair & Morris, 2009), these effects are not fully explained by modifications to traditional CVD risk factors (Green et al. 2003; Mora et al. 2007). Rather, exercise-induced improvements in traditional cardiovascular risk factors (e.g. weight status, blood pressure, lipid profile) only explain ~50% of the reduction in CVD risk (Green et al. 2008). It is now recognized that exercise provides direct anti-atherogenic effects on the blood vessel wall (Joyner & Green, 2009), a novel mechanism by which transient increases in blood flow and shear stress provides physiological stimuli important for blood vessel function and remodeling (Tinken et al. 2009).

The following sections will cover vascular structure and measures of vasodilator function, followed by a review of the effect of HIIE on vasodilator function and a discussion of the mechanisms involved.

### 1.3.1 Vascular System

The arterial network contains vessels of different calibers, and it is at the larger peripheral vessels where cardiovascular function can be measured directly. The research in this thesis investigates the conduit artery function, specifically the superficial femoral artery (SFA).

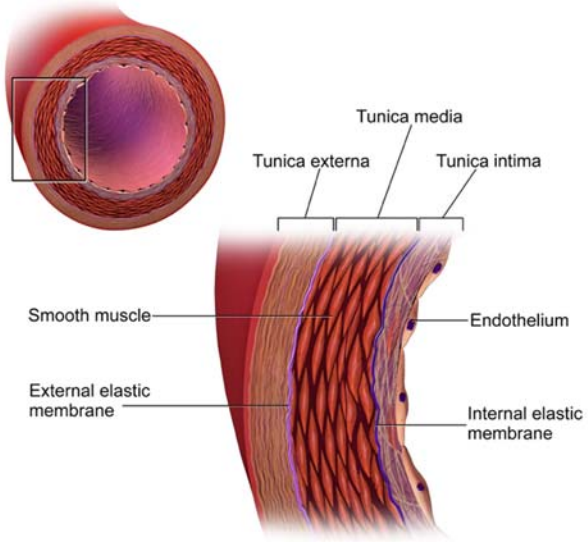



**Figure 1.5 Cross-sectional image of a conduit artery.** The endothelium is the inner-lining of the blood vessel and through chemical reactions influences smooth muscle tone which influences arterial diameter. Reproduced from Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". Wikiversity Journal of Medicine 1 (2): 10. doi:10.15347/wjm/2014.010. ISSN 2002-4436, ©permission not required.

Conduit arteries are elastic arteries with many collagen and elastin filaments in the tunica media which permits stretch in response to the pulse pressure created from the cardiac cycle, allowing optimal regulation of pulsatile flow while maintaining a constant pressure (i.e. the Winkessel effect). Conduit arteries are the largest arteries in the body (>1000μm) and gradually increase diameter with age from children to young adults through natural growth and development (see Figure 1.6). Artery diameter is positively associated with height and weight in children (Hopkins et al. 2015) in-part due to increased metabolic requirements associated with growth and development necessitate increases in arterial size (Kontos et al. 2015). Arterial diameter continues to increase into old adulthood (Green et al. 2010; van den Munckhof et al. 2012) possibly due to increased systolic pressure (van den Munckhof et al. 2012) leading to wall thickening, requiring a larger diameter as a compensatory mechanism (Green et al. 2010; van den Munckhof et al. 2012). Increasing age also leads to loss of elastic fibres (van der Heijden-Spek et al. 2000) and stiffening of the vasculature (Schmidt-Trucksass et al. 1999) while a lower age is associated with higher elastin content, maintaining recoil function (Fritze et al. 2012).

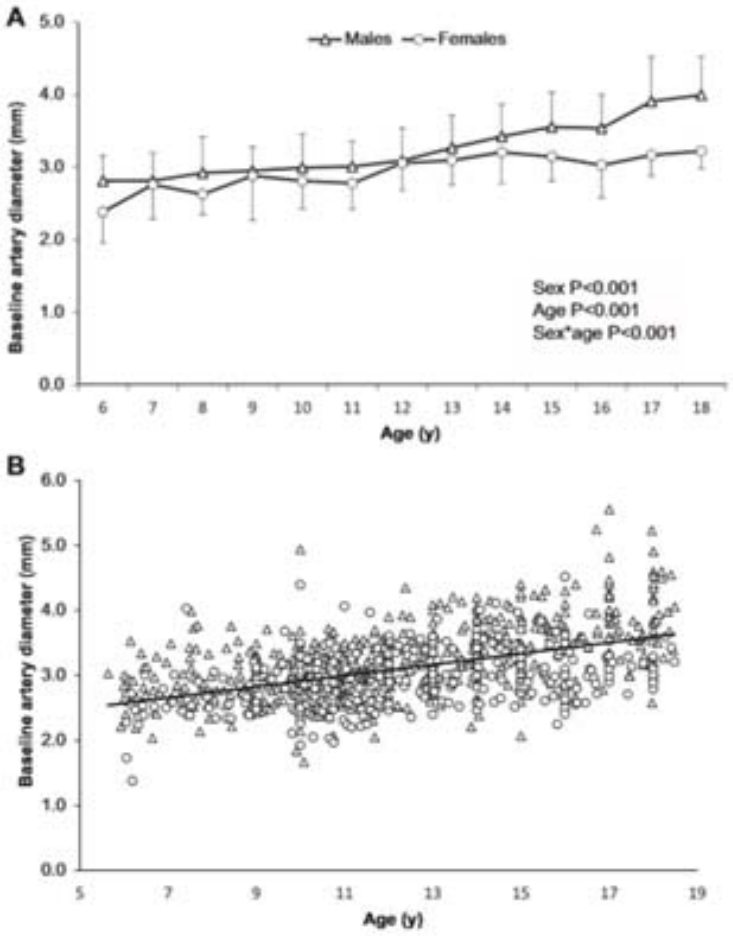



**Figure 1.6 Brachial artery growth with age.** Conduit arteries have a linear increase in size from 6- to 18-years-old in boys and girls. Panel A displays mean and SD while panel B displays individual data. Reproduced from Hopkins et al. 2015, ©permission not required upon request.

### Endothelium and Nitric Oxide

The inner-most layer of blood vessels is lined with the endothelium. This single-celled organ was once regarded as an inert layer until Furchgott and Zawadzki (1980) demonstrated that the in-tact endothelium lining of a rabbit thoracic aorta is necessary to induce relaxation of the artery using acetylcholine. They discovered the endothelium released a substance that relaxed blood vessels and termed it endothelium-derived relaxing factor (EDRF). This finding led to subsequent research identifying the endothelium as an important organ possessing autocrine and paracrine functions, which act to produce and release vasoactive substances.

Prior to Furchgotts work, Arnold and colleagues (1977) showed that nitric oxide (NO) activated guanylyl cyclase to effect smooth muscle relaxation and vasodilation. Following Furchgott's work it was realized that EDRF and NO both shared key properties. However, it was not until 1987 that Ignarro and colleagues definitively demonstrated that EDRF is NO, an important signaling molecule with powerful vasodilator effects. Ignarro, Furchgott and Murad received the Nobel Prize in Physiology or Medicine for their research. Rubanyi, Romero, & Vanhoutte (1986) advanced these findings by discovering blood flow through the arteries acts upon the endothelium to stimulate the release of EDFR/NO. The primary mechanism of this response was attributed to the mechanical stimulus of blood flow on shear stress sensitive ion channels within the endothelium (Lansman, Hallam, & Rink, 1987; Olesen, Clapham, & Davies, 1988). These findings established the primary mechanisms of the endothelium, successively leading to research of endothelium function in vivo.

Healthy endothelial cells respond to changes in blood flow by secreting vasoactive substances (i.e. NO, the primary vasodilator released). An increase in blood flow through the vessel lumen increases the frictional drag (i.e. shear stress) on the wall and stimulates the enzyme endothelium nitric oxide synthase (eNOS) which synthesizes NO from L-arginine. This causes NO-mediated vasodilation to normalize the shear stress on the vessel wall, regulate vascular tone, and adjust blood flow (Joannides et al. 1995; Kooijman et al. 2008; Di Francescomarino et al. 2009; Rauramaa & Hassinen, 2011). Therefore, vasodilator function as per this thesis refers to the degree of vasodilation of an artery in response to an increase in shear stress, which is endothelium-dependent and represents NO bioavailability.

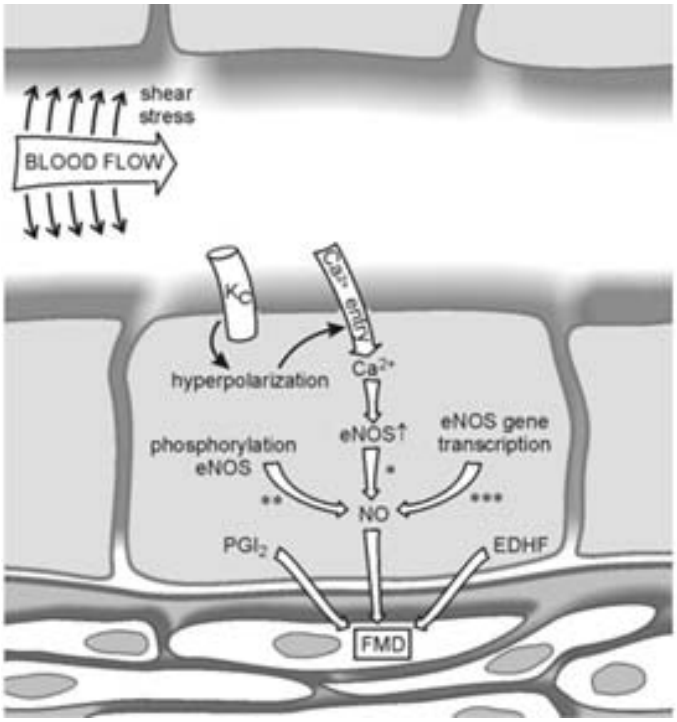



**Figure 1.7 Short- and long-term effects of shear stress on endothelial cells.** The endothelium acts as a mechanotransducer, sensing changes in shear stress and converting mechanical stimuli into vasodilatory chemicals. Shear stress opens specialized ion channels (calcium-activated potassium channels) in the endothelial cell membrane which causes the endothelial cells to become hyperpolarized, increasing calcium entry, activating eNOS, thereby generating NO to produce vasodilation. **short-term changes (seconds), **short-term changes (minutes), ***long-term changes (minutes to hours)*. Reproduced from Moens et al. 2005, ©permission received.

During exercise, NO functions to decrease sympathetic vasoconstriction in skeletal muscle (Hansen, Jacobsen, & Victor, 1994; Thomas & Victor, 1998), a process which is augmented via exercise training and to a greater degree with high-intensity exercise (Jendzjowsky & DeLorey, 2013). NO also mediates sustained vasodilation during recovery from contractions to maintain capillary-myocyte oxygen flux through improved matching of oxygen delivery to oxygen uptake (Shoemaker et al. 1997; Radegran & Saltin, 1999). Therefore, NO may play a crucial role in recovery from high-intensity bouts of interval exercise.

**Shear Rate and Patterns**

The endothelium is continually exposed to varying degrees of hemodynamic stimulus that exert forces upon the vessel wall. Shear stress is a biomechanical force that is determined by blood flow, vessel geometry, fluid viscosity, and is expressed in units of dynes/cm (Resnick et al. 2003). Frictional force generated by blood flow creates shear stress on the endothelium (Davies, 1995; Davies, Spaan, & Krams, 2005) which is the stimulating factor responsible for NO production, arteriogenesis (vascular remodeling), and angiogenesis (blood vessel formation) (Uematsu et al. 1995; Resnick et al. 2003). Shear stress cannot be measured directly non-invasively, for this reason shear rate will be used throughout this thesis. To derive an estimation of shear rate in vivo it is assumed that blood is a Newtonian fluid with a constant viscosity and steady laminar (straight) flow in a straight, cylindrical, inelastic vessel (Papaioannou & Stefanadis, 2005). Shear rate is defined by the equation:

$$\dot{y} = \frac{4 \cdot \mathrm{v\ (cm/s)}}{d\ \mathrm{(cm)}}$$

where $\dot{y}$ is shear rate expressed as reciprocal seconds ($s^{-1}$), $v$ is blood velocity, and $d$ is the vessel diameter. Due to children's smaller arteries, their endothelium is continually exposed to higher rates of shear.

The pulsatile nature of blood flow through conduit arteries causes a variation in the magnitude and direction of shear that can be explained by quantitative and directional assessment of shear rate and shear patterns. Blood flow through conduit arteries under resting conditions flows in an oscillatory pattern; there is a large antegrade (forward) flow during systole and a small retrograde (backward) flow during diastole (Thijssen, Green, & Hopman, 2011; see Figure 1.8). Normal antegrade flow is associated with anti-atherogenic effects, which act to maintain a normal vasodilator response (Cunningham & Gotlieb, 2005). Shear patterns that are non-laminar (i.e. larger retrograde flow) are involved in the pathogenesis of atherosclerotic plaque because it produces an endothelial cell phenotype that leads to a pro-atherogenic state (for reviews see Cunningham & Gotlieb, 2005; Harrison et al. 2006; Laughlin, Newcomer, & Bender, 2008; Newcomer, Thijssen, & Green, 2011).

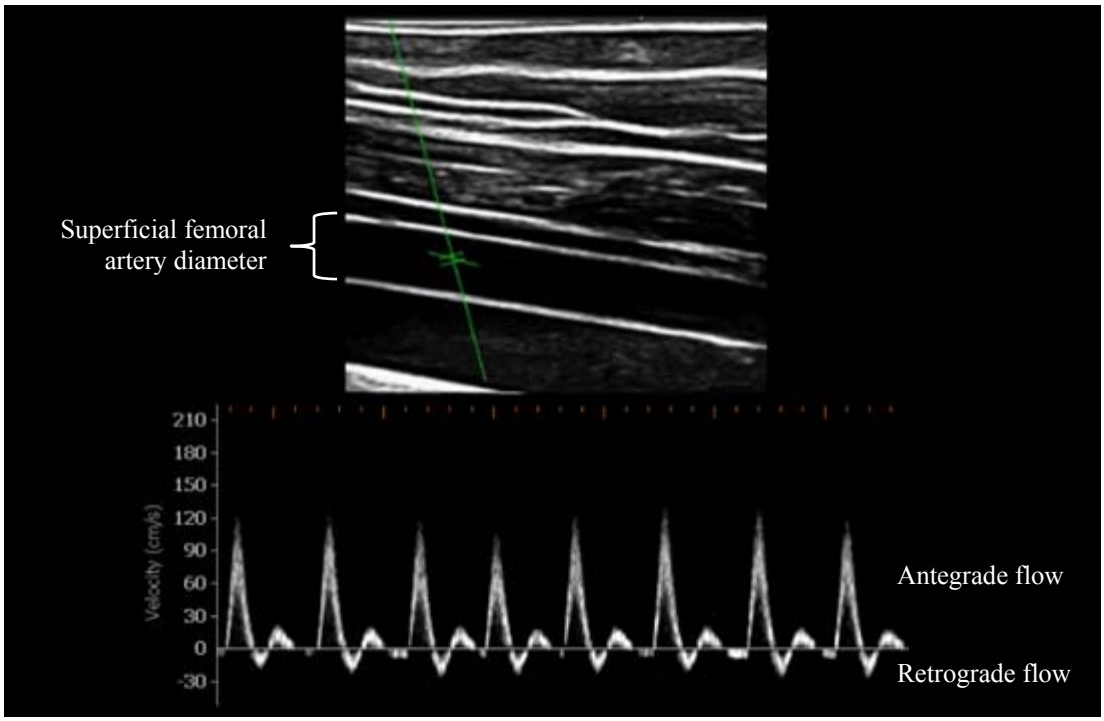



**Figure 1.8 Shear patterns in the superficial femoral artery using ultrasound Doppler imaging.** B-mode produces real-time images of the conduit artery, represented in the top half of the figure. The cursor (*green cross in the center of the vessel*) provides a measure of the blood flow velocity via spectral Doppler analysis, represented in the bottom half of the figure.

The shear pattern relationship can be described in terms of a ratio of antegrade to retrograde shear, termed oscillatory shear index (OSI) that indicates the magnitude of shear oscillation or shear reversal (McManus et al. 2015). High values of OSI are associated with endothelial dysfunction in adults (He & Ku, 1996) due to the diminished antegrade flow or increase retrograde flow.

$$OSI = |\text{retrograde shear}| \,/\, (|\text{antegrade shear}| + |\text{retrograde shear}|)$$

(Newcomer et al. 2008)

*During Exercise and Recovery*

In the transition from rest to maximal exercise skeletal muscle blood flow can increase 20-fold to supply the metabolic demand of active muscle beds (Olver, Ferguson, & Laughlin,

2015). Vasodilation and augmentation of blood flow ensues due to increases in NO production (Wang, Wolin, & Hintze, 1993; Tinken et al. 2009) via increases in antegrade shear stress on vascular endothelial cells (Rauramaa & Hassinen, 2011), a shear response exaggerated with higher intensity exercise (Birk et al. 2013; Atkinson et al. 2015a). Increased NO production occurs due to upregulation of eNOS (Rubanyi, Romero, & Vanhoutte, 1986) through mechanical and chemical stimuli in response to muscular contraction. Elevated levels of eNOS inhibit sympathetic vasoconstriction of the arterial soft tissue to allow vasodilation and a decrease in vascular resistance to improve blood flow to the active muscles (Ohyanagi, Nishigaki, & Faber, 1992; Thomas et al. 2003; Jendzjowsky & DeLorey, 2013).

Mean, antegrade, and retrograde shear has not been reported during or post-exercise in children which limits understanding of hemodynamic recovery. In adults, immediately following cycling exercise brachial shear is lower than during exercise but remains above that observed during rest and returns to pre-exercise levels with 30-60 minutes post-exercise in MIE (Atkinson et al. 2015b; Katayama et al. 2016) and HIIE (Menetrier et al. 2015). To our knowledge there is no research detailing the recovery patterns of shear rate in children or in the SFA in response to lower body exercise.

*Shear Differences in Limbs*

Conduit arteries are similar in nature, although shear and vasodilator function is not homogenous between the upper and lower limbs. At rest, while the brachial artery has inherently higher shear, vessels of the legs are exposed to lower antegrade and higher retrograde shear (Newcomer et al. 2008). This is due to different anatomical locations and hydrostatic pressures, leading to preserved vascular function and a protective effect from atherosclerosis in the vessels of the upper body (Wray et al. 2005). On the other hand, the legs are subject to lower shear stress and greater hydrostatic pressure because of gravity and postural positions of the legs, leading to a larger incidence of atherosclerosis in vessels of the lower body compared to arms (Ross et al. 1984; Kroger et al. 1999; Malek, Alper, & Izumo, 1999). During lower body exercise the musculature of the arms are not directly used, however cycling exercise produces an increase in antegrade shear with large increases in retrograde shear in the brachial artery (Green et al. 2002, 2005; Tinken et al. 2009; Padilla et al. 2011;

Birk et al. 2013; Atkinson et al. 2015b). Conversely, handgrip exercise is associated with higher rates of brachial artery antegrade shear along with minimal changes or decreases in retrograde shear (Green et al. 2005; Tinken et al. 2010; Atkinson et al. 2015a) due to a decrease in distal vascular resistance in the active limb (Green et al. 2005). Therefore, shear patterns in the exercised and non-exercised limbs are dissimilar and influence the acute adaptations in endothelial function.

With an understanding of the structures and pathways involved in arterial vasodilator function, the next section will cover the theoretical basis and application of vascular measurement.

### 1.3.2 Flow-Mediated Dilation (FMD)

Dilation of an artery that occurs due to an increase in luminal flow and subsequent increases in internal-wall shear is termed flow-mediated dilation (FMD). Measurement of vasodilator function in this thesis involves vascular occlusion at supra-systolic blood pressures to restrict blood flow and provide an ischemic challenge to a distal limb for a given time (5 min). Occlusion creates rapid dilation of downstream arterioles due to metabolic and prostaglandin dilators and accumulation of catabolites during the ischemic period, decreasing vascular resistance (Berg, Cohen, & Sarelius, 1997). When the cuff is released, the decreased resistance to flow results in hyperemia and an increase in shear. Shear stress triggers the endothelium wall to release NO, causing FMD (i.e. the difference between baseline and peak diameters; Joannides et al. 1995; Kooijman et al. 2008). Thorough details of the measurement technique are detailed in Section 2.3.2. Vasodilator function.

The FMD measurement used herein was first described by Celermajer and colleagues (1992). Their paper was the first to detail in-vivo measurement of vasodilator function, a landmark discovery utilizing non-invasive means to detect cardiovascular risk in children and adults. Of their most important findings, they found SFA and brachial artery FMD was impaired in asymptomatic children and adults who have risk factors for vascular disease (i.e. hypercholesterolaemic). It was already established that early vasodilator dysfunction is correlated to future atherosclerotic development (Fish et al. 1988; McLenachan et al. 1990) but now there existed a non-invasive means to observe cardiovascular risk: a 1% decrease in FMD

is associated with an 8-13% increase in cardiovascular even risk (Inaba, Chen, & Bergmann, 2010; Ras et al. 2013). Normal endothelial cells support cardiovascular function by promoting vasodilatation in response to blood flow. In contrast, impaired FMD reflects endothelial dysfunction and is characterized by a decreased production and/or local bioavailability of NO (Moens et al. 2005). In the decades since Celermajer's original work, technological advancements and improved methodological approaches have allowed superior imaging with more reliable and standardized measures (Thijssen et al. 2011a). As well, more recently the application of FMD has expanded from clinical diagnosis and risk stratification to also be used to investigate therapies that can improve vasodilator function (Watts et al. 2004, 2005), while determining the mechanisms involved (Moens et al. 2005; Green et al. 2011a).

Flow-mediated dilation (FMD) is higher in children compared to adults (Celermajer et al. 1994; Thijssen et al. 2009a). Hopkins and colleagues (2015) revealed FMD declines from age 6- to 18-years-old (n = 978, $P < 0.05$), with the more prominent changes occurring late pubertal development (age = ~17-18 y; see Figure 1.9). The age-related decrease in FMD is likely due to accelerated growth producing a larger vessel size which negatively influences FMD (see Figure 1.10). The negative relationship between FMD and age (Celermajer et al. 1994; Black et al. 2009; Thijssen et al. 2006) may be due to a reduction in NO bioavailability (Singh et al. 2002) or through vasoconstrictor pathways. For instance, the endothelin-1 pathway is more active with older age (Thijssen et al. 2007) and is negatively associated with endothelial-dependent dilatation (Donato et al. 2009). Also, a reduced antioxidant capacity (Donato et al. 2015) and increased inflammation within the blood vessel (Donato et al. 2008) may also be age-dependent mediators of FMD. However, it is important to consider child-adult differences in the structural characteristics of conduit arteries (i.e. diameter) and the interaction with hemodynamic stimuli, which are primary determinants of FMD.

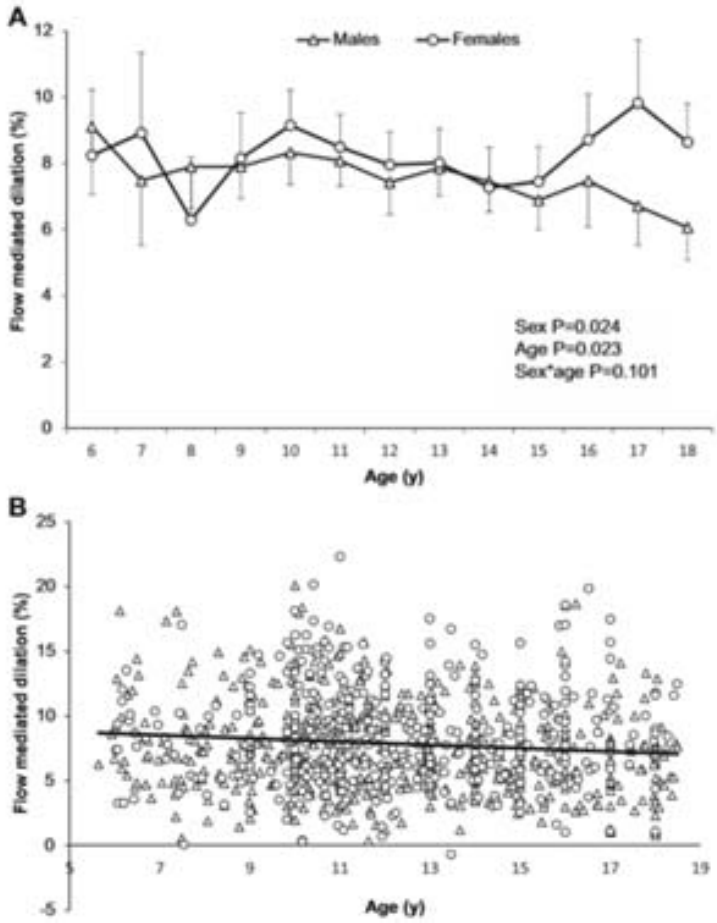



**Figure 1.9 Change in FMD (%) through childhood and adolescence.** Although there is a main effect for sex and age with FMD from ages 6- to 18-years-old, differences appear to align with maturational development for girls (age = ~14-16 y) and boys (age = ~16-18 y). The direction of change is in-part due to different effects of estrogen and testosterone during puberty as estrogen has beneficial effects on endothelial function (i.e. estrogen is linked to greater eNOS expression; Kellawan et al. 2015). Panel A displays mean and SD while panel B displays individual data. Reproduced from Hopkins et al. 2015, ©permission not required.

### Determinants of FMD: Baseline Diameter and Shear Rate

*Baseline Diameter*

There is a strong inverse relationship between baseline artery diameter (i.e. artery diameter prior to cuff inflation) and FMD (Thijssen et al. 2008; Atkinson & Batterham, 2013; see Figure 1.10). Furthermore, a positive relation exists between wall-to-lumen ratio and

vasodilator responses of upper and lower limb vessels (Thijssen et al. 2011b). Smaller arteries are inherently more sensitive because of an increased wall-to-lumen ratio which causes exaggerated vascular reactivity due to larger amounts of smooth muscle relative to elastic elaminae (i.e. 'Folkow effect'; Thijssen et al. 2008). Alternatively, attenuated vasodilator function in larger arteries may be a façade, a consequence dependent on size. Atkinson and Batterham (2013) addressed this issue by utilizing allometric scaling to correct for differences in baseline diameter in adults and children in the original work by Celemajer and colleagues (1992). They found the original observation of a reduced femoral FMD in adults compared to children was non-existent and due to inappropriate scaling when comparing diameters of different sizes. Hence, structural differences may be the reason children have augmented dilator capacity compared to adults. This matter becomes imperative when assessing FMD following exercise since exercise typically causes an increase in baseline diameter, and this will remain an important theme throughout the remainder of this thesis. Specific analyses for correcting FMD (%) for baseline diameter are detailed in Section 2.3.3.

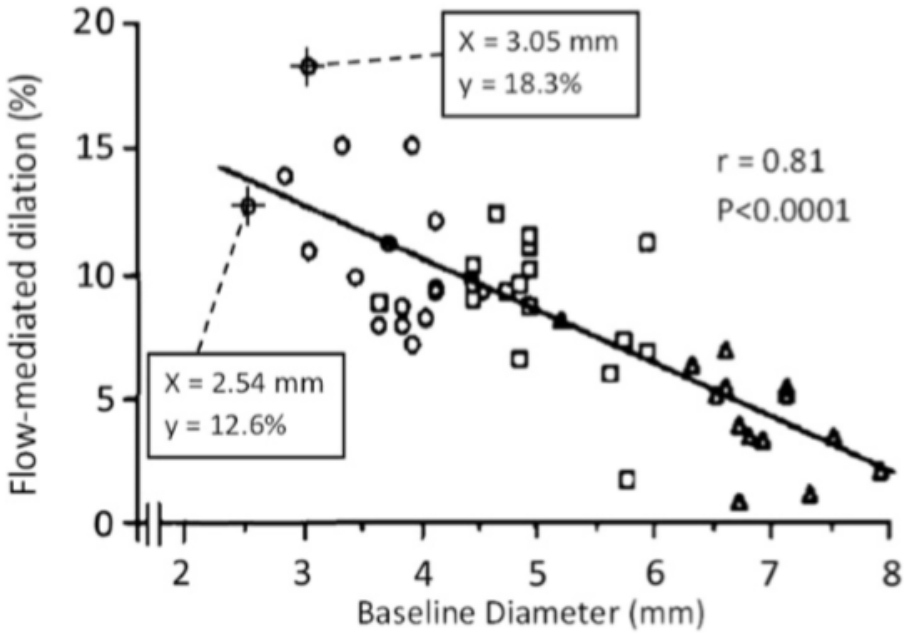



**Figure 1.10 Relationship between FMD (%) and baseline diameter (mm).** A larger baseline diameter leads to a smaller FMD (%) in the superficial femoral artery in adults (*triangles*), children (*squares*), and brachial artery in adults (*circles*). Reproduced from Atkinson & Batterham, 2013, ©permission received.

*Shear Rate*

A key stimulus for achievement of peak diameter during FMD is the shear stimulus upon cuff release, presented as shear rate area under the curve ($SR_{AUC}$). However, unlike adults there is no relationship between $SR_{AUC}$ and FMD in children (Thijssen et al. 2009a; see Figure 1.11). This is in line with previous studies in children that have found no relationship between shear and FMD (Hopkins et al. 2015) and further supports the contention of maturation-dependent influences of shear on endothelial cells (McManus et al. 2015). Therefore, it is possible that the shear stimulus during the exercise bout may act dissimilarly on children's endothelial cells compared to adults, resulting in different adaptations. Like baseline diameter, careful interpretation of $SR_{AUC}$ post-exercise is needed due to the large increases of blood flow and other confounding mechanisms that may influence FMD (Llewellyn et al. 2012). These will be discussed in the following sections, and the matter of maturational-dependent shear-sensitivity will an area of focus in the later chapters.

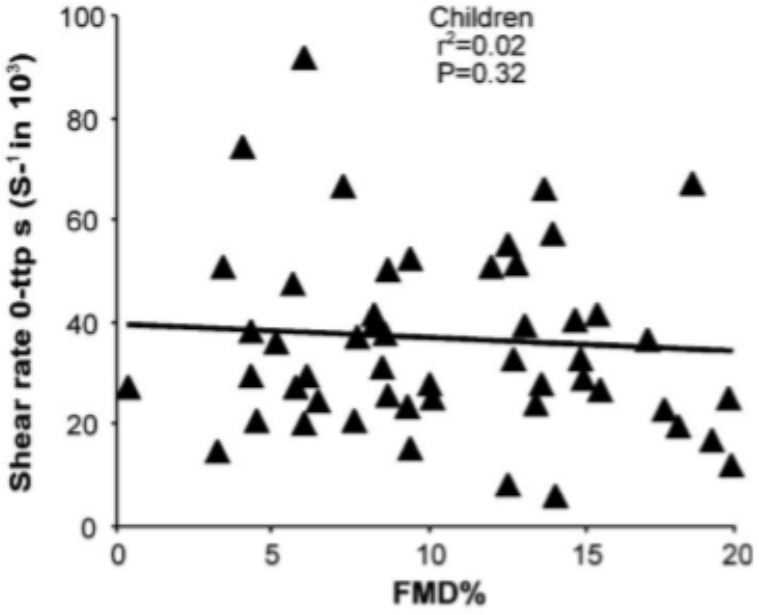



**Figure 1.11 Correlation between brachial artery FMD (%) and shear rate in children.** Shear rate post-cuff deflation is the main stimulus for FMD in adults, however there is no relationship between shear rate and the resulting vasodilation in children. This suggests that other processes mediate the FMD response aside from shear-dependent mechanisms. Reproduced from Thijssen et al. 2009a, ©permission not required.

### 1.3.3 Effect of Exercise on FMD

Exercise training can improve FMD in obese and overweight youth (for reviews see Watts et al. 2005; Dias et al. 2015) and healthy adolescents (Bond et al. 2015a; Yu et al. 2016). However, limited data exists on the effects of exercise training on vasodilator function in healthy children. This is unfortunate since the time-course of training-induced vascular alterations can provide valuable information on the relationship between early and latent vascular adaptations. Notable findings in healthy adults utilizing measures of brachial/popliteal artery vasodilator capacity (surrogate measure of conduit artery structure, Naylor et al. 2005) and FMD through 8 weeks of cycling and running exercise (30 min, 3x/week @ ~80% $HR_{max}$) have identified augmented FMD at weeks 2-4 and returned to baseline values through weeks 4-8, however structural improvements occurred as function returned to pre-training levels (Tinken et al. 2008; Birk et al. 2012; see Figure 1.12). Likewise, handgrip exercise training caused the same response in brachial artery adaptation (Tinken et al. 2010). This reveals improvements in vasodilator function precede longer-term adaptations in vascular remodeling (Miyachi et al. 2001; Prior et al. 2003). Increases in FMD are obligatory to normalize the repetitive increases in frictional shear stress on the artery wall before structural changes can assume this responsibility (Green et al. 2004). Therefore, short-term improvements in vasodilator function provide the foundation for long-term adaptation in arterial structure and provide insight into early adaptations noted with exercise training (Dawson et al. 2013).

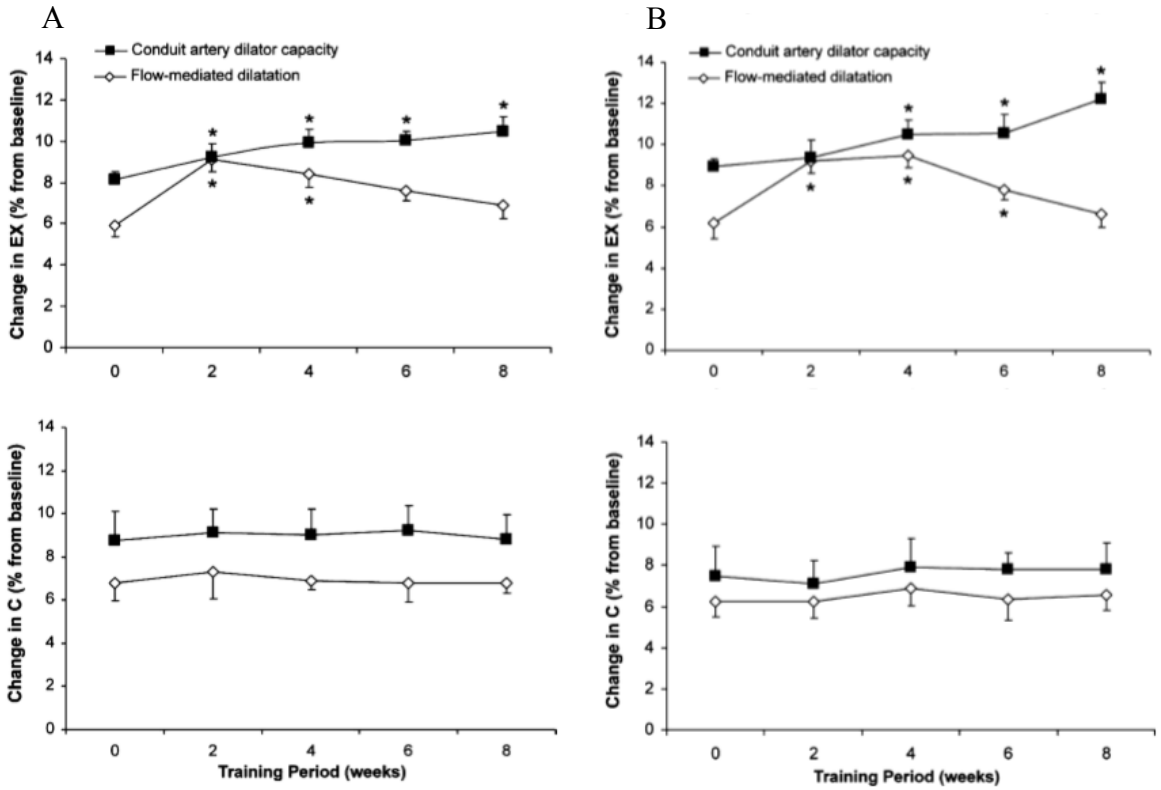



**Figure 1.12 Functional and structural vascular adaptations to 8 weeks of exercise training in adults.** Cycle and running exercise augments brachial (column A) and popliteal artery (column B) FMD (*open triangles*) in the early stages of training which are then succeeded by structural adaptations (*solid squares*), with no change when antegrade shear is blunted (*bottom graphs*). Reproduced from Tinken et al. 2008, ©permission received.

To date, only 2 studies have investigated the acute effects of exercise on vasodilator function in children. Mills and colleagues (2013) measured FMD before and after 15 minutes of high- and low-intensity active video game play in 15 pre-pubertal children (n = 15, age = 10 y, 8 males) and reported an attenuated FMD following the high- but not low-intensity condition. Contrary to this, comparison of different intensities of HIIE cycling (8 x 20:10 s @ 100, 130, or 170% $\dot{V}O_{2peak}$) in lean and obese pre-pubertal children found increased FMD immediately following exercise at 170% (Chuensiri, Tanaka, & Suksom, 2015). Although these conflicting data suggest higher intensity exercise may be a more potent stimulus for adaptation, it is necessary to carefully consider the methods employed. The high-intensity condition utilized by Mills and colleagues (2013) elicited an energy expenditure equivalent to

3.6 ± 2.5 metabolic equivalents (METs), classifying it as moderate-intensity exercise. Additionally, measurement of FMD occurred only immediately following exercise cessation which does not allow observations of further vascular adaptations that may have taken longer to transpire. For example, Bond and colleagues (2015c) compared MIE (~30 min cycling @ 90% GET) and HIIE (8 x 60:75 s cycling @ 90%$W_{max}$) in 20 adolescents (age = 14.1 ± 0.3 y, 10 male) and found FMD was unchanged following MIE, however following HIIE FMD was attenuated immediately post-exercise with subsequent increases above pre-exercise values when measured 1 and 2 hours post-exercise.

Similar to research in children, studies involving healthy adults are conflicting: 1 bout of exercise has been shown to lead to a decrease in FMD (Dawson et al. 2008; Rognmo et al. 2008; Jones et al. 2010; Gonzales et al. 2011; Phillips et al. 2011; Bailey et al. 2012; Birk et al. 2013; Hwang et al. 2012; Lleyellyn et al. 2012; Katayama et al. 2013; Atkinson et al. 2015b; Katayama et al. 2016), while others report an increase (Padilla et al. 2006; Tinken et al. 2009, 2010; Tyldum et al. 2009; Phillips et al. 2011; Rooks, McCully, & Dishman, 2011; Johnson et al. 2012; Johnson, Padilla, & Wallace, 2012; Atkinson et al. 2015a; Paiva et al. 2016; Siasos et al. 2016), or no change (Zhu et al. 2007; Dawson et al. 2008; Rognmo et al. 2008; Jones et al. 2010; Gonzales et al. 2011; Phillips et al. 2011; Birk et al. 2013; Hwang et al. 2012; Johnson, Padilla, & Wallace, 2012; McClean et al. 2015). These disparate results are highly dependent on the timing of FMD measurement, characteristics of the exercise bout, and methods used.

**Timing of Measurement**

In adolescents and adults, short-term alterations in FMD are dependent on the timing of measurement following the exercise bout, revealing a bi-phasic response after exercise that has yet to be investigated in children (Dawson et al. 2013; see Figure 1.13). Therefore, it is pertinent to discuss studies that repeat the FMD measure at multiple time points following exercise (Zhu et al. 2007; Rognmo et al. 2008; Tyldum et al. 2009; Atkinson et al. 2015a, 2015b; Birk et al. 2012; Johnson, Padilla, & Wallace, 2012; Katayama et al. 2013; Bond et al. 2015c; McClean et al. 2015; Katayama et al. 2016; Paiva et al. 2016) while carefully considering those that report FMD at specific discrete time intervals. Most (Rognmo et al. 2008; Birk et al. 2012; Atkinson et al. 2015b; Bond et al. 2015c; Katayama et al. 2016) but not

all (Johnson, Padilla, & Wallace, 2012; McClean et al. 2015) reveal an initial decrease in FMD that returns to pre-exercise values when measured 1-2 hours post-exercise. In agreement with this, studies that measure FMD at a discrete time point directly after exercise (within 30 minutes) report a decrease (Jones et al. 2010; Gonzales et al. 2011; Phillips et al. 2011; Bailey et al. 2012; Hwang et al. 2012; Lleyellyn et al. 2012), although not all agree (Johnson et al. 2012; Tinken et al. 2009; Tinken et al. 2010; Siasos et al. 2016). Furthermore, some have reported augmented FMD above pre-exercise values when measured 1 hour following exercise (Padilla et al. 2006; Tyldum et al. 2009; Rooks, McCully, & Dishman, 2011; Johnson, Padilla, & Wallace, 2012; Atkinson et al. 2015a; Bond et al. 2015c).

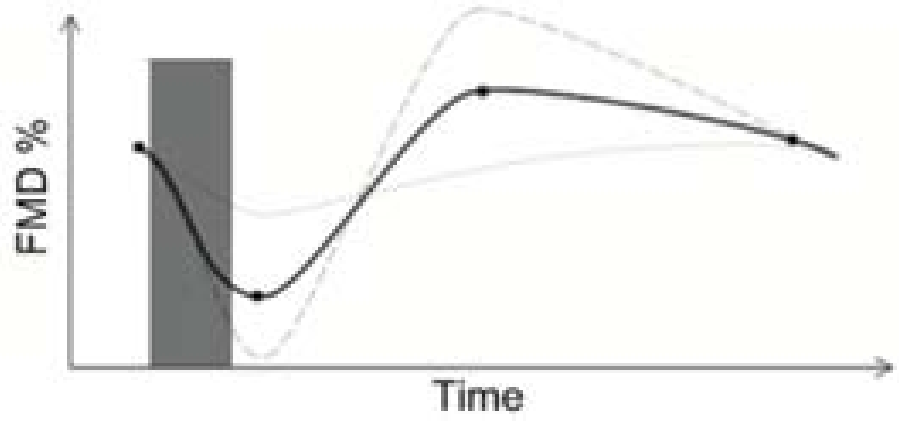



**Figure 1.13 Temporal response of FMD post-exercise.** Following exercise (*grey box*) a biphasic response in FMD is present that is dependent on the prior exercise intensity (*dotted grey line lower intensity, dashed grey line high-intensity*). Reproduced from Dawson et al. 2013, ©permission not required upon request.

The paradoxical nature of this response is intriguing: exercise training is effective at reducing cardiovascular events through anti-atherogenic effects on the vascular system, yet individual exercise bouts provide a transient pro-atherogenic stimulus that can lead to temporarily impaired vasodilator function (Green et al. 2004; Kojda & Hambrecht, 2005). Originally, the initial decrease in FMD was suggested to represent "exercise-induced vascular fatigue" that may not lead to an improvement in vasodilator function (Dawson et al. 2008). However, the initial decrease in FMD indicates the start of an adaptive process and represents a concept known as hormesis (Suvorava & Kojda, 2007; Padilla et al. 2011). Hormesis pertains

to many physiological processes and suggests that a seemingly negative stimulus may contribute to long-term adaptation and improvement. For instance, brief periods of exercise-induced vasodilator impairment may represent the activation of signaling responses that provide the stimulus leading to positive alterations in vascular function and structure (Padilla et al. 2011). When repeated, recurring periods of increased shear stress, cyclic strain, and oxidative stress can improve the tolerability of endothelial cells to deal with the negative responses from exercise and create a protective effect (Padilla et al. 2011).

**Intensity of Exercise**

The bi-phasic nature of the FMD response to acute exercise is influenced by the intensity of exercise. Birk and colleagues (2013) witnessed intensity dependent attenuations in FMD following cycling at 70 and 85% predicted $HR_{max}$ with no change during exercise at 50% $HR_{max}$. Furthermore, maximal intensity exercise has been shown to decrease FMD post-exercise (Bailey et al. 2012; Hwang et al. 2012; Llewellyn et al. 2012), contrary to evidence in children suggesting a positive relationship between supramaximal exercise intensity and post-exercise vasodilator function (Chuensiri, Tanaka, & Suksom, 2015). One of the few studies to compare two exercise intensities matched for total workload in adolescents (MIE vs HIIE, see page 47 for exercise protocols) found FMD was attenuated immediately post-exercise following HIIE and unchanged in the MIE condition (Bond et al. 2015c). These data agree with evidence of post-exercise attenuation in FMD in children during higher-intensity exercise with no effect of low-intensity exercise (Mills et al. 2013). However, these authors provided serial measures of FMD and reported an increased FMD greater than pre-exercise when measured 1 and 2 hours post-exercise (Bond et al. 2015c). This supports adult data which has reported larger increases in FMD during higher intensity exercise when measured 60 minutes post-exercise (Atkinson et al. 2015a). Conversely, exercise at moderate intensities has been shown to lead to no change or an immediate increase in FMD post-exercise (Zhu et al. 2007; Tinken et al. 2009, 2010; Tyldum et al. 2009; Rooks, McCully, & Dishman, 2011; Johnson et al. 2012; Johnson, Padilla, & Wallace, 2012; Atkinson et al. 2015a; Paiva et al. 2016; Siasos et al. 2016). Augmented FMD immediately following low- to moderate-intensity exercise which returns to pre-exercise levels may not lead to positive chronic adaptation with exercise training (Bailey et al. 2017). Instead, high-intensity exercise may provide a more potent

stimulus to create positive adaptations in vasodilator function (Dawson et al. 2013; Ramos et al. 2015) although this has yet to be properly addressed in children.

### Acute FMD Adaptation in the Exercised Limb

Flow-mediated dilation (FMD) in the brachial artery is not representative of SFA FMD (Thijssen et al. 2008). Studies measuring FMD following lower body exercise have almost exclusively assessed adaptations in the upper limbs. Considering the upper and lower limbs are exposed to different magnitudes and directions of blood flow, shear stress, and metabolic perturbations during lower body exercise (Green et al. 2005) it is important to address how the vessels of the legs adapt to lower body exercise.

No research in children has investigated the effect of exercise on vasodilator function in the exercised limb. In adults, vasodilator function in the exercised limb has been observed to increase (Tinken et al. 2009, 2010; Atkinson et al. 2015a; Paiva et al. 2016), decrease (Dawson et al. 2008; Gonzales et al. 2011; Katayama et al. 2013), or present no change (Gonzales et al. 2011; Atkinson et al. 2015a) dependent on the vessel imaged and intensity of exercise. Most have measured brachial artery FMD following upper-body exercise (Tinken et al. 2010; Gonzales et al. 2011; Katayama et al. 2013; Atkinson et al. 2015a; Paiva et al. 2016) and to our knowledge, only one measuring SFA FMD following lower body exercise (Dawson et al. 2008). Dawson and colleagues (2008) examined FMD in the brachial artery and SFA 1-hour post-marathon in 15 adults (age = 32 ± 10 y) and observed a significant decrease in SFA FMD with no change in the brachial artery. This is contradictory to others who reported an immediate increase in brachial artery FMD following handgrip exercise (Tinken et al. 2009, 2010; Paiva et al. 2016). Paiva and colleagues (2016) studied the effect of handgrip exercise on brachial artery FMD and observed an increase in FMD 15 minutes post-exercise which returned to pre-exercise levels 60 minutes after exercise. The dissimilar responses in SFA and brachial artery FMD following lower- and upper-body exercise may be due to altered shear stress patterns and differences in hemodynamic stimuli. While handgrip exercise is associated with increased brachial antegrade flow, cycling leads to higher brachial antegrade and retrograde flow which creates an oscillatory pattern and attenuates FMD (Green et al. 2005; see Section 1.3.4. Shear Patterns). Handgrip exercise requires small muscle mass and a low

cardiovascular/hemodynamic load which may not lead to substantial adaptations in healthy subjects. Therefore, initial improvements in brachial artery FMD following handgrip exercise which return to baseline values 1 hour following exercise cessation may not lead to long-term improvements due to lack of stimulus (Bailey et al. 2017). Exercise with larger muscle mass (i.e. running or cycling) and at a higher intensity may be necessary to observe the bi-phasic FMD response in the exercised limb, although this remains unclear in children.

**Data Processing**

Proper analysis of FMD can greatly improve the sensitivity and reliability of the technique. Utilizing semi-automated edge-detection and wall-tracking software makes the analysis less subjective to researcher bias (Green et al. 2002), and other techniques may result in large measurement error or bias selection of areas of the vascular wall. Those that utilized edge-detection and wall-tracking software (Dawson et al. 2008; Tinken et al. 2008; Tinken et al. 2009; Jones et al. 2010; Tinken et al. 2010; Birk et al. 2013; Atkinson et al. 2015a, 2015b) are considered to have the most methodologically sound approach for analysis. Of these studies, several other authors note a decrease in FMD post-exercise when intensity is above 70% $HR_{max}$ (Birk et al. 2013; Atkinson et al. 2015b) while low-intensity cycling exercise is associated with an increase in brachial artery FMD (Tinken et al. 2009). As well, those measuring the exercised limb during handgrip exercise consistently report an increase at higher intensities (Tinken et al. 2009, 2010; Atkinson et al. 2015a).

*Summary:* The effect of a single bout of exercise on post-exercise vasodilator function is highly dependent on the time-course of measurement, the intensity and mode of the exercise bout, and the vessel measured relative to the exercise stimulus. While low- to moderate-intensity exercise may lead to initial augmentation in FMD post exercise, this may not necessarily represent positive adaptation in vasodilator function or subsequent vascular remodeling. Rather, exercise at higher intensities (>70% $HR_{max}$/$\dot{V}O_{2peak}$) may cause an attenuated FMD immediately post-exercise with super compensation 1-2 hours following. As this remains unclear in children, evidence from this thesis will provide the first look at a potential bi-phasic, intensity-dependent response in vasodilator function post-exercise.

**Table 1.3** Summary of FMD response post-exercise in healthy children and adolescents.

| Citation | Participant Characteristics[a] | Study and Exercise Design[b] | Edge-detection Wall-tracking software (CV)[c] | Time commitment (min)[d] | Change in FMD[e] |
|---|---|---|---|---|---|
| Chuensiri, Tanaka, & Suksom, 2015 | 35 NW and OB children (18 NW, 0 girls, age = 8-12 y) | BA FMD pre, post<br>HIIE: 8 x 20 s @ 100, 130, 170% $VO_{2peak}$ w/ 10 s unloaded recovery | ✘ | 4 min | 100%: no change<br>130%: no change<br>170%: ↑ post* |
| Mills et al. 2013 | 15 NW children (7 girls, age = 9-11 y) | BA FMD pre, post<br>15 min of HI or LO exergaming | ✘ | 15 min | HI: ↓ post*<br>LO: no change |
| Bond et al. 2015b | 20 NW adolescents (10 girls, age = 12-15 y) | BA FMD pre, post60 HFM<br>HIIE: 8 x 60 s @ 90% $W_{max}$ w/ 75 s recovery @ 20 W<br>MIE: ~26 min cycling @ 90% GET (matched to HIIE) | ✘ (10.5%) | HIIE: ~18 min<br>MIE: ~26 min | HIIE: ↑ post60*†<br>MIE: no change |
| Bond et al. 2015c | 20 NW adolescents (10 girls, age = 12-15 y) | BA FMD pre, post, post60, post120<br>HIIE: 8 x 60 s @ 90% $W_{max}$ w/ 75 s recovery @ 20 W<br>MIE: ~26 min cycling @ 90% GET (matched to HIIE) | ✘ (9.7%) | HIIE: ~18 min<br>MIE: ~26 min | HIIE: ↓ post*†, ↑ post60*† and post 120*†<br>MIE: no change |

a NW – normal weight, OB – obese, sample size after exclusion

b HIIE – high-intensity interval exercise, MIE – moderate-intensity exercise, MIIE – moderate-intensity interval exercise, HI – high-intensity, LO – low-intensity, CON – control group, pre – pre-exercise, post – immediately post-exercise, post60 – 60 minutes post-exercise, post# – number displayed following post are minutes post-exercise, BA – brachial artery, SFA – superficial femoral artery, FMD – flow-mediated dilation, $VO_{2peak}$ – peak oxygen uptake, $HR_{max}$ – maximal heart rate, GET – gas exchange threshold, W – Watts, MVC – maximum voluntary contraction, HFM – high fat meal

c CV – coefficient of variation, only included if reported

d Does not include warm-up or cool-down, time commitment per session including rest periods

e Directional change in FMD for each group

* Significant difference compared to pre-exercise

† Significant difference compared to other exercise condition

**Table 1.4** Summary of FMD response post-exercise in healthy adults.

| Citation | Participant Characteristics[a] | Study and Exercise Design[b] | Edge-detection Wall-tracking software (CV)[c] | Time commitment (min)[d] | FMD (%)[e] |
|---|---|---|---|---|---|
| **1 time point** | | | | | |
| Johnson et al. 2012 | 12 NW adults (0 females, age = ~26 y) | BA FMD pre, post<br>20 min supine cycling @ 90 W | ✗ | 20 min | ↑ post* |
| Jones et al. 2010 | 10 NW adults (0 female, age = ~28 y) | BA FMD pre, post<br>3 x 10 min semi-supine cycling @ 70% $\dot{V}O_{2peak}$ w/ 10 min passive recovery | ✓ | 30 min | ↓ post* |
| Padilla et al. 2006 | 8 NW adults (3 females, age = ~26 y) | BA FMD pre, post120<br>45 min treadmill walking @ 60% $\dot{V}O_{2peak}$ | ✗ (2.22%) | 45 min | ↑ post120* |
| Rooks, McCully, & Dishman, 2011 | 24 NW and OW adults (24 female, age = ~20 y) | BA FMD pre, post<br>30 min cycling @ 50% $\dot{V}O_{2peak}$ | ✗ (32%) | 30 min | ↑ post* |
| Siasos et al. 2016 | 20 NW adults (0 females, age = ~23 y) | BA FMD pre, post<br>HIIE: 30 x 30 s @ 100% $W_{max}$ w/ 30 s passive recovery<br>MIE: 30 min cycling @ 50% $W_{max}$ (matched to HIIE) | ✗ | HIIE: 15 min<br>MIE: 30 min | HIIE: ↑ post*<br>MIE: ↑ post* |
| Tinken et al. 2009 | 10 NW adults (0 females, age = ~28 y) | BA FMD pre, post<br>MIE: 30 min cycling @ 80 W | ✓ | 30 min | ↑ post* |
| **Time Course** | | | | | |
| Atkinson et al. 2015b | 10 NW adults (0 females, age = ~28 y) | BA FMD pre, post, post60<br>30 min semi-supine cycling @ 75% $HR_{max}$ | ✓ | 30 min | ↓ post* |
| Birk et al. 2013 | 10 NW adults (0 females, age = ~22 y) | BA FMD pre, post, post60<br>30min cycling @ 50, 70, 85% predicted $HR_{max}$ | ✓ | 30 min | 50%: no change<br>70%: ↓ post*† (compared to 50)<br>85%: ↓ post*† (compared to 50) |

| Citation | Participant Characteristics[a] | Study and Exercise Design[b] | Edge-detection Wall-tracking software (CV)[c] | Time commitment (min)[d] | FMD (%)[e] |
|---|---|---|---|---|---|
| **Time Course** | | | | | |
| Johnson, Padilla, & Wallace, 2012 | 10 NW adults (0 females, age = ~26 y) | BA FMD pre, post, post60, post120<br>MIE: 30 and 60 min treadmill running @ 50% $VO_{2peak}$<br>HI: 17-22 and 30 min treadmill running @ 80% $VO_{2peak}$ (HI 17-22 min matched to MIE 30 min) | ✘ | 30-60 min | HI 17-22: ↑ post*<br>HI 30: no change<br>MIE 30: ↑ post*<br>MIE 60: no change |
| Katayama et al. 2016 | 7 NW adults (0 females, age = ~23 y) | BA FMD pre, post, post30, post60<br>30 min cycling @ 60% $VO_{2peak}$ | ✘ (6.2%) | 30 min | ↓ post* |
| McClean et al. 2015 | 16 NW and OW adults (0 females, age = ~27 y) | BA FMD pre, post, post90, post180<br>LO: 30 min treadmill running @ 55% $VO_{2peak}$<br>MIE: 20 min treadmill running @ 75% $VO_{2peak}$<br>HI: 5 min treadmill running @ 100% $VO_{2peak}$ | ✘ | 5-30 min | LO: no change<br>MIE: no change<br>HI: no change |
| Rognmo et al. 2008 | 7 NW adults (0 females, age = ~25 y) | BA FMD pre, post60<br>HIIE: 5 x 5 min treadmill running @ ~90% $HR_{max}$ w/ 2 min recovery @ 60-70% $HR_{max}$ | ✘ | 35 min | HIIE: no change |

| Citation | Participant Characteristics[a] | Study and Exercise Design[b] | Edge-detection Wall-tracking software (CV)[c] | Time commitment (min)[d] | FMD (%)[e] |
|---|---|---|---|---|---|
| **Time Course** | | | | | |
| Tyldum et al. 2009 | 8 NW and OW adults (0 females, age = ~42 y) | BA FMD pre, post16-18 h, post30-240 HFM<br>HIIE: 4 x 4 min treadmill running @ 85-95% $HR_{max}$ w/ 3 min recovery 50-60% $HR_{max}$<br>MIE: 47 min treadmill walking @ 60-70% $HR_{max}$ (matched to HIIE) | ✘ | 28 min | HIIE: ↑ all time-points*†<br>MIE: ↑ post16-18 h*<br>CON: no change |
| Zhu et al. 2007 | 11 NW adults (0 females, age = 19-26 y) | BA FMD pre, post, post60, post120, post180, post240<br>45 min treadmill running @ 60% $VO_{2peak}$ | ✘ | 45 min | no change |
| **Exercised Limb** | | | | | |
| Atkinson et al. 2015a | 11 NW adults (0 females, age = ~27 y) | BA FMD pre, post, post60<br>30 min handgrip w/ 30 contractions/min @ 5, 10, 15% MVC | ✓ | 30 min | 5%: no change<br>10%: no change<br>15%: ↑ post60* |
| Dawson et al. 2008 | 15 NW adults (0 females, age = 23-63 y) | BA and SFA FMD pre, post60<br>42.2 km marathon | ✓ | Not given | SFA: ↓ post*<br>BA: no change |
| Gonzales et al. 2011 | 14 NW adults (8 females, age = 21-34 y) | BA FMD pre, post30<br>30 min handgrip slow (0.2 $m \cdot s^{-1}$) and fast (0.5 $m \cdot s^{-1}$) contraction velocities w/ 3 contractions/min @ 65% $W_{max}$ w/ 1-2 min rest each 5 min | ✘ | 30 min | slow: ↓ post*<br>fast: no change |
| Katayama et al. 2013 | 8 NW adults (0 females, age = ~24 y) | BA FMD pre, post, post30, post60<br>30 min arm crank @ 30% $\dot{V}O_{2peak}$ | ✘ (3.9%) | 30 min | ↓ all time-points* |

| Citation | Participant Characteristics[a] | Study and Exercise Design[b] | Edge-detection Wall-tracking software (CV)[c] | Time commitment (min)[d] | FMD (%)[e] |
|---|---|---|---|---|---|
| **Exercised Limb** | | | | | |
| Paiva et al. 2016 | 9 NW and OW adults (0 females, age = ~28 y) | BA FMD pre, post, post60<br>20 min handgrip w/ 15 contractions/min @ 60% MVC | ✘ | 20 min | ↑ post* |
| Tinken et al. 2009 | 10 NW adults (0 females, age = ~28 y) | BA FMD pre, post<br>30 min handgrip w/ 30 contractions/min @ 1-2 kg | ✓ | 30 min | ↑ post* |
| Tinken et al. 2010 | 10 NW adults (0 females, age = ~28 y) | BA FMD pre, post<br>30 min handgrip w/ 30 contractions/min @ 30% MVC | ✓ | 30 min | ↑ post* |

a NW – normal weight, OW – overweight, OB – obese, sample size after exclusion

b HIIE – high-intensity interval exercise, MIE – moderate-intensity exercise, MIIE – moderate-intensity interval exercise, HI – high-intensity, LO – low-intensity, CON – control group, pre – pre-exercise, post – immediately post-exercise, post60 – 60 minutes post-exercise, post# – number displayed following post are minutes or hours (denoted by h) post-exercise, BA – brachial artery, SFA – superficial femoral artery, FMD – flow-mediated dilation, $VO_{2peak}$ – peak oxygen uptake, $HR_{max}$ – maximal heart rate, W – Watts, MVC – maximum voluntary contraction, HFM – high fat meal

c CV – coefficient of variation, only included if reported

d Does not include warm-up or cool-down, time commitment per session including rest periods

e Directional change in FMD for each group

* Significant difference compared to pre-exercise

† Significant difference compared to other exercise condition

### 1.3.4 Mechanisms of Acute FMD Adaptations

#### Sympathetic Nervous Activity

The influence of sympathetic nervous activity (SNA) on FMD has not been addressed in children, however in adults SNA negatively impacts vasodilator function (Hijmering et al. 2002; Thijssen et al. 2014). Atkinson and colleagues (2015b) found that 30 minutes of cycling exercise at 75% $HR_{max}$ with ingestion of adrenoreceptor blockade (prazosin) negated the decrease in FMD post-exercise observed in the placebo condition, suggesting sympathetic vasoconstriction competes with endothelium-dependent dilation. Higher rates of SNA following exercise may restrict conduit artery dilation due to increased vasoconstrictive mechanisms such as increased levels of endothelin-1 and angiotensin II (Okamoto, Masuhara, & Ikuta, 2008; Green et al. 2010). This may be linked to altered shear patterns since lower-body-negative-pressure increases SNA with concurrent increases in retrograde shear (Thijssen et al. 2014). It is not completely understood how exercise intensity influences SNA and how this contributes to intensity-dependent alterations in FMD, and how this may differ in children. Albeit, research in animal models has found that vasoconstriction during sympathetic stimulation is greater in type II compared to type I muscle fibers due to their sensitivity to norepinephrine (Folkow & Halicka, 1968; Gray, 1971; Hilton, Jeffries, & Vrbova, 1970). Therefore, the possibility exists that differential proportions of fibre types in children and adults may lead to dissimilar activation of SNA post-exercise, although this remains unclear.

#### Oxidative Stress and NO bioavailability

Elevations of shear stress during exercise initiates an increase in vascular oxidative stress through production of reactive oxygen species (ROS) in the circulation (Kojda & Hambrecht, 2005; see Figure 1.14). Higher levels of oxidative stress tip the redox balance from anti- towards pro-oxidant status and alter the critical balance between the redox signaling pathways and NO, leading to acute attenuation in vasodilator function. Specifically, the most abundant exercise-induced ROS is superoxide ($O_2$-) which has important immune functions and, under healthy resting conditions, is catalyzed by superoxide dismutase (SOD). However, during exercise $O_2$- scavenges NO and forms peroxynitrite (ONOO-) which decouples eNOS, leading to a reduction in NO bioavailability, thereby influencing the FMD response (Cai &

Harrison, 2000; Harrison et al. 2006). These pathways have been observed in-vivo using exogenous anti-oxidants such as vitamin C to prevent a decrease in FMD post-exercise (Johnson et al. 2013).

Higher intensity exercise may lead to more oxidative stress (Johnson, Padilla, & Wallace, 2012) and this is claimed to be a primary reason high-intensity exercise leads to an exaggerated immediate decrease in FMD with a return to baseline or supercompensation when measured 1 hour after (Birk et al. 2013; Bond et al. 2015c) while exercise of lower intensities leads to no change or an increase in FMD due to minimal ROS generation (Asano et al. 2012). This compliments the paradoxical nature of the post-exercise FMD response, being that exercise produces an acute shift to a pro-oxidant state which, when repeated, leads to an increase in total anti-oxidant capacity through augmented expression of antioxidant defense enzymes (Lauer et al. 2005; Phillips et al. 2011) leading to up-regulation of eNOS and NO bioavailability (Hambrecht et al. 2003; Laughlin, 2004).

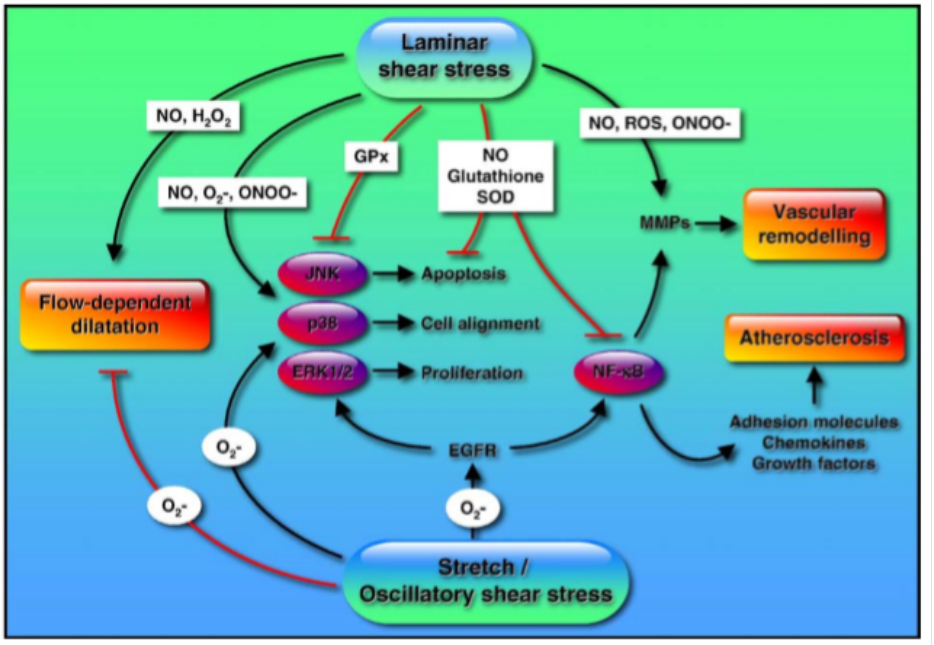



**Figure 1.14 Anti- and pro-oxidant pathways associated with laminar and oscillatory shear stress**. Oscillatory shear increases superoxide ($O_2$-) that upregulates the MAP kinases (JNK, p38, ERK1/2) and NF-nB and directly impairs FMD when NO is quenched by excessive $O_2$-. Laminar (i.e. antegrade) flow also generates $O_2$-, however this is equipoised by NO and

anti-oxidant production, offsetting activation of MAP kinases and NF-nB. However, high rates of laminar shear during high-intensity exercise may still cause ROS production and a tip towards pro-oxidant status. Reproduced from Lehoux, 2006, ©permission received.

## Shear Patterns and Blood Pressure during Exercise

### *Shear Patterns*

The direction and magnitude of shear is a necessary stimulus to induce adaptations in vascular function. In vivo elevations of antegrade shear increases vasodilator function whereas elevated retrograde shear leads to a dose-dependent decrease in vasodilator function (Tinken et al. 2009; Johnson et al. 2012; Johnson et al. 2013). Antegrade shear up-regulates eNOS activity and increases NO bioavailability (Pyke & Tschakovsky, 2007; Naylor et al. 2010; Schuler, Adams, & Goto, 2013) whereas higher retrograde shear can stimulate ROS production and impede FMD. The former was shown by Tinken and colleagues (2009, 2010) who measured brachial artery FMD pre- and post-cycling and handgrip exercise while using unilateral arm occlusion to sub-systolic pressures to blunt an increase in antegrade shear while maintaining similar retrograde patterns. They found FMD increased only in the arm exposed to increased antegrade shear whereas the arm with blunted shear did not change. Utilizing the same technique with higher occlusion pressures to increase retrograde shear during exercise (Johnson et al. 2012; Paiva et al. 2016) and rest (Thijssen et al. 2009c; Schreuder et al. 2014; Totosy de Zepetnek, Jermey, & MacDonald, 2014), a dose-dependent decrease in FMD in both the brachial artery and SFA was observed. Regarding shear magnitude, Atkinson and colleagues (2015a) observed increases in brachial artery FMD with concurrent higher levels of antegrade shear only at the highest intensity of handgrip exercise (15% MVC). Contrary, Birk and colleagues (2013) compared cycling intensities of 50, 70, and 85% and observed higher shear with the higher intensities (70 and 85%) with these two conditions being associated with a decreased brachial artery FMD post-exercise (see Figure 1.15). However, as detailed previously the non-exercised limb is exposed to higher rates of retrograde flow and the oscillatory pattern observed by Birk et al. (2013) may have induced ROS generation thereby acutely decreasing NO bioavailability. Therefore, high-intensity exercise may cause greater impact on eNOS gene expression due to higher levels of shear stress (Hambrecht et al. 2003;

Green et al. 2004) which may eventually lead to superior vascular benefits (Atkinson et al. 2015a; Bond et al. 2015c). However, as detailed previously, the relationship between shear and vaso-activity in children is weak, and a possibility exists that the patterns of shear may not influence FMD as it does in adults.

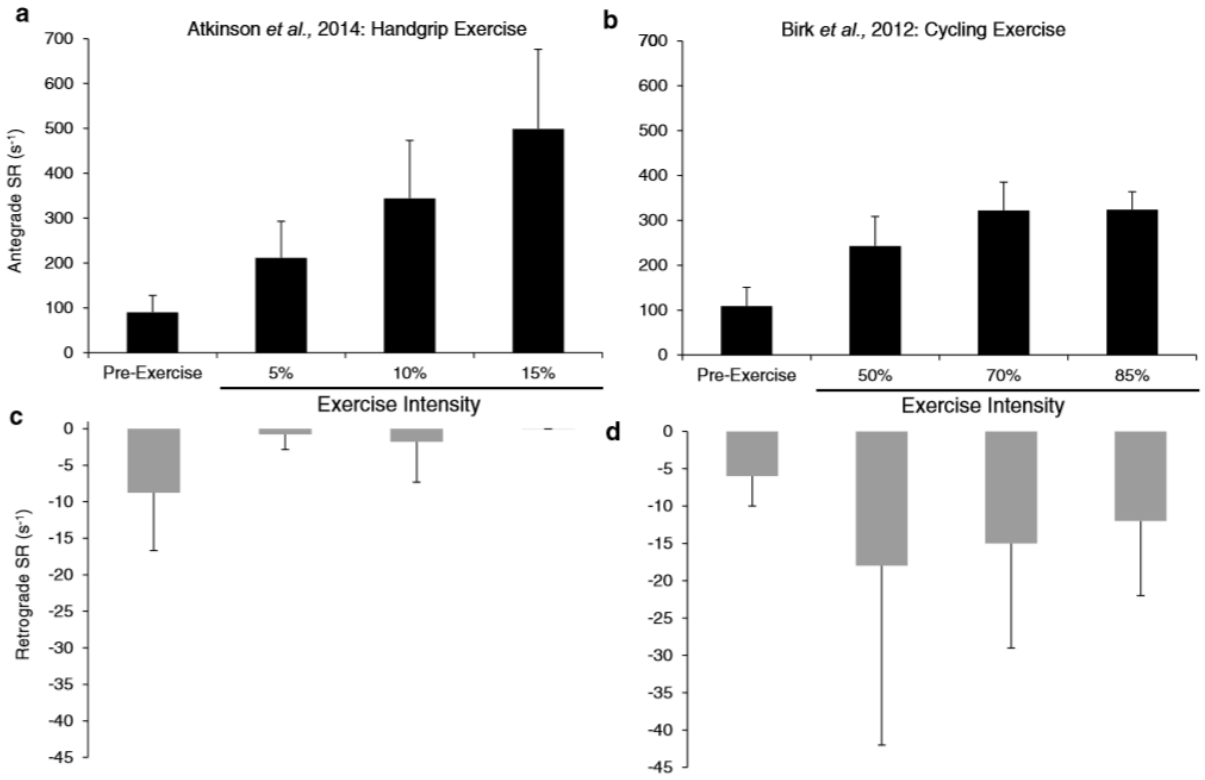



**Figure 1.15 Brachial artery shear patterns during upper and lower body exercise.** Brachial artery antegrade shear rate (*black bars*) increases with increasing work intensities during handgrip exercise (*panel a*) and is much larger compared to cycling exercise (*panel b*). Conversely, brachial artery retrograde shear rate (*grey bars*) diminishes at all exercise intensities during handgrip exercise (*panel c*) and is much lower compared to cycling exercise (*panel d*). Clear differences exist in both the magnitude and directionality of blood flow and shear in response to handgrip and cycle exercise, ultimately impacting the FMD response post-exercise. Reproduced from Atkinson et al. 2015a, ©permission received.

*Blood Pressure*

During exercise, there is an increase in Q accompanied by a linear increase in arterial blood pressure with increasing workloads (Astrand & Rodahl, 1977). With each cardiac cycle endothelial cells are exposed to cyclic strain and intraluminal stretch which increases in magnitude during exercise. Studies involving weight lifting (Jurva et al. 2006), handgrip exercise (Gonzales et al. 2011), and non-exercise methods (Millgard & Lind, 1998) have observed a decrease in endothelial function with concomitant increases in blood pressure. Although cyclic strain increases eNOS transcription in cultured endothelial cells (Awolesci et al. 1994) and isolated arteries (Laughlin, Newcomer, & Bender, 2008), it is also responsible for increased production of ROS (Sorescu et al. 2004) and adhesion molecules (i.e. VCAM-1, ICAM-1, E-selectin, and MCP-1; Wung et al. 1997; Cheng et al. 1998) which can impair vasodilator function. However, recurring bouts of hypertension and cyclic strain during exercise improve the ability of endothelial cells to tolerate the hypertension- induced negative stimuli (Padilla et al. 2011). It is currently unclear how exercise hypertension influences FMD in children, although lower resting and exercise blood pressure (Wanne & Haapoja, 1988) may suggest the negative influence of acute hypertension on FMD may be reduced compared to adults.

## 1.4 Summary and Objectives

Together, the data presented in Section 1.2 support the contention that HIIE may be an ideal exercise strategy for health benefits in children due to the child's unique metabolic characteristics. Children possess superior oxidative and attenuated glycolytic energy contribution at rest and during high-intensity exercise compared to adults, most likely originating from differences in muscle morphology, energy metabolism, local oxygen delivery/utilization, rates of resynthesis of energetic substrates, and enhanced removal/utilization of muscle metabolites. These characteristics lead to high tolerability of HIIE in children and appear to necessitate higher intensity exercise to generate an adaptive response to exercise training. Although cardiorespiratory and metabolic recovery is enhanced in children, the recovery of the peripheral vasculature remains unclear as no studies have

addresses post-exercise recovery of arterial vasodilator function and hemodynamics in children.

In Section 1.3.3 it is shown low- to moderate-intensity exercise in adolescents and adults may lead to initial augmentation in FMD post-exercise is provided, although this may not necessarily represent positive adaptation in vasodilator function. Rather, interval-style exercise at higher intensities (>70% $HR_{max}$/$\dot{V}O_{2peak}$) causes an attenuated FMD immediately post-exercise with subsequent improvements as early as 1 hour following exercise. This intensity-dependent recovery of FMD represents the initiation of an adaptive process to enhance peripheral vasodilator function to deal with repetitive increases in shear stress, obligatory for impending alterations in arterial structure. Section 1.2.2 highlights the importance of HIIE in terms of cardiovascular health benefits, specifically the improvements in $\dot{V}O_{2peak}$ in children with HIIE training and the mechanisms of adaptation, unlike those in adults, seem to be centrally mediated (increased Q, SV) with limited peripheral adaptations possibly due to enhanced oxidative capacity at the muscle (higher proportion of type I muscle fibres, oxidative enzymes, lipid oxidation and glycogen sparing, faster ability to recover from HIIE). Inference from this work may suggest children are well equipped peripherally to deal with the hemodynamic and metabolic stimulus of HIIE and may not require functional, and subsequently structural, adaptations do deal with repetitive increases in shear stress. Also, the mechanisms influencing post-exercise FMD in adults (Section 1.3.4) occur in the peripheral vasculature, and some evidence suggests these mechanistic pathways may differ in children. However, this remains unclear as no research has accurately determined how HIIE acutely influences FMD in children and how this compares to MIE.

Therefore, the objective of this study was to determine the effects of HIIE on vasodilator function in children by comparing the recovery of SFA diameter, shear patterns, and FMD following acute bouts of total external work-matched HIIE and MIE.

# Chapter 2 Effects of High-Intensity Interval Exercise on Vasodilator Function in Children.

## 2.1 Rationale

Exercise-induced improvements in traditional cardiovascular risk factors such as blood pressure or lipid profile, collectively only explain about 50% of the reduction in cardiovascular disease risk (Green et al. 2008). It is now recognized that exercise provides a direct anti-atherogenic effect on vascular health (Green et al. 2017). While there is evidence of improved vascular endothelial function with exercise training in adolescents (Watts et al. 2004; Bond et al. 2015a), little is known about the optimal exercise for vascular benefit in the child.

The response of the vascular endothelium to acute bouts of exercise provides insight into early endothelial adaptations noted with exercise training (Dawson et al. 2013). The rise in blood flow to the active muscle at the onset of exercise increases shear rates, causing endothelium-dependent vasodilation (Tinken et al. 2010). At least in adults and adolescents this response appears to be intensity-dependent, with greater elevations in shear rate (Bond et al. 2015c) and optimized shear patterns in the exercised limb (Atkinson et al. 2015a) following higher intensity exercise. A greater vasodilator response to higher intensity exercise has also been noted in adults (Birk et al. 2013; Atkinson et al. 2015a) although in adolescents the increase in baseline diameter was similar following both moderate- and high-intensity exercise (Bond et al. 2015a). In both adults and adolescents, a bi-phasic intensity dependent response in FMD exists, with decreases in FMD immediately after high- but not moderate-intensity exercise, then subsequent upregulation of FMD one or more hours after the cessation of high-intensity exercise (Birk et al. 2013; Atkinson et al. 2015a; Bond et al. 2015c). Although the mechanisms underlying the temporal response in FMD following high-intensity exercise remain unclear, there is some evidence in adults that sympathetic vasoconstriction may compete with endothelium-dependent vasodilation causing post-exercise reductions in FMD (Atkinson et al. 2015b). Equally, the intense pulsatile shear stimulus induced by higher intensity exercise may result in increased oxidative stress, leading to an immediate reduction in NO bioavailability (Goto et al. 2003), and hence a greater immediate decline in FMD. The increases in FMD in the late phase of recovery from high-intensity exercise may result from

eNOS upregulation (Davies, Spaan, & Krams, 2005), and this is believed to be an important transitory response that mediates longer-term FMD augmentation (Padilla et al. 2011).

Adaptation in vasodilator function to exercise of differing intensities in children is less clear. Work documenting the vasodilation and FMD responses to acute bouts of active video game play of differing intensities has shown a decrease in brachial artery FMD immediately following high, but not low, intensity play in 10-year-olds, but this was not attributable to differences in baseline diameter which remained constant across the conditions (Mills et al. 2013). In contrast, an increase in FMD following supra-maximal exercise has been demonstrated (Chuensiri, Tanaka, & Suksom, 2015). Importantly, the exercise bouts in both studies were not matched for workload, which is critical given that the volume of exercise also influences FMD (Johnson, Padilla, & Wallace, 2012). Furthermore, the time-course of the post-exercise FMD response has not been explored in children, so whether a transient bi-phasic response is also evidence in children following high-intensity exercise is unknown.

The purpose of this study was to compare the time-course of vasodilator responses of the SFA following acute bouts of workload-matched HIIE and MIE in children. Our hypotheses were (i) baseline diameter will be increased following both HIIE and MIE, returning to pre-exercise values 60 minutes post-exercise, (ii) HIIE will result in a decreased FMD immediately after exercise, with a subsequent increases above pre-exercise values 60 minutes following exercise, whereas FMD will be unaltered at all time-points following MIE, (iii) antegrade shear rate will be higher and retrograde shear rate lower following HIIE compared to MIE and (iv) blood flow and hyperaemic shear responses will be higher immediately post HIIE compared to MIE and will return to pre-exercise values 60 minutes post-exercise in MIE but not HIIE.

## 2.2 Methods

Twelve children (age = 7-12 y; 7 girls) were recruited, none of whom were currently training (exercise >10 hours per week), obese (according to child specific cut-offs [Cole et a. 2000]), or had a physical limitations or health condition that would impact vascular function or the ability to exercise (determined via a health history questionnaire completed by parents). This sample size is comparable to related research on the effects of exercise intensity on FMD,

and we estimated that a 2% change in FMD would be detected with 10 participants (assuming a standard deviation of 2%) with statistical power of 80% (Birk et al. 2013). Pubertal status was assessed using parental-assessment which entailed parents indicating pubic hair, genital (boys), and breast (girls) development on a pictorial scale of the Tanner stage (Rasmussen et al. 2015). Written participant assent and parental consent were obtained prior to participation in the study. Experimental procedures were approved by the University of British Columbia clinical research ethical review board.

### 2.2.1 Experimental Design and Procedures

We used a crossover trial with two experimental conditions: HIIE and MIE. Following an unloaded 3-minute warm-up, HIIE consisted of six 1-minute sprint intervals at 90% $W_{max}$ (90% of the peak power attained at the end of the $\dot{V}O_{2max}$ test), each separated by 1-minute of active recovery pedaling at 20% $W_{max}$. Following the same warm-up MIE entailed 15 minutes of cycling at a cadence of 70-90 rpm at 44% $W_{max}$, which was total external work-matched to HIIE. External work-matching was achieved using the square wave method. In brief, this entails summing the workload for the HIIE sprint and rest intervals (90%, 20%, 90%, 20%, etc.), then dividing the 15-minute exercise time for MIE by the total HIIE workload to give the MIE workload (44%). The order of completion was randomized and the conditions were completed on two separate days, separated by at least 48 hours of recovery.

Prior to the experimental trials the children attended the laboratory for familiarization and to complete anthropometric measurements and a $\dot{V}O_{2max}$ test. The children returned to the laboratory at the same time of day (either morning or afternoon) on two separate occasions to complete the two experimental conditions and were asked to abstain from vigorous exercise and caffeine for 24 hours prior to each visit and not to eat at least 2 hours before the visits. Parents were asked to provide identical food on testing days and avoid foods containing fat and vitamin C, which may acutely effect endothelial function (Vogel, Corretti, & Plotnick, 1997; Taddei et al. 1998).

During the two experimental visits participants completed pre-exercise FMD in the semi-supine position followed by the HIIE or MIE. FMD was measured immediately after (<10 minutes) and 60 minutes following exercise in the semi-supine position.

### 2.2.2 Experimental Measures

#### Body Composition

Stature and body mass were measured to the nearest 0.1 cm and 0.1 kg using a stadiometer (Seca 217) and electronic scale (Tanita TBF-410), respectively. Body mass index (BMI) was calculated as $kg/m^2$.

#### Blood Pressure and Heart Rate

Systolic (SBP) and diastolic blood pressure (DBP) were measured twice using a manual sphygmomanometer (Prestige Medical 79-BLK standard aneroid, Northridge CA) to the nearest mmHg on the right arm prior to each FMD measurement (pre, post, and 60 minutes post) and at the end of the exercise bouts. Mean arterial pressure (MAP) was calculated as (1/3) × SBP + (2/3) × DBP. Heart rate (HR) was measured continuously at rest and during exercise using HR telemetry (Polar Vantage NV, Polar Electro Oy).

#### Maximal Oxygen Uptake

Participants performed a ramp maximal test with supramaximal verification to determine power output at $\dot{V}O_{2max}$. The test began with 3 minutes of unloaded pedaling followed by a workload increase depending on the stature of the child (<110cm 5 $W \cdot min^{-1}$, 110-125cm 10 $W \cdot min^{-1}$, 125-150cm 15 $W \cdot min^{-1}$, >150cm 20 $W \cdot min^{-1}$). A pedal cadence between 70-80 rpm was maintained throughout the test and continued until volitional exhaustion, which was defined as a drop in cadence below 60 rpm for 5 consecutive seconds. At the end of the test the children pedaled unloaded for 5 minutes, then rested for 10 minutes before the supramaximal verification of maximum. The supramaximal test began with a 2-minute warm-up followed by a 'step' transition to 105% of the $W_{max}$ achieved during the ramp test. This intensity was maintained until exhaustion at which point a 3-minute unloaded cool-down was completed.

Respiratory gases and ventilation were assessed breath-by-breath using an online gas analyzer (Oxycon Pro, Carefusion). Data were subsequently time-aligned to second-by-second and processed using 15 second epochs. $\dot{V}O_{2max}$ was defined as less than 5% difference in the

slope of increase in $\dot{V}O_2$ between the maximal and supramaximal tests (Barker, Williams, & Armstrong, 2011).

**Vasodilator Function**

Superficial femoral artery (SFA) blood flow, diameter, and FMD were assessed via high-resolution Doppler with B-mode images with a 15 MHz Broadband Multi Frequency Linear Array Transducer (Terason USMART3300$^{TM}$; Teratech, Burlington, MA, USA). Following 15 minutes of supine rest a blood pressure cuff controlled with a fast deflating aneroid (Hokanson SC5, Bellevue, WA) was placed ~5 cm above the knee, distal to the ultrasound probe. Once optimal imaging of the lumen-arterial wall interface was obtained, baseline SFA diameter, blood flow, and shear rates (mean, antegrade, retrograde) were continuously measured for 1 minute prior to cuff inflation. The cuff was inflated to 50 mmHg above resting systolic blood pressure for 5 minutes, with measurement resuming 30 seconds prior to cuff deflation and continuing for 5 minutes post cuff deflation. Probe placement was measured from the medial epicondyle of the femur to ensure similar measurements across experimental conditions.

Semi-automated edge-detection and wall-tracking software, which is largely independent of investigator bias was used. This software provides continuous and simultaneous measurement of diameter and velocity, blood flow (lumen cross-sectional area and Doppler velocity [$v$]; [4*velocity cm.s$^{-1}$]/diameter cm) and shear rate; as well as post hoc calculation of FMD. Velocity and flow were calculated from the Doppler envelope. Antegrade and retrograde blood flow and shear rates were calculated from antegrade and retrograde area under the curve data that were subsequently averaged from positive or negative data points respectively (Thijssen et al. 2011a). OSI was calculated as an indicator of the magnitude of shear oscillation, which is associated with endothelial dysfunction in adults (Newcomer et al. 2008).

$$OSI = |\text{retrograde shear}|/(|\text{antegrade shear}|+|\text{retrograde shear}|)$$

FMD was not corrected for $SR_{AUC}$, in agreement with previous recommendations (Atkinson et al. 2009), but was adjusted for baseline diameter using a log-linear approach

(Atkinson & Batterham, 2013). A single researcher conducted all FMD measurements and analysis and was blinded to the study codes and participant files. The between-trial coefficients of variation for FMD (%) and baseline diameter were 8.2% and 3.0%, respectively.

### 2.2.3 Statistical Analyses

All data are presented as means ± SD. Within (pre, post, post60) and between (HIIE, MIE) condition main effects for baseline diameter, flow, shear rates, and uncorrected SFA FMD (%) were examined using repeated measures analyses of variance (RM ANOVA). Where necessary, main effects were deconstructed using *t*-tests with Bonferroni correction. A log-linear mixed model, with baseline diameter as the time-varying covariate, was used to examine the effect of exercise intensity (HIIE, MIE) on FMD during recovery (pre, post, post60). Mean differences were back-transformed to the original units of FMD (%), providing corrected SFA FMD (%). Mean differences in heart rate between the HIIE and MIE trials were assessed using independent samples *t*-test. Statistical significance was set at $P \leq 0.05$. Statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, IL, USA).

## 2.3 Results

Several participants were excluded from the final analysis. Two did not present to each visit in the same prandial state, and one child did not achieve $\dot{V}O_{2max}$. Of our remaining cohort (n = 9), the mean age was 10.5 ± 1.5 years-old (range = 7.2 – 11.8 y). Seven were pre-pubertal (Tanner stage 1 for breast or genital and pubic hair) and 2 were early-pubertal (2 girls, Tanner stage 2 for breast and pubic hair development). Mean BMI was 17.1 ± 2.3 $kg \cdot m^2$ (range = 13.8 – 21.9 $kg \cdot m^2$), with one overweight participant (Cole et al. 2000). Mean body mass and stature were 36.1 ± 8.2 kg and 144.3 ± 10.2 cm respectively. Cardiorespiratory data at rest are presented in Table 2.1. $\dot{V}O_{2max}$ values were as expected for the age group (1.65 ± 0.22 $L \cdot min^{-1}$, range = 1.40 – 2.10 $L \cdot min^{-1}$).

**Table 2.1** Cardiorespiratory data at rest.

| | Mean ± SD | Range |
|---|---|---|
| Resting HR (beats·min$^{-1}$) | 85 ± 8 | 73 – 97 |
| SBP (mmHg) | 105 ± 8 | 92 – 116 |
| DBP (mmHg) | 73 ± 8 | 58 – 80 |

n = 9. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure

Physiological responses during the two exercise conditions are presented in Table 2.2. There was no difference in the total external work completed between the exercise trials. Mean HR during interval sprints was 164 ± 11 bpm, increasing from 145 bpm during sprint 1 to 175 bpm during sprint 6. Mean HR (% $HR_{max}$) during HIIE (including interval and recovery periods) was higher compared to MIE ($t(16) = 4.211$, $P < 0.01$). HR immediately post-exercise was elevated in both HIIE and MIE, but higher in HIIE ($t(16) = 2.446$, $P < 0.05$); however, values returned to pre-exercise levels 60 minutes after exercise in both trials.

**Table 2.2** Physiological responses during HIIE and MIE.

| | HIIE (n = 9) | MIE (n = 9) |
|---|---|---|
| Mean work rate (W) | 109 ± 22* | 53 ± 11 |
| Total external work completed (AU) | 796 ± 163 | 796 ± 163 |
| Mean HR (% $HR_{max}$) | 78 ± 5* | 70 ± 4 |
| HR post (beats·min$^{-1}$) | 99 ± 10* | 88 ± 9 |
| HR post60 (beats·min$^{-1}$) | 79 ± 7 | 75 ± 7 |

AU, arbitrary units; HR, heart rate.

Data are mean ± SD. *Significant difference between HIIE and MIE, $P < 0.05$.

There were no significant differences in baseline diameter or FMD (%) prior to the HIIE or MIE conditions between the children starting with the HIIE condition or those starting with the MIE condition, verifying that the washout period between trials was sufficient to eliminate any possible carry-over effects.

### 2.3.1 Vasodilator, Blood Flow, and FMD Response

SFA diameters at baseline and with reactive hyperaemia prior to and following the HIIE and MIE trials are presented in Table 2.3. Baseline diameter increased to a similar extent following both HIIE and MIE ($F(1.318,21.089) = 27.108$, $P < 0.001$; $\eta = 0.629$), returning to pre-exercise values 60 minutes after. Delta change in baseline diameter from Pre-to-Post and Pre-to-Post60 are presented in Figure 2.1, panel 2.

SFA FMD (%) decreased following both HIIE and MIE ($F(2,32) = 8.145$, $P < 0.01$; $\eta = 0.337$), returning to pre-exercise values 60 minutes post-exercise (see Table 2.3). Delta change in FMD (%) Pre-to-Post and Pre-to-Post60 are shown in Figure 2.1, panel A. When FMD (%) was corrected for changes in baseline diameter, there was no longer a decrease post-exercise ($P = 0.34$, see Table 2.3).

Baseline SFA blood flow increased following both HIIE and MIE ($F(1.172,18.749) = 89.172$, $P < 0.001$; $\eta = 0.848$). Flow remained elevated 60 minutes after both HIIE and MIE (see Table 2.3). Under hyperemic conditions SFA blood flow increased post HIIE and MIE ($F(1.282,20.510) = 46.695$, $P < 0.001$; $\eta = 0.745$), remaining elevated 60 minutes after the two exercise conditions (see Table 2.3).

**Table 2.3** Baseline and reactive hyperemic responses of the superficial femoral artery before (Pre), after (Post) and 60 minutes after (Post60) HIIE and MIE.

| | | HIIE (n = 9) | | | MIE (n = 9) | | |
|---|---|---|---|---|---|---|---|
| | | Pre | Post | Post60 | Pre | Post | Post60 |
| Baseline | Diameter (mm) | 4.25 ± 0.42 | 4.76 ± 0.58† | 4.25 ± 0.36 | 4.29 ± 0.49 | 4.61 ± 0.67† | 4.28 ± 0.53 |
| | Flow ($ml \cdot min^{-1}$) | 138.8 ± 34.5 | 512.3 ± 163.9† | 221.6 ± 51.2† | 157.4 ± 46.5 | 384.2 ± 139.9† | 218.7 ± 88.0† |
| | OSI (AU) | 0.12 ± 0.07 | 0.00 ± 0.00† | 0.03 ± 0.02† | 0.10 ± 0.06 | 0.01 ± 0.01† | 0.03 ± 0.03† |
| Reactive hyperemia | Peak diameter (mm) | 4.60 ± 0.45 | 5.03 ± 0.51† | 4.62 ± 0.37 | 4.65 ± 0.53 | 4.84 ± 0.57† | 4.67 ± 0.57 |
| | FMD (%) | 8.42 ± 2.10 | 5.90 ± 3.25† | 8.82 ± 1.87 | 8.24 ± 2.34 | 5.43 ± 4.78† | 8.85 ± 2.46 |
| | Corrected FMD (%) | 7.68 ± 2.74 | 7.57 ± 2.74 | 8.00 ± 2.74 | 7.68 ± 2.74 | 6.29 ± 2.74 | 8.22 ± 2.74 |
| | $SR_{AUC}$ ($ml \cdot min^{-1}$) | 32511 ± 11027 | 51524 ± 15486† | 39494 ± 9541† | 33206 ± 7609 | 53880 ± 9901† | 38291 ± 9964† |
| | $Flow_{AUC}$ ($ml \cdot min^{-1}$) | 525 ± 127 | 1075 ± 224† | 648 ± 130† | 561 ± 159 | 1045 ± 353† | 632 ± 131† |

FMD, flow-mediated dilation; SR, shear rate; $_{AUC,}$ area under curve; AU, arbitrary units.

Data are mean ± SD. *P* values were obtained from repeated measures ANOVA. *Significant difference between HIIE and MIE, $P < 0.05$. †Significant difference compared to Pre, $P < 0.05$.

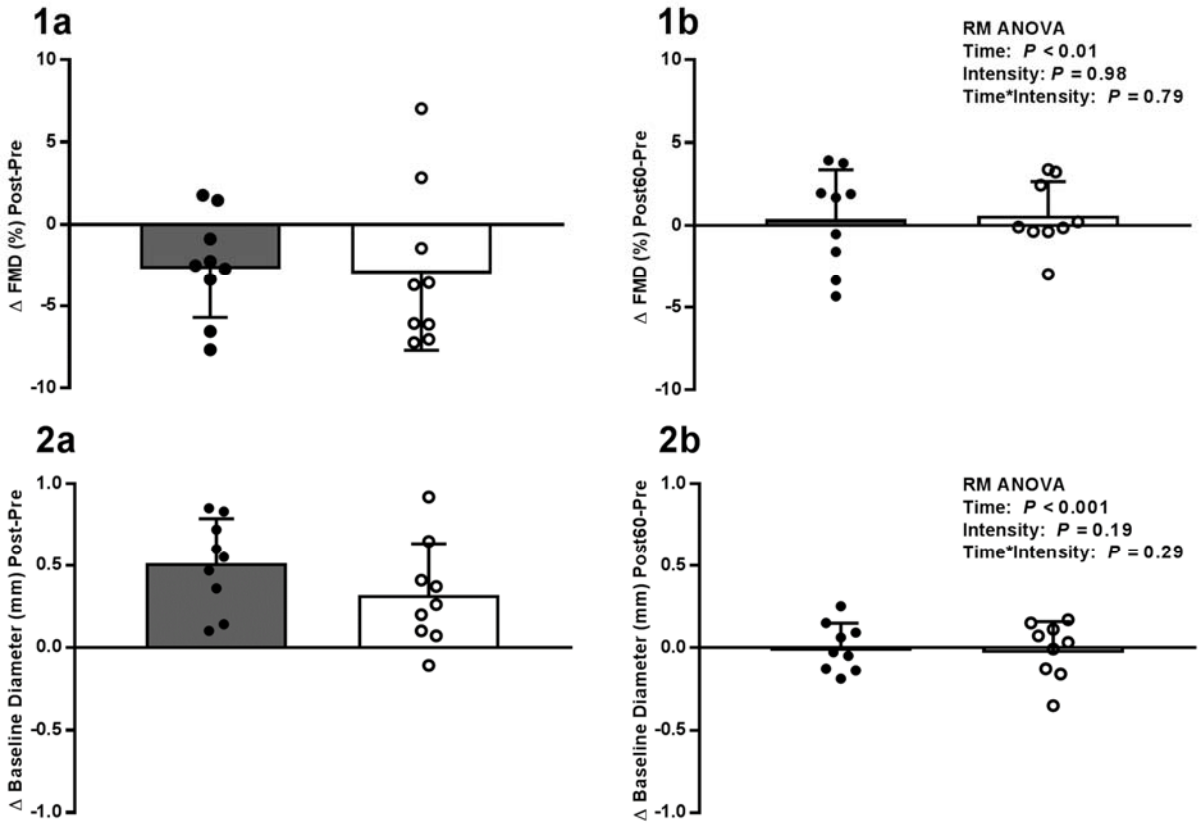



**Figure 2.1** Delta change in FMD (*panel 1*) and baseline diameter (*panel 2*) from pre-exercise to post-exercise (a) and from pre-exercise to 60 minutes post-exercise (b) following HIIE (*grey bars*) and MIE (*white bars*). Data are mean ± SD. Dots represent individual data points.

### 2.3.2 Shear Rates

Baseline mean, antegrade, and retrograde shear rates are presented in Figure 2.2. Mean shear rate increased following both HIIE and MIE ($F(2,32) = 190.101$, $P < 0.001$; $\eta = 0.922$), but to a greater extent after HIIE ($F(2,32) = 7.941$, $P < 0.01$; $\eta = 0.332$). Likewise, antegrade shear rate increased following HIIE and MIE ($F(2,32) = 151.601$, $P < 0.001$; $\eta = 0.905$), with a greater increase after HIIE ($F(2,32) = 7.310$, $P < 0.01$; $\eta = 0.314$). Mean and antegrade shear rates remained augmented 60 minutes after HIIE and MIE compared to pre-exercise values (see Figure 2.2). Retrograde shear was attenuated to a similar extent following both HIIE and MIE ($F(1.319,21.109) = 35.998$, $P < 0.001$; $\eta = 0.692$) and remained so 60 minutes post-exercise (see Figure 2.2). Oscillatory shear rate was reduced post-exercise ($F(1.253,20.040) =$

41.688, $P < 0.001$; $\eta = 0.723$), remaining lower than the pre-exercise values for both HIIE and MIE 60 minutes post exercise (see Table 2.3).

Hyperemic $SR_{AUC}$ was elevated following both HIIE and MIE ($F(1.410,22.568) = 30.122$, $P < 0.001$; $\eta = 0.653$), remaining elevated above pre-exercise values 60 minutes post-exercise in both conditions (see Table 2.3).

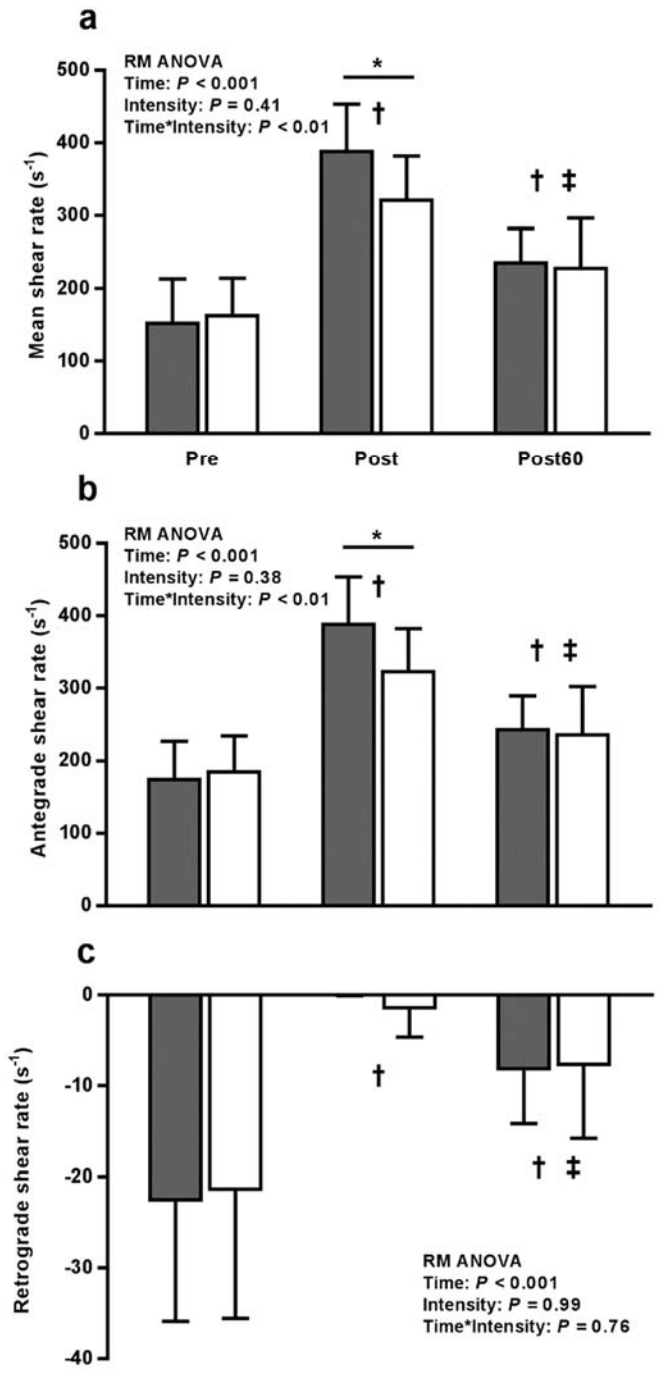



**Figure 2.2** Mean shear rate (*panel a*), antegrade (*panel b*), and retrograde (*panel c*) shear before (Pre), after (Post) and 60 minutes after (Post60) HIIE (*grey bars*) and MIE (*white bars*). Data are mean ± SD. *Significant difference between HIIE and MIE, $P < 0.05$. †Significant difference from pre-exercise, $P < 0.001$. ‡Significant difference between Post and Post60, $P < 0.001$.

## 2.4 Discussion

These findings demonstrate that blood flow and antegrade shear rate were increased following both HIIE and MIE, with a concomitant increase in the diameter of the SFA. Interestingly, the increases in flow and antegrade shear rate at baseline were greater following HIIE and while the increase in dilation of the SFA was similar following both exercise conditions, this was reversed 60 minutes after exercise, whereas blood flow and shear rates remained elevated an hour after exercise. Contrary to our hypothesis, SFA FMD (%) decreased after both HIIE and MIE, returning to pre-exercise values within one hour. This post exercise reduction was negligible after correcting FMD for the increase in SFA baseline diameter.

The post-exercise SFA vasodilation noted in this study is likely mediated by the increase in antegrade shear rate following the increase in blood flow in the exercising limb (Green et al. 2005). The changes in shear patterns we note are typical of the exercised limb (Atkinson et al. 2015a) and would, at least in adults, be expected to augment FMD 60 minutes following exercise at a higher intensity, due to decreases in retrograde shear leading to an improved oscillatory shear pattern and upregulation of eNOS (Davies, Spaan, & Krams, 2005; Green et al. 2005). The lack of an augmented FMD, despite optimised oscillatory shear patterns an hour after exercise corroborates previous findings that factors regulating FMD may differ in children compared to adults (Thijssen et al. 2009a).

This study was intended to extend earlier findings in children (Mills et al. 2013; Chuensiri, Tanaka, & Suksom, 2015) by observing the temporal vasodilatory and FMD response, as well as appropriately scaling FMD for baseline diameter at the various time-points. Uncorrected FMD can lead to erroneous conclusions, given that baseline diameter has an inverse relationship with FMD (Thijssen et al. 2008). We demonstrate that the post-exercise nadir in FMD observed after HIIE and MIE was negligible once FMD was corrected for baseline diameter. In contrast, post-exercise attenuation in FMD typically noted in adolescents and adults remains even when FMD has been adjusted for increases in baseline diameter (Birk et al. 2013; Katayama et al. 2013; Bond et al. 2015c). It is worth noting methodological differences between our study and prior studies. We assessed vasodilator function in the exercising limb, while the adult and adolescent studies report adaptations in the non-exercised

limb and the impacts on baseline diameter may not be as large. Vessels in the non-exercised limb experience an increase in retrograde shear (Green et al. 2005; Padilla et al. 2011) which can increase production of ROS (Hwang et al. 2003) and decrease eNOS (Ziegler et al. 1998), causing a decrease in NO bioavailability. In contrast, we show cycling exercise, irrespective of intensity, caused a reduction in retrograde shear in the SFA.

Exercise-induced oxidative stress has been shown to be attenuated in children compared to adults due to a blunted cytokine and immune response (Timmons, Tarnopolsky, & Bar-Or, 2004). Moreover, considering the negative relationship between exercise hypertension and FMD (Gonzales et al. 2011), the lower hypertensive response during exercise noted in children (Wanne & Haapoja, 1988) could diminish any strain-mediated ROS generation. Exercise also produces large increases in SNA in the exercising muscle (DiCarlo, Chen, & Collin, 1996), which impairs vasodilator function in adults due to competition between SNA-mediated vasoconstriction and endothelium-dependent vasodilation (Atkinson et al. 2015b). Pre-pubertal children possess a greater proportion of type I muscle fibres compared to adolescents and adults (Oertel, 1988) and have a lower density of vascular α-adrenergic receptors and a smaller adrenergic response to exercise compared to adults (Timmons, Tarnopolsky, & Bar-Or, 2004; Rubin et al. 2014). These characteristics lead to a smaller response to norepinephrine, lesser adrenergic vasoconstrictor tone, and greater vasodilatory influence compared to glycolytic type II fibres (Behnke, Armstrong, & Delp, 2011). Furthermore, the larger contribution of oxidative phosphorylation during exercise of higher intensities in children attenuates peripheral metabolic perturbations (Barker et al. 2010), which can impact upon plasma anti- and pro-oxidant status (Ghiselli et al. 2000), and lead to lower muscle lactate production, a blunted catecholamine response and lower efferent sympathetic excitation (Tolfrey & Armstrong, 1995; Amano et al. 2017). This may explain the maintenance of SFA blood flow 60 minutes following exercise due to a lack of vascular constrictive tone, leading to a reduction in peripheral vascular resistance through the maintenance of vasodilation in the distal arterioles (Behnke, Armstrong, & Delp, 2011).

Another possibility for the initial decline in uncorrected FMD is the larger cardiovascular load during cycling exercise. Exercise with a lower cardiovascular load (<100 beats·min$^{-1}$) is associated with minimal effect on FMD (Tinken et al. 2010; Atkinson et al.

2015a), regardless of the vessel imaged, while initial attenuation in FMD in healthy individuals is typically reported following exercise at intensities at or above 70% $HR_{max}$ (Birk et al. 2013; Atkinson et al. 2015b; Bond et al. 2015c). Our exercise conditions elicited mean intensities of 78 and 70% $HR_{max}$ for HIIE and MIE respectively, so it is possible that the initial decrement in uncorrected FMD could be attributed to these cardiovascular loads. It is important to note that the average heart rate of 78% $HR_{max}$ during the 1-minute HIIE protocol may not be considered high-intensity, suggesting the children did not work hard enough. Also, heart rate during HIIE increased from sprint 1 to sprint 6 (145 to 175 bpm) and ranged from 134-154 bpm during the 1-minute recovery periods. Therefore, considering the need of higher intensities to cause adaptation in children (>85% $HR_{max}$ for improving $\dot{V}O_{2max}$; Armstrong & Barker, 2011), the lack of a sustained high cardiovascular load may be a reason for the absence of an upregulated FMD at 60 minutes post-exercise. More sprint repetitions or shorter, higher intensity bouts (i.e. repeated Wingates) may be necessary to elicit the bi-phasic response noted in adolescents and adults.

**Methodological Considerations**

The strengths of the current study include utilizing external work-matched exercise trials, assessing the time-course of vasodilation, FMD, and shear rate recovery from exercise, and appropriate scaling for changes in baseline diameter across the multiple time points. This research is also strengthened by utilizing semi-automated edge-detection and wall-tracking software to determine FMD and hemodynamic responses, and rigorously following international guidelines for measurement (Thijssen et al. 2011a). Nonetheless, some limitations still exist. Unfortunately, we were unable to measure SFA diameter and shear patterns during exercise due to the technical difficulties obtaining this measure while cycling. Furthermore, we did not assess endothelial-independent vasodilation via nitroglycerine, therefore our results reflect global vascular change and cannot be ascribed definitively to the endothelium or smooth muscle cells. We did not take measures in the fasted state as typically done in adults; however, we did track consistency of foods across the conditions and the avoidance of foods that could influence vascular function. Last, with the loss of three participants from the final data analyses, the study is likely under-powered; however, this is unlikely to change the main findings from this study.

In conclusion, external work-matched exercise, irrespective of intensity, exerts a similar impact on artery diameter and FMD in children. The lack of an increase 60 minutes after the cessation of HIIE in FMD in children suggests the mechanisms which govern the FMD response to acute exercise are likely developmentally divergent.

## Chapter 3 Conclusion

The response of the endothelium after exercise demonstrates the unique nature of vascular adaptation to exercise where, at least in adults and adolescents, the diminished FMD following high-intensity exercise reflects temporary vascular impairment which initiates the transitory response leading to latent improvements. The paradoxical nature of this response is intriguing and, the present study too demonstrates a decrease in FMD following exercise. However, this was not followed by the transitory increase one hour later and when corrected for changes in arterial diameter, the decrement in FMD post-exercise is negligible. These disparate findings may result from our study measuring the exercised limb and/or inherent maturational differences in the vascular response to exercise.

Considering the first option of arterial measurement differences, large increases in shear rate in the exercised limb (Green et al. 2005) may lead to a substantial vasodilatory effect on the SFA which could encroach on maximum vessel diameter, not allowing further vasodilation to occur (Gori et al. 2010), thereby limiting FMD. This unequivocally supports the necessity to accurately account for any changes in vessel diameter that may occur following exercise. Conversely, reduction in FMD observed in the children tested in this study may also reflect inherent age or maturational differences in the adaptive process of vasodilator function. FMD and shear have been shown to be uncoupled in children. Firstly, $SR_{AUC}$ is the main stimulus for FMD, however the relationship between $SR_{AUC}$ and FMD is weak in children (Thijssen et al. 2009a). Thus, it was unsurprising that increases in $SR_{AUC}$ 60 minutes following exercise did not lead to a larger FMD. Secondly, in adults, sitting induced decrements in FMD are mediated by a blunted shear rate (Restaino et al. 2016) whereas sitting-induced FMD reductions in children are not shear mediated (McManus et al. 2015). These observations highlight the inherent differences in shear-dependent vaso-activity between children and adults. Based on our observations of enhanced shear profiles throughout the recovery period (increased antegrade shear, decreased retrograde shear), the influence of shear-dependent mechanisms on vascular function may differ in children. This could be due to smaller vessel diameters and higher resting heart rates in children, resulting in continuous high levels of shear at rest and during exercise. The possibility exists that endothelial cells may have a threshold dependent response on absolute shear levels rather than relative changes. Therefore, higher

shear rates may cause inherent endothelial cell desensitization, leading to a chronically overstimulated endothelium which can influence vasoactive responses (Gori et al. 2010). However, we unaware of any research directly manipulating shear rate and patterns while determining how this influences FMD in children, so this hypothesis remains speculative.

The mechanisms underlying exercise induced changes in vascular function in healthy children are unclear. It is possible that in children measurable vascular adaptations may only be observed in those with endothelial dysfunction (Green et al. 2004). There is no evidence in healthy children to show FMD increases with longer-term exercise training, therefore we are unaware if FMD can actually be optimized in the healthy child population. Although speculative, our results could suggest healthy children do not require normalization of shear stress on the vessel wall and therefore do not display short-term adaptations in peripheral vascular function which would eventually lead to structural remodelling. This theory is deduced from other experimental models which show that children's superior oxidative and attenuated glycolytic energy contribution during exercise limits peripheral adaptations whereas adults improve markers of peripheral oxidative function following 1 bout of exercise. The ability of children to better match oxygen delivery to oxygen demand may mean there is less need to improve local blood flow to the working muscles, thereby limiting peripheral adaptations which may improve local oxygen delivery and uptake (i.e. enhanced vasodilation, structural remodelling). However, Hopkins and colleagues (2009, 2011) have reported high-intensity physical activity is associated with higher FMD in children and recommend higher intensity activity should be prioritized when the goal is improvements in vascular health outcomes (Hopkins et al. 2011). Although correlational, *regular* exercise at higher intensities may lead to improved vascular health, although a HIIE training study is needed to confirm this. It is important to consider therefore that definitive statements on the type of exercise strategy for optimal vascular health during childhood are not evidence-based.

### 3.1 Methodological Limitations

Our cohort of children was healthy and relatively fit with higher endothelial function *a priori* (FMD = ~9%). This limits the generalizability of these findings to children with impaired endothelial function due to disease such as obesity. Furthermore, participants were a

sample of volunteers who most likely enjoy exercise, biasing recruitment to those who regularly participate in physical activity. We lacked metabolic measures during the two exercise conditions or $\dot{V}O_2$ during recovery, which could have better explained the recovery process following exercise. In particular, understanding EPOC would provide insight into the energetic contribution and substrate utilization for the two exercise conditions. That said, EPOC and substrate utilization measures post-HIIE are limited because of $CO_2$ retention and bicarbonate buffering which decreases RER, and may provide unreliable conclusions. Furthermore, we were unable to measure FMD beyond 60 minutes of exercise and it is possible that we missed latent improvements since protein expression and phosphorylation of eNOS can take a few hours (Fisslthaler et al. 2000).

The difficulty of enforcing 6+ hours of fasting on children means the children in our study had eaten up to 2 hours prior to the start of the protocol. This may affect the measures we observed; however, we were diligent in asking parents to restrict foods that could impact endothelial function and removed 2 subjects who did not present to each visit in the same prandial state, which noticeably impacted FMD. We also matched the foods and time of intake between days so we were certain both days were identical in the timeframe and substance of meals eaten.

The HIIE protocol used in this thesis did not produce a large cardiovascular load, suggesting the children did not work hard enough during the sprint exercise and questions the reliability of calling it 'high-intensity'. It would be interesting to investigate different types of HIIE protocols (e.g., repeated Wingates, Tabatas, resistance) to ensure high-intensity efforts to differentially stress oxidative and glycolytic pathways and investigate if alterations in the protocol results in distinct adaptations.

## 3.2 Future Directions

The evidence provided by this thesis generates questions which should be a priority to fully understand the adaptive mechanisms underlying the impact of exercise on vasodilator function in childhood. This puts the onus on researchers to design experiments and utilise techniques to adequately address these questions. Vascular adaptations in the exercised limb is an area worthy of continued research, specifically how the lower limbs adapt and how this

differs from the upper body in children. It is largely unknown how SFA function acutely adapts to lower body exercise in adults, and this information could help us understand if our results are specific to children or the limb measured. In children, it is important to understand how shear rate during exercise influences exercise vasodilation and if there is a dose-dependent response in shear and vasodilation like there is in adults (Carter et al. 2013). This can be accomplished using handgrip exercise at varying intensities with partial occlusion to blunt the shear increase while measuring brachial artery dilation. Similarly, using cuff occlusion at rest and during exercise to either blunt shear or manipulate shear patterns (i.e. increase retrograde shear) can provide evidence on how shear stress impacts FMD. This model can also be used during a longer-term training study to observe functional and structural adaptations and how blunted/altered shear impacts these. This work has been completed in adults which therefore offers interesting developmental comparisons (Tinken etl. 2008, 2010). Furthermore, administering antioxidants (i.e. vitamin C) during acute exercise can be used to delineate the influence of oxidative stress on FMD, while other investigations will need to utilize innovative designs to manipulate SNA. Completing these studies in both lean and obese children can directly inform us of the adaptive responses and how these might differs depending on health status. This would be a large undertaking, but the knowledge provided would be invaluable.

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# Appendices

## Appendix A Participant Consent Form

a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

Participant Consent for:

**THE IMPACT OF EXERCISE OF DIFFERING INTENSITIES ON VASCULAR FUNCTION IN CHILDREN AND ADULTS.**

(Exercise and blood vessel health in children and adults)

**Principal Investigator:** Dr.Ali McManus, Ph.D.

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Victoria Armstrong, MSc student- School of Health & Exercise Sciences, UBC

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**Investigation Site:** Pediatric Exercise and Inactivity Physiology Laboratory, Centre for Health, Lung and Blood vessel Health, ART 183, University of British Columbia, Okanagan Campus

**Sponsors:** National Science and Engineering Research Council Discovery Grant (F14-04841)

**Emergency Telephone (24 hrs/day):** 250-864-3513 (Ali McManus)

**If you are a parent or legal guardian of a child who may take part in this study, permission from you and the assent (agreement) of your child is required. When we say "you" or "your" in this consent form, we mean you and/or your child; "we" means the researchers and other staff involved in the project.**

**Invitation**

Thank-you for your interest in this research study! You are being invited to participate in this study because you are a healthy boyor girl between the ages of 8-10 years or a healthy male or female between the ages of 18-30 years.

By participating in this study, your data will help us understand how exercise improves the blood vessels in our body. This project will include 40 participants in total (20 children and 20 adults).

Please take time to read through this document carefully, and discuss any questions you may have with the investigator, your family, your doctor, or any others before you decide to participate in this study.

**Your participation is voluntary**

Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research

involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.
If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you do not have to provide any reason for your decision not to participate nor will you lose the benefit of any medical care to which you are entitled or are presently receiving.

Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

**Who is conducting the study?**
This study is being conducted by a team of researchers from the University of British Columbia in the Okanagan. This team specialises in studying child health and blood vesselfunction.

**Background of the study**
As we age blood vessel function steadily declines which poses future cardiovascular risk(i.e. heart disease). This blood vessel impairment can be observed in children with no symptoms, which can be indicative of the beginning of atherosclerosis(i.e. disease of the blood vessels which causes them to become blocked).Exercise provides acute improvements in blood vessel function which when measured, gives us information on how the individual exercise bouts may lead to long term adaptation and therefore enhanced blood vessel health.
Although we understand the effects of exercise on blood vessel function in adults, there is limited information in healthy children. We do not know if children demonstrate the same adaptive response to exercise that adults do, and this information can give us valuable information for interventions aimed at improving blood vessel health in children with disease.Also, there is no research documenting the effects of exercise in the legs or brain blood vessels of children, which can impact future intervention for improving health outcomes. This will be the first study to investigate the acute effects of exercise on children's blood vessel

healthin the legs and brain and determine the time at which these adaptations take place over two exercise bouts. Details of this study are presented below.

**What is the purpose of the study?**

The purpose of this study istwo-fold: 1) To determine the acute changes in blood vessel function in the legs and brain following exercise in children and adults; and 2) To explore the time-course for adaptation in the leg and brain.

We will collect a range of non-invasive physiological data including heart rate, blood pressure, blood vessel dilation, and blood flow velocity. The results of this investigation will assist us in determining how the child's blood vessels adapt to exercise and whether this differs from adults.

All results will be kept confidential.

**Who can participate in this study?**

Healthy human volunteers from the local community can participate in this study if

- You are a boy or girlaged between 8-10 years or a male or female aged between 18-30 years.
- All participants must have a health history clear of cardiorespiratory and cerebrovascular diseases and must not be taking any form of medication.
- Are comfortable communicating in English.

**Who should not participate in the study?**

You will not be eligible to participate in this study if:

- unable to exercise on a stationary exercise bike
- have congenital abnormalities (i.e. tetralogy of Fallot)
- have cardiovascular disease (i.e. atherosclerosis, coronary heart disease)
- have known respiratory disease (i.e. asthma)
- are active smokers
- have known high blood pressure
- cannot understand English.

Participants will not be excluded from participation on the basis of weight status however, we will exclude obese individuals from the final data analyses. Obese adults are classified as those with a BMI at or above 30. Obese children are classified as having a BMI 2 standard deviation above the WHO growth standard median for BMI. Data from children and adults who are currently training for sport or competition (>10 hours/week) will also not be included in the final analyses.

**What does the study involve?**

All studies will be conducted in the Pediatric Exercise and Inactivity Laboratory at the University of British Columbia (ART Building, Room 183), Kelowna, British Columbia, Canada. Forty volunteers will be enrolled in this study.

Overview of the Study:

If you decide to participate, you will be asked to come into the laboratoryin sports clothes (t-shirt, shorts and running shoes). Your participation in this study will involve 3 visits to the laboratory, which will include orientation and testing. The total time commitment for this study will be approximately 8 hours over the 3 visits (1-2 weeks).

The specific details and procedures of the laboratory visits are outlined below.

*Measured Variables of the Study:*

Blood vessel dilation: You will rest on a bed for 10 minutes followed by placing a blood pressure cuff around your thigh above your knee. A Doppler ultrasound will be used to image your superficial femoral artery. The cuff will inflate for 5 minutes to temporarily limit blood flow to your lower leg. The cuff will then deflate while measurements continue for 5 more minutes. Doppler ultrasound systems are a safe, non-invasive measurement technique.

Transcranial Doppler ultrasound: The speed of blood flow (velocity) in the cerebral arteries will be measured by small ultrasound probes attached to a small helmet, which sits on your head.

Vascular ultrasound and Applanation Tonometry of the carotid arteries: The carotid and vertebral arteries will be measured at rest. This is non-invasive, pressing a small probe onto the surface of the skin.

Near-infrared spectroscopy: A small black box will be strapped to the leg, which measures how much oxygen is in the blood. This will be completed during the blood vessel dilationprotocol listed above.

Heart rate and blood pressure: heart rate will be measured using 3 sticky pads connected to your chest and stomach. Beat-by-beat blood pressure will be measured at your finger with a mini blood pressure cuff.

Aerobic fitness test: Oxygen uptake and carbon dioxide output of your bodywill be measured via a mouthpiece while cycling until maximal effort. The resistance on the bike will gradually increase until you cannot maintain a pedal speed of 70 rpm. Heart rate data will be collected continuously using the 3 sticky pads on your chest and stomach. This test will last ~8-12 minutes.

High-intensity interval exercise:This exercise will be performed on a cycle ergometer. From the aerobic fitness test we have your intensity for the exercise bouts. You will begin with an easy 3-minute warm-up and then alternate between 1 minute of heavy cycling and 1 minute of easy cycling, repeated 8 times. You will then cool down for 3 minutes. This exercise will last 16 minutes.

Moderate-intensity exercise: This exercise will begin with a 3-minute warm-up followed by ~20 minutes of moderate-intensity cycling determined from the aerobic fitness test.

*Procedures of the Study:*

Prior to the visits it is required that you abstain from heavy exercise for 24 hours and caffeine for a period of 12 hour sand arrive to the laboratory in sports clothes. You will be asked to abstain from food for a period of 2 hours, and upon arriving you will be given a standardized snack.

Visit 1(~1.5 hour): Upon arrival at the laboratory, participants will be given the health-screening questionnaire to determine eligibility. If eligible, measures of height and weight will be recorded. Participants will then lie down and complete the brain blood flowand velocity tests. Participants will then complete a measure of aerobic fitness to determine aerobic fitness and exercise intensity.

Visit 2 and3(3 hours each): On these visits you will complete either high-intensity interval exercise or moderate-intensity exercise. The order that these occur will be randomized, in other words half the participants will start with high-intensity interval exercise and half with moderate-intensity exercise.

When you arrive 2 hours fasted you will eat your standardized snack. After 15 minutes of lying on your back, measures of heart rate, blood pressure, and blood vessel dilationwill begin. A blood pressure cuff will be placed around the calf below the knee. Using an ultrasound Doppler to image the superficial femoral artery, the cuff will inflate for 5 minutes. Upon release measurements will continue for 5 more minutes. Following this you will complete either high-intensity interval exercise or moderate-intensity exercise,which will be randomly determined. Within 10 minutes of finishing exercise another blood vessel dilationwill be completed. Following 60 minutes of rest after exercise, the last blood vessel dilationwill be measured.

The total time required for this study will be ~8 hours with breaks.

**What are my responsibilities?**

Please do not perform any high-intensity exercise the day before any of your laboratory visits (e.g., running fast for at least 20 minutes, or playing a competitive soccer or hockey game).
Please eat a light meal 2 hours prior to yourarrival and eat this same meal before each visit.

Please eat the same meal the day before testing.

Please do not drink or eat caffeine 12 hours prior to testing.

**What are the possible harms and discomforts?**

The ultrasound and tonometry procedures are non-invasive and pose no risk. The cuff during the blood vessel dilation procedures may be uncomfortable for a small period of time, but this

poses no risk and this feeling goes away after cuff release. This technique is frequently used and well tolerated in children.

The high-intensity interval exercise and aerobic fitness test are safe but some people may experience sore legs and light-headedness when they finish cycling. This is perfectly normal and will subside. If, however, the subject feels uncomfortable they are able to stop the test to sit or lie down and the feelings will go away.

**What are the potential benefits of participating?**

You will not directly benefit from this study. Participation in this experiment will contribute to the understanding of how exercise leads to acute blood vessel adaptation in children, particularly how short term training leads to longer term adaptation in the exercising limb. This will provide knowledge to direct intervention aimed at improving these variables in children with complications due to disease or environment.

**What happens if I decide to withdraw my consent to participate?**

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of your information collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data will not be able to be withdrawn for example where the data is no longer identifiable (meaning it cannot be linked in any way back to your identity) or where the data has been merged with other data. If you would like to request the withdrawal of your data, please let your study doctor know. If your participation in this study includes enrolling in any optional studies, or long-term follow-up, you will be asked whether you wish to withdraw from these as well.

**What happens if something goes wrong?**

A physician will be on-call during all experimental sessions should any complications arise. In the unlikely event of any complication, such as fainting, an emergency medical response will be immediately initiated. The on-call physician and campus/hospital emergency rooms will be alerted and the response time is less than 10 minutes. We are located 14km (~20 minutes) from Kelowna General Hospital and the response time for an ambulance is ~20 minutes. All investigators are certified to perform cardiopulmonary resuscitation and in the use of an

automated external defibrillator (located in the laboratory ART 183) and will follow standard emergency protocols.

Individuals can differ in their responses to experimental procedures and you are encouraged to report any unusual sensations or symptoms to the investigator. You are allowed to end any of the tests at any time for any reason. If you do experience undesirable symptoms during the experiment, immediate care will be provided. All the procedures we use to collect physiological data will pose no risk to your continued health and wellbeing. In case of a serious medical emergency as a result of this study, please report to an emergency room and inform them that you are participating in a research study and that the following person can be contacted for further information: Ali McManus at telephone number: (250) 864-3513.

By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

**Can I be asked to leave the study?**

If you are not able to follow the requirements of the study, you may be asked to withdraw from the study. If you are asked to leave the study, the reasons for this will be explained to you and you will have the opportunity to ask questions about this decision.

**What will the study cost me?**

All of the tests that you complete during your participation in this study will be provided at no cost to you. We will provide $20 for each trip for the child and accompanying adultor adult participant. This will be reimbursed on the test day. We do not require receipts but ask you to sign a sheet to say you have received reimbursement.

**How will my taking part in this study be kept confidential?**

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator and the University of British Columbia Clinical Research Ethics Board for the purpose of monitoring

the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.
You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

The data on which the results of the project depend upon will be retained in secure storage for 5 years, after which they will be destroyed. If you completed the Health Screening Questionnaire but not the rest of the study, this information will be destroyed immediately.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected. You also have the legal right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

**Who do I contact if I have questions about the study during my participation?**
Please feel free to contact us at any time with questions and concerns you may have about participating in this research study.
If you wish to contact an independent person regarding any aspect of your participation in this study please contact:

Dr. Gordon Binsted
Dean, School of Health and Exercise Sciences
Faculty of Health and Social Development
University of British Columbia
3333 University Way, Kelowna, B.C.

V1V 1V7
Office: 250-807-9642

**Who do I contact if I have any questions or concerns about my rights as a participant?**
If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598).

**Study Title: THE IMPACT OF EXERCISE OF DIFFERING INTENSITIES ON VASCULAR FUNCTION IN CHILDREN AND ADULTS.**

**Participant Consent:** My signature on this consent form means:

1. I have read and understood the information in this consent form.
2. I have explained the information contained in this consent form to my child to the extent that he/she is able to understand it.
3. I have been able to ask questions and have had satisfactory responses to my questions.
4. I understand that all of the information collected will be kept confidential and that the results will only be used for scientific purposes.
5. I understand that participation in this study is entirely voluntary.
6. I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change the quality of care that I receive.
7. I understand that I am not waiving any of my legal rights as a result of signing this consent form.
8. My child has assented to participating in this research study.

**Signatures**

The participant or parent(s)/guardian(s)/substitute decision-maker (legally authorized representative) and the investigator are satisfied that the information contained in this consent form was explained to the child/participant to the extent that he/she is able to understand it, that all questions have been answered, and that the child/participant assents to participating in the research.

I consent to (my name or my child's name) _____participating in this study.

_____ _____ _____

*Name of Participant /Parent(s)* *Signature* *Date*

_____

*Participant / Parent/Legal Guardian Contact telephone*

| _____ | _____ | _____ | _____ |
|---|---|---|---|
| *Person obtaining consent* | *Signature* | *Study Role* | *Date* |

**Appendix B  Assent Form**

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**ASSENT FORM –Exercise and blood vessel health in children and adults**

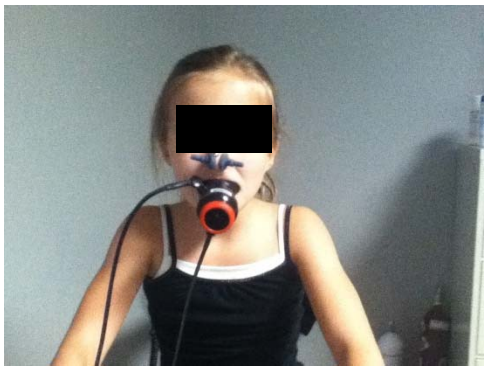
**1. Invitation**

I am being invited to be part of a research study. It is up to me if I want to be in this study. No one will make me be part of the study. Even if I agree now to be part of the study, I can change my mind later. No one will be mad at me if I choose not to be part of this study.

**2. Why Are We Doing This Study?**

We are researchers at the University and we are interested in finding out more about how the body works. This study will help us learn more about how your blood vessels respond to exercise. We would like to find out if exercise helps your blood vessels function better. Blood vessels are tubes inside of you that deliver blood to your body to keep you healthy.

**3. What Will Happen in This Study?**

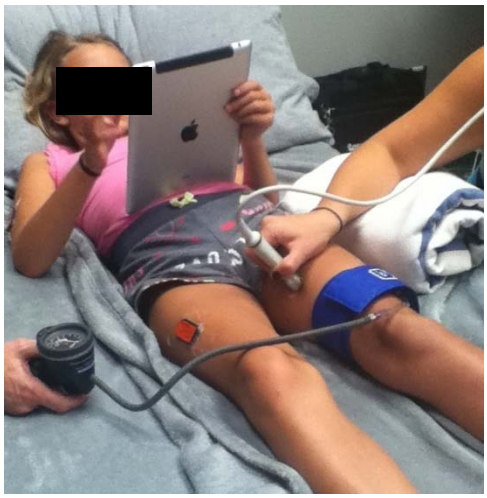


If I agree to be in this study, I will visit the University3 times. The first visit will take about 1.5hours, the second and third visit will take about 3 hours each. This means I will spend approximately 8 hours at the university in total. During the first visit I will have my height and weight measured. I will then lay down while having my neck blood vessels measured while I wear a crown on my head that takes pictures of my brain blood vessels. I will also do an exercise test. The exercise test involves me cycling on a stationary bicycle.  At

the beginning of the test it is easy, but it gets harder – like cycling up a hill. I will cycle until the hill gets too steep and I feel I cannot pedal anymore. This involves putting a mouthpiece into my mouth – this a small rubber device like a snorkel which my teeth fit into. I will also wear a special clip on my nose so all the air I breathe can be measured

The second and third time I go to the lab I will have my heart rate, blood pressure, the amount of oxygen in my leg and leg blood vessels measured while resting on a bed. The blood vessels and blood flow are measured using a special device that looks like a TV remote that is placed on my skin.

When my blood vessels are being measured I will also have a small black box on my leg which measures the oxygen in the blood in my leg.



After this I will either cycle hard on the same exercise bike or cycle at a medium pace. Immediately after I have exercised and 60 minutes after I have exercised I will have my leg blood vessels and oxygen measured.

**4. What is Expected of Me?**

If I decide to join this study I will visit the University three times. In total I will spend about 8 hours being a part of this study.

When I visit the University I will not exercise a lot the day before. I will wear shorts and a t-shirt and a pair of sports shoes and eat a light meal at least 2 hours before. I will eat a snack when I arrive at the University. I will not drink caffeine the day I visit the University.

**5. Can Anything Bad Happen to Me?**

Sometimes the exercise test and training I will do make's children like me feel tired and out of breath. But those feelings should go away within a few minutes of finishing the test. Sometimes the cuff that gets placed around my leg feels tight, but I know nothing bad can happen to me and this feeling goes away very soon after the cuff deflates.

**6. Who Will Know I Am in the Study?**

Only the people who are involved in the study will know I am in it. When the study is finished, Dr. McManus will write a report about what was learned. This report will not say my name or that I was in the study. My parents and I do not have to tell anyone I am in the study if we don't want to.

**7. Do I have to be in this study**

It is entirely up to me if I decide to join this study. If I do decide to take part, I can stop at any point and do not have to explain why I want to stop. If I do not want to join the study I don't have to explain to anyone why.

**8. Who is doing this Study?**

**Dr. Ali McManus** is doing this study. She will answer any questions I have about the study. I can call her at **250-864-3513**, if I am having any problems or if there is an emergency and I cannot talk to my parents.

**9. When Do I Have To Decide?**

I have 2 weeks to decide if I want to be part of the study. I have also been asked to discuss my decision with my parents.

**10. My Name**

*If I put my name at the end of this form, it means that I agree to be in this study.*

_____ _____

*My name* *Date*

**Appendix C Health Screening Questionnaire**

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**Health Screening Questionnaire Children**

Thank you for considering participation in our study. We would be most grateful if you could take the time to complete this questionnaire in relation to the health of your child, which should take about5 minutes to complete.

**Please return this completed questionnaire with the consent and assent forms when you visit the laboratory.**

**Completing the questionnaire**

• This questionnaire should be completed by the main caregiver for the child.
• Please answer the questions in relation to your child's current health.
• Please indicate your answers by ticking the relevant box or by writing your answer in the space provided

**Confidentiality**

All information about you and your child will be kept in the strictest of confidence. Your responses will only be seen by members of the research team and will be made anonymous. If you do not meet eligibility requirements based on this screening, any data collected from this questionnaire will be destroyed immediately.

**Why we are collecting this information**

The purpose of this form is to ensure we provide every child with the highest level of care. For most children, exercise provides an opportunity to have fun and promotes the basis for good health and an enhanced quality of life for the future. However, there are a small

number of children who may be at risk when exercising. There are some health conditions, such as of heart, blood vessel, metabolic (e.g., type 1 diabetes) or lung and breathing problems or diseases, that make participation difficult or unsafe and we need to be sure all the children participating in our study are able to complete the procedures safely.

## Contact us

If you have any questions about the study during your participation you may contact the Pediatric Exercise Research Laboratory at (250) 807-9873.

## Child's details

**Child's gender:**
(Please tick one box)

☐ Male

☐ Female

**Your relationship to this child**:
(please tick one box)

☐ Mother

☐ Father

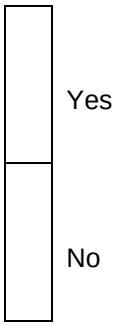
☐ Other (*please describe*)

The changes in hormones with pubertal development cause changes to many aspects of bodily function including blood flow. Some girls and boys may have started pubertal development between the ages of 8 and 12 years of age. So we can consider this when we

look at the results of the study we would like you to assess your child's current stage of pubertal development by answering the following questions.

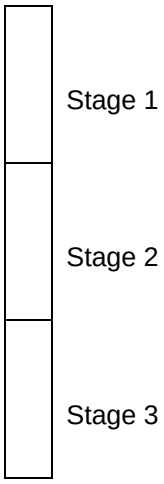
**1. If you child is a girl, has she had her first menstruation:**

(Please tick one box)




**2. The pictures below illustrate the different stages of pubertal development in girls and boys. Please look at these and consider which one best describes your child's stage of development.**

(Please tick one box)



| | Girls | | Boys | |
|---|---|---|---|---|
| | **Breast** | **Pubic Hair** | **Genitals** | **Pubic Hair** |
| **Stage 1** | Small nipples. No breast.  | No pubic hair.  | No signs of puberty. Scrotum, testes, and penis as in childhood.  | No pubic hair.  |
| **Stage 2** | Breast and nipples have just started to grow. The areola has become larger. Breast tissue bud feels firm behind the nipple.  | Initial growth of long pubic hairs. These are straight, without curls, and of light color.  | Initial growth of scrotum and testes. The skin on the scrotum has become redder, thinner, and more wrinkled. The penis may have grown a little in length.  | Few hairs around the root of the penis. The hairs are straight, without curls, an of light color.  |
| **Stage 3** | Breast and nipples have grown additionally. The areola has become darker. The breast tissue bud is larger.  | The pubic hair is more widespread. The hair is darker, and curls may have appeared.  | The penis has now grown in length. Scrotum and testes have grown. The skin of the scrotum has become darker and more wrinkled.  | Hairs are darker and curlier and still sparse, mostly located at the penis root.  |

**Parents/guardian of girls & boys complete questions 1 to 11**

1. **Does your child have or has your child ever had:** (Please tick the box if the answer is yes)

| | Yes |
|---|---|
| A heart condition (please specify) | |
| Cystic fibrosis | |
| Diabetes (please specify type 1 or type 2) | |
| High blood pressure | |
| Unexplained coughing during exercise | |
| Breathing problems or shortness of breath (please specify) | |
| Seizures or epilepsy (please specify whether at rest or during exercise) | |
| Dizzy spells | |
| Fainting | |
| Increased bleeding (e.g., haemophilia) | |

2. **Does your child take any medication for any of these conditions or another condition? If yes please provide details:**

| Name of medication and purpose: |
| --- |
| |

3. **In the last 6 months has your child had any muscular pain while exercising?** (Please tick the box if the answer is yes 'pain in the back of the right heel' or 'pain on the inside of the right elbow)

**Yes**

| |
| --- |
| |

4. **In the last 6 months has your child had any joint pain or pain in the bones while exercising?** (Please tick the box if the answer is yes and explain where the pain is eg. Behind my knee)

**Yes**

| |
| --- |
| |

5. **Has your child broken any bones in the past 12 months ?** (Please tick the box if the answer is yes and explain where the break was eg. Lower leg)

**Yes**

| |
| --- |
| |

6. **Does your child or has your child ever had difficulty or problems with the following:** (Please tick the box if the answer is yes)

| | **Yes** |
|---|---|
| Vision | ☐ |
| Hearing | ☐ |
| Speech /language | ☐ |
| Motor/sensory skills | ☐ |
| Poor balance | ☐ |
| Sleep apnea | ☐ |

7. **Has your child ever had a brain or spinal cord injury?** (Please tick the box if the answer is yes and specify)

**Yes**

☐

8. **Does your child have any difficulty climbing up or down stairs?** (Please tick the box if the answer is yes and specify)

**Yes**

| |
|---|
| |

9. **Does your child have any allergies?** (Please tick the box if the answer is yes and specify)

**Yes**

| |
|---|
| |

10. **Does your child have any special dietary needs?** (Please tick the box if the answer is yes and specify)

**Yes**

| |
|---|
| |

11. **Are you aware of any other reason why your child should not exercise or take part in this study?** (Please tick the box if the answer is yes and specify)

**Yes**

| |
|---|
| |

**If there is an emergency, specify the person who should be contacted and their emergency phone number:**

Name.....

Telephone.....

**Future Contact**

Would you like to be contacted about participating in future studies? Please indicate by ticking the appropriate box below:

**YES** ☐

## Appendix D  Ethics Clearance Form

*The University of British Columbia*
*Office of Research Ethics*
*Clinical Research Ethics Board – Room 210, 828*
*West 10th Avenue, Vancouver, BC V5Z 1L8*

# ETHICS CERTIFICATE OF EXPEDITED APPROVAL

| PRINCIPAL INVESTIGATOR: | INSTITUTION / DEPARTMENT: | UBC CREB NUMBER: |
|---|---|---|
| Ali McManus | UBC/UBCO Health & Social Development/UBCO Health and Exercise Sciences | H16-00077 |

**INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:**

| Institution | Site |
|---|---|
| UBC | Okanagan |

**Other locations where the research will be conducted:**
N/A

**CO-INVESTIGATOR(S):**
Thomas J. Warshawski
Nathan Sletten
Philip Ainslie

**SPONSORING AGENCIES:**
- Natural Sciences and Engineering Research Council of Canada (NSERC) - "Exercise Oxidative Metabolism in Children "

**PROJECT TITLE:**
THE IMPACT OF EXERCISE OF DIFFERING INTENSITIES ON VASCULAR FUNCTION IN CHILDREN AND ADULTS.

**THE CURRENT UBC CREB APPROVAL FOR THIS STUDY EXPIRES: May 13, 2017**

**The UBC Clinical Research Ethics Board Chair or Associate Chair,** has reviewed the above described research project, including associated documentation noted below, and finds the research project acceptable on ethical grounds for research involving human subjects and hereby grants approval.

This approval applies to research ethics issues only. The approval does not obligate an institution or any of its departments to proceed with activation of the study. The Principal Investigator for the study is responsible for identifying and ensuring that resource impacts from this study on any institution are properly negotiated, and that other institutional policies are followed. The REB assumes that investigators and the coordinating office of all trials continuously review new information for findings that indicate a change should be made to the protocol, consent documents or conduct of the trial and that such changes will be brought to the attention of the REB in a timely manner.

| DOCUMENTS INCLUDED IN THIS APPROVAL: | | | APPROVAL DATE: |
|---|---|---|---|
| Document Name | Version | Date | |
| **Protocol:** | | | |
| Protocol | 0.2 | May 11, 2016 | |
| **Consent Forms:** | | | **May 13, 2016** |
| Consent | 0.2 | May 11, 2016 | |
| **Assent Forms:** | | | |
| Assent | 0.2 | May 11, 2016 | |

| | | | |
|---|---|---|---|
| **Advertisements:** | | | |
| Adult recruitment | 0.2 | May 11, 2016 | |
| Flyer | 0.2 | May 11, 2016 | |
| Child recruitment | 0.2 | May 11, 2016 | |
| **Questionnaire, Questionnaire Cover Letter, Tests:** | | | |
| Health Screening children | 0.2 | May 11, 2016 | |
| Health Screening Adult | 0.2 | May 11, 2016 | |
| **Other Documents:** | | | |
| YMCA invitation to recruit | N/A | February 9, 2016 | |

CERTIFICATION:
**In respect of clinical trials:**
*1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.*
*2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.*
*3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.*

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

*Approval of the Clinical Research Ethics Board by:*
Dr. Stephen Hoption Cann,
Chair