THE SYSTEMIC EFFECTS OF EXERCISE IN PRE-PUBERTAL GIRLS AND WOMEN ON MUSCLE AND BONE GROWTH *IN VITRO*:

TRANSLATING PEDIATRIC EXERCISE SCIENCE WITH ANIMATION

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TITLE: The systemic effects of exercise in pre-pubertal girls and women on muscle and bone growth *in vitro*: Translating pediatric exercise science with animation

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ABSTRACT

The systemic environment in children is characterized by factors that facilitate muscle and bone development. These systemic factors may help to regulate the growth of these tissues particularly when mechanical loading is minimal, as observed with low-impact exercise. Low-impact exercise induces an alteration in the systemic environment in children and adults, however the direct implications of these changes on muscle and bone development are unclear. The focus of this thesis was to examine the effects of exercise on systemic regulators of muscle and bone growth in prepubertal girls and women, and to determine whether changes in these systemic regulators can influence osteoblast and myoblast proliferation and differentiation. In addition, this thesis will also highlight the importance of translating knowledge in the form of an animation video about the effects of exercise on bone health.

Our first study demonstrated that an acute bout of moderate intensity exercise elicits similar responses in systemic regulators (CX3CL1, FGF-2, and IGF-1) of muscle and bone growth in prepubertal girls and women, with exception of a higher inflammatory response (IL-6) in women.

In our second study, we show that exercise does not elicit a proliferative response myoblasts and osteoblasts *in vitro* after treatment with serum collected from prepubertal girls and women. However,

proliferation of osteoblasts and myoblasts was higher in women post-exercise relative to prepubertal girls. Moreover, there was no exercise effect on myotube formation in prepubertal girls and women, however mineralization decreased post-exercise in both groups.

In our final study, we developed an animation video that summarizes the findings of this thesis to school-age children and explored the educational utility of this knowledge translation tool. Four salient viewpoints were identified in our cohort of children, each with varying degrees of engagement and attitudes towards the video. All participants expressed an improved understanding how exercise influences bone as evidenced by their consensus statement.

Altogether, our data suggests that inflammatory responses induced by exercise are attenuated in children relative to adults, which can have an effect on myoblast and osteoblast proliferation. The decrease in mineralization observed after exercise may be indicative of increased bone remodeling followed by an anabolic bone response. Finally, the development of a knowledge translation tool proved to be feasible and beneficial in promoting awareness about the benefits of exercise on bone health.

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DEDICATION

To Mama, Baba, Ahmed and Addel:
I would not be where I am today had it not been for Allah's will and your endless love and support,

To Lulu, for starting this journey with me and forever remaining in my heart.

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LIST OF ABBREVIATIONS

Akt Protein kinase B
ALP Alkaline phosphatase
ANOVA Analysis of variance
BMD Bone mineral density
BAP Bone alkaline phosphatase
BMI Body mass index
BSA Bovine serum albumin
BSI Bone strength index
CAM Calmodulin
CCL2 C-C Motif chemokine ligand 2
CO_2 Carbon dioxide
CPC Cetylpyridinium chloride
CX3CL1 CX3 Chemoligand-3
DAPI 4',6-diamidino-2-phenylindole
DMEM Dulbecco's Modified Eagle's Medium
ELISA Enzyme-linked immunosorbent assay
ETDA Ethylenediaminetetraacetic acid
EX Serum or plasma sample collected immediately following exercise
CX3CR CX3C chemokine receptor 1
DEXA dual x-ray absorptiometry
eMHC Embryonic myosin heavy chain
FBS Fetal bovine serum
FEV1 Forced expiratory volume in 1 sec
FFM Fat-free mass
FGF2 Fibroblast growth factor 2
GH Growth hormone
GM Growth media
HGF Hepatocyte growth factor
IGF-1 Insulin-like growth factor
IL-1 Interleukin 1
IL-6 Interleukin 6
IL-6Rα IL-6 receptor alpha
IL-10 Interleukin 10
JAK Janus Kinase
LBM Lean body mass
MFI Myonuclei fusion index
Fox Forkhead box

gp130 Glycoprotein 130
HS Human serum
ICTP Type 1 collagen fragments
IGF-BP Insulin growth factor binding protein
IL-8 Interleukin-8
IRS Insulin receptor substrate
JAK Janus kinase
kDa Kilodaltons
KT Knowledge Translation
MAPK Mitogen-activated rotein kinase
MICE Moderate intensity cycling exercise
MMP Matrix metallopeptidase
MRF4 Myogenic regulatory factor 4
MRFs myogenic regulatory factors
mRNA Messenger ribonucleic acid MHC Myosin heavy chain
MPC Myogenic precursor cells
mTor Mammalian target of rapamycin
MTS Tetrazolium
MVPA Moderate-vigorous physical activity
Myf5 Myogenic factor 5
MyoD Myogenic differentiation antigen
Pax7 Paired box 7
PFA Paraformaldehyde
pSTAT3 Phosphorylated STAT3
NF-kB Nuclear factor kappa-light-chain enhancer
NTX N-termina telopeptide
O_2 Oxygen
OPG Osteoprotegerin
PBS Phosphate buffered saline
PICP Procollagen 1 carboxyterminal propeptide
PIK Phosphoinositide 3-kinase
PINP Procollagen type 1 N-terminal peptide
pQCT Peripheral quantitative computed tomography
RANKL Nuclear factor-kB ligand
RCF Relative centrifugal force
REST Serum or plasma sample collected before exercise
REC Serum or plasma sample collected 1 hour after exercise
RPM Revolution per min
RT-PCR Reverse transcription polymerase chain reaction
SD Standard deviation
Runx2 Runt-related transcription factor 2

SC Satellite cells
SE Standard error of mean
SOCS3 suppressor of cytokine signaling 3
STAT3 Signal transducer and activator of transcription 3
TNF-α Tumor necrosis factor alpha
TRAP5B Tartrate-resistant acid phosphatase
VO_2max Maximal oxygen uptake

PREFACE

This thesis is prepared in the “sandwich” format as outlined in the “Guide for the preparation of Master’s and Doctoral Theses” from McMaster University. Chapter 1 serves as a rational and general literature review of this thesis. Chapter 2-4 each represent an independent study manuscript, one of which has been published, and one in preparation to be submitted at the time of this thesis submission. All published, submitted, and prepared manuscripts were written by the author of this thesis, who is also the first author on all manuscripts. The preamble section preceding each chapter describes the contributions of other authors to the multi-authored work. Finally, Chapter 5 serves as the discussion section used to summarize overall finding and discuss implications.

Chapter 1: LITERATURE REVIEW

1.1 Introduction

Physical activity elicits many benefits on muscle and bone which improve the quality and strength of these tissues (Vicente-Rodríguez, 2006). There appears to be a positive correlation between muscle and bone adaptations to physical activity, indicating a possible relationship between the two tissues as well (Schoenau, 2005). For example, increased muscle cross-sectional area is observed with increased bone mineral in children who are involved in high-impact physical activity (Vicente-Rodríguez, 2006). Similarly, muscle contractile force and bone strength are also positively correlated in healthy pediatric populations (Schoenau et al., 2000). These findings indicate that correlations between muscle and bone are reflective of the structure and function of both tissues, thereby coining the widely used term in musculoskeletal physiology, the muscle-bone unit (Harold M. Frost, 2003).

Many of the studies examining the effects of exercise on muscle-bone attribute the effects to the ability of these tissues to detect mechanical stimuli (Schoenau, 2005). Indeed, high impact exercise involves high mechanical strain which elicits increased muscle and bone remodeling, which is well demonstrated using plyometric exercises in pediatric populations (Kish et al., 2015). Studies assessing low-impact exercise show

that activities such as swimming or cycling may also result in improved muscle and bone indices, such as muscle strength and bone quality (Gómez-Bruton et al., 2013), however these effects may be linked to systemic regulation. Indeed, exercise triggers many changes in the systemic environment, such as increased mobilization of cytokines and growth factors, which may target muscle and bone to produce these responses (Cianferotti & Brandi, 2014). The effects of exercise on systemic responses are widely addressed in adult populations, however there is a lack of research investigating these responses in children, particularly girls. While there is some research that assesses the effects of puberty on the exercise response in adolescents, there are fewer studies assessing pre-pubertal stages. In order to understand the effects of exercise on systemic responses in females, and how these responses influence muscle and bone, it is important to address this investigation at different stages of development. This will also help to address the gender gap between females and males in the exercise literature.

In addition to addressing the gender gap, another important objective of this work is to address the gap between research and the community. Knowledge translation is defined as a dynamic and iterative process that includes synthesis, dissemination, exchange and ethically-sound application of knowledge to improve the health of Canadians, provide more

effective health services and products and strengthen the health care system (CIHR, 2020). There are multiple ways to apply knowledge translation to research, one method is to promote awareness on health issues (Sullivan et al. 2007), such as the effects of exercise on musculoskeletal health. Animated videos provide effective means to deliver knowledge to children, and this is demonstrated with favourable outcomes in knowledge retention and increased motivation to adopt healthy behaviours in pedagogical and health promotion research (Grassi et al.,2005; Barak et al. 2011). Thus, another aim of this thesis is to explore the utility of an animation videos in informing children about exercise and the associated benefits on musculoskeletal health. The following literature review was written to explain the rationale for the aims of this thesis and to discuss current gaps in the literature.

1.2 Determinants of pediatric muscle and bone growth and development

Muscle and bone growth during the pediatric years is shaped by multiple intrinsic and extrinsic factors. During the early onset of puberty, growth hormones drive rapid growth of bone mineral accrual and elongation. Peak height velocity, which is a period where child experiences their fastest upward growth in stature, is achieved in girls and boys near the ages of 12

and 13.5 years, respectively (Kindler et al. 2015). Following PHV, peak lean mass velocity is achieved and is followed months later by peak bone mineral content velocity. These timepoints are marked by alterations in hormonal activity due to the dramatic stimulation of the hypothalamic-pituitary-gonadal axis (HPG). Elevations in growth hormone and insulin growth factor (IGF-1) play an important role in facilitating the changes leading to the pubertal growth spurt. This growth is mediated by sex hormones, estrogen and testosterone, which also undergo heightened production in response to the HPG stimulation (Casazza et al. 2010). Collectively, these hormones define specific trajectories for peak muscle and bone acquisition.

Nutrition plays an important role in mediating muscle and bone growth. Over 99% of the body's calcium is found in skeletal stores, which plays an essential role in calcium homeostasis (Gordon et al. 2016). This is emphasized in longitudinal studies showing calcium intake improves bone mineral density (BMD) measures in children (Gordon et al. 2016), which is also demonstrated in adults as well. Vitamin D is also an essential nutrient that mediates musculoskeletal growth by optimizing intestinal absorption of calcium, which ensures the normal calcification of the growth plate and mineralization of trabecular and cortical bone (Maggioli & Stagi 2017). Adequate levels of these nutrients are required for the maintenance of

normal blood levels of their concentrations and growth and maturation of muscle and bone.

Physical activity is a critical determinant of muscle and bone growth. Childhood and adolescence represents a sensitive time for increased musculoskeletal remodeling and bone turnover, which is characterized by bone formation and resorption. These processes are influenced by mechanical and biochemical triggers of physical activity and exercise. During this time of childhood and adolescence, physical activity adds bone mass to periosteal surface that enhance bone strength (Gordon et al. 2017). The accretion of bone at an early age is a critical step towards achieving peak bone mass, which is also a well-known determinant for osteoporosis (Maggioli & Staggi 2017.). Therefore, the prevention of geriatric conditions begins by optimizing musculoskeletal gains throughout childhood.

In addition to these factors, the use of oral contraceptives at a young age is also shown to influence bone development (Polatti et al. 1997). The use of oral contraceptives amongst adolescent females is associated with suboptimal levels of bone accrual and declines in bone mineral density (Polatti et al. 1997). These findings are linked to the effects of oral contraceptives on the hypothalamic-pituitary-ovarian axis, which in turn decrease the production of estradiol, a critical estrogen with anabolic effects on bone growth (Casazza et al. 2010, Gordon et al. 2017).

1.3 The Muscle-Bone Unit

The average human body consists of at least 600 muscles (Kamibayashi & Richmond, 1998). The primary function of muscle is to provide mobility and stability through contractions. Muscles also contribute to stability through their proprioceptive functions, which are coordinated with the vestibular system. In addition to their kinesthetic functions, muscles also play intrinsic functions related to circulation, digestion, and reproduction (Martini et al. 2009).

Bones provide structural support for the human body by acting as points of anchor for muscle attachment, hence facilitating movement. Bones also provide protection to vital organs, as seen with the cranium and thoracic rib cage which house the brain and abdominal organs, respectively. Bones also play pivotal roles in immunology by providing sites of blood and immune cell production specifically in the bone marrow (Martini et al. 2009).

Muscle and bone are distinct organs with different functions that are found to be within close proximity throughout the human body, which allows for their coordinated function (Brotto & Bonewald, 2015). However, over the past two decades it has become apparent that this intimate proximity serves more purposes beyond points of attachment, such that the function of muscle and bone and codependent on the health and function of each other (Hamrick, 2011).

1.3.1 The Mechanostat theory

Preliminary evidence of a possible relationship stems from literature on the mechanostat qualities of bone, which states that bones respond to varying strains imposed by increases or decreases of mechanical loading to keep bone deformation within safe limits (Lang, 2011). These loads can vary between ground reactive forces (as experienced with impact), tension forces (as experienced with movement), and oppositional acting forces (as observed with muscle contractions). It is further implied that mechanical strains guide biological mechanisms in time and space, such that bone achieves its architecture (H. M. Frost, 2001). Moreover, the mechanostat is supported by observations stating that bones are equipped with infrastructure (i.e., trabeculae) that enables them to undergo peak voluntary loads without experiencing fractures (Harold M. Frost, 2003).

According to Frost et al. (2001), bone remodeling is initiated when a mechanical load or strain overcomes a mechanical threshold, consequently increasing bone strength as well as the threshold range (H. M. Frost, 2001). As such, additional loads that do not exceed bone strength continue to result in increased bone formation that allows the skeleton to withstand peak loads. However, when these strains are below the remodeling threshold, this results in disuse remodeling which is characterized by a reduction of bone marginal to the site of bone marrow (H. M. Frost, 2001). Moreover,

this causes disuse pattern osteopenia, which is characterized by less trabecular bone, an enlarged marrow cavity, and a thinned cortex due to the latter (H. M. Frost, 2001). Thus, in order to prevent osteopenia or its progression, the remodeling threshold needs to be overcome by mechanical loads (H. M. Frost, 2001).

Theoretically, muscle contractions impose the largest physiological loads on bone, thereby putting several fold larger stresses on the skeleton than the simple effect of gravity and positioning muscle to play a critical role in the structure and function of bone (Rauch et al., 2004). As muscles contract, they generate force and strain on bone that modify cortical and trabecular structure and shape, thereby shaping bone to withstand everyday tasks. As such, this association has led to the establishment of the muscle-bone unit (Rauch et al., 2004).

1.3.2 Correlations across the lifespan

During growth, muscle development is a leading force that drives bone development (Rauch et al., 2004). It is postulated that muscle contraction in the developing fetus even contribute to skeletal growth and development and that skeletal adaptations in early post-natal life are driven by changing mechanical forces (Brotto & Bonewald, 2015). Moreover, it is reported in children that the peak lean body mass velocity precedes peak bone mineral

content velocity (Rauch et al., 2004). Furthermore, this relationship is also supported by cross-sectional studies assessing the correlations of muscle and bone surrogate measures using densitometry techniques, such as dual X-ray absorptiometry (DEXA) or quantitative commuted tomography (pQCT) (Fricke & Schoenau, 2007). For example, cortical bone mineral density (BMD) and bone cross sectional area (CSA) are positively correlated with lean body mass and muscle CSA in both children and adults between the ages of 2-60 years (Schoenau, 2005; Slizewski et al., 2013). In addition, studies with children have confirmed significant, positive correlations between parameters of muscle strength and bone strength (Schoenau, 2005). For example, one study found that bone strength index (BSI) to be positively correlated with grip strength in the same population; while another study found elbow flexor muscle force to be positively correlated with radial BSI at the midshaft (Schoenau, 2005). In all cases, the correlations between muscle and bone were strongest for children, while the ratio gradually plateaued in adults (Fricke & Schoenau, 2007; Rauch et al., 2004). These findings indicate a functional relationship between muscle and bone that extends beyond their physical proximity.

1.4 Development of the muscle-bone unit

The relationship between bones and muscle is established in the early stage of life: the embryo. During embryonic development, mesenchymal cells originating from the mesoderm give rise to bone and muscle precursor cells (Matsuoka et al., 2005). Initially, bone and muscle cells develop independently prior to forming attachments to one another. Bone formation begins as mesenchymal cells differentiate into osteoblasts (bone forming cells) that lay down the matrix of bone in cartilage. As bones grow during limb morphogenesis, muscle precursor cells known as somites migrate to extremity buds where they differentiate into myoblasts (Sachidanandan & Dhawan, n.d.). Hence it is after the sixth week of development that the muscle-bone unit is first formed (Sachidanandan & Dhawan, n.d.)

Muscle and bone originate from the same precursor cells, however they go on to develop into distinct cell types and organs. Muscle is comprised of satellite and myoblast cells, and bone is comprised of osteoblasts, osteoclasts and osteocytes. Each of these cells provide different functions, which in turn lead to the comprehensive development and function of each organ.

1.4.1 Myogenesis

Skeletal muscle is marked by its dynamic ability to remodel, regenerate and repair (Le Grand & Rudnicki, 2007). There are at least 600 skeletal muscles in the human body with main functions to support motor control and stability, both of which are made possible due to the contractility of skeletal muscle (Chal & Pourquié, 2017). Contractility of the skeletal muscle is dictated by the organization of cellular structures of the muscle fibre and their integration of with intermembrane environment comprising of neural and vascular tissue, all of which facilitate excitation-contraction and nourishment of skeletal muscle (Le Grand & Rudnicki, 2007; Matsuoka et al., 2005).

Myoblasts

During the 8th week of embryonic development, somites migrate to the paraxial dermomyotome of the embryo to form the templates of muscle generation. This localization is mediated by signaling pathways involving sonic hedgehog and Wnt signaling proteins, which give rise to localized expression of a family of transcription factors know as myogenic regulatory factors or MRFS (Sachidanandan & Dhawan, n.d.). Four MRFS play a critical role in the early establishment of myogenic cells: Myf5, MyoD, myogenin and MRF4. Myf5 and myoD are important for the establishment of proliferating myoblasts, and are expressed earliest in myogenic

progenitor cells. Migration of myogenic progenitor cells from the dermomyotome ends with an upregulation of Myf5 and myoD, which causes myoblasts to proliferate. Myoblasts are mononucleated cells, characterized by dendritic extensions. After multiple rounds of proliferation, myoblasts undergo differentiation into myotubes, which are made up of fused myoblasts, thereby resulted in a multinucleated appearance. The differentiation of myotubes is mediated by myogenin, and maturation is facilitated by MRF4. Other proteins are involved but there four MRFs are the most universal across human and animal studies and therefore, most consistent (Ceafalan et al., 2014; Le Grand & Rudnicki, 2007, Asfour et al. 2018).

Throughout the progression of myotube formation, there is an orchestration of adhesion molecules, which include cadherin, CAM, and integrin families. There are two types of fusion for muscle cells: primary fusion of myoblasts, and secondary progressive fusion, both of which lead to the formation of myofibers. Myofibers are fused myotubes, and also make up the basic unit of a muscle. Following these two stages, differentiation is characterized by diversification of myofibers, which leads to the development of different types of muscle fibers, namely fast twitching and slow twitching fibers. (Sachidanandan & Dhawan, n.d.; Asfour et al. 2018).

Satellite Cells

During early postnatal development, proliferating myoblasts cease prior to entering differentiation. Some of these cells, however, will leave the cell cycle and transition into quiescence, which is characterized by cellular dormancy. In postnatal development up until adulthood, these cells remain quiescent unless 'damage to the tissue, which results in a return to both the cell cycle and the capacity to differentiate.' These quiescent cells are known as satellite cells (Le Grand & Rudnicki, 2007, Asfour et al. 2018).

Satellite cells (SC) were first identified in amphibian studies due to their location on the myofiber (Matsuoka et al., 2005). By definition, SCs are identified by their position relative to myofiber extracellular matrix, and are first detected in muscle only after the basal lamina is formed (Le Grand & Rudnicki, 2007). Although they are in close proximity to myofibers, SCs do not contain the same MRF identifiers as neighbouring myonuclei, suggesting these cells come from a different lineage. Amongst the regulators of SC are Pax7, which is a paired box transcription factor. SCs make up 35-45% of muscle nuclei at birth, however this pool is reduced to 1-4% in adulthood. These self-replenishing cells will undergo proliferation and differentiation to increase mass of pre-existing muscle fibres during postnatal development and injury (Chal & Pourquié, 2017; Zammit 2015).

In postnatal biology, the activation of SC relies on injury to a myofiber (Ceafalan et al., 2014). Injured myofibers produce an array of factors that facilitate this activation, namely proteases and chemoattractants such as leukocytes and myogenic progenitor cells. This is also accompanied by an infiltration of the damaged myofibers with phagocytic cells (i.e macrophages), which incite debris removal and cytokine production. Upon activation, SC undergo gene alterations which cause an upregulation of MRFS. Under the influence of Myf5 and MyoD, SC enter the cell cycle and proliferate for 2-3 days prior entering differentiation. This new population of myogenic precurusor cells undergo differentiation through the influence of myogenin and MRF4. Extrinsic factors that are released in the environment will also contribute to the fusion of myotubes, such IGF-1 and FGF2 released by resident non-muscle cells (ex. endothelial and fibroblast) (Chal & Pourquié, 2017). These triggers cause SC to undergo proliferation and maturation into terminally differentiated myofibers, while, a subpopulation of proliferating myogenic cells will replenish the SC population so preserve this pool of renewing cells for future use (Zammit 2015; Chal & Pourquié, 2017).

Myoblast proliferation and differentiation can be influenced by alterations in their upstream regulators. Similarly, changes in proliferation and differentiation modulate downstream effects, namely muscle growth (Podbregar et al. 2013; Asfour et al. 2017). Therefore, understanding how

these processes respond to specific stimuli provides insight on the influences of muscle growth.

1.4.2 Osteoblastogenesis

Bone remodeling is a multicellular process. Bone resorption and bone formation are conducted through the communication of several cell types, however the two key cells that are responsible for these functions are the osteoclasts (bone resorption cells) and osteoblasts (bone formation cells) (Datta et al., 2008). Both osteoclasts and osteoblasts undergo several morphological phases before they differentiate into their active resorption and formation phase. These cells originate from different precursors, perform different functions on bone tissue, and are activated by separate molecular pathways, yet they are well- coordinated not only by local signals but also by each other to maintain the structure of bone (Kular et al., 2012).

The common ancestor cells of osteoblasts are mesenchymal stem cells, which are multipotent cells derived from the bone marrow and periosteum (Datta et al., 2008). The differentiation of mesenchymal stem cells into osteoblast precursors is reliant on specific stimuli such that these cells do not differentiate into other cell types, such as adipocytes, myocytes, of chondrocytes (Robling et al. 2006). Specifically, the expression of runt-related transcription factor-2 (Runx2), a bone specific transcription factor, is required for mesenchymal cells to differentiate into the osteoblast lineage

(Robling et al. 2006). There are four functional phases within the osteoblast lineage: preosteoblasts, mature osteoblasts, bone lining cells, and osteocytes (Sims & Gooi, 2008).

Once activated by Runx2, mesenchymal cells differentiate into preosteoblasts. These cells proliferate and express fibronectin, collagen, and matrix proteins such as osteopontin (Datta et al. 2008). Preosteoblasts share many commonalities with osteoblasts in terms of function and morphology. For example, they secrete type 1 collagen (PICP), which is the basic building block of bone, in addition to bone alkaline phosphatase (BAP), which is an enzyme required for bone formation (Robling et al. 2006). Unlike osteoblasts, preosteoblasts do not produce mineralized tissue and are therefore not surrounded by a bony matrix. Rather, these cells reside along the bony surfaces of active bone formation, after which they differentiate into mature osteoblasts.

Mature osteoblasts are post-mitotic cells, but are not terminally differentiated (Sims & Gooi, 2008). These cells have a cuboidal morphology and produce factors similar to those produced in the preosteoblast lineage, such as produce type 1 collagen and BAP, and also form bone. However, in addition to these factors, osteoblasts are also able to form bone by depositing unmineralized matrix (Robling et al., 2006). Collectively, newly formed matrix and osteoblasts are referred to as osteons, which are the

multicellular units of bone. Each osteon retains collagen strand orientation and the individual cells remain alive and interconnected during the life of the functional bone unit. Osteoblasts that remain on the surface of bone are called bone-lining cells (Kular et al., 2012). These cells are quiescent and may undergo apoptosis, however they can also play an important role in initiating the formation of the remodelling compartment. By releasing a digestive enzyme called collagenase, bone-lining cells digest unmineralized matrix in preparation for the underlying mineralized matrix to be resorbed by osteoclasts (Sims & Gooi, 2008).

Once osteoblasts become completely engulfed by bony matrix, they differentiate into osteocytes (Datta et al., 2008). Osteocytes are terminally differentiated cells that are found dispersed throughout the matrix, making up approximately 95% of the adult skeleton (Bloomfield, 2001). These cells have extensive cell processes that extend outwards via minute channels in the bone matrix called canaliculi, which connect with adjacent lacunae (Bloomfield, 2001). Osteocytes are often described as inert cells, however their functions are key to bone remodelling. Specifically, osteocytes have the ability to detect mechanical loading imposed on the skeleton and communicate this information to neighbouring bone cells (Datta et al. 2008). Osteocytes use their dendritic cell processes to communicate with other osteocytes, osteoblasts, and osteoclasts via gap junctions. This elaborate

network of communication allows osteocytes to partly regulate bone formation and bone resorption by producing chemokines that influence osteoblast and osteoclast activity (Sims & Gooi, 2008).

Osteoblast proliferation and differentiation can be influenced by alterations in their upstream regulators. Similarly, changes in proliferation and differentiation modulate downstream effects, namely proliferation and mineralization (Datta et al. 2008). Therefore, understanding how these processes respond to specific stimuli provides insight on the influences of bone growth.

1.5 Regulation of the muscle-bone unit

The development of both muscle and bone is tightly mediated by time-specific cues, including local and humoral factors. Common genetic factors seem to affect muscle tissues and bone metabolism concurrently because both osteogenic and myogenic cells are differentiated from mesenchymal cells (Kawao & Kaji, 2015). Given the common origin, many of these factors will act on both tissues simultaneously, potentially causing similar growth effects as evidenced by overlapping signaling pathways. However, the pleiotropy observed in the early stages of muscle and bone is also evident in contexts beyond development. Humoral and endocrine profiles at different stages of growth and aging have implicated effects on muscle and

bone (Kawao & Kaji, 2015) and diseases characterized by unique inflammatory profiles also have holistic effects on muscle and bone health (Brotto & Bonewald, 2015). As such, these findings suggest that factors related to the systemic and local environment play a critical role in muscle and bone health, and may concurrently act with mechanical loading to drive muscle and bone development.

1.5.1 Systemic Regulation of the muscle-bone unit

The evidence of systemic effects on muscle and bone is most evident in disease, when the systemic profile is characterized by inflammatory factors**.** Patients with chronic inflammatory states, such as metabolic syndrome or inflammatory bowel disease, can experience muscle loss that is accompanied by bone loss (Kawao & Kaji, 2015). This is due to increased levels of inflammatory mediators in their circulation, which can facilitate bone loss through the inhibition of osteoblast differentiation and enhanced osteoclastic resorption, along with the suppression of myogenic proliferation and differentiation, and increase in muscle degradation (Tagliaferri et al., 2015). This is also evident in cases of osteosarcopenia. For instance, patients with osteoporosis express a reduced level of Akt, which is a component of the IGF-1/PIK/Akt pathway. This abnormality in IGF-1 signaling may explain the negative effects experienced in muscle and bone

wasting in these patients (Kawao & Kaji, 2015). This is also exemplified during the decline of sex hormones during older age, namely estrogen and testosterone. Due to lower estrogen levels, postmenopausal women present an increased risk of developing sarcopenia and osteoporosis (Tobias & Compston, 1999). Men who suffer from androgen deficiency will also experience reductions in muscle size and strength as well as increased risk of fracture due to declines of testosterone (Mohamad et al., 2016).

A number systemic factors are produced in distant tissues; however these factors may overlap with factors that are produced by muscle and bone, which may act on each other locally. This is exemplified by animal studies which have shown that muscle activity aids in the process of fracture healing (Hamrick, 2012). When the fracture injury is covered by muscle flaps, bone repair significantly improves (Hamrick, 2012; Brotto 2015). Indeed, these studies indicate that systemic factors, whether produced locally or by distant tissues, are significantly implicated in the development of muscle and bone throughout life. Despite the cumulative evidence on systemic regulation of muscle and bone, there remains to be unanswered questions about the extent to which these systemic factors mediate these tissues, respectively. Furthermore, the scope of research on systemic regulation primarily focuses on the independent effects of these factors on muscle and bone, irrespective of each other. However, over the past decade

it has become evident that key systemic factors act on muscle and bone concurrently, as observed human, animal, and *in vitro* models. The elucidation of these systemic effects on muscle and bone relationships serves great implications on the understanding of the musculoskeletal system and pathology. A perquisite to understanding these effects is identifying key regulators for which evidence points out a significant role in muscle and bone growth, namely interleukin 6 (IL-6), insulin growth factor-1 (IGF-1), chemoligand-1 (CX3CL1), and fibroblast growth factor-2 (FGF-2).

1.5.1.1 Interleukin-6 (IL-6)

IL-6 is a widely studied cytokine that is associated with a wide range of functions. It is a single glycoprotein chain of 26 kDa and consists of 212 amino acids (Barton 1997). IL-6 can be produced by monocytes, macrophages, fibroblasts, endothelial cells, and B cells (Barton 1997). IL-6 is also considered a myokine as it can be produced by skeletal muscle as well (Pederson 2011). The receptor for IL-6 is a membrane bound heterodimer consisting of an alpha and beta subunit. The beta subunit, also known as gp130, is responsible for signal transduction. IL-6 receptors are expressed by a variety of cells, including lymphocytes, monocytes, fibroblasts, endothelial cells and pituitary cells (Barton 1997). IL-6 is also

produced by a number of tissues in the body, such as the liver, muscle, and bone (Pedersen & Febbraio, 2012).

Due to the pleiotropic nature of IL-6, the effects of this cytokine on muscle are variable (Pelosi et al., 2014). An acute increase in IL-6 will result in increases anabolic responses in muscle, which is demonstrated in vivo and in vitro studies. For example, treating satellite cells with IL-6 results into an increase in myoblast proliferation, which occurs in a dose-response manner (Otis et al., 2014). IL-6 also upregulates myogenic differentiation, namely through the JAK/STAT pathway (Muñoz-Cánoves et al., 2013). Myoblasts derived from mice that are null in IL-6 expression show reduced capacity for differentiation, indicating the importance of IL-6 in myogenic activity (Muñoz-Cánoves et al., 2013). Similarly, the knockout of IL-6 expression in mice with reduces the extent of myoblast differentiation and fusion, while the addition of exogenous IL-6 augments the expression of muscle-specific genes, supporting the myogenic function of IL-6 (Muñoz-Cánoves et al., 2013). The anabolic effects of IL-6 on myoblast proliferation and differentiation indicate the role of this cytokine on muscle regeneration and hypertrophy (Pelosi et al., 2014). In non-pathological conditions, IL-6 can also be synthesized by skeletal muscle in response to increased workload and secreted locally or into the blood stream, where it might act in hormone like manner stimulating the hepatic gluconeogenesis and

increasing adipose tissue lipolysis (Pelosi et al., 2014). However, in cases of chronic elevated IL6, studies show that this cytokine induces atrophy of muscle and impedes myogenic differentiation (Pedersen & Febbraio, 2012). These findings suggest that the effects of IL-6 on muscle are mediated by spatial and temporal interactions between this cytokine and the target tissue (Pelosi et al., 2014).

Studies show that the effects of IL-6 on bone appear to be cell-dependent. That is, the effects of IL-6 on osteoblasts and osteoclasts differ with respect to proliferative and differentiative capacities. For example, *in vitro* studies show that treating MC3T3-E1 osteoblasts with IL-6 does not influence proliferation, however, differentiation is significantly impaired by the downregulation of ALP activity and expression of osteoblastic genes such as Runx2, osterix, and osteocalcin (Kaneshiro et al., 2014). Similarly, treatment of osteoblasts will result in a reduction of OPG and mineralization of the extracellular matrix (Bakker et al., 2015; Kaneshiro et al., 2014). However, the treatment of committed osteoblastic cells with IL-6 may also promote differentiation and expression of ALP, suggesting that the effects of IL-6 on osteoblast may also be dependent on the stage of growth (Bakker et al., 2015; Bellido et al., 1997). Studies suggest that the effects of IL-6 on bone may primarily be driven by the regulation of osteoclast activity (Lombardi et al., 2016). IL6 is closely associated with expression of receptor

activator of NF-kb ligand (RANKL) in osteoblast; as such, IL-6 acts indirectly on osteoclastogensis by stimulating the release of RANKL by cells within bone tissues such as osteoblasts (Kaneshiro et al., 2014). Indeed, the treatment of osteoblast with IL-6 results in an increase in RANKL production, while IL-6 transgenic mice present with decreased osteoblast and increased osteoclasts populations (Lombardi et al., 2016). Moreover, basal concentrations of IL-6 are negatively correlated with BMD and muscle strength in post-menopausal women, indicating that chronic levels of IL-6 may simulate catabolic effects on muscle and bone (Lombardi et al., 2016).

1.5.1.2 Chemoligand-1 (CX3CL1)

CX3CL1, also known as fractalkine, is a member of the CX3C chemokine family. Chemokines are a class of cytokines that have the ability to induce migration of cells such as lymphocytes, monocytes and macrophages (Ferretti et al., 2014). CX3CL1 acts on target cells by binding to a G-protein coupled receptor, defined as CX3CR, however it has also been shown to bind to related receptors of the CX3CL chemokine family, such as CCR, CR, or CXCR (Ferretti et al., 2014). CX3CL1 exists in two forms, one that anchors to the membrane where it functions as an adhesion molecule, and another other form that sheds as a soluble chemoattractant (Strömberg et al., 2016). The adhesive molecule, which is induced on activated primary

endothelial cells, promotes strong adhesion to leukocytes, while the soluble form chemoattracts T cells and monocytes (Bazan et al., 1997). CX3CL1 expression has been reported in many cell types of hematopoietic origin (e.g. endothelial and epithelial cells, lymphocytes) and nonhematopoietic origin including neurons, microglial cells, myoblasts, and osteoblasts (Ferretti et al., 2014).

Though CX3CL1 is becoming increasingly well-known in physiological and pathological literature, the functions of CX3CL1 on myoblasts have only recently been established (Catoire et al., 2014). CX3CL1 is well known for its role in trafficking and recruiting of immune cells to muscle tissue during inflammation (Ferretti et al., 2014; Griffin et al., 2010). *In vitro* stimulation of human myoblasts with CX3CL1 induces the production of IL-8, CCL2 and IL-6, all of which are proangiogenic factors and chemotactic mediators, thereby the role of this chemokine in promoting monocyte recruitment to skeletal muscle (Strömberg et al., 2016). Downstream effects of CX3CL1 increases production of IL6, TNFa, both of which have proliferative effects on myoblasts suggesting that CX3CL1 may also act as a mitogen for these cells (Strömberg et al., 2016). In addition to immune function, CX3CL1 also acts on myoblasts to support cell adhesion ie. myotube differentiation (Griffin et al., 2010; Strömberg et al., 2016). For example, CX3CL1 stimulation of myotubes in vitro induces MMP9, which

affects collagen turnover in skeletal muscle and regulates the bioavailability of various growth factors by proteolytic cleavage of extracellular matrix molecules (Strömberg et al., 2016). CX3CL1 also plays a role in myogenesis by regulating myoblast migration and fusion, such that the deficiency of chemokine receptor-ligand can impair terminal differentiation (Griffin et al., 2010). This is also further supported by the increased upregulation of CX3CL1 and associated chemokines during different phases of myogenesis

Similarly, the role of CX3CL1 in bone physiology is increasingly studies in clinical literature assessing the pathology of arthritis (Matsuura et al., 2017). Similar to myoblasts, CX3CL1 also mediates migration of bone cells, specifically osteoclast precursors. In vitro stimulation of a co-culture bone marrow derived mononuclear cells (which are osteoclast-like) and murine osteoblasts with CX3CL1 stimulates paracellular and transcellular migration of osteoclast-like cells across the osteoblast monolayer (Koizumi et al., 2009; Matsuura et al., 2017). In addition to cell migration, CX3CL1 also stimulates osteoclastogenesis. Neutralization of CX3CL1 inhibits osteoclastogenesis in vitro (Koizumi et al., 2009; Matsuura et al., 2017) , while resulting in significant increases in trabecular and cortical bone thickness in CX3CL1-deificient mice owing to reduced number of osteoclasts and osteoid formation. Studies with CX3CL1 deficient mice also

show an downregulation of osteoclast-mediating markers in osteoblasts such as NF-kb, TRAP5B, MMP3 and MMP13, thereby providing further evidence for the effects of this chemoligand on bone resorption (Hoshino et al., 2013). CX3CL1 also influences osteoblast directly. CX3CL1 is also significantly involved in the early stages of osteoblast differentiation, whereas expression of this ligand is reduced at later stages. The expression of this ligand and associated receptors also varies in bone tissue, specifically across trabecular and cortical bone, where expression is highest in trabecular. Similarly, in cultured MC3T3E1 cells, CX3CL1 and its receptor are highly expressed in the pre-confluent state, but is downregulated in their subconfluent state and in later stages, suggesting that CX3CL1-CX3CR1 axis plays role in an early stage of osteoblast differentiation (Hoshino et al., 2013). Cultures of CX3CL1 deficient osteoblasts also show reciprocal changes in osteoblast differentiation markers, leading to spatiotemporally disordered bone matrix deposition that eventually results in impaired deposition (Hoshino et al., 2013).

1.5.1.3 Insulin growth factor-1 (IGF-1)

IGF-1 is a hormone that belongs to insulin related family. The name of IGF-1 comes from the resemblance to proinsulin on its discovery in 1975 from the extraction of human serum (Laron, 2001). IGF-1 is a small protein with

a weight of 70 amino acids and 7649 Da. IGF-1 is produced by many tissues, however the majority of IGF-1 is produced by the liver (Laron, 2001). IGF-1 is primarily mediated by growth hormone, which a potent stimulator of IGF-1 secretion and action. GH is produced by the pituitary gland, and acts on the hepatocytes in the liver to synthesize and release IGF-1. Circulating IGF-1 then inhibits the further release of GH from the pituitary gland, thereby completing a negative feedback loop. The GH-IGF-1 axis is the principle endocrine system that regulates the development of growth plates on bones and linear growth in children by activating the MAPK and PI3K pathway (Blum et al., 2018). However, it is important to note that IGF-1 can also act in an independent manner from GH (Blum et al., 2018). Furthermore, IGF-1 can be produced locally or secreted into the systemic environment. At least 90% of systemic IGF-1 is bound to proteins known as IGF-1 binding proteins. These proteins mediate the bioactivity and availability of free IGF-1 to tissues, and thus play a major role in mediating IGF-1 muscle and bone relationships. To date, six BPs have been identified, with IGFBP3 being the most abundant. The growth promoting effects of IGF-1 are primarily mediated via interactions with the type 1 IGF-1 receptor (Tahimic et al., 2013). The IGF-1 receptor is a heterodimer composed of two extracellular spanning alpha and transmembrane subunits. The IGF-1 receptor is expressed in almost all tissues, including muscle and bone.

IGF-1 has many mitogenic effects on muscle growth. IGF-1 increases expression of myogenic factors, as it has been demonstrated in animal studies through the increased expression of MRFs and Pax7 (Yu et al., 2015). Consequently, IGF-1 signaling have been implicated in the mediation of satellite cells, contributing to both their proliferation and differentiation (Philippou et al., 2009). IGF-1 also downregulates inhibitors of myogenic activity, such as myostatin (Yu et al., 2015). IGF-1 acts on muscle through the PI3K and MAPK pathways, which are involved in cell cycle progression and cell survival. IGF-1 is also critical for muscle regeneration, by activating satellite cell proliferation, increasing protein synthesis, and promoting differentiation as observed with in vitro and in vivo studies (Ascenzi et al., 2019; Philippou et al., 2009; Yu et al., 2015). Expression of IGF-1 induces muscle hypertrophy in childhood and adulthood, and maintains of muscle mass and functionality during aging and in animal models of neuromuscular disease (Yu et al., 2015). The actions of IGF-1 on muscle may vary depending on whether they are local or systemically induced. Indeed, the exogenous administration of IGF-1 in animal models increases body weight and muscle mass, while augmenting skeletal myoblast proliferation in vivo (Yu et al., 2015).

The roles of IGF-1 on bone development are multifaceted. In vitro application of IGF-1 in osteoblast cultures stimulates survival, proliferation,

differentiation, and matrix production (Tahimic et al., 2013). Osteoblast efficiency, osteocyte survival, and maturation is also improved with the treatment of IGF-1 in vitro. Due to the nature of osteoblast-osteoclast coupled function, the loss of IGF-1 can negatively influence osteoclasts by reducing both osteoclast proliferation and osteoblast mineralization in culture (Tahimic et al., 2013). The importance of IGF-1 on cellular activity is also exemplified by the reduction in osteoblast and osteoclast numbers upon the deletion of IGF-1 gene. Downstream effectors of IGF-1 have been shown to play critical roles in osteoblast and osteoclast function. For example, deletion of insulin related substrate (IRS) genes and Forkhead group of transcriptional factors (Fox), both of which act downstream to IGF-1, results in increased apoptosis of osteoblasts and reductions in bone mass (Rosen, 1999). mTOR, which is also a downstream target of IGF-1 through the PI3-Akt pathway, regulates a number of processes critical for bone, including osteoblast differentiation and matrix formation (Rosen, 1999). The activation of mTOR in vitro leads to an increase in osteoblast differentiation markers like osteocalcin and osteoblast transcription factors such as Runx2, while the treatment of rapamycin, an mTOR inhibitor, inhibits these processes (Rosen, 1999). The effects of IGF-1 skeletal growth is also demonstrated in animal and human studies. For example, IGF-1 mutations and in humans result in notable phenotypes characterized by smaller

skeleton, severe osteopenia of the lumbar spine, reduced mineralization and fewer trabeculae (Tahimic et al., 2013). A global knockout mouse model results in dwarfism phenotype, a hypomineralized skeleton, and growth plate defects characterized by reduced chondrocyte proliferation and differentiation and increase chondrocyte apoptosis (Tahimic et al., 2013). Bone abnormalities are also implicated in interruptions of IGF-1 receptors, which is exemplified by severe reductions in bone formation following deletion of the type 1 IGF-1 receptor in vivo (Rosen, 1999).

1.5.1.4 Fibroblast growth factor-2 (FGF-2)

FGF-2 was amongst the first to be isolated from the brain and pituitary gland in 1979 (Yun et al., 2010).The FGF family spans a series of 18 factors which are grouped in 6 subfamilies based on differences in sequence homology and phylogeny (Beenken & Mohammadi, 2009). FGF-2 belongs to the FGF1 subfamily (comprising of FGF1 and FGF2), 'which lack classical secretory signal peptides but are nevertheless readily exported from cells by direct translocation across the cell membrane (Ornitz & Itoh, 2015). The mechanisms by which FGF-2 transits through the cell are poorly understood, but are thought to require binding to and activating cell surface tyrosine kinase fibroblast growth factor receptors (FGFRs) located chiefly on the cell surface, with heparin/HS as a factor and interaction with HSP90

(Ornitz & Itoh, 2015). The high affinity that FGF-2 has for heparin/HS allows this factor to act in a localized manner near its source of expression (Beenken & Mohammadi, 2009). FGF-2 binding activates downstream events primarily with the phospholipase C, RAS/MAP kinase pathway, and PI3K-Akt pathway (Yun et al., 2010). FGF-2 is widely expressed in cells and tissues of epithelial and mesenchymal origin, and regulates many key cell behaviours such as proliferation, migration, differentiation, and survival (Yun et al., 2010). Most notably, FGF-2 plays a key role in mediating angiogenesis by regulating proliferation and epithelial cells and inducing mitogenesis of smooth muscle cells and fibroblasts towards the development of large collateral vessels with adventitia. Other physiological roles of FGF-2 include tissue regeneration and repair and mediating biological activities of other cell types including muscle and bone (Yun et al., 2010). The most abundant FGFs that show paracrine activity are FGF-1 and FGF2 (Granchi et al., 2013).

FGFs are abundantly expressed in skeletal muscle. The roles of FGF-2 on skeletal muscle have been studied extensively with respect to muscle repair (Kästner et al., 2000). In vitro studies assessing targeted gene delivery systems on wound healing show that FGF-2 enhances the muscle angiogenic response and subsequently arteriogenesis (Doukas et al., 2002). These findings are similar to an *in vivo* study in which impairment of

the FGF-2 gene in rat skeletal muscle causes ischemia and delayed wound healing (Liu et al., 2006). Furthermore, positive correlations are found between the levels of FGF-2 expression and speed of muscle regeneration (Liu et al., 2006). FGF-2 is also reported to significantly influence satellite cell activation and proliferation during embryogenesis and post-natal development (Yun et al., 2010). This is demonstrated by the ability of FGF-2 to stimulate inactive satellite cells to enter the cell cycle, which has been observed in cultures extracted from young and old rats, respectively (Yun et al., 2010). In addition to satellite cells, FGF-2 stimulates the proliferation of cultured myoblasts and multipotent cells, and reduced concentrations yields fewer myogenic progenitor cells (Dyke & Suzuki, 2014). The proliferative role of FGF-2 is also observed in pathological contexts, as observed with Duchenne muscular dystrophy by a four-fold increase in proliferation of cultured satellite cells from mdx mice (Bizario et al., 2009). Although FGF-2 is a potent proliferation agent, there are controversies regarding the role of FGF-2 in myogenic differentiation (Yun et al., 2010). FGF-2 promotes early differentiation of multipotent cells, and stimulate the expression of myogenic specific markers such as MyoD and myogenin in comprised tissue cultured treated with dexamethasone (Adhikary et al., 2019), however it will also inhibit terminal differentiation of embryonic satellite cells and myoblasts (Dyke & Suzuki, 2014; Liu et al., 2006)

Moreover, FGF2 inversely modulates the expression of myostatin in skeletal muscle cells, suggesting the importance of this factor in muscle hypertrophy (Adhikary et al., 2019).

Similar to the effects on muscle, FGF-2 induces osteoblast proliferation (Naganawa et al., 2006). Studies utilizing MC3T3E1 cells and bone mononuclear cells (BMNCS) report increased osteoblast proliferation through Brdu incorporation (Adhikary et al., 2019; Takei et al., 2015). Moreover, FGF-2 also promotes differentiation by upregulating osteogenic transcription factors for bone morphogenic protein-2 (BMP2), Runx2, and osteocalcin. This is also observed in pathological contexts where the administration of FGF-2 to osteoblast cultures after dexamethasone treatment leads to an upregulation of BMP expression and bone nodule formation (Adhikary et al., 2019; Takei et al., 2015). Osteogenic effects of FGF-2 on BMNCs is also exemplified by the upregulation of Wnt/b-catenin pathway upon treatment. One study demonstrates that the application of FGF-2 to MC3T3E1 does not induce mineralization effects, however the co-treatment with vitamin D it does lead to mineralization (Yun et al., 2010). This suggests that FGF-2 can be incubated with something else to make something good! The osteogenic properties of FGF-2 are also evident using in vivo studies. FGF-2 can reverse osteopenia in ovariectomized mice by modulating the trabecular and cortical microarchitecture of long bones and

vertebra (Adhikary et al., 2019). Similarly, mice that are FGF-2 present a phenotype characterized by decreased bone mass and bone formation. Clinical studies also show that low levels of FGF2 were indicative of poor recovery. These findings suggest the important of FGF-2 on physiology of bone (Granchi et al., 2013).

1.6 Effects of exercise on systemic regulation of muscle and bone

Exercise is described as a planned, structured and repetitive movement of the human body. The benefits of exercise on muscle and bone health are reported extensively in the literature, and include effects such improved bone mineral density, increased muscle hypertrophy, and improved muscle and bone strength (Banfi et al., 2010; Brotto & Bonewald, 2015). Exercise is also associated with a wide array of systemic changes, many of which are associated with the effects of exercise on muscle and bone (Hamrick, 2012). These changes include mobilization of factors, ligand secretion, clearance, and changes in receptor binding densities (Alon Eliakim & Nemet, 2010; Lombardi et al., 2016; Timothy P. Scheett et al., 1999a). These changes may be stimulated by the body's endeavour to meet the energy demands of exercise (ie. increased heart rate, breathing, vascularization) or are consequently caused by the exercise stimulus (ie. microdamage in the muscle). As a result, the nervous, endocrine, and

immune system, and circulatory system work collectively to generate these dynamic systemic changes (Gibala & McGee, 2008)

One of the most common approaches to assessing exercise-induced systemic changes has been the enzyme-linked immunosorbent assay (ELISA). ELISA is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones from various bodily samples such as serum, plasma, saliva, or urine (Wacharasindhu et al., 2002). Given their sensitive and versatile application, ELISAs are an accurate and practical means to gather proteomic information on subjects before and after performing bouts of exercise from serum or plasma, thereby providing insight on the effects of exercise on the systemic environment (Mezil et al., 2015). One of the early systemic regulators of muscle and bone to be assessed in the context of exercise is IL-6. Studies in children and adults show that IL-6 generally increases rapidly after exercise, returning back to baseline within hours after the exercise bout (Pedersen & Febbraio, 2012). The response of IL-6 to exercise however can vary depending on other factors inherit of the exercise and individual (Petersen & Pedersen, 2005). In general, longer exercise durations and greater exercise intensity result in a larger increase in IL-6 (Edwards et al., 2006).

The understanding of how IL-6 behaves in response to exercise provides researchers with an improved understanding of the general functions of this cytokine. However there remain to be many gaps in terms of how this cytokine behaves in certain populations, particularly those with variable levels of fitness, and oppose sexes, and exercise durations, which leads to questions about the impact of IL-6 on muscle and bone in these situations. There is even a greater scarcity in research looking at other systemic regulators, namely IGF-1, FGF-2, and CX3CL1, which have not been studied as extensively with respect to exercise. As such, their roles in muscle and bone remain unclear. Furthermore, there are also multiple factors to take into consideration when assessing the effects of exercise on these systemic regulators, such sex, ethnicity, and development.

1.6.1 Sex

Currently there is limited research on the sexual dimorphisms of the systemic response to exercise (Gillum et al., 2011). At homeostatic levels, there have been conflicting findings of IL6 levels in men and women. No differences in IL-6 are detected in men and women after a 1.5hr marathon or cycling at 55%, 75%, and 85% VO2peak (Gillum et al., 2011). However, one study by Edwards et al. 2005 documents the effects of maximal exercise on IL-6 concentrations in 12 men and 12 women (Edwards et al.,

2006). The study shows that women experience higher IL-6 concentrations following a maximal exercise protocol. In addition, circulating IL-6 peaks at 30 minutes in men into recovery, while IL-6 in women continues to increase after this time (Edwards et al., 2006). These discrepancies are possibly due to differences in methodological approaches, such as variances in oral contraceptive use amongst participants. Indeed, the use of oral contraceptives is shown to increase the IL-6 response to exercise, particularly during the follicular phase (Timmons, 2005).

During pre-pubertal stages, there are no significant differences in serum IGF1 between boys and girls (Yüksel et al., 2011). During adolescence, female circulating IGF-1 tend to be slightly higher than their male counterparts (Yüksel et al., 2011). On the contrary, exercise studies show discrepant findings between males and females. Two studies utilizing the same 5-week endurance training protocol for boys and girls show that IGF-1 decreases in girls, while no changes are observed in boys (Alon Eliakim & Nemet, 2010; T. P. Scheett, 2002). The exercises ranged between running, jumping, and competitive sports and involved different intensities and durations. In adulthood, basal concentrations of systemic IGF-1 is lower in women than men despite higher growth hormone production (Alon Eliakim et al., 2014). In the context of exercise, free IGF-1 reaches peak concentrations earlier in young adult females than males. Free IGF-1

increases at a higher fold in men than women with exercise, this is possibly due to the availability of marginal pools of systemic IGF1 available in men than women (Alon Eliakim et al., 2014).

Unlike IL-6 and IGF-1, research on sexual dimorphisms in exercise-induced responses of FGF-2 are limited. At basal conditions, there are no differences in circulating FGF-2 between boys and girls (Granchi et al., 2013), however in adulthood, women express higher circulating levels of FGF-2 in comparison to men (Larsson et al., 2002). During aerobic exercise, postmenousal women do not experience changes in circulating FGF-2 (Brenner et al., 2019), while FGF-2 declines after one bout of anaerobic exercise in older men (Amir et al., 2007) and unilateral wrist flexion exercise in young healthy men (A. Eliakim et al., 2000). FGF-2 also declines in young men following a bed rest protocol coupled with resistance exercise (Clarke et al., 1998). Similarly, the effects of exercise on circulating CX3CL1 levels are scarce and nonexistent in children. An acute bout of endurance exercise on a cycle ergometer at 50% maximal wattage increases mRNA and plasma levels of CX3CL1 middle-aged men, however no changes on observed after 12 weeks of training (Catoire et al., 2014). Similarly, one study in middle-aged women reports no changes in circulating CX3CL1 after 6-months of aerobic exercise (Verheggen et al., 2016). The differences in exercise protocols and samples used in these studies poses a challenge to make

conclusive remarks sexual dimorphisms in exercise, as it seems that this factor is more sensitive the level of strain and impact of the exercise.

Differences between males and females in the systemic response to exercise is linked to the differences in their sex hormones. Estrogen has the capacity to mediate several systemic factors including cytokines and growth factors. For example, estrogen suppresses IL-6 in women by increasing the expression of suppressors of cytokine signaling proteins (SCOS) (Casazza et al. 2010). Indeed, IL-6 concentrations are found to be highest in women when estrogen concentrations are the lowest, reflecting an inverse relationship between these factors. Although estrogen may not appear to have anabolic effects per se, it influences the catabolic effect of proinflammatory cytokines. Similarly, estrogen lowers IGF-1 concentrations by acting directly on the hepatic production of IGF-1 and/or inhibiting signaling of growth hormone in a dose-dependent manner (Casazza et al. 2010). These effects of estrogen can impact muscle and bone by mediating their growth and repair. Moreover, estrogen is shown to increase the mechanical sensitivity of bone by acting directly on estrogen receptor-α (ER α) strain response pathways in osteoblasts (Tobias 2003). The knockout of estrogen as demonstrated in murine studies results in lower indices of muscle and bone, supporting the role of estrogen in muscle and bone health (Tobias 2003). This is also further supported by trends of

muscle and bone loss occurring during menopause, a period characterized by a decline in estrogen and therefore protective effects on muscle and bone.

1.6.2 Development

Systemic levels of IL-6 increases as a function of chronological and biological age (Timmons, 2005). Studies investigating the IL-6 response to cycling show that children's responses are approximately 50% lower than what is reported in adults under similar exercise conditions (Timmons 2005). IL-6 peaks in children and is generally followed by a quick recovery, however adults exhibit elevated levels of IL-6 for longer durations than children (Timmons et al., 2006). The difference in the IL-6 response in children and adults is most notably linked to differences in physical attributes, such lean body mass. Given that contractile skeletal muscle is an important source of IL-6 production (Pedersen & Febbraio, 2012), the greater muscle mass observed in adults may explain why children exhibit lower concentrations. Additionally, differences in metabolism may explain alterations in IL-6. For example, children preferentially oxidize fat rather than carbohydrate as a source of endogenous fuel during exercise, which reduces the demand for circulating IL-6 during exercise (Rubin et al., 2014; Timmons, 2005).

Given the association of IGF-1 with growth hormone, the role of this factor in of development has been studied extensively in children and adults (Timothy P. Scheett et al., 1999b). Circulating levels of IGF-1 increase slowly with age in childhood, reach a peak level at puberty, and decrease with age thereafter (Yüksel et al., 2011). With exercise, however, circulating levels of IGF-1 in children tend to decrease or remain unchanged. As described earlier, circulating levels of IGF-1 decline or show no change after a 5-week endurance training program in pre-pubertal children (Timothy P. Scheett et al., 1999b). This decline is also observed in adolescent boys after single acute bouts of high intensity exercise (Nemet, Oh, et al., 2002). This is generally coupled with an increase in pro-inflammatory cytokines, such as IL-6 and TNF-α, which has been used to explain the catabolic effects of exercise in children (Nemet, Oh, et al., 2002). In adults, however, the total IGF-1 response has been less consistent (Amir et al., 2007; A. Eliakim et al., 2000). No change in IGF-1 is observed in males after a 30s sprint or 30 minutes of cycling (Frystyk, 2010; Wallace et al., 2000), while an increase in IGF-1 is observed in healthy 67-80 year-old men after strength training exercise (Berg & Bang, 2004; Frystyk, 2010) and in young trained males after a Wingate test (Alon Eliakim & Nemet, 2010) These findings indicate that the IGF-1 response to exercise is adults is reliant on other factors such as fitness levels and exercise frequency (ie. acute vs training). These

research findings are primarily established in males as there are fewer studies investigating the IGF-1 response to exercise in females. One study using resistance training in healthy young women documents an increase in IGF-1 following one bout of resistance exercise (Gregory et al., 2013). In addition to acute effects, IGF-1 levels were higher at 8 weeks of resistance training relative to baseline levels (Gregory et al., 2013), which is contrary to another study that reported no change in IGF-1 after 16 weeks of aerobic exercise in young sedentary women (Arikawa et al., 2010). Furthermore, many of these findings remain inconclusive because total IGF-1 was usually measured. Additional research is required to characterize the effects of exercise in adults, particularly in females.

During growth, arteriogenesis increases in children to facilitated physical development. Consequently, children express higher concentrations of circulating FGF-2 in comparison to adults at resting conditions (Przewratil et al., 2010). The increase in FGF-2 observed in children is also linked to increased rates of bone turnover and skeletal muscle remodeling that is also associated with physical development (Granchi et al., 2013). Surprisingly, there are no studies that examine the effects of exercise on circulating FGF-2 levels in children. The majority of pediatric literature with respect to FGF-2 focuses on clinical populations given the association of this growth factor with various conditions such as

chronic kidney disorders, autism spectrum disorder, and cancer (Esnafoglu & Ayyıldız, 2017; Przewratil et al., 2010). Furthermore, FGF-2 levels in healthy controls were collected from male subjects, and circadian rhythms were not considered (Esnafoglu & Ayyıldız, 2017). Although research on the FGF-2 response to exercise in adults is also scarce, some findings that show FGF-2 declines with exercise. For example, a decrease in FGF-2 in older men and women is observed after 45 minute and 3-hour bouts of moderate and high intensity exercise of varying modalities, including kayaking, running, and hiking (Bruserud et al., 2005). However, another study shows that circulating FGF-2 increases after a 10-minute bout of unilateral wrist flexion in young men and women (Nemet, Hong, et al., 2002). FGF-2 concentrations were elevated in serum collected from both arms, ruling out local production of FGF-2 (Nemet, Hong, et al., 2002). More studies are required to clarify the FGF-2 response to exercise, especially in children in order to understand to role of this factor in development.

The two studies that examine the effects of exercise on CX3CL1 in adults report that this chemokine increases after an acute bout of exercise, while no changes are observed after long term training (Catoire et al., 2014; Verheggen et al., 2016). No studies have yet to measure circulating CX3CL1 in children at resting or exercise conditions.

1.6.3 Ethnicity

In addition to sex and development, ethnicity may also influence the response of systemic regulations of muscle and bone. An acute bout of running shows that both Caucasians and African Americans present with an increase in pro-inflammatory cytokines, with African Americans demonstrating higher concentrations of circulating IL-6, IL-1-, IL-4 and IL-1 (Starzak et al. 2015). Another study using a similar running protocol found no differences in circulating cytokines (McKenzie et al. 2014). The first study was conducted in young male athletes while the second was conducted in young untrained females, possibly indicating an interaction between differences in ethnicity, sex, and training status. However, that African Americans can demonstrate higher inflammatory responses in comparison to Caucasians is also consistent with other physiological contexts, such as disease susceptibility (McKenzie et al. 2014).

Although there is a lack of studies that assess systemic profiles in children of varying ethnicities, there is evidence to support variations on bone growth and development. For example, in a study of 748 pubertal girls, it was shown that Asian girls had lower BMC and BMD than Caucasian and Hispanic girls at all sites, whereas bone mass of Hispanic girls was generally similar to that of Caucasian girls (Weaver et al. 2007). Similar trends were also observed in another cohort study of 423 boys and girls,

where Asian girls presented with significantly lower femoral neck BMD, total BMD, and total BMC/height ration than Hispanic and Caucasian girls. It seems that ethnicity influences BMD by influencing bone size, however, as Weaver et al. (2007) states it is also possible that other factors may contribute to these findings, such as differences in nutrition and levels of recreational physical activity.

Table 1: The effects of short- and long-term exercise on systemic regulators of muscle and bone in children and adults

Authors	Systemic regulators	Sample	Exercise	Outcome	Time points	Results
Shojaei et al. 2011	IL-6, CRP, and IL10	10 healthy, active men, mean age 21±1.2 yr	45 minutes moderate intensity cycling at 50% VO_2max	S	Immediately before/after exercise	↑ IL-6, CRP, and IL-10 immediately after exercise.
Stromberg et al. 2016	CX3CL1, CCL2, and CCL22	17 moderately active males and females. divided in exercise group (n=12) and control (n=5).	1-h of cycle exercise consisting of 20 min at a workload corresponding to 50% VO2max and 40-min at a work load corresponding to 65% of VO2max, while control group rested.	B	Baseline,30 min, 2h, 6h and 24 h after cycling/resting.	↑ CX3CL1 at mRNA levels (30 min post-exercise) and protein levels (2-hr post) in human skeletal muscle after one bout of exercise. CX3CL1 returned to resting at 24-hour post. No difference between males and females in mRNA levels.
Scheett et al. 1999	IGF-1, TNF α, IL1-β, IGF-1 IGFBP1, IL-6, IL-1rα, IL-4, and IL-10	17 healthy children, mean age 9.7±1.2 yr (4 F). Tanner 1-2	1.5-h soccer practice (40 min constituted of vigorous aerobic type exercise).	P	Baseline and within 15-min after soccer practice	↑ TNF-α, IL-6, and IGFBP1 after exercise ↓ IGF-I after exercise

S = serum, P = plasma, B = muscle biopsy

Scheett et al. 2002	VO2 peak, IGF-1m IGFBP 1,2, and 3, GHBP, TNFα, IL1-β, IL-6, and IL1rα	29 healthy pre-pubertal and early pubertal boys (8-11 years); 8 Tanner 1, 18 Tanner 2; randomized into control (n=14), and training (n=12)	Control – four 45-min sit down courses; training group – 90 min of aerobic type activity interspersed throughout the 3 h each day, 5days a week for 5 weeks. Activities included: sports drills (football, rugby, soccer, etc). and games such s running, jumping, aerobic dance. No control of physical activity beyond study.	S	Baseline and 2-3 days after completion of training intervention	GHBP was (+) correlated with body weight, BMI percentile, and negatively correlated with normalized peak VO_2/kg. (+) correlations were also found between IGF-1 and peak VO_2, whereas IL1rα was (-) correlated with normalized peak VO_2/kg (baseline). ↓ IGF1 and IGFBP3 post-training ↑ IGFBP2, TNF-α, IL1-β post-training No change in IL-6 and IGFBP2
Eliakim et al. 2014	IGF-1, GH, blood lactate	Young men (12) and women (16), age range 24-34 years. Participants were noncompetitive athletes, training approximately 8 hours a week Females exercised in the follicular phase	Wingate anaerobic test (WAnT). Each participant cycled 30 seconds against constant resistance. Resistance was set to 0.05 kg per female participant's kilogram body weight and 0.06 kg per male participant's kilogram body weight.	S	Baseline, and 20, 30, 40, and 60 minutes after exercise	↑ GH in both men and women after WAnT Peak exercise GH was greater in women than men Post-exercise GH peak occurred significantly earlier in females Levels returned to baseline values 60-minutes post exercise

S = serum, P = plasma, B = muscle biopsy

						↑ IGF-1 after exercise (in males)
Eliakim et al. 2001	GHBP, IGF-1, IGFBPs 1-6	Pre and early pubertal 39 girls 8-10 years Tanner 1 and Tanner 2. Summer school program Randomized to a control (20) and training group (19)	45-minute endurance type training for 5 consecutive weeks. Training included running, jumping, aerobic dance, competitive sport. Intensity varied throughout the week.	S	Baseline and 5 weeks after the beginning of the intervention	IGF-1 (+) correlated with peak VO_2 and muscle volume IGFBP2, 4 and 5 were also (+) correlated with muscle volume ↑ IGF-1 in control but not trained subjects ↓ IGFBP3 and GHBP in the training group
Eliakim et al. 2001	IGF-1 and FGF-2, GH, and IGFBP3	10 healthy adult males (20-36 years)	Unilateral wrist flexion of the nondominant arm, 10 minutes	S	Baseline, 5 min into exercise, at the end of exercise, and every 10 minutes leading up to 50 min of recovery	↓ FGF-2 immediately after exercise in both arms ↑ IGFBP3 both arms immediately after exercise ↑ IGF-1 levels in both the resting and exercising arm at 10 minutes into recovery ↑ GH 20 minutes after exercise in both resting and exercising arm IGFBP3 returned to baseline in exercising arm but was significantly higher

S = serum, P = plasma, B = muscle biopsy

						in resting arm 40 minutes into recovery.
Nemet et al. 2002	Total IGF1, bound IGF, unbound IGF1, and insulin	11 healthy adolescent boys aged 14-18 years	A single 1.5 hr wresting practice session	S	Blood was collected before and after the session	↑ TNFα, IL1 β, IL1-r α, and IGFBP-1 increased after exercise ↓ Total IGF-1, bound IGF-1 and insulin after exercise No change in unbound IGF-1
Nemet et al. 2002	IL-6, GH, FGF2, VEGF2	15 men and 8 women, 22-36 years	Unilateral wrist flexion of the nondominant arm, 10 minutes	S	Baseline, end of exercise, 10, 20, 30, 60 and 120 minutes after exercise	↑ GH and VEGF after 20 minutes of exercise in both arms ↑ IL-6 increased significantly after exercise and there was no difference between exercising and resting arm ↓ FGF-2 with exercise
Edwards et al. 2005	IL-6	12 male and 12 female (9 of which were on birth control) recreationally active adults, 20-27 years	Cycling at 70-80% of peak power for 4 minutes, followed by cycing at 50% peak power for 25 minutes	P	Baseline, immediately after exercise, 30 min, 60 min after completion	↑ IL-6 immediately after maximal but not submaximal exercise Compared to control, ↑ IL6 increased at 30 and 60 min after both exercise tasks Il6 response was greater in women than in men 60 min after maximal exercise

S = serum, P = plasma, B = muscle biopsy

Brenner et al. 2019	FGF-2, VEGF	386 previously inactive but healthy postmenopausal women aged 50-74 years of age	Women were randomized to 150 or 300 minutes per week of aerobic exercise at 65-75% of maximal heart rate reserve for 12 months.	S	Blood samples were drawn as baseline, 6 and 12 month and after a 24 hour of complete abstinenence from exercise and alcohol intake and at least 10 h since last food intake	↑ FGF-2 after 12 months of exercise in higher fitness at baseline levels No differences in growth factor levels related to increasing doses of exercise
Amir et al. 2007	IGF-1, FGF-2	24 healthy older males, 12 higher fit and 12 lower fit; 57-60 years	30 second all-out Wingate anaerobic test; 40 g/kg body weight	S	Samples collected before, immediately after, and 50 min into recovery	Pre-exercise IGF-1 was lower, and FGF-2 was higher in males who were more fit. ↓ FGF-2 following exercise, in both groups ↑ IGF-1 following exercise in fit males
Catoire et al. 2014	CX3CL1, CCL2	30 male subjects, 59+-3.7	12 subjects did a 1 h one legged acte endurance exercise intervention and 18 males did a 12 week exercise training intervention	B, P	Before and after acute exercise or exercise training	↑ CX3CL1 and CCL2 after exercise training No change in plasma iL6
Verheggen et al.	IL-6, CX3CL1	10 healthy women, 48-50 years	6 months of aerobic exercise (cycling) training	P	Baseline and 6 months after exercise	Exercise training did not change plasma levels of cytokines in both groups (CX3CL1 and IL-6)
Gregory et al. 2013	IGF-1, IGFBP1, IGFBP2, and IGFBP3, GH, total IGF-1 and free IGF-1	46 women, 20-22 years	Women were randomized into endurance training, resistance training, and combined training or control for 8 weeks	S	Blood samples were obtained at rest, after the third set and immediately postexercise and at 15 min and 30 min after	↑ all factors except free IGF-1 after resistance exercise ↑ total IGF-1 and ↓ IGFBP1 after 8 weeks of

S = serum, P = plasma, B = muscle biopsy

					exercise on week 0, and again on week 8	training in resistance and combination exercise
Arikawa et al. 2010	IGF-1, IGFBP3	319 sedentary women; 18-30 years, no oral contraceptives;	16 weeks of aerobic exercise; 30 minutes of weight bearing aerobic exercise, 5 times per week; exercise intensity increased weekly to reach 80-85% HRmax	S	Blood was collected at baseline and 16 weeks	↓ IGFBP3 in controls and ↑ IGFBP3 in exercisers at the end of 16 weeks No effects on other IGF-1 proteins
Bruserud et al. 2005	FGF-2	14 Healthy young athletes (all males, 18 years) and elderly individuals (9 M, 11 F, 68-88 years)	Young – 45 minute activity with heart rate range of 178-184 beats per min (kayaking, running); old- mountain walks	S	Before and within 15 minutes after the activity	↓ FGF-2 after 45 min of intensive physical activity in young athletes ↓ FGF-2 after a 3-h mountain walk in the elderly

S = serum, P = plasma, B = muscle biopsy

1.7 Systemic Effects of Exercise and the Muscle-Bone Unit

Studies assessing the systemic effects of exercise highlight that there are number of key mediators that are involved in the exercise response, and that IGF-1, IL-6, CX3CL-1, and FGF-2 can exhibit significant responses which are reliant on factors such as sex and development. These factors may translate their effects onto myoblasts and osteoblasts, as demonstrated by *in vitro* and *in vivo* studies. Given the significant systemic responses observed in children and adults, and the chemical responsiveness of muscle and bone to the systemic environment, it can be postulated that the exercise effects observed in muscle and bone are mediated partly by these systemic regulators. Indeed, clinical studies provide evidence that in which alternations of systemic factors are highly associated with particular conditions (such as FGF-2 and cancer, IGF-1 and diabetes, etc) (Arikawa et al., 2010). The dysregulation observed in these conditions highlights the importance of systemic involvement on muscle and bone health.

To assess the systemic effects of exercise on muscle and bone, it is important to acknowledge the responsiveness of both tissues to mechanical loading (Brotto & Bonewald, 2015). Muscle and bone are constantly loaded with mechanical forces to perform basic locomotive functions, however these loads can range from high mechanical forces to those that impose

smaller strains on muscle and bone (Tagliaferri et al., 2015). Similarly, exercise may involve high impact loads which will expose muscle and bone to great degree of mechanical loading, or low impact which will produce a lesser degree. Examining the systemic effects of exercise using a high impact protocol, such as running or plyometrics, may present as a challenge as the effects of the exercise on muscle and bone may are likely to be an amalgamation of high mechanical loads and systemic regulation. Thus, in order to adequately assess the systemic regulation of muscle and bone, understanding the effects of low impact exercise provides the opportunity to assess this while minimizing mechanical loads.

1.7.1 Low-impact exercise and the muscle-bone unit

Low impact exercise involves motion that typically places less mechanical stress on the body and reduce oppositional forces on the joints (Abrahin et al., 2016; Guillemant et al., 2004). Low-impact exercises such as swimming and cycling are highlighted as particularly beneficial exercises for patients with joint abnormalities given the minimal weight bearing involved, which translates into less wear-and-tear on the joints and connective tissue (Abrahin et al., 2016). However, the assessment of the effects of these exercises n younger, healthy populations yield discrepant results and are generally less favourable towards these exercises for improving muscle and

bone strength. In a systematic review of 10 studies, 9 showed that professional cyclists presented with low levels of bone mineral density (BMD), and another 18 studies reported that low-impact exercise does not cause positive effects on BMD (Abrahin et al., 2016). Although cycling is associated with increased muscle strength of the thigh muscles due to pedaling activities, researchers report that this is possibly linked to training effects (Abrahin et al., 2016). Similarly, though not to the same extent, researchers report that muscle hypertrophy is delayed in cycling relative to high-impact activities (Ozaki et al., 2015). These findings lead researchers to conclude that low-impact exercise does not cause positive effects on muscle or bone relative to sedentary controls, and/or that the benefits of exercise on muscle and bone is achieved primarily from high mechanical loads. However, there are multiple indices that reflect muscle and bone strength that go beyond BMD and hypertrophy (Gómez-Bruton et al., 2013), such as bone quality and muscle function (Rauch et al., 2004). Indeed, this is discussed in a systematic review assessing the effects of swimming on bone health using multiple indices of bone health, including BMD and quantitative computerized tomography qCT (Gómez-Bruton et al., 2013). Based on the analysis of 64 studies, the researchers report that swimmers have a higher bone turnover than controls, resulting in a different structure which in turn results in higher resistance to fracture indexes despite lower

values in BMD (Gómez-Bruton et al., 2013). Similar findings were reported in another study assessing the effects of exercise of various mechanical loads (swimming, soccer, volleyball, and body building) on markers of muscle differentiation (Vitucci et al., 2018). Specifically, exercise-induced serum from trained participants was used to treat human myoblasts. Based on the findings of the study, serum from aerobic exercised subjects (swimming) had a greater positive effect on late differentiation marker (MyHC-B) expression than serum from the other exercises (Vitucci et al., 2018). Consequently, circulating IGF-1 levels were higher in swimming serum, suggesting that benefits of low-impact exercise may be driven by systemic factors.

1.8 Assessment of the muscle-bone unit

The effects of exercise on the muscle-bone unit can be assessed in multiple ways. Among these are *in vivo* methods, in which organisms are studied as a whole. In vivo methods for assessing muscle and bone can involve quantifiable measures of muscle and bone growth, such as MRI to assess muscle hypertrophy (Sorichter et al., 1995; Nijholt et al. 2017), or DEXA and qCT to assess bone mineral density and spatial growth (Gabel et al., 2015). *In vivo* methods can also involve the collection of tissue as observed with muscle and bone biopsies, which can be followed by staining procedures

that quantify physiological processes such as satellite cell expansion or mineralization (Catoire et al., 2014). There are also *in vitro* studies that refer to the use of cell culture. Cells used for cell culture studies can be primary cells or a cell line. Primary cells are isolated from muscle biopsies and are then cultured. Researchers can either obtain the muscle biopsies and isolate the primary cells independently (Conboy et al., 2005; Johnston et al., 2010; Cai et al. 2018), or purchase these primary cells from various companies (Lonza, 2015). Skeletal muscle cell lines are composed of what were once primary cells, originally taken from skeletal muscle, but have been immortalized allowing for indefinite growth and proliferation. This comes at the cost of cell lines being genetically abnormal. In addition, maintaining cell lines in an artificial environment for prolong period of time may altered their function (Owens, Moreira, & Bain, 2013).

The purpose of this thesis is to study the effects of systemic factors on the development of the muscle-bone unit; therefore an *in vitro* method is deemed more suitable as it allows for the direct exposure of systemic factors (ie. serum) to skeletal muscle and bone cells. Using a cell line provides an alternative solution to collecting pediatric muscle and bone biopsies, which can be associated with multiple difficulties.

Ideally, the use of a human skeletal muscle and bone cell lines would be more applicable given the use of pediatric human systemic factors for

this thesis. However, human cell lines are known to be more difficult to work with than a rat or mouse cell line, as such, it is more feasible to use a murine lines for our experiments. The findings of this dissertation can provide information for future work that involves human cells.

C2C12 cells are myoblasts that have the capacity to proliferate and differentiate, making them ideal to study skeletal muscle growth. C2C12 myoblasts originate from the skeletal muscles of C3H mice (Yaffe & Saxel, 1977). MC3T3E1 cells are osteoblasts established from newborn mouse calvaria, and like C2C12, have the ability to proliferate and differentiate. They are used widely as a osteogenic model as they exhibit developmental stages of osteoblast, including the ability to lay down mineral deposits (Yan et al., 2014). The benefits of using the C2C12 and MC3T3-E1 cell lines include the relative ease in maintenance and growth of cells, as well as the lower cost compared with other lines. The well-established characterization and utility of these cell lines make them viable candidates for addressing the aims of this thesis.

1.9 Communicating the research to the target audience

The importance of investing the *in vivo* and *in vitro* effects of exercise creates multiple implications. It improves our understanding of the physiological adaptations that occur in muscle and bone in response to exercise, which can help to identify key players that involved in the health

benefits. Evidence may also be used to further advocate the use of exercise in a clinical setting (Brotto & Bonewald, 2015). Collectively, exercise research has the potential to benefit the community, provided that the research is communicated to the target audience. In 2016, Statistics Canada released the *Canadian 24-Hour Movement Guidelines for Children and Youth*, indicating that only a third of kids are meeting physical activity recommendations (Government of Canada, 2017). This statistic is alarming as it indicates a trend of sedentary lifestyles amongst children, which may lead to short-term and long-term health implications. Though there are several factors that may attribute to decline in physical activity, such as nutrition, technology, and parenting styles, one possible explanation is related to the children's understanding of how physical activity benefits their health. Specifically, children's lack of understanding of the benefits of physical activity can attribute to their views towards it, and their resulting behaviour (Ferguson, 2012). As such, there is a dire need for research to be communicated such that children and families are aware of the effects of exercise of muscle and bone health and understand the benefits and implications.

Currently there is a gap between clinical pediatric science and family knowledge of pediatric health. This gap stems from challenges existing in the methodological aspects of pediatric research, such as ethical

implications that are involved in pediatric testing. There are unique ethical concerns around working with pediatric samples and recruiting participants for semi-invasive work (e.g. blood draws), therefore research in pediatrics is often derived from studies that utilize small sample sizes (Wittmeier et al. 2015). Consequently, the lag created by limited research may delay the delivery of findings to medical personnel, policy makers, and families. Despite these challenges, the field of clinical pediatric sciences is growing alongside supportive networks that aim for universal, high calibre clinical pediatric practice. Thus as this field continues to expand, it is presented with another challenge linked to the implementation of findings (Ashish et al. 2015). Implementing pediatric research such that it reduces the gap between the science and knowledge of families requires an interdisciplinary approach and mixed methodologies to meet a larger audience (Wittmeier et al. 2015, Radom-Aizik & Cooper, 2016). In addition to establishing supportive networks and collaborations within clinical pediatric science, cross-talks are needed between pediatric scientists and interdisciplinary experts, such as those in marketing, anthropology, and ethnography, etc (Wittmeir et al. 2015). More importantly, the involvement of families and collection of their feedback about research practices and optimal methods of engagement is also necessary to reduce the gap between the pediatric science and knowledge.

1.9.1 Knowledge Translation

Knowledge translation (KT), as defined by CIHR, is *the exchange, synthesis and ethically-sound application of knowledge—within a complex system of interactions among researchers and users—to accelerate the capture of the benefits of research for Canadians through improved health, more effective services and products, and a strengthened health care system* (CIHR, 2020). In this way, KT addresses the underutilization of scientific findings in the scientific and clinical sector such that it is made accessible for public consumption. There are multiple terms that have been used interchangeably with KT, such as knowledge transfer, knowledge exchange, and knowledge mobilization to name a few. However, each of these terms deals with unique processes that allow for knowledge to be communicated to the end user. The four main components that distinguish KT from other modes of knowledge communication are: knowledge synthesis, dissemination, exchange, and application. *Knowledge synthesis* refers to the contextualization of research findings into a larger body of knowledge. Knowledge *dissemination* involves the identification of the appropriate audience and tailoring the message and medium to that audience. *Knowledge exchange* refers to the interaction between the knowledge user and the researcher, resulting in mutual learning. Finally, *application* refers to the iterative process by which knowledge is put into

practise (CIHR, 2020). Collectively, these components establish a series of interactions between researchers and knowledge users that may vary in intensity, complexity and level of engagement depending on the nature of the research and the findings as well as the needs of the particular knowledge user.

There are multiple considerations to take when planning for KT, such as identifying the knowledge user (anyone who consumes the knowledge), partners, and team expertise. Typically, KT teams are reflective of individuals from interdisciplinary fields, such as science, consulting, marketing, etc. The goals of the KT initiative need to be clearly defined, as well as the key message to the knowledge users (CIHR). The message should be concise and applicable. The process KT planning also needs to be determined, that is whether the KT is integrated within the research grant or is delivered at the end of grant (CIHR). With integrated KT, researchers and knowledge users will collaborate to shape the research process, such as setting the research questions, deciding the methodology, involvement in the data collection and tool development, interpretation of findings, and dissemination of research results (SickKids). End of grant KT typically takes place upon completion of the research process, such that the goal of the researcher is to develop and implement a plan for making knowledge users aware of the knowledge that was gained during the project (CIHR). KT

planning may also adopt both approaches, such that the researcher develops a program to drive knowledge user awareness, while collaborating with knowledge users on how to improve the project (CIHR).

Delivering KT to the knowledge user can take one of many forms. Art-based approaches to KT offer viable ways of engaging knowledge users, such as children and parents, in a meaningful manner (Archibald et al. 2018). Arts-based KT is defined as the use of any art form to communicate knowledge (Archibald et al. 2018). This form of KT can be delivered in a form of music, theatrical performance, illustration, and other forms of art. Arts-based KT can dynamically influence the portrayal of data and promote empathetic understanding and emotion to influence attitudinal, knowledge and behavioural change. Arts-based KT can also address social and cultural issues through the use of a visual depictions such the characters and setting (Levy 2018). As such, communicating KT through art presents a universal and inclusive approach that may engage audiences with little to no background on the scientific matter at hand, which positions art as an attractive means to communicate to children.

1.9.2 Challenges of knowledge translation in pediatrics

When learning about the benefits of physical activity, children are often told to exercise because it makes their bodies stronger, faster, and better (*3*

Ways to Build Strong Bones, n.d.; *AboutKidsHealth*, n.d.; *How Exercise Benefits Your Whole Body*, n.d.; Bilich, 2005). While these terms are used to represent the benefits of physical activity, they do not provide children with insight about how physical activity leads to these benefits, or in other words, what happens in their bodies when they move. In focusing on the benefits of physical activity (ie. stronger, faster, better), children's understanding becomes limited to the end results as opposed to the dynamic physiological process that bring about these benefits. However, it is in the latter where the story of physical activity is found.

A major challenge for researchers is with transferring the knowledge generated by their research into meaningful information for the children and families (Reid et al., 2017) . Knowledge translation efforts can become even more challenging when research involves complex physiological processes, such as the case with studying the physiological response to exercise. One aspect of our research is studying the stimulation of cytokine release, bone turnover and muscle maturation in response to exercise for children. Such a study creates a significant amount of information, and unless delivered in a simplified and engaging manner, would be difficult for a typical child to understand. Knowledge translation efforts are often reduced to scientific publications or static information on a website – which does not necessarily reach the audience, especially those with limited accessibility as in the case

of children. Despite the presence of these materials online, their limited accessibility to children presents a barrier to their understanding and adaptation of healthy behaviours. Consequently, these efforts to translate knowledge may only partly meet their potential of reaching the target audience. Thus, a vehicle is needed for knowledge translation that overcomes these challenges, by bringing the target audience to the initiative, and creating an environment in which the content is applicable and relevant (Azimi et al., 2015).

1.9.3 Education as a vehicle for KT

The educational system provides an excellent platform for implementing KT initiatives related to health and exercise. Schools provide accessibility to children, which allows for the delivery of KT material to the target audience (Pinfold et al., 2005). Unlike static websites, schools provide a consistent, interactive environment for which children are able to communicate their ideas with their peers and educators on a daily basis. The presence of educators also allows for KT initiatives to be assessed for their effectiveness in promoting awareness and/or generating change in understanding or behaviour (Grassi et al., 2016). Furthermore, promoting the benefits of exercise in schools provides opportunities to build healthy and active life-styles that are supportive to the well-being (Pinfold et al., 2005). Research

shows that the implementation of health promotion in school-based systems yields positive effects on understanding and adoption of healthy behaviours (Grassi et al., 2016). For example, health promotion research shows that media-based education can be used to improve the dietary habits of elementary-aged children. Using weekly newsletters and production campaigns, children are able to understand the implications of healthy eating, and are likely to improve their fruit and vegetable intake as seen in this intervention study (Grassi et al., 2016). As such, the implementation of similar health initiatives in schools is not only beneficial to promoting awareness but may also help to promote application of knowledge translation.

One of the emerging ways to promote health awareness in schools is utilizing media-based tools (Ferguson et al. 2012). There are multiple examples where schools will use media-based tools to deliver information to children, such as sex education, nutrition education, and physical education (Grassi et al., 2016; Siskos et al., 2005). Tools such as posters and infographics can be used by educators to convey the messaging of their content while simplifying it in an engaging and understandable manner (Iqbal Shah & Muhammad Khan, 2015; Takacs & Bus, 2016). Similar findings are reported in other forms of pediatric KT research. Specifically, the use of illustrations and animations in the forms of infographics, videos,

and games are successful in conveying information in multiple fields in pediatrics, such as pain management, childhood disability, education around acute and chronic conditions, and general health and well-being (*AboutKidsHealth*, n.d.; *Home • EchoKT*, n.d.) Videos in particular can provide promising results, as they are often accompanied with engaging elements such as animations, narrations, and interactive presentation (Arroyo et al., 2011; Ferguson, 2012). The presence of a storyline or narrative in these projects is an effective approach to enabling the content to be relatable to the target audience, and thus more applicable (Ferguson, 2012). These findings present an opportunity to utilize media and narrative as an approach to enhance children's understanding of physical activity, thereby promoting awareness about the benefits of a healthy, active lifestyle.

1.9.4 Science Animated Videos

The promotion of videos in school can be observed in multiple disciplines. In elementary schools, subjects such as physics, chemistry, and math will sometimes include video presentations to enhance students learning of specific topics (Cheung et al., 2017; Slavin et al., 2014; Ward et al., 2011). In addition, these videos are often accompanied with post-activities to address student learning and promote interaction in class. Videos are also utilized in secondary and post-secondary education to facilitate student

learning throughout a variety subjects, which indicates the educational utility of this tool (Carmichael et al., 2019.).

1.9.4.1 Effects on learning

Scientific subjects contain abstract phenomenon that often cannot be seen or felt; therefore, visual representations are required in order to facilitate their explanation to an audience or classroom (Barak et al., 2011). Animation videos in particular are effective for this, as they utilize the pictorial representation of information to visualize concepts and convey information (Türkay, 2016). Specifically, animation videos utilize multi-senses for the construction of knowledge, such as visual (ie. animated characters), and auditory (ie. music) stimuli, which collectively promotes meaningful learning (Türkay, 2016). The ability of animations to distort of realism, in particular, can help the understanding of cause-effect relationships between abstract events in a system or process, such as the release of cytokines and growth factors in the systemic environment (Türkay, 2016). Furthermore, it is suggested that one of the fundamental principles behind multimedia learning is that people learn better from words and pictures than from words alone (Mayer et al., 2005). Indeed, the use of words, including written and spoken text, and pictures, including static

images and video, allows the brain process more information in working memory as suggested by cognitive learning theories (Mayer et al., 2005).

The utility of animation videos to deliver content to children makes them an optimal teaching tool for an educational setting. For example, animations can be presented to classrooms such that the topics are in alignment with the curriculum, providing children with additional resources to process new knowledge. Animations also provide the opportunity for knowledge application, which can be practised by providing children with an assessment in conjunction to the animation experience to assess their understanding and ability to transfer the content independently. This practice can be very helpful for subjects that present complex processes (ex. exercise physiology), and/or subjects that do not rely heavily on text education (ex. physical education). This is exemplified by a by Sisko et al., who utilizes animation platforms to teach children about physical education (Siskos et al., 2005). In their study, students utilizing the animated platform were found to produce superior results in learning performance as opposed to those exposed to traditional learning methods, which was demonstrated by improved knowledge retention and motivation to learn (Siskos et al., 2005).

1.10 Q-methodology

Exploring attitudes or points of view around a particular topic can be achieved using Q-methodology (Herrington & Coogan, 2011). Q-methodology combines qualitative and quantitative methodologies to investigate subjectivity under a scientific framework (Brewer-Deluce et al., 2019.). Studies that employ the use of Q-methodology range from those exploring the perceptions of meanings in natural environments, child experiences and emotions, and parent-child relationships (Brewer-Deluce et al., 2019.; Herrington & Coogan, 2011) The use of Q-methodology in psychological and social research allows for the exploration social constructs towards interventions or phenomena, including perceptions. To achieve this, Q-methodology employs a by-person factor analysis in order to identify groups of participants who make sense of (also called a Q 'sort') a pool of items in comparable ways (Watts & Stenner, 2005). As such, this method explores correlations between persons or whole aspects or persons, which reveals patterns of thought within a given sample (Herrington & Coogan, 2011).

1.10.1 Process of Q-methodology

One of the fundamental aspects of Q-methodology is that utilizes participant perceptions to explore correlations without testing participants nor imposing

a *priori* meaning (Herrington & Coogan, 2011). This is done through the development of a concourse, which is a series of statements related to the phenomenon of study that are generated by the participants. Upon the development of a concourse, participants rank these statements in order of agreement, which are organized into a Q-sort table (Akhtar-Danesh, 2018). Consequently, the rankings of the statements will result in a forced normal distribution. Unlike Likert Scales, which can result in bimodal or skewed distribution (with clustering of responses towards either or both extremes), the distribution of Q-methodology allows for a more sophisticated analysis of respondent data (Brewer-Deluce et al., 2019). Upon analysis, the rankings reveal characteristic perceptions, based on the grouping of similar rankings Q-sort statements. This information becomes valuable as it demonstrates how each subset within the sample perceives an intervention, thereby providing insight on how the perceptions differ with respect to the intervention.

Additional insight is also provided by an exit interview, where each participant provides a reasoning for the selection of their polarizing statements, specifically the statement they agreed with most and disagreed with most. This further characterizes the viewpoints, and incorporates the qualitative aspect of the assessments, thereby allowing researchers to

better understand the factors that lead to the effectiveness of the intervention (Akhtar-Danesh, 2018).

1.10.2 Using Q-methodology in Education

The ability of Q-methodology to address participant perceptions provides the opportunity to address many pedagogical issues in the education system, yet there are very few studies that utilize Q-methodology in the context of education. To date, one study utilizes Q-methodology in the context of post-secondary education to explore student perceptions of a pathoanatomy course at the Anatomy Education Program at McMaster University (Brewer-Deluce et al., 2019). This study makes multiple observations on how students viewed the program and shed insight on their perceptions of various components, which also lead to the characterization of their attitudes towards the course. This has implications on developing the course for student learning outcomes, which rectifies the use of q-methodology as a model to assess student learning at earlier stages such as elementary school (Brewer-Deluce et al., 2019).

Using the same approach can also shed light on the effectiveness of animation videos and their influence on student perceptions. By comparing Q-sorts and assessing exit interviews, we can identify multiple perceptions in the sample and understand why certain groups may be more engaged

with the video**.** Ultimately, this allows the researcher to revise the video and its use in an effort to make it engaging as to many viewpoints as possible, such that everyone in the classroom is able benefit from the messaging of the video.

1.11 Gaps in the literature

There are multiple studies that assess the effects of exercise on systemic profiles, and this has been well-demonstrated particularly in adult populations. Studies in children are still less abundant, and significantly lacking at earlier stages of development. Assessing the effects of development on the systemic exercise response is made more challenging when studies assessing children and adults separately. Differences in study methodologies, including exercise protocols, subject recruitment, outcome measurements across these studies limits the direct comparisons between children and adults across different studies. Therefore, more studies are required such as that both children and adults to assessed using the same methodologies in order to reduce variability and produce consistent results.

There remains to be discrepancies in the acute effects of exercise on muscle and bone, with some studies presenting with increased growth indicators of these tissues and others a lack thereof. This may be partly due to the models used in these studies. Specifically, recombinant versions of

factors that typically increase with exercise have previously been used to treat cell culture models to address exercise effects. The limitation of this approach is that different studies may utilize different concentrations, thereby yielding variability and possibly different results from study to study. In addition, treating cells with recombinant factors provides direct insight on the effects of these independent factors as opposed to their collective effective on cells, the latter providing a better representation of an exercise-induced systemic environment.

In addition to research gaps, there are also barriers between different bodies of literature around exercise, specifically between experimental or clinical and translational exercise science. There is limited collaboration between exercise scientists, translational researchers, and educators, which may otherwise facilitate the promotion of exercise and physical activity to children. One of the means of promoting awareness and education to this population has been through the means of animations, and these tools have been used extensively a variety of sciences including chemistry, physics, and mathematics. However the effectiveness of animations on attitudes towards exercise and promoting active lifestyles remain to be unclear.

1.12 Objectives and Hypotheses

The general objectives of this thesis are to assess the effects of low-impact exercise on the systemic regulation of muscle and bone in girls and women, and to develop a knowledge translation tool to communicate these findings to children.

1.12.1 Specific Objectives

The specific objectives of the studies in this thesis are as follows:

1. To assess the effects of an acute bout of moderate intensity exercise on systemic factors that regulate muscle and bone growth in girls and women.

2. To assess the effects of an acute bout of moderate intensity exercise on myoblast and osteoblast proliferation in girls and women.

3. To explore children's knowledge retention and viewpoints towards a research video that translates the effects of exercise on muscle and bone.

1.12.2 Specific hypotheses

The specific hypotheses of the studies in this thesis are as follows:

1. Girls will experience a greater increase in systemic regulators of muscle and bone than women due to time of rapid bone accrual and muscle development.

2. Myoblasts and osteoblast will increase in proliferation and differentiation after low-impact exercise in both girls and women.

3. Knowledge retention and viewpoints expressed towards the video will address the potential utility of the video as an educational tool.

CHAPTER 2

The Systemic Effects of Exercise on Regulators of Muscle and Bone in Girls and Women

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Dr. Brian Timmons, Dr. Sandeep Raha, Dr. Thomas Hawke and I contributed to the design of the study. I was responsible for participant recruitment and blood sample collection, with assistance from Dr. Joyce Obeid. I completed data collection, with the assistance of undergraduate thesis students Alexis Bullock, Kylee Innes, and Evelina Zebrowski, who are acknowledged in the publication. I was responsible for the analysis of the data presented. Dr. Timmons assisted with statistical analyses. I drafted the manuscript, with support from Dr. Brian Timmons, Dr. Sandeep Raha, Dr. Thomas Hawke and Dr. Joyce Obeid.

ABSTRACT

Purpose: The purpose of this study is to assess, in pre-early pubertal girls and women, the systemic effects of an acute bout of moderate intensity exercise on factors that are known to regulate muscle and bone growth. **Methods:** Twelve pre-early pubertal girls (8-10 y) and 12 women (20-30 y) cycled at 60% VO_2max for 1 h followed by 1 h recovery. Blood samples were collected at rest (REST), mid-exercise (EX1), end of exercise (EX2), mid-recovery (REC1) and end of recovery (REC2). Plasma was analyzed for IL6, CX3CL1, FGF-2, total IGF-1, and free IGF-1 using ELISA assays. **Results:** Both groups had similar concentrations of systemic factors at baseline with the exception of free IGF-1, which was higher in girls (p=0.001). IL-6 response was lower in girls vs women (p=0.036), with a difference of +105.1% at EX2 (p< 0.001), +113.5% at REC1 (p=0.001), and +93.2% at REC2 (p=0.022). Girls and women exhibited significant declines in CX3CL1, FGF-2, and total IGF-1 during recovery. **Conclusion:** Compared to women, an acute bout of moderate intensity exercise in girls elicits a lower inflammatory response, suggesting that other mechanisms may be more important for driving the anabolic effects of exercise on muscle and bone in children.

Key words: muscle, bone, systemic, exercise, females, IL-6, CX3CL1, IGF-1, FGF-2.

BACKGROUND

Throughout the human body, muscles and bones are located alongside arteries and veins, and as such, these organs are constantly exposed to the systemic environment (Brotto & Bonewald, 2015). Exercise induces a dynamic cascade of factors secreted into the systemic environment, ranging from immune cells, inflammatory mediators, growth peptides, all of which closely interact with muscle and bone (Hamrick, 2012). Effects of systemic factors that are known to increase with exercise have recently been studied in relation to muscle and bone adaptations using *in vivo* and *in vitro* models (Brotto & Bonewald, 2015; Forbes et al., 2012; Hamrick, 2011). IL-6, one of the most recognized markers known to increase with exercise, induces transient inflammation to drive hypertrophy and enhance osteoclastogenesis (Samee et al., 2008). Systemic factors such as chemokines (chemokine ligand 1, CX3CL1) and growth peptides (insulin growth factor-1, IGF-1 and fibroblast growth factor-2, FGF-2) can induce anabolic changes in muscle and bone models such as enhanced osteoprogenitor differentiation (Koizumi et al., 2009), myotube formation (Al-Shanti & Stewart, 2012), and mineralization (Naganawa et al., 2006). However, the effects of exercise on these systemic factors are not well understood in females due to a lack of studies in prepubertal and early pubertal girls and inconsistent findings with adult females as a result of

different exercise protocols, durations, and sampling time of the menstrual cycle (Arikawa et al., 2010; Eliakim et al., 2014; Northoff et al., 2008).

In addition to the above systemic factors, maturation will also modulate muscle and bone activity. The effects of growth and sex hormones associated with maturation, drive anabolic function of muscle and bone towards peak height velocity and peak bone mass in adolescence (Carson & Manolagas, 2015; Venken et al., 2007). The importance of these hormones on muscle and bone health is also observed in populations where their concentrations decline, such as the case with estrogen and post-menopausal women (Tobias, 2003). Pre-pubertal girls have low levels of estrogen, however unlike post-menopausal women, their mechanical responsiveness to exercise is sensitive as they are undergoing a dynamic period of musculoskeletal maturation (Vicente-Rodríguez, 2006). Thus, studying the effects of exercise in this subset of the population provides an opportunity to investigate the mechanisms of exercise that are independent of growth and sex-related hormones.

Though the child-adult differences in resting concentrations of these hormones are well established in the literature (Carson & Manolagas, 2015; Esnafoglu & Ayyıldız, 2017; Juul et al., 1997; Venken et al., 2007), it is not clear whether these differences will also translate into the systemic responses to exercise. Exercise-induced inflammation has been previously

reported to be smaller in pre-pubertal children in comparison to adults (Dan Nemet, Oh, et al., 2002); however, whether this is the case with anabolic regulators of muscle and bone is not certain. Understanding the impact of maturation on the systemic effects of exercise may identify differential mechanistic roles of regulators of muscle and bone growth.

Thus, the primary objective of this study was to assess the effects of an acute bout of moderate intensity exercise on systemic factors that are known to regulate muscle and bone growth. The secondary objective was to assess whether there are any differences in this response between children and adults. We hypothesize that children will exhibit a higher anabolic response in their systemic regulators in comparison to adults. Due to the scarcity of data in females pertaining to the systemic effects of exercise, we focused only on girls and women.

MATERIALS AND METHODS

Participants

Twelve girls aged 8-10 years (Tanner stages 1 and 2) and twelve women aged 20-30 years participated in this study. Participants were recruited from the local Hamilton and Niagara Region community through poster advertisements and social media announcements. Participants were included if they were healthy, physically active (recreationally or

competitively; with at least 2 hours of moderate to vigorous activity a week), were not taking any medications and had no diagnosed medical conditions. Pubertal status of the girls was self-assessed using breast development according to Tanner to confirm prepubertal and early pubertal staging (Medeiros et al., 2014). Only women with regular menstrual cycles who were not using oral contraceptives in the past 6-months were included in this study. All participants and parents/guardians provided written informed consent and assent, respectively, prior to enrollment in this study, which was approved by the Hamilton Integrated Research Ethics Board.

Exercise and blood sampling

Participants completed two study visits. During the first visit, anthropometric variables were collected, including height, weight, body composition using bioelectrical impedance analysis. Height, weight, and body mass index (BMI) percentiles were calculated using reference values for weight-for-age and stature-for-age from the Centers for Disease Control and Prevention. Aerobic fitness, defined as maximal oxygen uptake (VO_2max), was assessed using the McMaster All-Out Progressive Continuous Cycling Test on a cycle ergometer (Lode Corival, Groningen, The Netherlands). Gas collection for the measurement of O_2 and CO_2 was completed using a breath-by-breath analysis on a calibrated metabolic cart (Vmax29, Yorba Linda, USA). VO_2max was defined as the highest volume of oxygen uptake

over a 20-sec period, and normalized to fat-free mass. The criteria for attaining VO_2max included: a heart rate of ≥195 beats per minute, a respiratory exchange ratio (RER) ≥ 1.0, and an inability to maintain the prescribed pedaling cadence in spite of strong verbal encouragement (Samaan et al., 2013). All women completed their first study visit on approximately the 20th day of their menstrual cycle.

The second visit was scheduled one week after the first visit. For the women, the second visit took place during the early follicular stage of their menstrual cycle (ie. 1-6 days after the first day of menstruation). All visits were scheduled during the same time of day for girls and women, which was at 4 PM. Participants were asked to refrain from consuming any food or liquid with the exception of water, 2 hours prior to the visit. They also refrained from participating in any strenuous physical activity for at least 24 hours before the visit. This was confirmed by questionnaire at the study visit. Upon arrival to the laboratory, participants were asked to rest in a supine position for a minimum of 10 minutes. An indwelling catheter was placed in a vein in the antecubital region of the arm for ease of blood collection. A resting blood sample (REST) was collected with the participant in the supine position. Participants then completed 2 x 30-minute bouts of moderate intensity cycling exercise (MICE) at a constant pace of 60 to 80 revolutions per minute and an intensity equivalent to 60% of their VO_2max. A total of 5

blood samples were collected: REST, mid-exercise (EX1), end of exercise (EX2), mid-recovery (REC1, 30-min post-exercise) and at the end of recovery (REC2, 60-min post-exercise). Blood samples were collected into chilled EDTA-coated vacutainers that were placed on ice. Samples were then centrifuged for 20 min at *2000* RCF and 4°C. All plasma samples were aliquoted and stored at -80°C for future analysis.

Plasma Analysis

Plasma samples were analyzed for IL-6, CX3CL1, FGF-2, total IGF-1, and free IGF-1 using high-sensitivity enzyme-linked immunosorbent assays (ELISA) from R&D Systems (Human IL-6 Quantikine HS ELISA Kit, Human CX3CL1/Fractalkine Quantikine ELISA Kit, Human FGF Basic Quantikine HS ELISA Kit, Human IGF-1 Quantikine ELISA Kit, and Human Free IGF-1 ELISA Kit, Minneapolis, MN). The average intra-assay and inter-assay coefficients of variation for IL-6, CX3CL1, FGF-2, total IGF-1, and free IGF-1 were 7.4%, 5.1%; 6.6%, 3.4%; 3.0%, 5.8%; 8.4%, 7.8%; and 4.4%, 3.3%, respectively.

Statistical Analyses

Statistical analyses were run using SPSS Software (Version 20) and Statistica (Version 22.0).

To determine whether girls and women had similar marker values at rest, independent t-tests were used to compare resting values of IL-6, CX3CL1, FGF-2. total IGF-1, and free IGF-1.

Absolute change values were calculated by subtracting each time point from the concentration at rest. All girls (n=12) and women (n=12) were included in the analysis. Normality was met across all changes in markers relative to their baseline values which was assessed using the Shapiro-Wilk test. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for the two-way interaction for IL-6 [$\chi^2(5)=12.923$, $p=0.024$], total IGF-1 [$\chi^2(5)=11.810$, $p=0.038$], and free-IGF-1 [$\chi^2(5)=25.604$ $p=0.000$], therefore the Greenhouse-Geisser estimate was used to determine the presence of an interaction in these analytes.

A repeated measures ANOVA was carried out to determine the effects of exercise on the absolute change in markers in both groups. The two factors examined were group (girls, women) x time (EX1, EX2, REC1, and REC2). To control for differences at baseline, a repeated measures ANCOVA was carried out to analyze the effects of exercise on the absolute change in free IGF-1, while treating baseline as a covariate. Values were expressed mean ± SD unless stated otherwise. Significance was set a $P \leq 0.05$.

RESULTS

Participant Characteristics

The baseline characteristics for the participants are outlined in Table 1. Girls and women differed significantly across all characteristics, including aerobic fitness normalized to fat-free mass.

Resting values of IL-6, CX3CL1, FGF-2, total IGF-1, and free IGF-1 in girls and women are presented in Table 2. Both groups were similar prior to exercise with the exception of free IGF-1, which was significantly higher in girls than women ($p<0.001$).

The effects of exercise on the on the absolute change of IL-6, CX3CL1, FGF-2, total IGF-1, and free IGF-1 concentrations relative to rest are presented in Figure 1.

There was a significant two-way interaction for the change of IL-6 between group and time, $F(2.126, 46.766)=3.482$, $p=0.036$. The change in IL-6 was not significant in girls compared to women at EX1, $F(1, 22)=0.0748$, $p= 0.154$. However, the change in IL-6 was lower in girls compared to women at EX2 ($F(1, 22)=0.296$, $p<0.001$), REC1 ($F(1, 22)=0.340$, $p=0.001$), and REC2 $F(1, 22)=0.508$, $p=0.022$), representing an increase of +105.1%, +113.5%, and +93.2%, respectively.

There was no significant two-way interaction for the change of CX3CL1 between group and time, $F(3, 66)=0.586$, $p=0.627$. There was no

main effect for group (girls vs. women), $F(1, 22)=0.655$, $p=0.427$. A significant main effect for time was observed for CX3CL1, $F(3, 66)=7.558$, $p<0.001$. Post-hoc tests with Bonferroni correction indicated that the decline in CX3CL1 concentration at REC1 was significantly greater than declines observed at EX1 ($p<0.001$) and EX2 ($p=0.003$) relative to baseline, respectively, representing decreases of -2.8% and -10.1% in girls and women, respectively.

There was no significant two-way interaction for the change of FGF-2 between group and time, $F(3, 66)=0.362$, $p=0.780$. There was no main effect for group (girls vs. women), $F(1, 22)=2.576$, $p=0.123$. A significant main effect for time was observed for FGF-2, $F(3, 66)=7.558$, $p<0.001$. Post-hoc tests with Bonferroni correction indicated that the decline in FGF-2 concentration at REC2 was significantly greater than the decline observed at EX2 ($p=0.014$) relative to baseline, representing decreases of -18.3% and -39.7% in girls and women, respectively.

There was no significant two-way interaction between group and time for the change in total IGF-1, $F(2.201, 48.427)=0.400$, $p=0.692$. There was no main effect of group ($F(1, 22)=0.000$, $p=0.995$), but a significant effect of time for the change of total IGF-1 concentrations ($F(2.201, 48.427)=10.754$, $p<0.000$) was observed. Post-hoc tests with Bonferroni correction indicated that the decline in total IGF-1 concentration at REC1 was significantly

greater than the increase observed at EX1 (p=0.007) relative to baseline, representing decreases of -9.6% and -12.5% in girls and women, respectively. Similarly, the decline of total IGF-1 at REC2 was greater than the changes observed at EX1 (p=0.001) and EX2 (p=0.013) relative to baseline, representing decreases of -9.5% and -14.6% in girls and women, respectively.

There was no significant two-way interaction between group and time for free IGF-1, $F(1.927, 40.475)=1.123$, $p=0.333$). There was no main effect of group ($F(1,21)=1.185$, $p=0.289$) and time ($F(1.927, 40.475)=1.666$, p=0.202).

DISCUSSION

We set out to study the effects of exercise on systemic regulators of muscle and bone growth in pre-pubertal girls and women. Understanding how systemic regulators respond to exercise provides valuable and practical information that can be used to develop exercise programs for these conditions by assessing muscle and bone regulation. Furthermore, investigating these responses in children and adults provides insight on how these regulators change, or remain the same, throughout the lifespan.

Contrary to our hypothesis, girls did not experience a greater anabolic response as reflected by the lack of increase in systemic regulators

of muscle and bone relative to women. Rather, girls expressed an attenuated IL-6 response relative to women; however, they responded similarly in CX3CL1, FGF-2, total IGF-1 and free IGF-1.

With the exception of free IGF-1, girls and women shared similar values at baseline prior to the exercise. Free IGF-1 was significantly higher in girls, which can be explained by their pubertal development. Plasma IGF-1 levels rise sevenfold from very low concentrations at birth (20 to 60 ng/mL) to peak values at puberty (Juul et al., 1997). During adolescence and adulthood, these concentrations fall rapidly, reaching values that are 40 to 50 percent of the maximum pubertal levels by age 20. These patterns in IGF-1 are largely governed by age-dependent changes in GH secretion. (Vestergaard et al., 2014)

That the response of IL-6 was found to be lesser in girls than in women is consistent with our hypothesis (See Figure 1a). Although IL-6 is expressed systemically, this cytokine is also expressed locally by multiple organs including fat, bone, and skeletal muscle (Hamrick, 2012; Pedersen & Febbraio, 2012). Considering the notable differences in fat free mass and percent body fat between the women (42.27 kg, 24.7%) and girls (28.11 kg, 14.4%) in this study, it is possible that increased IL-6 expression in women is largely attributed to these physical characteristics (Timmons, 2005). Furthermore, the differences in IL-6 responsiveness between children and

adults with exercise has been illustrated in multiple studies (Dan Nemet, Oh, et al., 2002; Tirakitsoontorn et al., 2001). Similar exercise protocols consisting of 1 hour cycling at a moderate intensity result in an exaggerated IL-6 response in adults that is roughly 50% greater than what is reported in children (Ploeger et al., 2009; Tirakitsoontorn et al., 2001). In addition to physical characteristics, children and adults also exhibit differential exercise metabolism, specifically higher reliance on fat oxidation in children relative to adults, which may reduce the need for intra-cellular signaling of IL-6 in children (Timmons, 2005).

The reduction in CX3CL1 concentrations at REC1 relative to baseline in girls (-0.04 ng/ml) and women (-0.10 ng/ml) was markedly greater than what was observed at EX1 and EX2 (See Figure 1B). This is the first study to assess the levels CX3CL1 in children; as such there is no reference to compare the sample of girls. However, there are two studies to date that document the effects of an acute bout of exercise on changes in CX3CL1 in adults (Catoire et al., 2014; Strömberg et al., 2016). Following a 45-minute bout of moderate intensity cycling in a sample of healthy middle-aged men, CX3CL1 was elevated in plasma and skeletal muscle mRNA (Catoire et al., 2014). Similarly, elevated levels CX3CL1 in plasma and skeletal muscle mRNA were observed at 30 minutes and up to 2 hours after exercise comparing to resting values in adults (Strömberg et al., 2016). The

same study found that *in vitro* administration of CX3CL1 to a myotube culture induces cells to produce IL-6 and TNF-α, which supports the role of CX3CL1 in promoting exercise-induced immune cell infiltration and muscle repair (Strömberg et al., 2016). CX3CL1 administration in osteoclast cultures and neonatal mice also induces bone growth specifically through increased bone resorption and osteoclastogenesis, both of which are generally increased immediately after exercise (Mezil et al., 2015). These findings illustrate the role of CX3CL1 in orchestrating the crosstalk between the immune system and muscle and bone after exercise, which further indicates the involvement of this chemokine in mediating inflammatory and growth pathways in musculoskeletal disease such as arthritis or osteoporosis.

In our analysis, FGF-2 declined in both girls and women. The decline was significant at REC2 relative to EX2. Considering that FGF-2 is upregulated by inflammation and that this growth factor exhibits anabolic effects on muscle and bone, such as the induction of muscle hypertrophy and osteoblastogensis (Hamrick, 2011; Kim et al., 2016), we hypothesized that it would increase after exercise. Indeed, biopsies of the gastrocnemius and vastus lateralis muscles show an upregulation of FGF-2 mRNA after one bout of resistance exercise in rats and humans, respectively (Breen et al., 1996; Hanssen et al., 2013). However, no such increase in FGF-2 has

been observed in plasma after exercise in healthy populations (Bruserud et al., 2005; J.-W. Gu et al., 2004; Dan Nemet, Oh, et al., 2002; Wahl et al., 2011). Plasma FGF-2 declines immediately after acute bouts of wrist flexion, running, and kayaking in adults, ranging from low to high intensities (Bruserud et al., 2005; Dan Nemet, Hong, et al., 2002). FGF-2 is an important regulator of arteriogenesis, and studies have shown that this process of vascular remodeling can occur immediately after exercise (Olver et al., 2016). Receptors for FGF-2 and extended members of FGF family are expressed robustly on the surface of endothelial progenitor cells of smooth muscle and bone marrow (Aviles et al., 2003). In this way, it is possible that exercise stimulates the binding of FGF-2 to these surface receptors to modulate arteriogenesis, thereby reducing the concentration of the soluble form in circulation. In addition, urine concentrations of FGF-2 increase significantly after treadmill exercise, suggesting an increased glomerular filtration of this factor post-exercise (J. W. Gu et al., 1997). Thus, the local upregulation of FGF-2 as observed in muscle biopsies suggests a compensatory mechanism to sustain levels of FGF-2 after exercise despite increased clearance.

In children, most studies have found that total IGF-1 declines or is unresponsive with exercise irrespective of the intensity and duration (Eliakim et al., 2001; Dan Nemet, Oh, et al., 2002; Timothy P. Scheett et al.,

1999). Indeed, our findings are consistent with previous studies reporting that acute bouts of exercise (i.e., intermittent cycling, soccer, and wrestling) result in a decrease in total IGF-1 (D. Nemet et al., 2004; Timothy P. Scheett et al., 1999; Tirakitsoontorn et al., 2001). In adults, however, the total IGF-1 response has been less consistent and seemingly dependent on the type of exercise, fitness, and sex (Amir, n.d., p. 1; Eliakim et al., 2014). Despite the reduction in total IGF-1 in this study, the **concentration** of free IGF-1 in girls and women did not change after exercise or recovery. This differential response between total and free IGF-1 is possibly linked to changes in IGF-1 binding proteins (IGFBPs), which are a family of six-high affinity proteins that make up the tertiary complex as observed with total IGF-1 (Eliakim et al., 2014). Therefore, the sustained levels of free IGF-1 as observed in our study may be a net result of exercise-induced clearance (Timothy P. Scheett et al., 1999), coupled with increased availability upon dissociation from IGFBPs. IGFBPs, however, were not measured in this study, therefore further research is required to clarify the mechanism post-exercise.

One of the plausible mechanisms of total and free IGF-1 clearance from circulation is linked to increased receptor binding (Nindl & Pierce, 2010). It has been shown that IGF-1 receptor densities increase in skeletal muscle fibres after exercising (Urso et al., 2005), indicating that free IGF-1 is likely taken up by receptive organs to induce an anabolic response. This

could also explain why children participating in exercise training exhibit lower levels of free IGF-1 despite developing increased muscle mass, which is facilitated by the GH-IGF-1 axis (T. P. Scheett, 2002). Bone tissue, however, is more susceptible to circulating levels of IGF-1 relative to muscle tissue, which is predominantly regulated by local IGF-1 production (Bikle et al., 2015). Indeed it has been shown that exercise-induced bone formation is compromised in IGF-1 receptor deficient rodents, while systemic administration of recombinant IGF-1 induces increased bone mass and osteoblastogenesis in ovariectomized rats and osteoporotic patients, respectively (Boonen et al., 2002; Tian et al., n.d.). The importance of these findings can be expanded to the bioavailability of circulating IGF-1 in women, which has been shown to correlate with estradiol concentrations and relate closely with years since menopause than to chronological age (Boonen et al., 2002). Thus assessing the levels of total and free IGF-1 at baseline and post-exercise may prove to be a clinical index of osteoporosis risk, which is greater in women relative to men (Bilek et al., 2016).

It is important to highlight that this study is not without limitations. Given that circulating IL-6 concentrations are also affected by fitness level (39), it is possible that the attenuated pro-inflammatory response observed in girls is attributed to their greater level of fitness relative to the women in this study. However, given that there were no significant differences in IL-6

concentrations between girls and women at rest, any potential effects of fitness on the pro-inflammatory response in this study may have been minimal. Indeed, it seems more likely that this difference is attributable to lower absolute fat-free mass in girls compared with women. Second, hematocrit was not measured therefore changes plasma volume could not be elucidated. Participants were well hydrated throughout exercise and recovery, therefore any changes in plasma volume were expected to be minimal and not different between girls and women.

In summary, a single bout of moderate intensity cycling induced systemic responses that are reflective of downstream anabolic effects on muscle and bone in girls and women. The differences in the responses of IL-6 in this study suggest that the systemic effects of exercise are less driven by pro-inflammatory factors in children than adults, suggesting other mechanisms that may drive the anabolic effects on muscle and bone growth, such as growth factors. Future directions of this study would be to address the exercise-induced effects on additional factors related to muscle and bone growth beyond those included in this study (e.g., binding proteins) and to assess whether these responses hold true for girls at different stages of maturity (i.e., post-pubertal) and women in different stages of their menstrual cycle (i.e., luteal phase).

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Table 1: Participant Characteristics

Age (years)	10.0 ± 0.8	23.2 ± 2.3	<0.0001
Height (cm)	140.6 ± 7.2	162.1 ± 6.8	<0.0001
Height percentile	57.6 ± 18.4	N/A	
Weight (kg)	32.9 ± 3.17	56.2 ± 8.0	<0.0001
Weight percentile	47.1 ± 14.3	N/A	
BMI	16.8 ± 1.2	21.6 ± 2.7	<0.0001
BMI percentile	45.2 ± 19.8	N/A	
% Body Fat	14.4 ± 5.3	24.7 ± 7.4	0.0004
Fat Free Mass (kg)	28.1 ± 2.6	42.27 ± 7.3	<0.0001
Maturity*	Tanner 1: n= 6 Tanner 2: n=6	N/A	
VO2max (mL/kg$_{FFM}$/min)	59.6 ± 11.2	49.05 ± 8.4	0.016

Values expressed as mean ± SD

BMI: body mass index; FFM: fat-free mass; N/A: Not Applicable

*Based on breast development.

Table 2: Resting Markers in Girls and Women

IL-6 (pg/ml)	1.25 ± 1.72	0.92 ± 0.71	0.553
CX3CL1 (ng/ml)	0.75 ± 0.31	0.64 ± 0.18	0.298
FGF-2 (pg/ml)	9.86 ±7.91	6.07 ± 4.77	0.172
Total IGF-1 (ng/ml)	1.55 ± 0.57	1.30 ± 0.27	0.181
Free IGF-1 (ng/ml)	1.55 ± 0.66	0.72 ± 0.15	0.001

Values displayed as mean ± SD.

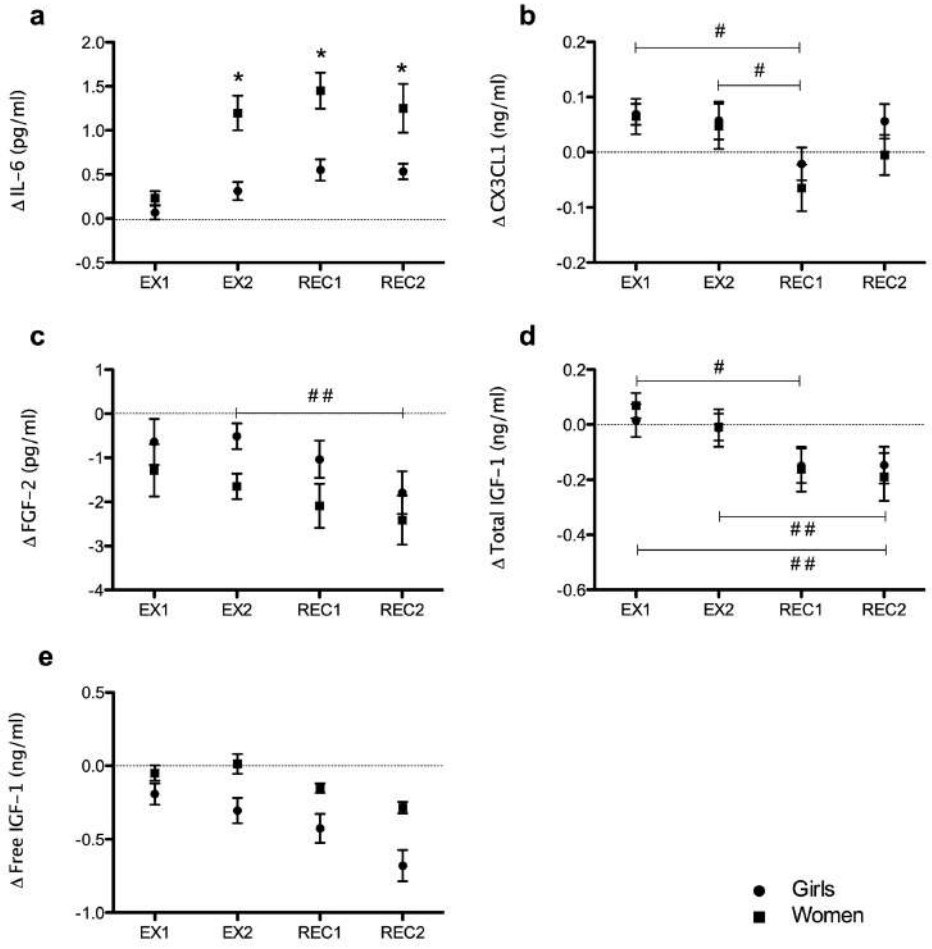

Figure 1: Changes in muscle and bone regulator concentrations in girls and women. (a) ΔIL-6 (pg/ml), (b) ΔCX3CL1 (ng/ml) (c) ΔFGF-2 (pg/ml), (d) Δ total IGF-1 (ng/ml), and (e) Δ free IGF-1 (ng/ml). (●): Girls, (■): Women, EX1: midpoint of exercise, EX2: end of exercise, REC1: midpoint of recovery, REC2: end of recovery. Values are displayed as mean ± SE. (*) denotes significant interaction between girls and women, (#) denotes significant difference from change at REC1 for both girls and women, (# #) denotes significant difference from change at REC2 for both girls and women. Significance was denoted at $p<0.05$.

CHAPTER 3

The effects of exercise on myoblast and osteoblast proliferation and differentiation in prepubertal girls and women

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This article is currently being prepared for submission.

Dr. Brian Timmons, Dr. Sandeep Raha, Dr. Thomas Hawke and I contributed to the design of the study. I was responsible for participant recruitment and blood sample collection, with assistance from Dr. Joyce Obeid. I completed data collection, with the support of Inna Ushcatz and Pasha Kulinich (who is acknowledged in the current article). I was responsible for the analysis of the data presented. Dr. Timmons and Dr. Joyce Obeid assisted with statistical analysis. I drafted the manuscript, with support from Dr. Brian Timmons, Dr. Sandeep Raha, Dr. Thomas Hawke, Dr. Joyce Obeid and Inna Ushcatz.

ABSTRACT

Purpose: The purpose of this study is to assess, in pre-early pubertal girls and women, the effects of an acute bout of moderate intensity exercise on myoblast and osteoblast proliferation. **Methods:** Twelve pre-/early pubertal girls (8-10 years old) and 12 women (20-30 years old) cycled at 60% VO_2max for 1h followed by 1h recovery. Blood samples were collected at rest (REST), mid-exercise (EX1), end of exercise (EX2), mid-recovery (REC1) and end of recovery (REC2). Serum from each respective time point was used to treat C2C12 myoblasts and MC3T3E1 osteoblasts. Cells were incubated in 5% (v/v) serum in media for 1h, after which they were left to incubate for 24h (myoblasts) and 36-h (osteoblasts) hours to examine proliferation using an MTS assay. Cells were also incubated for 6d (myoblasts) and 21d (osteoblasts) in human serum to examine myotube formation using immunofluorescent staining and mineralization using alizarin red, respectively. **Results:** Exercise did not affect myoblast and osteoblast proliferation. Girls exhibited lower cell proliferation relative to women at EX2 (osteoblasts, p=0.041; myoblasts, p=0.029) and REC1 (osteoblasts, p=0.010*).* Mineralization was lower at REC2 relative to REST (p=0.014) in both girls and women. Myotube formation was not affected by exercise or group. **Conclusion:** The systemic environment following one acute bout of low impact moderate intensity exercise (MICE) in girls and

women does not elicit anabolic responses in osteoblast and myoblast activity. Differences in proliferation of osteoblast and myoblasts between girls and women are suggestive of systemic factors possibly influenced by growth.

BACKGROUND

Exercise plays an important role in muscle and bone development. It facilitates locomotion of the musculoskeletal system, increases weight loading, and generates impact, all of which promote growth and accrual of muscle and bone, most significantly in prepubertal children (Burrows, 2007). Data from cross-sectional and longitudinal studies report prepubertal children who are more active present with improved indices of muscle and bone strength and acquisition, such as cross-sectional area, lean mass, bone mineral content, and structural distribution compared with their less active peers (Falk et al., 2003; G Vicente-Rodriguez et al., 2005). However, limitations to previous research in this area include a lack of understanding of the cellular processes involved and a lack of studies devoted to understanding the female response.

The physiological mechanisms responsible for improving indices of muscle and bone development have are primarily attributed to the high mechanical sensitivity observed in prepubertal children (German Vicente-Rodriguez, 2006). Satellite cells and osteocytes both respond to mechanical strain by inducing differentiation and activating remodeling processes that involve different cells (e.g., osteoblasts), all of which drive anabolic responses in muscle and bone (Bazgir et al., 2017; Kouvelioti et al., 2018). However, structural and functional indices of muscle and bone also improve

after exercises characterized by low mechanical strain (Gómez-Bruton et al., 2013; Peitz et al., 2018). For example, sports such as cycling and swimming, both of which involve minimal external mechanical loads on muscle and bone relative to high-impact sports, have been associated with improved muscle strength (Gäbler et al., 2018), higher bone turnover, and lower fracture indices (Gómez-Bruton et al., 2013). These improvements have been linked to mediators in the systemic environment such as inflammatory and anabolic factors, (Hamrick, 2012) which fluctuate with exercise modality (i.e., intensity, duration) (Petersen & Pedersen, 2005). As such, these findings highlight the possible role of the systemic environment in mediating the effects of low-impact exercise on muscle and bone in children.

In addition to the exercise modality, the responsiveness of muscle and bone growth to exercise appears to be sex-specific, as strenuous exercise increases bone mineral density (Lang, 2011) and muscle protein synthesis (Smith et al., 2008) to a greater extent in older men relative to older women. This sexual dysmorphism is largely connected to the age-associated decline in estrogen levels in women, which attenuate musculoskeletal responsiveness to exercise (Lang, 2011). In younger women, the greater anabolic effects of estrogen on muscle and bone (Tobias, 2003) may interact with the exercise-induced systemic

environment to promote cell proliferation and differentiation. However, there is limited and inconsistent literature on the effects of moderate intensity exercise on muscle and bone responsiveness in young women, and a scarcity of information in girls.

A common approach to understanding muscle health in the context of exercise is to acquire muscle biopsies. Given the ethical limitations associated with obtaining biopsies in children, an alternate approach is required. We have previously used a cell culture model incubated with cell-free serum collected from children before and after exercise. Isolating the systemic environment can also provide insight on additional characteristics that influence the muscle and bone growth response to exercise, such as those that are inherit to maturity (Casazza et al., 2010). Here, we extend our previous work by studying both muscle and bone cell responses to the exercise systemic environment of exercise while focusing on females, specifically comparing prepubertal girls and adult women, to distinguish between the effects of exercise and development on muscle and bone growth *in vitro.*

METHODS

Participants

Twelve girls aged 8-10 years (Tanner stages 1 and 2) and twelve women aged 20-30 years participated in this study. Participants were recruited from the local Hamilton and Niagara Region community through poster advertisements and social media announcements. Participants were included if they were healthy, physically active (recreationally or competitively; with at least 2 hours of moderate-to-vigorous activity a week), were not taking any medications and had no diagnosed medical conditions. Only women with regular menstrual cycles who were not using oral contraceptives in the past 6-months were included in this study. All participants and/or parents/guardians provided written informed assent and/or consent, as appropriate based on age, upon enrolling in this study, which was approved by the Hamilton Integrated Research Ethics Board.

Exercise and blood sampling

Participants completed two study visits. During the first visit, anthropometric variables were collected, including height, weight and body composition using bioelectrical impedance analysis. Height, weight, and body mass index (BMI) percentiles were calculated using reference values for weight-for-age and stature-for-age from the Centers for Disease Control and Prevention. Pubertal status of the girls was self-assessed using breast development according to Tanner to confirm pre-pubertal (Tanner 1) or early pubertal (Tanner 2) staging (Medeiros et al., 2014). Aerobic fitness,

quantified as maximal oxygen uptake (VO_2max), was assessed using the McMaster All-Out Progressive Continuous Cycling Test on a cycle ergometer (Lode Corival, Groningen, The Netherlands) (Nguyen et al., 2014). Gas collection for the measurement of O_2 and CO_2 was completed using a breath-by-breath analysis on a calibrated metabolic cart (Vmax29, Yorba Linda, USA). VO_2max was defined as the highest volume of oxygen uptake over a 20-sec period and expressed normalized to fat-free mass (mL/kg/min). All women completed their first study visit in the late luteal stage of their menstrual cycle (approximately the 20th day of their menstrual cycle).

The second visit was scheduled one week after the first visit. For the women, the second visit took place during the early follicular stage of their menstrual cycle (ie. 1-6 days after the first day of menstruation). All visits were scheduled at 4 PM for both the girls and women, in order to minimize diurnal hormonal variation. Participants were asked to refrain from consuming any food or liquid with the exception of water, 2 hours prior to the visit. They also refrained from participating in any strenuous physical activity for at least 24 h before the visit. This was confirmed by questionnaire at the study visit. Upon arrival to the laboratory, participants were asked to rest in a supine position for a minimum of 10 minutes. An indwelling catheter was placed in a vein in the antecubital region of the arm for ease of blood

collection. A resting blood sample (REST) was collected with the participant in the supine position. Participants then completed 2 x 30-minute bouts of moderate intensity cycling exercise (MICE) at a constant pace of 60 to 80 revolutions per minute and an intensity equivalent to 60% of their VO_2max. After completion of a single 30-minute bout, participants received a 5-7-minute break, at which time the mid-exercise (EX1) sample was collected. A total of 5 blood samples were collected: REST, EX1, end of exercise (EX2), mid-recovery (REC1, 30-min post-exercise) and at the end of recovery (REC2, 60-min post-exercise). Blood samples were collected into clear serum separator tubes (BD Vacutainer) and allowed to clot for 30 minutes at room temperature. Samples were then centrifuged for 20 min at 2000 RCF and 4°C. All serum samples were aliquoted and stored at -80°C for future analyses.

Physical Activity Assessment

Each participant was outfitted with an ActiGraph GT1M accelerometer (The ActiGraph, Pensacola, FL), which is a small device that provides objective and valid measures of habitual physical activity (Evenson et al., 2008). Accelerometers were initialized to sample data in 3-sec intervals. Participants were instructed to wear the device over the right hip during all waking hours, with the exception of water activities, for 9 consecutive days. Levels of MVPA were determined and reported in minutes per day and

minutes per hour of monitoring time. Participants were included in the analysis if they wore the device for ≥10 h on ≥ 4 days, including 1 weekend day.

Osteoblast and myoblast cultures

MC3T3E1 osteoblast and C2C12 myoblast cell lines were purchased from American Tissue Type Collection (Bethesda, MD, USA). Cells were grown on 100-mm cell culture dishes in growth media (GM), which consisted of Dulbecco's Modified Eagle Medium (DMEM; C2C12) or alpha Minimum Essential Medium (α-MEM; M3CT3E1), supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin. Cells were incubated at 37°C in 5% CO_2 and passaged once they reached a confluence of approximately 70%.

Cell Treatment and Proliferation Assay

Prior to cell treatments in each experiment, serum samples from each time point were thawed at room temperature, shortly after which they were heat-inactivated at 56°C for 30 minutes (Tateishi et al., 2008). Once serum returned to room temperature, cells were immediately treated.

To assess cell proliferation, a colorimetric assessment was carried out using the MTS Assay (CellTiter 96® AQ_{ueous}, Promega). First, cells were seeded in 96-well plates at a density of 3000 cells per well (osteoblasts) and 1000 cells per well (myoblasts). After cell attachment (approximately 2 h

after seeding), cells were treated with media containing only 1% (v/v) penicillin-streptomycin for 12 h to onset serum deprivation and thus enhance cell synchronization (Langan & Chou, 2011). Cells were then treated with the heat-inactivated serum samples collected from girls and women at a concentration of 5% (v/v) in DMEM (myoblasts) or α-MEM (osteoblasts). Cells were cultured in serum treated media for 1 h to mimic the exercise-induced systemic environment stimulated by 1 h of low impact MICE. Cells were cultured with serum-treated media from each time point. After incubation, the media was replaced with GM and cells were allowed to incubate for 24 hours (myoblasts) and 36 hours (osteoblasts) to accommodate one doubling cycle, respectively. At the end of incubation, cells were treated with the MTS reagent at a volume of 20 mL per cell for 1hr and 30 minutes. The absorbance was used as the indicator of cell proliferation, which was measured at 490 nm using a Thermo Scientific Multiskan Spectrum Reader (Vintaa, Finland).

Myotube Differentiation

Myoblasts were seeded in 96-well plates at a density of 8000 cells per well. After cell attachment (approximately 2 hours after seeding), cells were serum-deprived for 12 hours with media only containing 1% (v/v) penicillin-streptomyocin to enhance cell synchronization. Cells were then treated with the heat-inactivated serum samples collected from girls and women at a

concentration of 5% (v/v) in DMEM (myoblasts). Cells were cultured with serum treated-media from each respective time point for 1 hour, after which serum-treated media was replaced with differentiation media (2% (v/v) horse serum and 1% (v/v) penicillin-streptomyocin in DMEM). Differentiation media was replaced each day for 6 consecutive days.

After differentiation, cells were washed with 100 mL of 0.01% Tween in phosphate-buffered saline (PBS) and subsequently fixed with 4% paraformaldehyde (PFA) in PBS for 10 minutes. Cells were then washed three times with 100 □L of 0.01% Tween in PBS (PBS-T). Cells were permeabilized for 5 min with 100 □L of 0.1% Triton-X in PBS, after which they were washed twice with 100 □L of 0.75% glycine in PBS-T. Cells were washed with 100 □L of PBS-T and then treated with blocking buffer for 2 hours, which comprised of 10% (v/v) goat serum and 1% (v/v) bovine serum albumin (BSA) in PBS-T. Cells were treated with 60 µL of primary antibody against embryonic myosin heavy chain (eMHC, 1:12, Developmental Studies Hybridoma Bank, University of Iowa) and incubated overnight at 4°C in the dark. After incubation, cells were washed with PBS-T and subsequently incubated in secondary antibody (Alexa flour goat anti-mouse 594, 1:1000, Invitrogen, Carlsbad, CA) for 1 hour at room temperature in the dark. Cells were washed in 100 mL PBS-T and subsequently incubated with DAPI (Sigma Aldrich, 1:500 dilution) to stain nuclei for 5 minutes in the

dark. Incubation was followed by 3 washes with 100 µL of PBS, after which cells were air dried and stored at 4°C until future analysis.

Myonuclei fusion index (MFI) was used as a marker of differentiation. Five random fields of view were captured with a Nikon Eclipse Ti microscope at 10X magnification for the each well (Nikon Instrument Inc. Melville NY, U.S.A). The 5 images obtained from each well were viewed using NIS-Element AR 3.2 64-bit software and the total number of myonuclei and nuclei were counted using ImageJ (ImageJ version 1.51, U.S. National Institutes of Health, Bethesda, MD). The sum of the myonuclei and nuclei count for all 5 images was considered representative of the total number of myonuclei and nuclei within each well. The myonuclei and nuclei were not included in the count unless the entirety of its shape was captured in the photograph. Additionally, myonuclei were only counted when 2 or more nuclei were present in a myotube. MFI was calculated as: MFI = (total number of myonuclei/total number of nuclei) * 100.

Osteoblast Differentiation

Osteoblasts were seeded in 24-well plates at a density of 30,000 cells per well. After cell attachment (approximately 2 h after seeding), cells were serum-deprived for 12 h with media only containing 1% (v/v) penicillin-streptomyocin to enhance cell synchronization. Cells were then treated with the heat-inactivated serum samples collected from girls and women at a

concentration of 5% in α-MEM (osteoblasts). Cells were cultured with serum-treated media from each respective time point for 1 h, after which serum-treated media was replaced with mineralization media which comprised of α-MEM supplemented with 10% (v/v) Hyclone™ FBS (Fisher Thermo Scientific, USA), 50 μg ascorbic acid, 10 mM β-glycercol phosphate, and 1% penicillin-streptomyocin in α-MEM. Mineralization media was replaced every 48 hours for a period of 21 days to allow for mineral nodules to form.

To detect mineralization, the alizarin red stain was used to bind to mineral nodules formed in culture after 21 days. Wells were washed twice with 300 μL PBS and were subsequently fixed with cold 4% of PFA/PBS for 15 minutes at 4°C. Wells were then washed twice with 300 μL distilled water, after which they were stained with 300 μL of 40 mM of Alizarin Red S (Sigma Aldrich, Germany) which was prepared in distilled water at pH 4.2. After staining, the plates were placed on a plate shaker and set to incubate at room temperature for 30 minutes at a speed of 500 RPM in the dark. This incubation was followed by 2 washes with distilled water. To facilitate the removal of excess stain, the plates were tilted at a 45° angle for 2 minutes on the bench counter top and left to air dry. Images of the wells were taken prior to stain extraction.

To quantify mineralization, the residual alizarin bound to mineral nodules was extracted by adding 500 mL of 100 mM cytelpyridinium chloride (CPC) (Sigma Aldrich, Germany) to each well. Plates were then placed on a plate shaker and were set to incubate overnight at room temperature at a speed of 200 RPM in the dark. Following the incubation, 100 μL of the CPC extract was removed from each well in the 24-well plate and transferred to wells in a 96-well plate. The plates were read at an absorbance of 561 nm. All samples were run in duplicates, and osteoblast differentiation was run in triplicate to ensure reproducibility.

Statistical Analysis

Statistical analyses were run using SPSS Software (Version 20) and Statistica (Version 22.0). All data were normally distributed as indicated by the Shapiro-Wilk tests for normality.

To determine whether there were any differences at baseline in girls and women, independent t-tests were used to compare resting values of myoblast and osteoblast proliferation and differentiation, respectively.

A repeated measures ANOVA was carried out to determine the effects of exercise on cell proliferation and differentiation in both groups. The two factors examined were group (girls, women) x time (REST, EX1, EX2, REC1, and REC2). A linear regression was also run to identify the relationship between myoblast and osteoblast activity, using separate

analyses for proliferation and differentiation. Values were expressed mean ± SD unless stated otherwise. Significance was set a $P \leq 0.05$.

RESULTS

Participant Characteristics

The baseline characteristics for the participants are outlined in Table 1. Girls and women differed significantly across all characteristics, with girls exhibiting higher aerobic fitness normalized to fat-free mass and MVPA relative to women.

Cell Proliferation

Myoblast and osteoblast proliferation are presented in Figure 1A and Figure 1B. There were no group differences in myoblast proliferation at REST, $t(1,22)=0.576$, $p=0.643$. A significant main effect was observed for group (girls vs. women), $F(1, 22)=4.476$, $p=0.046$, with women exhibiting higher levels of proliferation than girls (6.25%, 6.12%, 4.16%, and 4.16%) at all time points relative to baseline. No significant main effect for time was observed in myoblast proliferation, $F(1,22)=0.832$, $p=0.372$. There was no statistically significant two-way interaction between group and time, $F(4, 88)=0.332$, $p=0.856$.

There were no group differences in osteoblast proliferation at REST $t(1,22)=1.062$, $p=0.476$. A significant main effect was also observed for

group (girls vs. women), $F(1, 22)=4.300$, $p=0.050$, with women exhibiting higher levels of proliferation than girls (9.11%, 11.9%, 13.2%, and 4.5%) at all time points relative to baseline, No significant main effect for time was observed in osteoblast proliferation, $F(4, 88)=2.104$, $p=0.088$. There was no statistically significant two-way interaction between group and time, $F(4, 88)=1.775$, $p=0.141$.

Myotube Differentiation

Myoblast differentiation is presented in Figure 2. There were no group differences in myotube differentiation at REST $t(1,22)=-1.646$, $p=0.845$. Myoblast differentiation was not different by group ($F(1,22)=1.262$, $p=0.273$) or by time ($F(2.938, 64.640)=1.468$, $p=0.232$). There was no statistically significant two-way interaction between group and time, $F(2.938, 64.640)=0.839$, $p=0.475$.

Osteoblast differentiation

Osteoblast differentiation is presented in Figure 3. There were no group differences in osteoblast differentiation at REST $t(1,22)=-1.427$, $p=0.138$. Mineralization was not different by group (main effect, $F(1,22)=0.563$, $p=0.203$). There was, however, a main effect of time ($F(4, 88)=2.602$, $p=0.041$). Post-hoc testing with Bonferroni correction indicated that mineralization was significantly lower at REC2 relative to REST ($p=0.014$).

There was no statistically significant two-way interaction between group and time, $F(4, 88)=0.916$, $p=0.458$.

Relationship between osteoblast and myoblast proliferation

The regression analysis indicated a linear correlation between osteoblast and myoblast proliferation at REST (r^2 =0.30, p= 0.005), EX2 (r^2=0.21, p=0.022), and REC1 (r^2=0.38, p=0.001) (Figure 4). No relationship was observed for differentiation outcomes.

DISCUSSION

We set out to investigate the systemic effects of exercise on osteoblast and myoblast proliferation and differentiation in girls and women. Osteoblast differentiation significantly decreased at the end of recovery for both girls and women (Figure 3), whereas no exercise or group effects were observed in myoblast differentiation (Figure 2). Proliferation of osteoblasts and myoblasts were significantly lower in girls than women post-exercise and during recovery (Figure 1). Our analysis also confirmed a direct linear relationship between osteoblast and myoblast proliferation at rest, post-exercise and recovery (Figure 4).

Our study is one of two studies that assessed the effects of human exercise-induced serum on skeletal muscle cell proliferation. Previous findings demonstrate that serum obtained from mid-pubertal children (n=11;

9 of which were male) after an acute bout of moderate intensity exercise elicits a proliferative response in C2C12 myoblasts at the end of exercise and recovery (37). The response was attributed to the systemic environment which demonstrated an increase in pro-inflammatory cytokines with exercise, particularly IL-6 (Nguyen et al., 2014). Though there are contradictory findings on the *in vitro* effects of IL-6 on myoblasts, the evidence predominantly supports that IL-6 is a potent proliferative agent in myoblast cultures (Podbregar et al., 2013) as seen with the upregulation of growth-related proteins, such as myf5, Pax7 and MyoD (Yu et al., 2015). However, in our study, myoblasts did not show enhanced proliferation with exercise suggesting that other circulating factors may hinder cell growth after exercise in pre-pubertal girls and adult women. For example, TGF- β increases after an acute bout of exercise (Czarkowska-Paczek et al., 2006), and has previously been observed *in vitro* experiments to inhibit myoblast proliferation (Kim & Lee, 2017). Alternatively, concentrations of other factors that stimulate cell growth may decrease in the systemic environment with exercise, as observed previously with in IGF-1 and FGF-2 in children and adults (Bruserud et al., 2005; Nemet et al., 2002).

Proliferative indicators are downregulated when differentiation markers are increased *in vitro*, suggesting that the inhibition of proliferation is an important step prior to differentiation (Dogra et al., 2006). However,

this was not the case in our study as demonstrated by the lack of exercise-induced changes in myotube formation in girls and women. This is consistent with a previous study by Nguyen et al. (2014) in which no changes were observed in mRNA levels of myogenin and SOCS3, markers of differentiation, in mid-pubertal children after an acute bout of MICE (Nguyen et al., 2014). Moreover, myoblast differentiation markers, such as myosin heavy chain-I (MHC) mRNA expression and satellite cell activation, do not change in response to an acute bout of moderate intensity cycling, as indicated by muscle biopsy collection in young healthy men (Snijders et al., 2012). These findings suggest that one bout of acute exercise may be insufficient to induce a change in myotube differentiation and repeated exercise training may be required to elicit acute benefits. Indeed, myotube formation was shown to increase after an acute bout of aerobic and anaerobic exercises in exercise trained individuals who have been training for three consecutive years (Vitucci et al., 2018). The effects of exercise on pronounced myotube formation were primarily attributed to increased levels of systemic IGF-1, which is known to increase with training (Vitucci et al., 2018).

Similar to myoblasts, our findings with osteoblast cultures indicate that the systemic environment induced by MICE does not elicit a proliferative response. Many of the markers that are upregulated acutely

after exercise are pro-inflammatory in nature (ie. IL-6, IL-1, TNF α) and have been shown to compromise bone growth by inhibiting osteoblast function in clinical conditions such as osteoporosis and arthritis (Malysheva et al., 2016). Although the concentration of these markers are high in pathological conditions, even modest increases in the concentration of pro-inflammatory cytokines such as IL6, as observed in response to exercise, have been shown to interfere with osteoblast activity *in vitro* (Bakker et al., 2014). This indicates a potentially causal relationship between moderate inflammation and bone status (Frost et al., 1997). Inflammatory markers are believed to influence osteoblast proliferation by inhibiting the Wnt signaling pathway and upregulating RANKL expression, which is an osteoclastogenesis-promoting factor that is produced by osteoblasts (Frost et al., 1997; Malysheva et al., 2016). Indeed, serum collection after acute bouts of exercise demonstrate increased levels of RANKL (Mezil et al., 2015), as well as related markers of bone resorption such as carboxyterminal telopeptide of type 1 collagen (Langberg et al., 2000) and osteocalcin (Patti et al., 2013). Moreover, osteocyte-derived factor release, such as sclerostin, which is triggered by exercise also inhibits osteoblast activity, suggesting that local regulators also prevent bone growth immediately after an acute bout of exercise (Kouvelioti et al., 2018).

Osteoblast mineralization changed with time for both girls and women, resulting in reduced mineralization at the end of recovery. This observation is in line with *in vivo* studies that have shown an increase in bone resorption marker concentrations (in serum or urine) shortly after acute bouts of exercise (Banfi et al., 2010). Concurrently, serum concentrations of bone alkaline phosphatase (BAP) and pro-collagen type 1 N-terminal propeptide, markers reflective of increased bone formation activity, have been shown to decrease or remain unchanged acutely after bouts of low- to moderate-intensity exercise, which may explain our findings (Guillemant et al., 2004; Malm et al., 1993). Moreover, the treatment of osteoblast cultures with recombinant IL-6 and CX3CL1, as observed after an acute bout of exercise, upregulates osteoclast activity while decreasing mineralization (Koizumi et al., 2009). In contrast, some studies indicate delayed increases in serum concentrations of BAP and osteoprotegerin hours to days after an acute bout of low-impact, high-intensity exercise suggesting delayed onset of bone formation (Scott et al., 2011; Tosun et al., 2006). Indeed, the same markers of bone formation are more likely to remain elevated after multiple bouts of exercise training (Erickson & Vukovich, 2010; Shibata et al., 2003), or after an acute bout of high-intensity exercise (Mezil et al., 2015; Rudberg et al., 2000; Scott et al., 2010). Training also leads to higher concentrations of growth factors while

attenuating the pro-inflammatory response, both of which improve osteoblast activity and, as noted above, promote myotube formation (Banfi et al., 2010; Berg & Bang, 2004; Vitucci et al., 2018). Taken together, the systemic environment plays an important role in mobilizing factors that regulate muscle and bone function when stimulated by exercise, and the magnitude of anabolic effects may depend on the frequency of exercise.

Despite a lack of exercise-induced effects on muscle and bone proliferation, we did observe main effects for group, with women demonstrating greater muscle and bone proliferation compared with girls. This suggests that in comparison to prepubertal girls, women may be predisposed to greater anabolic effects in muscle and bone due to the pro-proliferative factors that are inherent to their systemic environment, such as higher estrogen levels (Tobias, 2003). Estrogen increases osteoblast and myoblast proliferation *in vitro*, and is highly correlated with increases in muscle cross sectional area and bone accrual in adolescents and adults due to its ability to lower mechanical thresholds in both tissues (Schoenau, 2005; Tobias, 2003; Velders et al., 2010; Yin et al., 2015), As such, the differences in the systemic environment between prepubertal girls and women may explain why women doing similar low-impact exercises (such as swimming) may experience improvements in muscle and bone strength indices (Gómez-Bruton et al., 2013) while prepubertal girls engaging in the

same protocol may not experience the same effect (Courteix et al., 1998). In contrast, high-impact exercise (namely plyometrics) stimulates pronounced effects on muscle and bone turnover and strength indices in prepubertal children relative to postpubertal children, adolescents, and adults (Kish et al., 2015; Peitz et al., 2018). Taken together, these findings suggest mechanical strain may be largely responsible for muscle and bone growth during the prepubertal stages. However, with maturing age muscle and bone growth is regulated by the interactions of the mechanical and systemic input due to the emergence of physiological mediators in the systemic environment. To address this hypothesis, a future step would be to assess the influences of the systemic environment on myoblast and osteoblast proliferation in multiple maturational stages, while considering the differences between acute bouts and training exercise on muscle and bone growth.

In this study we utilized a novel cell culture approach to study the effects of an exercise-induced systemic environment on cellular processes, although our study is not without limitations. We acknowledge that the use of human serum on mouse cell lines may limit the application of our results since we investigated the effects of systemic factors from one species on another species tissue. However, relative to primary human osteoblast and myoblasts, both C2C12 and MC3T3E1 cells are more proficient in growth,

proliferation, and differentiation and thus serve as a good model to assess the physiological adaptations of muscle and bone. Another limitation to this study is that the results may be influenced by factors beyond exercise and development, such as differences in aerobic fitness and levels of habitual physical activity. The prepubertal girls in this study were more fit and physically active than women (Table 1), which may consequently influence their systemic environment by characterizing it with increased levels of anabolic factors due to their higher fitness levels (Berg & Bang, 2004). However, this anabolic effect was not translated *in vitro,* therefore further investigation is required.

In conclusion, the systemic environment following one acute bout of low impact MICE in girls and women does not elicit anabolic responses in osteoblast and myoblast activity. While our data do not provide signaling mechanisms whereby moderate intensity exercise affects muscle and bone, they do provide new insights into the potential role of development on the systemic regulation of muscle and bone growth *in vitro.*

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Table 1: Participant Characteristics

Age (years)	10.0 ± 0.8	23.2 ± 2.3	<0.0001
Height (cm)	140.6 ± 7.2	162.1 ± 6.8	<0.0001
Height percentile	57.6 ± 18.4	N/A	
Weight (kg)	32.9 ± 3.17	56.2 ± 8.0	<0.0001
Weight percentile	47.1 ± 14.3	N/A	
BMI	16.8 ± 1.2	21.6 ± 2.7	<0.0001
BMI percentile	45.2 ± 19.8	N/A	
% Body Fat	14.4 ± 5.3	24.7 ± 7.4	0.0004
Fat Free Mass (kg)	28.1 ± 2.6	42.27 ± 7.3	<0.0001
Maturity*	Tanner 1: n= 6 Tanner 2: n=6	N/A	
VO2max (mL/kg$_{FFM}$/min)	59.6 ± 11.2	49.05 ± 8.4	0.016
MVPA (min/day)	65.1 ± 20.2	40.3 ± 19.8	0.006
MVPA (min/hr wear time)	5.0 ± 1.5	3.2 ± 1.3	0.005

Values expressed as mean ± SD
BMI: body mass index; FFM: fat-free mass; N/A: Not Applicable
*Based on breast development.

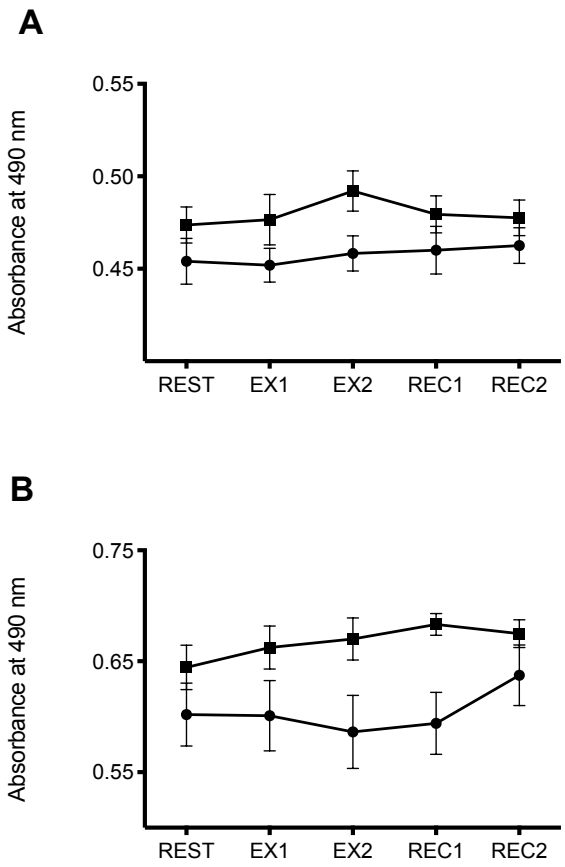

Figure 1: Systemic effects of exercise on proliferation of myoblasts (A) and osteoblasts (B) in girls (●) and women (■). REST: rest, EX1: midpoint of exercise, EX2: end of exercise, REC1: midpoint of recovery, REC2: end of recovery. (a) Significant time effects denoted by $p \leq 0.05$. Values are displayed as mean ± SE.

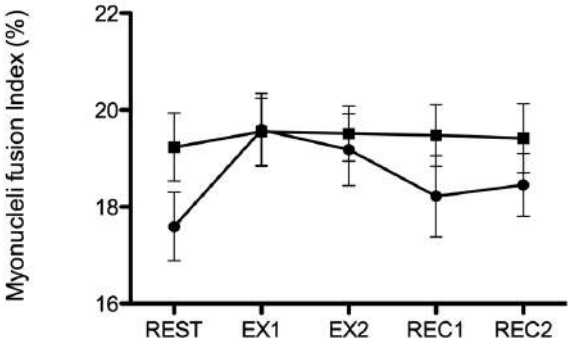

Figure 2: Systemic effects of exercise on myonuclei fusion index in girls (●) and women (■). REST: rest, EX1: midpoint of exercise, EX2: end of exercise, REC1: midpoint of recovery, REC2: end of recovery. (*) Significant time effects denoted by $p \leq 0.05$. Values are displayed as mean ± SE.

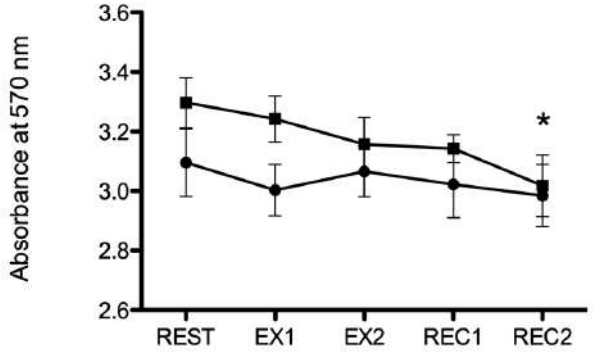

Figure 3: Systemic effects of exercise on osteoblast mineralization in girls (●) and women (■). REST: rest, EX1: midpoint of exercise, EX2: end of exercise, REC1: midpoint of recovery, REC2: end of recovery. Values are displayed as mean ± SE.

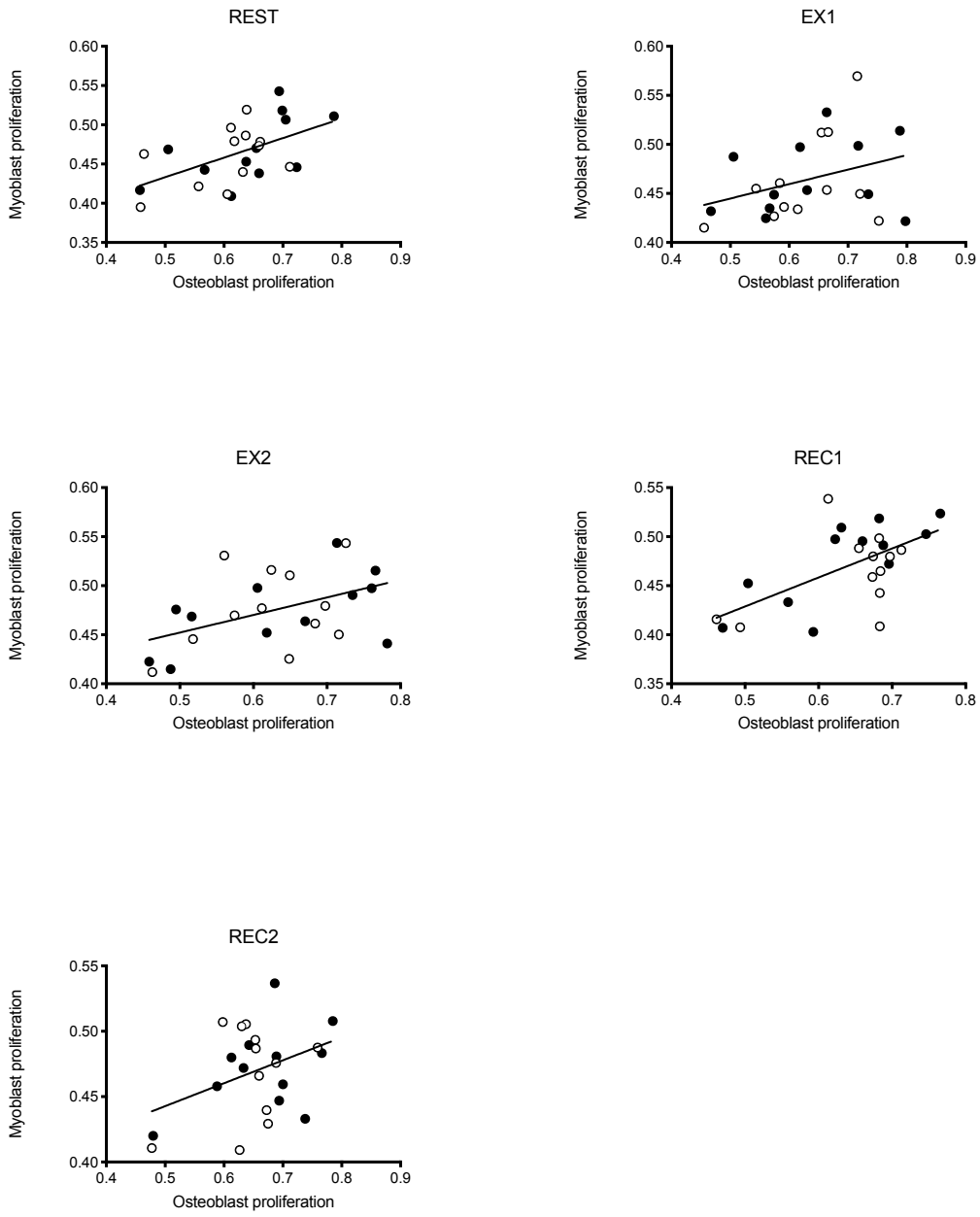

Figure 4: Linear regression for osteoblast and myoblast proliferation for girls (●) and women (○). All 24 participants were included, with each point representing an individual participant. Significant correlations were observed for REST, EX2 and REC1.

CHAPTER 4

Exercise Messengers: Exploring Learning Perceptions of a Science Animation Video Using Q-Methodology

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This article is currently being prepared for submission.

Dr. Brian Timmons, Dr. Sandeep Raha, Bhanu Sharma and I contributed to the design of the study. I was responsible for participant recruitment. I completed data collection, with the support of Bhanu Sharma and additional members of the Child Health and Exercise Medicine Program. I was responsible for the analysis of the data presented. Bhanu Sharma and Dr. Noori Akhtar-Danesh assisted with statistical analysis. I drafted the manuscript, with support from Dr. Brian Timmons, Dr. Sandeep Raha, Dr. Noori Akhtar-Danesh and Bhanu Sharma.

ABSTRACT

Purpose: The purpose of this study is to assess children's learning views and understanding of an animated chalkboard video about physical activity and bone health. **Methods:** The findings of Study I and Study II, along with pertinent literature, were summarized into a storyboard designed for children between ages 8-14 years. The storyboard was converted into an animation video using commercially available platforms: Procreate© for illustrations, EZGIF© and Powtoon© for animations, and Audacity© for character voice-overs. The resulting video was 9 min and 43 s. Upon video completion, children between 8-14 years were recruited to attend the video screening at McMaster University. Participants were assessed for their viewpoints towards the video using a q- method activity comprising of 25 statements about the video. Participants were tasked to rank their statements in order of their level agreement/disagreement, and explain their reasoning for choosing *strongly agree/disagree with* statements. After the q-method activity, participants completed a quiz about the video content which assessed their level of understanding of the video. Data was analyzed using Stata® (Version 16). **Results:** Thirty-one boys and girls with a mean age of 11.2 ± 2.0 years were recruited to participate in this study. Q-factor analysis produced 4 factors, which revealed multiple considerations for utilizing chalkboard animation videos for physical activity and/or science

education, such as level of engagement, enjoyment, and motivation. Moreover, a significant difference was noted between learning viewpoints and understanding (p=0.0200). No differences between demographics (sex, age) and learning viewpoints or understanding were observed. The feedback collected from participants was used to revise the video for future use. **Conclusion:** The use of animated chalkboard videos to convey knowledge on physical activity may serve to be educationally valuable for children with characteristic learning views.

BACKGROUND

Science education presents multiple challenges, amongst them are conveying complex physiological processes in a manner that is easy to understand. Presenting scientific knowledge in an understandable and engaging manner can be particularly tasking, more commonly with elementary school children (Barak et al., 2011). As such, science educators have turned to multiple modalities to supplement education, including the use of animations. Animations are a special form of multimedia materials that are characterized by their interactive nature and extensive use of pictorial representation of information (Türkay, 2016). Animated videos are effective in conveying knowledge because of their ability to present concepts in visual ways that are otherwise difficult to conceptualize, which has been demonstrated with complex subjects such as physiology, molecular biology, and physics (Barak et al., 2011).

Despite the increasing popularity of using science animations, studies on the utility of animation videos in science education has yielded mixed results, with some studies showing success in student engagement and performance, and others a lack thereof (Cheung et al., 2017). Some students may express higher engagement with animations, while others require additional tools for high-level engagement to be reached. While these discrepancies can be intrinsic to the type of video intervention, they

may also be a result of the diverse learning perceptions within a given classroom. Perceptions towards science may also influence learning outcomes and motivation to pursue related learning activities (Iqbal Shah & Muhammad Khan, 2015; Wyss et al., 2012). Thus, understanding of the perceptions within a given classroom may be an important perquisite to determining the effectiveness of a science-animated video on factors associated with learning, such as engagement and motivation.

In order to assess perceptions, a method is required that would allow for subjective viewpoints to be identified. Q-methodology combines qualitative and quantitative techniques to identify subjective viewpoints, thereby offering great potential to explore factors that may influence engagement with pedagogical material (Akhtar-Danesh et al., 2007; Brewer-Deluce et al., n.d.). The process of Q-methodology involves the development of a sample of statements that are related to the topic of interest, and rank-ordering them by a group of individuals from their points of views about the statements using a Q-sort table (a grid) with a quasi-normal distribution **(Figure 1)** (Akhtar-Danesh, 2017). When Q-sorts of several persons are analyzed, ‘factors’ indicate groups of persons who have ranked the statements in much the same way, thereby identifying viewpoints within a sample (Taylor et al., 1994). Indeed, the use of Q-methodology has been validated in multiple forms of psychosocial research

to understand elements of subjectivity, such as attitudes, perspectives, and beliefs within an audience (Ellingsen et al., 2014; Watts & Stenner, 2005). It has also been used to evaluate educational programs by identifying areas for course strength and improvement by means that are aligned with student needs in an evidence-based way (Brewer-Deluce et al., n.d.). Q-methodology has proven to be well-suited for children's participation in methodological research as it provides them with an easy and non-threatening way to express their views while taking into consideration their perceptions and cognitive abilities (Ellingsen et al., 2014; Taylor et al., 1994). The sorting nature of Q-methodology in particular creates a tangible stimuli that can encourage children to share their reflections and help them sort and express what they think and feel about a topic in a systematic manner (Ellingsen et al., 2014).

Thus, the primary objective of this study is to examine the educational utility of a science-animated video by using Q-methodology to explore student perceptions. The secondary objectives are to evaluate learning by assessing knowledge retention, and explore whether any relationships exist between the latter and perceptions.

METHODS

Development of the animation video, "Exercise Messengers"

The animation video follows the story of three children who are curious about how exercise makes their bones grow (Youtube© link: https://bit.ly/326NfYk). The storyboard was curated based on the findings of a doctoral thesis which investigated the acute effects of exercise on systemic regulators of muscle and bone in girls and women, along with existing literature pertaining to the subject (Hamrick, 2011, 2012). The illustrations and animations were designed using the commercially available application, Procreate© Version 4.3.4, and web application, Powtoon©, respectively.

The setting of the video takes place during science period in the classroom, where three children named Mai (girl), Arjun (boy), and PJ (boy) engage in an educational conversation with their teacher, Ms. Z. They begin by asking how exercise makes them grow, after which Ms. Z explains the benefits of exercise to their bones. As the discussion continues, Ms. Z explains physiological concepts to the children such as the systemic effects of exercise on bone remodeling **(Figure 2)**. To incorporate a narrative into the discussion, the animations focus on the journey of a cytokine as it ventures in the systemic environment to find bone cells and deliver information about an exercise stimulus. The script was developed to allow the physiological concepts to be explained in an age-appropriate form targeting 8-10 year-old children, for example, *cytokines* were alternatively

referred to as “exercise messengers”. Certain concepts were repeated in multiple ways, both visually and verbally, to aid understanding. At the end of the video, the children summarize what they learned in science class, such that any technical terms mentioned by Ms. Z were interpreted and explained in a way that children could understand. The script was revised by pediatric researchers and elementary school teachers, and three children between the ages of 8-14 years old to ensure that it met the readability of the target audience.

Development of the Q-methodology assessment

The first step to generating the Q-methodology assessment is to explore the educational themes of the video by generating a list of broad statements, otherwise known as the concourse (Brewer-Deluce et al., 2019). The aim of developing the concourse is to generate statements that are likely to be the broad representation of the relevant opinion domain, and this can be achieved from any number of sources (Watts & Stenner, 2005). We chose to develop the concourse with individuals who specialize with working with children in an educational and research context. As such, the concourse around this video was generated from the following:

(1) Pediatric exercise researchers: A shared document was generated for 10 pediatric exercise researchers at McMaster University to list down content-specific statements about the script of the video. Some of the researchers were also parents of children who met the target audience.
(2) Examples from related literature (Barak et al., 2011; Soffer & Yaron, 2017).

In addition, the document was also shared with the following individuals who also contributed to generating statements to the concourse:

(3) Two elementary school teachers
(4) Knowledge translation researcher, CanChild
(5) Science outreach worker, Scientists in School

A total of 49 statements were compiled from all of the sources listed above. The statements were reviewed for their uniqueness and clarity by a panel that was comprised of three to five pediatric researchers who participated in generating the concourse. After three rounds of revision, a series of 25 statements was drawn from the original concourse. These statements reflected attitudes towards the video and subject matter. The readability

score of the statements was checked using an online readability tool (ReadabilityFormulas.com ©) which calculated the scores based on 8 readability indices. The reading levels of the statements was appropriate for 10-11-year-old children, and was marked as fairly easy to read.

A Q-sort table was then developed with 25 cells, so that each of the statements could be ranked and ordered within the table to permit subsequent analyses. In addition, a short feedback questionnaire was developed for participants to explain their choices at either extreme (-4 and +4) by writing brief statement to contextualize their response.

Development of the knowledge retention assessment

To assess whether children understood the video, a questionnaire was developed pertaining to the specific content of the video. A total of 12 questions were developed: six multiple choice and six true or false statements. The questionnaire was reviewed by participating pediatric researchers to ensure that it was age-appropriate based on questionnaires that were regularly administered to children in the Child Health and Exercise Medicine Program. The reading levels of the questionnaire was appropriate for 10-11-year-old children, and was marked as fairly easy to read.

Participants

Thirty-one boys and girls between 8-14 years old were recruited to participate in this study (n=15 girls, and n=16 boys, with a mean age of 11.2 ± 2.0 years). A formal sample size calculation was not performed as it is not typical for Q-studies. The purpose of Q-methodology is to identify typologies within a cohort, rather than estimating proportions or making comparisons between subgroups, therefore low response rates do not bias results (Brewer-Deluce et al., 2019).

Children were recruited from the Hamilton community through announcements and social media advertisements. Announcements were made during the McMaster Child Youth University Family Lecture series, a science outreach program that aims to engage children in science inquiry. In addition, families who had previously participated in the Child Health and Exercise Medicine Program were also contacted through email to participate in the study. All participants and parents/guardians provided written informed consent and assent, respectively, prior to enrollment in this study, which was approved by the Hamilton Integrated Research Ethics Board (Project No. 5366).

Data Collection

Data was collected over the course of three sessions at McMaster University. Each session started with having the participants and their

parents fill out assent and consent forms, followed by collecting their demographic information (age and sex). Participants and their families were then guided to a lecture hall where they watched the video without any background introduction. Immediately after the video screening, participants were guided to a separate room to complete the Q-sort activity. The student investigator provided the instructions prior to the Q-sort activity, making sure they were consistent in each session. Participants were given hard copies of the 25 statements (each printed and cut out individually, allowing participants to physically manipulate and rearrange statements during the ranking process activity **(Figure 3)**. Participants were instructed to read all of the statements and then rank them (based on degree of agreement/disagreement) according to the Q-sort headings that were taped to the desk (Akhtar-Danesh et al., 2008). Statements were ranked under the "0" (zero) column reflected neither agreement nor disagreement, whereas the highest and lowest levels of agreement and disagreement were marked as "+4" and "-4", respectively**.** Participants were told that each column should contain the appropriate number of statements at the end of the sorting activity. Participants were informed that they could change the rank of any of their statements during the sorting activity as many times as needed, but the final, completed Q-sort table should reflect their viewpoints after consideration of all statements. Once participants completed the Q-

sort table, they were provided with the feedback questionnaire and instructed to write a brief statement to contextualize their responses at either extreme.

Participants who completed both portions of the Q-sort activity were then directed to a separate room to complete the content questionnaire. Participants were instructed to read the statements and circle the correct letter for the multiple choice and true or false sections.

Participants completed the Q-methodology assessment and content questionnaires within approximately 15-20 minutes and 5-10 minutes, respectively. There were 4-6 pediatric researchers in each session who facilitated the questionnaires and assisted the participants with reading the statements when needed.

Analysis

Raw data were manually entered into Microsoft Excel spreadsheet, and then imported into Stata software for subsequent analyses using the "qfactor" command in Stata (Akhtar-Danesh, 2018). An iterated principal axis factor extraction with varimax rotation was used for analysis. Factor scores for each statement were subsequently calculated using a weighted averaging to compare between factors (Brewer-Deluce et al., 2019).

Data from the Knowledge Retention Questionnaire was analyzed separately and in conjunction to factors identified in the q-analysis. Performance in this questionnaire was analyzed based on 3 components: performance on multiple choice questions, true or false statements, and overall performance. To assess whether the perceptions towards the video influenced knowledge retention (level of understanding), a one-way ANOVA was conducted between each factor and questionnaire component.

RESULTS

Q-methodology assessment

A by-person, iterated principal axis factor analysis and varimax rotation on the 31 participants extracted four factors representing four major perceptions of towards the Exercise Messengers Video **(Table 1)**. All Q-sorts analyzed were accounted for by these four factors, with each participant loading significantly on a single factor. It should be noted that n=6 participants did not load on to any factor, and were therefore excluded from this analysis, resulting in a final sample of n=25. There was no statistically significant relationship between the factors and any of the demographic variables (age and sex). Participant demographics, by factor, are noted in **Table 1**. It should be noted that the following naming (1: Engaged Learners, 2: Action Takers, 3: Interactive Learners, and 4:

Receptive Learners) and description of each factor is subjective but based on the interpretation made by the study team of the statements that loaded on to each factor.

The rankings of the distinguishing statements per factor noted in **Table 2.** Distinguishing statements (having statement scores that are significantly different between the highlighted factor and the other factors) are noted in factor columns in bold. Statements demonstrating consensus across factors (no significant differences) are noted by an asterisk.

Factor 1: Engaged Learners

This group actively understood the concepts explained in this video and promoted the use of the video as a learning tool in educational settings.

Factor 2: Action Takers

This group was able to reflect on the concepts in the video, and were motivated to change their behavior based on the messaging in the video.

Factor 3: Interactive Learners

This group engaged least with the video while expressing a greater preference to discuss the content with their teacher.

Factor 4: Receptive Learners

This group showed an openness to the video, but still prefer traditional learning (ie. books) despite seeing utility in sharing it to family and friends.

Consensus Statements:

Only one consensus statement was identified in our sample **(Table 2)**. All participants felt that the video helped them understand what happens to their body when they exercise. Rankings on this statement were relatively in agreement, ranking between 1-2.

Knowledge Retention

The average score of the Knowledge Retention Questionnaire was 78.7% ± 15.9%, with 20/31 participants performing over 80%. There were no significant relationships between understanding and age or sex.

All factors performed similarly on the multiple choice component, $F(3,24)=1.16$, $p=0.3494$. However, there were significant differences in the true or false component, $F(3,24)=6.56$, $p=0.0027$, and overall performance, $F(3,24)=4.07$, $p=0.0200$. Post-hoc tests with Tukey correction showed that Factor 3 (Interactive Learners) performed significantly lower than Factors 1 (Engaged Learners) and 4 (Receptive Learners) (HSD tests: 4.8259, 3.9485) **(Figure 4).**

DISCUSSION

We set out to explore the perceptions of elementary-aged children towards a science-animated video about the effects of exercise on bone physiology. We also assessed knowledge retention to further assess the utility of the video as an educational tool, and to determine whether there is a relationship between perception and knowledge retention.

We identified four salient perceptions within this sample, which were described as Engaged Learners, Action Takers, Interactive Learners, and Receptive Learners. We classified these perceptions based on student-ranked statements related to perceived understanding, engagement, action-based thinking, enjoyment, learning preferences, and endorsement. Engaged Learners actively understood concepts explained in the video and promoted the use of the video as a learning tool in educational settings. Action Takers were able to reflect on the concepts in the video, and were motivated to change their behavior based on the messaging of the video. Interactive Learners engaged least with a video while expressing a greater preference to discuss the content with their teacher. Lastly, Receptive Learners showed openness to the video, but still preferred traditional learning despite seeing utility in sharing the video with family and friends. Most notably, the perceptions shared similarities and differences according

to perceived level of understanding, support of the educational utility of the video, and intent to modify behaviour.

Perceived level of understanding

Generally, participants felt that they understood the video, which was reflected by their shared agreement of the consensus statement **(Table 2)**. Perceived understanding was also demonstrated in different ways across the factors, with some participants expressing that the video helped to learn something new (Action Takers) and/or the meanings of new words (Engaged and Interactive Learners), and others expressing that the visual aids used in the video (ie. cartoons and examples) helped to learn the content (Receptive Learners and Engaged Learners). Collectively, these responses indicate that perceived understanding and engagement with the content may have been facilitated by multiple elements of the video, which was addressed in the feedback provided by the participants:

> EL1 (F, 12y): “This video was very simple and easy to understand”
>
> EL2 (M, 10y): “It told a lot of detail why different types of exercise are good for my bones in the video”

The simplicity of the video promoted perceived understanding across different members of this group. The simple visualization and ease of use as demonstrated by other multimedia modalities (ie. animations and tablets) has been shown to promote perceived learning by encouraging student engagement, exploration, and attention to the material (Iqbal Shah & Muhammad Khan, 2015; Mitrovic & Suraweera, 2000; Ward et al., 2011). Perceived understanding can also be improved by the use of pictures (Iqbal Shah & Muhammad Khan, 2015), as indicated by the following participants:

> EL3 (F, 12y): “Video was very easy and clear, and all the little visual aids helped a lot”.
>
> AT1 (F, 13y): “The video helped me get more ideas of how our muscles work in order to make our bones healthy. This video shows pictures and also at the side they write the name of it. In class our teacher would just say it instead of explaining it properly.”

A fundamental principle behind multimedia learning is that students learn better from words and pictures rather than from words alone (Mayer et al., 2005). The combined use of words and pictures, including static images and video, allows the brain to process more information in working memory, and further enhances interest in the content (Carmichael et al., n.d.; Mayer et

al., 2005). The use of animations in particular can also enhance explanation ability (Barak et al., 2011), as demonstrated by the following:

> EL4 (M, 10y): "I got what the video meant when the 'clast' broke down the bone to make new stronger bones. I learned that there are 2 different types of cells in your bone and your bones are alive."

That participants were able to transfer knowledge (ie. clasts, cell types) was possibly facilitated by the combined use of animations and words, specifically those that were repeated multiple times in the video given the association between repetition and retention (Bishop et al., 2012). Although knowledge transfer is primarily treated as a learning outcome, it has also been shown to be influenced by perceptions on learning and competence (Hoogerheide et al., 2014). Indeed, the act of learning with the intention of explaining it to others further enhances engagement with the content (Hoogerheide et al., 2014), which may explain why Receptive Learners were in high agreement with statements around sharing the video with others and explaining what they have learned from the video to friends and family.

Educational Utility

The setting for watching the video revealed to be important for all factors. In general, factors did not demonstrate the likelihood to watch the video at home. Participants felt that they "would rather play", or "watch movies not related to health" at home. According to the 2018 Children and Parent's Media Tracker Report, at least 75% of children between the ages of 7-15 years spend their screen-time watching content associated with leisure ie. funny videos, cartoons, pranks (*Children and Parents: Media Use and Attitudes Report 2018*, n.d.), which provides contextual insight to the with feedback provided in this study. However, when participants were asked about the use of the video in school, the perceptions of some factors changed considerably. Two of the factors (Engaged Learners and Action Takers) reflected a strong agreement for the use of the video in a school setting. Their agreement was further supported by their feedback statements:

> EL5 (F, 12y): "I think all students should watch this so they understand how their bones grow"
>
> EL6 (F, 14y): "…if they showed this video in schools, I think all children will like it and learn new things"

Based on participant feedback, preference to watch educational videos at school may be reflection of the social environment, where students are accustomed to a wide variety of collective and novel learning experiences relative to home, which are more closely related to leisure activities (Kent & Facer, 2004). Watching videos as a class may assist with providing contextual information that students may otherwise not have access to, thereby fostering student interest in the subject matter, as seen with elementary and secondary groups (Petrilli, n.d.; Wyss et al., 2012). Thus, that participants encouraged the use of this video in school may potentially be a result of their previous experiences with similar tools.

It is important to note, however, that there have been controversial findings with the use of videos in school, primarily due to the lack of integration with educational content (Barak et al., 2011; Petrilli, n.d.). Without the inclusion of reflection or application, some students may not perceive the importance of the video messaging, or may be left with misconceptions that hinder their understanding of the subject (Barak et al., 2011). Therefore, in order to produce favourable learning outcomes for certain perceptions, effective use of videos is encouraged with is tied to the course content and supplemented with interactive activities such as group discussions and assignments (Barak et al., 2011; Türkay, 2016). This is exemplified by the following feedback:

> AT2 (F, 11y): “[This video] taught me something important…but I think that this video should be more interactive”.

The importance of interaction was highlighted in the Q-sorts of Interactive and Receptive Learners, who preferred their teacher’s explanation or reading over watching a video, and were in least agreement with supporting the use of the video in school, as supported by the following:

> IL1 (F, 9y): “My teacher would more time to explain it to me”.
>
> RL1 (M, 9y): “I prefer to learn about books instead of videos because I like to read”.
>
> IL2 (F, 13y): “I could ask [my teacher] more questions and learn even more”.

Based on these responses, it is likely that the lack of interaction or supplementary reading material might have also influenced other perceptions, such as perceived understanding. For example, although Interactive Learners felt that they understood certain concepts in the video (ie. the effects of different types of exercise on bones), they were also most likely to watch the video again in order to thoroughly understand the content

relative to the other factors **(Table 2).** These findings indicate that the implementation of interactive or reflective components may be necessary to help foster interest and reinforce concepts presented by a video for certain groups of learners.

Motivation to change behaviour

As a means of addressing the perceptions towards the subject matter of the video ie. exercise, participants were presented with statements related to exercise and potential interest to pursue the activity or learn more about it. Amongst the four factors, Action Takers highly agreed with statements that reflected the messaging of the video onto their own behaviours. For example, Action Takers highly agreed that this video encouraged them to ask their teacher more questions about exercise relative to the other factors. Most considerably, they were also motivated to exercise after watching the video, and felt that they should exercise in order to keep their bones strong and healthy.

That participants showed interest to exercise and inquire about the activity indicates that this video was able to influence their perceptions towards the activity. According to Eccles, the capacity of an activity to influence children's perspective can be generally broken down four basic components (1) interest in, or enjoyment of the activity, (2) perceived

importance of being good at the activity, (3) perceived usefulness of the activity for short- and long-range goals, and (4) the cost of engaging in the activity (Eccles et al., 1993). In our sample, it seems that the perceived usefulness of the exercise was shown to be one of the main components in driving the viewpoints of Action Takers, as indicated in the following statements:

> AT3 (M, 8y): “I write it because I don’t want my bones to get weak.”
>
> AT4 (M, 9y): “This video made me want to exercise because I don’t want my bones to shrink.”
>
> AT5 (F, 14y): “I chose [this statement] because the video made me realize how important exercising actually is. The fact that your bones can break when you’re older made me realize that exercising at a young age is important. Teaching children and even kids my age would help them know that not doing exercise can impact them later in life.”

The feedback indicated by participants showed that agreement with these statements was based on concern of developing poor bone health, which is most likely attributed to the distinction made in the video regarding the impact of active and sedentary lifestyles on bone strength. Indeed, it has been shown that the clear distinction between positive and negative behaviors is a critical element in generating effective health education tools

that promote knowledge and behavioural change (Ferguson, 2012). Seemingly, this distinction resonated with Action Takers (and Receptive Learners to a lesser extent), resulting in an increased interest in wanting to exercise as a result of watching the video.

Additional elements that contribute to motivation are those that are intrinsically linked to the video, such as the presence of a narrative (Ferguson, 2012), inclusive representation as reflected by the choice of characters and setting (Ashby Plant et al., 2009), emotional design (Türkay, 2016). Effective emotional design, such as appealing colours and graphics, has been shown to not only impact learning outcomes, but also lead to higher intrinsic motivation, and better comprehension of the content (Türkay, 2016). This is supported by previous studies where participants expressed variable levels of motivation towards content delivered in different ways ie. traditional teaching methods and animated methods (Papastergiou, 2009). Students using animations will generally present a greater interest to continue learning the content as well as increased self-efficacy to perform the content-related tasks (Hoogerheide et al., 2014). That this video led to an increased motivation to exercise in Action Takers may be linked to elements mentioned previously, leading to the ability to internalize the messaging of the video:

> AT6 (F, 13y): “I have chosen [this statement] because this video has made me feel to exercise and made me feel that maybe we should help out our bones and keep them healthy. I think more videos should be made so other people can feel the same feeling that I had and also be curious.”

It is important to point out that by assessing participants’ interest to engage in a behavior does not equate to the pursuit of this action (*Guide to Monitoring and Evaluating Health Information Products and Services | Management Sciences for Health*, n.d.). Rather, these findings support that this video is a useful reflective tool, one that is associated with perceived knowledge and motivation to adopt a behavior and/or learn about it. Although intrinsic motivation can serve as a predictor for modified behaviors (*Guide to Monitoring and Evaluating Health Information Products and Services | Management Sciences for Health*, n.d.), this would be best addressed with a follow-up study that measures these outcomes.

Knowledge Retention

Generally, participants performed well on the knowledge retention assessment, with average score of 79% (16% SD), and approximately 20/31 participants scored above 80% on the questionnaire.

On a closer examination, we found that students performed highest on three categories of questions: narrative, process, and cued questions. Narrative questions were specific to the events of the story, showing that children were engaged with the storyline of the video. Process questions were specific to physiological processes that were addressed in the video, such the process of bone formation and breakdown. Cued questions were specific to questions that were addressed directly by the characters in the video, such as “Why does bone break down after exercise?”, which was addressed by one of students in the video. That participants were better able to recall concepts that were explicitly addressed by characters indicates that video elements can be used to direct learners attention to important aspects of the learning material, which has also been observed in other learning tools such as whiteboard animations (Türkay, 2016). Cueing facilitates overt attentional allocation to direct attention to important content, making educationally important aspects salient (Türkay, 2016). Given the conventional learning association with blackboards, this medium was particularly chosen to cue important concepts to facilitate learning.

Questions that tended to be answered incorrectly were definition- or distinction- related questions. For example, in one of the multiple-choice questions, students were asked what the about the reference term for “exercise messengers”. While some students answered correctly with

"molecules", 42% of students selected "bone cells" or "I don't know" as the answer. Similarly, in a true or false question, students were presented with the following false statement "Osteoblasts break bone, osteoclasts build bone", 68% of whom marked is as true. That participants struggled with term specific questions is in line with literature showing young learners are least likely to recall newly learned vocabulary shortly after exposure (Baker-Ward et al., 1993; Bishop et al., 2012). However, this outcome can be improved by the implementation of application or knowledge transfer activities (ie. short discussion, peer activity), which promote optimal learning (Hoogerheide et al., 2014; Türkay, 2016).

Perceptions and Knowledge Retention

We conducted comparative analysis to determine whether there was a relationship between perception and knowledge retention. The rationale for doing so was to identify whether certain perceptions were more or less likely to retain knowledge acquired from the video. We found that Interactive Learners achieved the lowest scores on the assessment, specifically in the true or false section, and overall performance **(Figure 4).** Interactive Learners performed significantly less than Engaged Learners and Receptive Learners, while also demonstrating differential perceptions towards the video. Interactive Learners felt the need to watch the video

again to understand the content, while both Engaged Learners and Receptive Learners highly disagreed with this statement. The latter groups also felt that cartoons made it easier for them to understand the video, whereas Interactive Learners disagreed with this statement. The latter sentiment was shared by both Interactive Learners and Action Takers, which may explain why there were no significant differences between these two groups in their performance on knowledge retention.

Altogether, findings suggest a possible relationship between learning perceptions and knowledge retention of educational material delivered with an animation video. While one perception may learn the content as presented by the video, other perceptions may benefit from additional reinforcements such as targeted interaction, supplementary reading, and/or increased exposure.

LIMITATIONS

Given the primary goal of Q-methodology in identifying viewpoints within a cohort, a sample size is not required (Brewer-Deluce et al., n.d.). Although Q methodology uses statistical procedures for data analysis, it is not possible to generalize findings directly to a greater population (Ellingsen et al., 2014). However, by exploring perceptions of participants, these findings can be considered to generate new hypotheses about the educational utility

of animated science videos that may be explored in future quantitative research.

CONCLUSION

Using Q-methodology, we showed that a given group of elementary students can have multiple learning perceptions towards a science-animated video about exercise and bone health. We focused on themes of perceived understanding, educational utility, and motivation to change behaviour to provide insight on the comprehensiveness of these perceptions, and their similarities and differences. We also found that perceptions may be related to students actual understanding of the content, as determined by knowledge retention.

We conclude that identifying learning perceptions can help educators to generate learning tools that will effectively engage students with various types of perceptions while taking into consideration their learning preferences. With this information, we can refine science animation videos and/or supplement them with additional learning tools, thereby optimizing science education for every student in the classroom.

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-4	-3	-2	-1	0	+1	+2	+3	+4

Figure 1: Example of a Q-sort Table

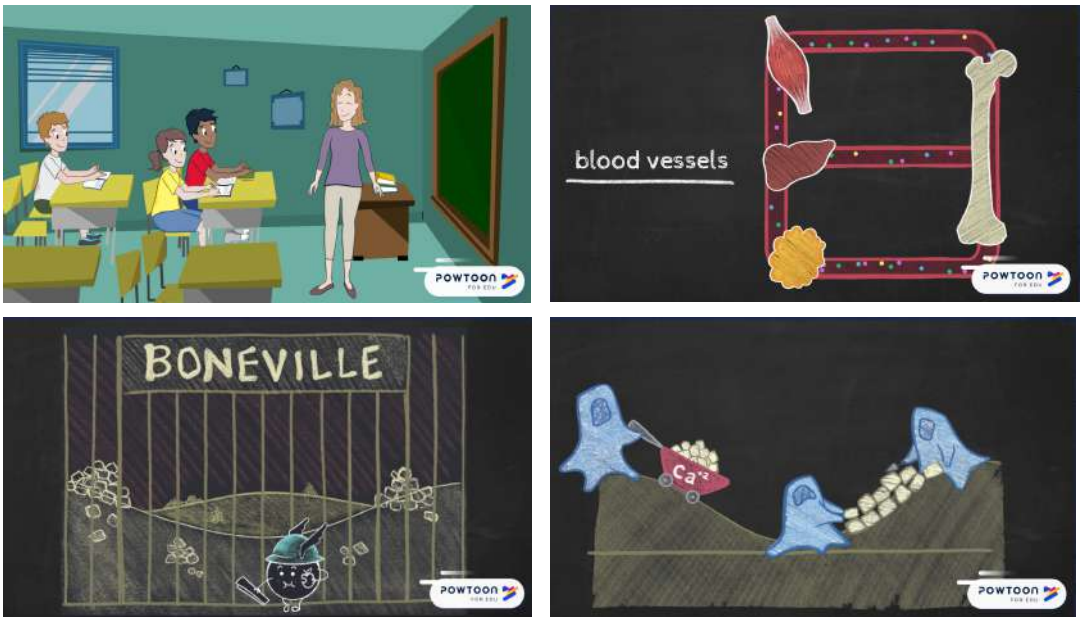

Figure 2: Screenshots of the *Exercise Messengers* video. The setting of the video takes place during science period in the classroom, where three children named Mai (girl), Arjun (boy), and PJ (boy) engage in an educational conversation with their teacher, Ms. Z. They begin by asking how exercise makes them grow, after which Ms. Z explains the benefits of exercise to their bones. As the discussion continues, Ms. Z explains physiological concepts to the children such as the systemic effects of exercise on bone remodeling. To incorporate a narrative into the discussion, the animations focus on the journey of a cytokine as it ventures in the systemic environment to find bone cells and deliver information about an exercise stimulus.

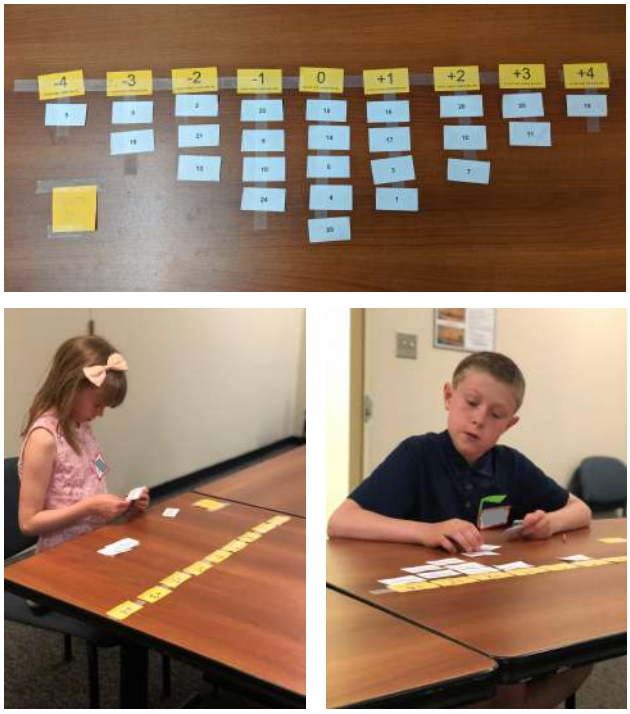

Figure 3: Q-sort activity. Participants were given hard copies of the 25 statements (each printed and cut out individually, allowing participants to physically manipulate and rearrange statements during the ranking process activity. Participants were instructed to read all of the statements and then rank them (based on degree of agreement/disagreement) according to the Q-sort headings that were taped to the desk (Akhtar-Danesh et al., 2008). Statements were ranked under the "0" (zero) column reflected neither agreement nor disagreement, whereas the highest and lowest levels of agreement and disagreement were marked as "+4" and "-4", respectively. Participants were told that each column should contain the appropriate number of statements at the end of the sorting activity. Participants were allowed to change the rank of any of their statements during the sorting activity as many times as needed, but were instructed that the final, completed Q-sort table should reflect their viewpoints after consideration of all statements. Once participants completed the Q-sort table, they were provided with the feedback questionnaire and instructed to write a brief statement to contextualize their responses at either extreme. There were 4-6 pediatric researchers in each session who facilitated the questionnaires and assisted the participants with reading the statements when needed.

Table 1: Participant Demographics by Factor

Characteristics	1: Engaged Learners	2: Action Takers	3: Interactive Learners	4: Receptive Learners	Total
Number of participants	9	8	4	4	25
Mean Age	11 $\pm$ 1.9	11 $\pm$ 2.6	11 $\pm$ 1.7	11 $\pm$ 2.7	11 $\pm$ 2.1
Sex; Male: Female Ratio	5:4	3:5	1:3	3:1	16:15

Table 2: Video Q-Sample Statements with Statement Rankings Grouped per Factor and Video Component Assessed

		1	2	3	4
Perceived Knowledge Attainment					
6	This video helped me understand why different types of exercise are good for my bones.	2	2	**3**	1
1	I learned something new from this video.	1	**3**	2	1
22	I was able to learn the meanings of new words from watching this video.	**1**	-1	2	-1
3*	This video helped me understand what happens to my body when I exercise.	1	2	1	1
2	I feel that I can explain what I've learned from this video to my friends/family.	-1	0	0	**2**
5	In order for me to understand this video, I would need to watch it again.	-4	-3	**-1**	-4
Video Engagement					

15	The examples in this video made it easier to learn how bone works.	0	0	0	**3**
18	I think the cartoons made it easy for me to learn about bones.	**3**	-2	-2	**0**
24	After watching this video, my feelings about exercise have changed.	-2	**1**	-2	-2
Perception-based Thinking					
23	This video made me want to exercise.	-1	**3**	0	-1
8	This video encourages me to ask my teacher more questions about exercise.	-3	**0**	-3	-3
14	This video makes me feel that I should exercise to keep my bones strong and healthy.	0	**4**	1	**2**
Video Enjoyment					
25	I liked watching this video	2	1	1	**-3**
Learning Preferences					
10	I would rather have my teacher explain exercise to me than watch a video.	**-1**	**-4**	0	0
21	I think learning about exercise is more fun when my friends and I do it together.	**-2**	**0**	4	4
9	I prefer to learn about exercise using videos instead of books.	**0**	**-3**	**2**	**-1**
Video Endorsement					
17	I would like to share this video with my family.	-3	**-1**	-2	**1**
20	I would like to watch videos like this at home.	-1	-2	**-4**	-2
16	I would like to share this video with my friends.	-2	-1	-3	**2**
7	I think this video and videos like this should be used in school.	**3**	1	0	0

*Consensus statement; Distinguishing statements by factor are in bold (indicate significantly different value from other factors, $p \leq 0.05$).

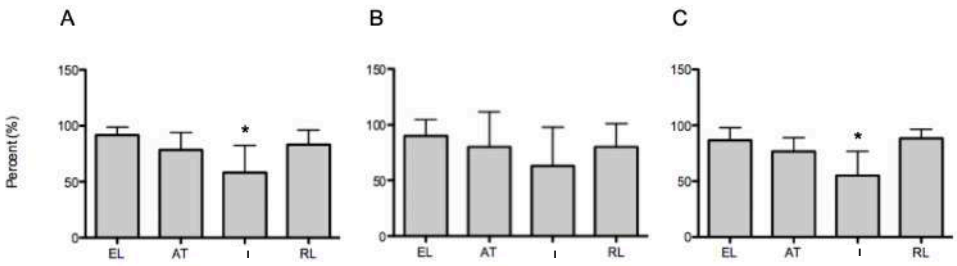

Figure 4: Performance on Knowledge Retention Per Factor. (A) Overall performance, (B) multiple choice, and (C) true or false. (*) denotes statistical significance at $p<0.05$ relative to performance of EL and RL.

CHAPTER 5: DISCUSSION

5.1 Objectives

The specific objectives of the studies in this thesis were as follows:

1. To assess the effects of an acute bout of moderate intensity exercise on systemic factors that regulate muscle and bone growth in prepubertal girls and women.
2. To assess the effects of an acute bout of moderate intensity exercise on myoblast and osteoblast proliferation and differentiation in prepubertal girls and women *in vitro.*
3. To explore children's knowledge retention and viewpoints towards a research video that translates the effects of exercise on muscle and bone.

5.2 Main Findings

In general, this study aimed to assess the effects of exercise on the systemic regulation of muscle and bone in prepubertal girls and women. Chapter 2 focused on the acute effects of moderate intensity exercise on responses of IL6, CX3CL1, FGF-2, total and free IGF-1 relative to baseline.

The main finding of this study was that exercise elicited a similar response in prepubertal girls and women such that both groups presented with similar declines in CX3CL1, FGF-2 and total IGF-1 post-exercise and recovery. In addition, a significant interaction was observed with the response of IL-6, where women experienced a higher response post-exercise than prepubertal girls. No difference was observed in free IGF-1 in between prepubertal girls and women. This indicates that the robust inflammatory response of IL-6 may play an important role in facilitating the effects of exercise in adults.

In Chapter 3 exercise serum was used to treat myoblasts and osteoblasts, in which cell proliferation and differentiation was assessed. One of the main findings of this study was that myoblast and osteoblast proliferation did not change with exercise in prepubertal girls and women. However, proliferation was greater at all time points after exercise and recovery in women relative to prepubertal girls. In addition, myoblast differentiation did not change with exercise or group, while osteoblast mineralization declined at the end of exercise for both prepubertal girls and women.

Chapter 4 focused on translating the findings of the study to children through the development of an animated video. To assess educational utility of this video, learning viewpoints and understanding of the content was

assessed. This study identified four salient learning perceptions within this sample, which were described as Engaged Learners, Action-Takers, Interactive Learners, and Receptive Learners. These perceptions were classified based on student-ranked statements related to video engagement, including knowledge attainment, action-based thinking, enjoyment, learning preferences, and endorsement. The knowledge retention assessment showed that students scored an average of 79% (SD=16%), with 20/31 students performing above 80% on the assessment.

5.3 The systemic effects of exercise on muscle and bone proliferation *in vitro*

We hypothesized that exercise would (1) induce an increase in all systemic regulators of muscle and bone and that this would (2) elicit significant proliferative effects on muscle and bone proliferation *in vitro*. Contrary to our first hypothesis, exercise did not increase all systemic factors, rather only IL-6 and CX3CL1 increased significantly with exercise in prepubertal girls and women, while free IGF-1 decreased (Figure 5.1). Furthermore, prepubertal girls experienced a smaller increase in IL-6 relative to women, while the response in CX3CL1 was similar across groups. An increase in

inflammatory mediators led us to speculate that the exercise-induced systemic environment would lead to an increase in proliferation.

Contrary to our speculation, exercise did not induce proliferative effects on osteoblasts and myoblasts in *vitro*. We predicted that the increases we observed for IL-6 and CX3CL1 in females, particularly at EX2, would serve as a driving factor in influencing myoblast and osteoblast growth in Chapter 3, however this was not the case. We then speculated whether the myoblast and osteoblast proliferative response was correlated with any of the systemic factors measured in Chapter 2, however only free IGF-1 was correlated negatively with osteoblast proliferation at EX1, EX2, and REC 1 (Table 5.1).

That exercise did not induce an increase in proliferation is contrary to findings that were published previously (Nguyen et al., 2014). A moderate bout of cycling in mid-pubertal children resulted in an increase in proliferation of myoblasts immediately after one hour of exercise and recovery, respectively. This discrepancy however may be explained by differences in methodology. For example, the prior study utilized pooled samples of human serum from the participants at each time point, which was used to treat myoblasts and assess proliferation. However, in this study, we used individual serum samples to treat the myoblasts and osteoblasts, resulting in a significantly attenuated exercise response.

Nguyen et al. also found that when cells were treated with serum from the individual participants, the exercise effects were no longer evident on myoblast proliferation (unpublished data). Thus, it is possible that by pooling samples, the effects of exercise on cells is amplified due to the additive effect on combining systemic responses to cells *in vitro.*

Another factor that may explain why exercise effects were not observed on muscle and bone proliferation *in vitro* may be related to the exercise intensity used in this study. The response of inflammatory mediators is augmented with increased intensities as shown in pediatric and adult literature (Ostrowski et al., 2000; Timmons, 2007). For example, a moderate bout of running exercise may induce a 25-fold increase in plasma IL-6 concentration, whereas running at a higher intensity may induce a 100-fold increase (Ostrowski et al., 2000). This observation may explain why pooling samples may result in increased proliferation as it combines concentrations of mitogenic factors from multiple participants into one treatment. Thus, although one bout of moderate intensity cycling exercise can stimulate a systemic response in prepubertal girls and women, these responses are not substantial to elicit an anabolic effect on muscle and bone *in vitro* when used as individual treatments.

5.4 The systemic effects of exercise on myotube formation *in vitro*

We hypothesized that (1) exercise would elicit an anabolic effect on myotube differentiation, and (2) that this increase would be greater in prepubertal girls relative to women. However, similar to proliferative phenotype, the systemic effects of exercise did not elicit any anabolic effect on myotube differentiation in either group. One factor that may have contributed to this response is the decline of anabolic factors in the systemic environment, such as FGF-2 and IGF-1, both of which promote myotube formation. No correlations were observed between the exercise response of IGF-1 and FGF-2 and myotube differentiation (Table 5.1)

That an acute bout of moderate intensity exercise did not result in an increase in myotube differentiation is consistent with the lack of proliferation in Chapter 3. Furthermore, it was also demonstrated by Nguyen et al. that this exercise modality fails to stimulate an increase in myotube formation *in vitro* using samples from healthy, mid-pubertal children. This is contrary to the findings expressed in another study, where serum from exercised subjects resulted in an increase in myotube differentiation using LHCN-M2 cells, a human myogenic cell line (Vitucci et al., 2018). In addition to increased myotube differentiation, Vitucci et al. reports that exercise led to an increased expression of early and late differentiation markers, such as creatine-kinase, myogenin, MyHC-beta *in vitro*. These differences in

reporting are likely attributed to differences in methodology (e.g cell lines, duration of treatment) and sample. The sample in the latter study is comprised of young, healthy adults who have been undergoing exercise training for a duration of three years. Furthermore, the results were categorized according to the type of training that was adapted by each group, namely aerobic (swimming), anaerobic (body building) and mixed-exercise (soccer and volleyball). Interestingly, serum from aerobic training resulted in a greater positive effect on myotube differentiation relative to other groups, and also expressed higher serum concentrations of IGF-1 at rest. These findings suggest that training status may play a predominant role in the muscle response to exercise, which may explain why both our study and Nguyen et al. did not observe a change in myotube differentiation after one bout of moderate intensity exercise.

5.5 The systemic effects of exercise on mineralization *in vitro*

We hypothesized that (1) exercise would elicit an anabolic effect on mineralization, and (2) that this increase would be greater in prepubertal girls relative to women. Contrary to our hypothesis, mineralization steadily declined throughout exercise, and exhibited significant reductions at the end of recovery in both girls and women. Considering that two of the potent inducers of mineralization, IGF-1 and FGF-2, also declined with exercise in

Chapter 2, the decrease in mineralization is supported by this data. However, no systemic factors were correlated with the change in mineralization (Table 5.1).

One factor that may explain the pro-resorptive effects of exercise on mineralization is the increase in inflammatory mediators in the systemic environment. IL-6 is a potent inhibitor of osteoblast activity and can influence bone mineralization by upregulating osteoclastogenesis. The relationship between this inflammatory mediator and bone resorption is also indicated by previous studies, where an increase in IL-6 post-exercise is positively correlated with increases in markers of bone resorption, such as RANKL (Steeve et al., 2004). Most recently, an increase in osteoclastogenesis is observed after incubation of osteoblast-osteoclast progenitor co-culture systems with muscle-derived IL-6 (Chowdhury et al., 2020). This is supportive of our observation that an acute bout of exercise in girls and women leads to a decrease in mineralization in osteoblasts *in vitro*, which was also observed with an increase in IL-6 in the systemic environment in both groups. This decrease in mineralization may present as an exercise adaptation preparing bone for remodeling, which is a sequential process that is initiated by osteoclast activity to remove old bone, after which osteoblasts lay down new bone. An exercise stimulus triggers bone remodeling, such that bone formation may occur hours to days after

the bout, as supported by previous exercise data (Tosun et al., 2006). Moreover, the pro-resorptive environment of acute exercise may also be linked to muscle adaptations. Using genetic epistasis in their training study, Chodhury et al. demonstrates that an exercise-induced increase in osteoclastogenesis also plays an important role in producing downstream mediators of muscle function, namely fatty acid uptake and catabolism.

5.6 Application of the muscle-bone unit *in vitro*

The rationale for investigating muscle and bone concurrently in this study was to assess the muscle-bone relationship (ie. muscle-bone unit) in the context of an acute bout of exercise. Given the positive association between muscle and bone growth as established in the literature (Brotto & Bonewald, 2015), we conducted Chapter 3 with the inherit hypothesis that similar responses would be observed between myoblast and osteoblast proliferation and differentiation, respectively. That is, an anabolic response in muscle would be met with in an anabolic response in bone, and vice versa.

Our results in Chapter 3 revealed that our hypothesis was partially met. Although exercise did not elicit anabolic effects in muscle and bone *in vitro*, we were still able to observe a positive correlation in proliferation between osteoblasts and myoblasts (Table 5.2). That is, increased osteoblast

proliferation induced by a systemic treatment was met with increased myoblast proliferation, and vice versa. Similarly, both myoblasts and osteoblasts exhibited increased proliferation (non-significant) upon incubation with human serum in comparison to growth media (Table 5.3). This muscle-bone pleiotropy is most likely attributed to the shared mesenchymal origin between myoblasts and osteoblasts, allowing them to express pleiotropic receptors and respond to stimuli in a similar manner.

Unlike proliferation, no correlations were observed between myoblast and osteoblast differentiation. More specifically, myonuclei fusion index did not change with exercise, while mineralization declined after an acute bout of exercise. That the responses were not coupled in myotube formation and mineralization may be due to the staging of outcome measures to assess cell differentiation. Both myonuclei fusion index and mineralization are considered late markers of differentiation, however mineralization also provides insight the functional capacity of osteoblasts while myonuclei fusion index relies on the quantified expression by myosin heavy chain. As such, we speculate that the measurement of late differentiation markers prior to mineralization, such as alkaline phosphatase or osteocalcin, is more aligned with the quantification of myosin heavy chain, possibly providing more consistent results in absence of exercise effects on myob. Mineralization is typically observed 21-days of culture while ALP and

osteocalcin can be observed within a week, a duration that is similar to the one used for the myotube formation. Alternatively, the inclusion of a functional measure of myotubes (e.g. distance of myotube contraction) may provide a more consistent measurement to mineralization, provided they are incubated for a similar duration.

5.7 The effects of maturation, fitness, and physical activity

One of the primary objectives of this thesis to compare the effects of exercise in girls and women with respect to proliferation and differentiation of myoblast and osteoblasts *in vitro.* We hypothesized that prepubertal girls would experience increased muscle and bone growth *in vitro* because of their dynamic development (Vincente-Rodriquez, 2006). Contrary to our hypothesis, however, exercise resulted in a significantly attenuated proliferation response in myoblasts and osteoblasts in serum collected from prepubertal girls relative to women. In addition to experienced attenuated proliferation, prepubertal girls also presented with an attenuated increase in IL-6 relative to women during exercise and recovery.

The IL-6 response to exercise increases with maturation, which is linked to differences in metabolism and body composition (Timmons, 2005). For example, female development is associated with increased body fat accumulation, which is typically distributed between the breasts, buttocks

and thighs (Charmas & Gromisz, 2019). We speculated that there would be a positive correlation between systemic IL-6 and body percent fat, however upon closer examination, we did not observe any baseline differences between prepubertal girls and women in the study, suggesting that IL-6 may be influenced by other factors beyond maturation (e.g. fitness). Moreover, there were no correlations between IL-6 and percent body fat (or other physical parameters such as fat free mass and fat free percent), which may explain why there were no differences between prepubertal girls and women at baseline. Therefore, increased proliferation found in women may be independent of changes in IL-6 and more representative of other factors that are closely associated with body fat, such as concentration of fatty acids or adipokines, which are reported to be expressed in lower concentrations in children than adults in other studies (Lombardi et al., 2016).

One aspect of this study that may have contributed to the attenuated effects of maturation was the presence of differences relating to fitness between prepubertal girls and women. Girls were significantly more fit than women and presented higher levels of MVPA. Increased physical activity and fitness are both closely linked to systemic profiles, as previously demonstrated in pediatric and adult populations (Green et al., 2014; Nielsen et al., 2016). One study assessing the physical activity levels of prepubertal girls reports that proinflammatory cytokines (e.g. IL6) are negatively

associated with MVPA (Nielson et a. 2016). In young women, MVPA is associated with systemic concentrations of TNF-α, but not IL-6 (Green et al., 2014). This may be related to the multipotent nature of this cytokine, as it is also expressed by skeletal muscle during exercise, and therefore may be positively be associated with MVPA. However, we did not observe any correlations between MVPA and systemic factors in this study, possibly attributed to variations in physical characteristics of samples across studies or sample size.

Although controlling for fitness and MVPA did not influence group effects on systemic regulators of muscle and bone (Chapter 2), it did negate the group effects on myoblast and osteoblast proliferation *in vitro* observed with exercise (Chapter 3). Therefore, individuals with higher fitness levels (as demonstrated by the prepubertal girls in this study) presented with a systemic environment that attenuated myoblast and osteoblast proliferation relative to participants with lower fitness levels. Indeed, participants that are more physically active demonstrate smaller resting concentrations of pro-inflammatory cytokines (Eliakim & Nemet, 2010; Kraemer & Ratamess, 2005). Many mitogenic factors are associated with inflammatory effects, which may suggest that fitness plays a protective role in exercise by minimizing basal inflammatory concentrations to desensitize tissue from “chronic-like” inflammation. Despite differences in fitness and MVPA

between the prepubertal girls and women this study, there were no significant differences in resting levels of inflammatory mediators (IL-6, CX3CL1), suggesting that other mitogenic factors may be involved beyond the ones investigated in this thesis.

5.8 Development of a video as doctoral thesis chapter

The findings of Chapter 2 and Chapter 3 in this thesis support that exercise elicits responses on systemic factors in girls and women, and that these effects may be observed *in vitro*. These results provide us with insight on potential mechanisms in which exercise influences muscle and bone growth. Moreover, the findings of Chapters 2 and 3 highlight the capacity of an acute bout of exercise to influence muscle and bone health in children and adults, which is information that may be used to promote exercise and physical activity for the community. Given the association of this information to the health and wellbeing of the community, translating the knowledge such that it is made accessible to the community is not only beneficial, but also an important mandate for all graduate students and researchers alike. Therefore, for the last chapter of this thesis, we aimed to develop a project that would relay the findings of Chapters 2 and 3 to the community, particularly school-aged children.

Children have a general understanding that exercise helps their muscles and bones, but they may not be as informed as to how these benefits arise (Bilich 2005). More specifically, the idea that messages are relayed to muscles and bones (ie. growth factors and cytokines) is foreign to children. Using the findings of this paper as well as supporting literature, we wanted to develop a tool that would convey this message to children. The rationale for doing this is to provide children with a explanation as to how exercise helps their bodies, in hopes that this will encourage children to view exercise in a proactive perspective. We decided to develop this too using a medium that is proven to be engaging in childhood education, an animation video (Barak et al. 2010).

The development of the video comprised of six elements, which are outlined in Table 5.2. In summary, these elements included the creation of the Storyboard, Script, Illustrations, Animations, Audio, and Integration of all elements. Each of these stages was associated with its own set of challenges, including the development of an engaging story, selection of relevant and relatable analogies to demonstrate physiological concepts, and optimizing the method of illustration and animation through a series of trouble-shooting attempts, all of which are typically conducted by different multiple members in a translational team. The challenges faced with creating an animation video in Chapter 4 may appear to be vastly different

from those experienced through designing an exercise study and series wet lab experiments in Chapters 2 and 3, however, they are unified by the primary objective of achieving an optimized study design. Every element of the video was meticulously chosen to ensure accuracy with the research findings and supporting literature, thereby resulting in multiple stages of revision before the final version of the video was published and showcased to children.

5.9 The education utility of animation videos on research awareness in children

We hypothesized that Chapter 4 would address the educational utility of an animation video about exercise for school-aged children. We defined educational utility as the capacity of a tool to be used in an educational setting to enhance students learning experiences (Ashby Plant et al., 2009). Our analysis revealed multiple learning perceptions towards the video which allowed us to collect insight on important elements to consider when designing an animation video for educational purposes.

One of the elements of the animation video that stood out to participants was the depiction of process. This is supported by the findings generated from the factorial analysis in Chapter 4, which identified the following consensus statement: *This video helped me understand what*

happens to my body when I exercise. Irrespective of the viewpoints towards the video (ie. Action-Takers, Interactive Learners, etc.), all participants shared a similar positive ranking of this statement, indicating the global importance of this element when creating an animation video. This was also supported by the Retention assessment where participants scored highly on questions related the sequential order of events referenced in the video. Indeed, the ability of animations to distort realism and convey abstract messages provides the opportunity to facilitate the understanding of cause-effect relationships between events in a system (Türkay, 2016), as depicted in this thesis. This is also supported by exit interview feedback from the participants in Chapter 4, as outlined below:

"By watching this video, it really helped me to understand what actually happens to my bones when I exercise. It really goes into depth about the monucles (molecules) and names of the processes of what happen in your body. After watching the video you will walk away knowing about what actually happens to your body."

- Female, 11 years old

"Before watching the video, I knew that exercise was good for your bones but I didn't know why. Now I know why its good and what happens to your bones when you exercise."

- Female, 12 years old

"The video helped me get more ideas of how our muscles work in order to make our bones healthy."

- Female, 13 years old

It is worth mentioning that participants were able to comment on understanding the video while acknowledging the depth of the content. This observation is particularly insightful as it points out that knowledge translation efforts need not to be overly simplified in order for children to understand. Rather, the knowledge can be simplified enough such that the delivery of the content is facilitated by elements of the animation or tool chosen to convey the message. As it was demonstrated by this animation, breaking down concepts into a sequential visuals accompanied with sound and text may be instrumental in facilitating the comprehension of this complex knowledge for children.

Using animation to break down scientific processes can facilitate knowledge delivery in multiple education settings. One example can be to

utilize the animation in science class to depict abstract processes to children, as depicted by the story of the animation. Indeed, using animations and videos concurrently with traditional learning introduces added value to learning by providing another dimension of active learning (Barak et al. 2010). The learning component can be further enhanced with the inclusion of interactive tools, especially for participants who prefer more interactive means of learning by nature (Interactive Learners, Chapter 4). Another example of utilizing a video is incorporating it in courses where contextual information and/or instruction may be lacking, such as physical education. In a report published by Mandigo (2010), it is found that schools across the country are not meeting their requirements of delivering physical education instruction. Some of the challenges experienced in this area include insufficient time, lack of qualified personnel to teaching physical education, and a lack in up-to-date information about physical education, both contextually and in practice (Mandigo, 2010). Thus, using 'scientifically reviewed' animations in an educational context may help with addressing these issues as it provides a cost effective means of delivering relevant content without having to rely on qualified persona, and includes up-to-date information about exercise and bone health that is aligned with recent research practises. While this may not necessarily solve all issues around the delivery of physical education, the use of animations can at least provide

an additional means to promote physical activity for children within and beyond the context of a classroom. Indeed, the support of having this animation administered in educational settings was also implied by participants as observed in the feedback below:

"Just by watching that 10 min video I understand so much more about exercise. If they showed this video in schools I think all children will like it and learn new things."

- Female, 14 years old

"I think all students should watch this so they understand how their bones grow. As well as keeping the video short is easy to understand."

- Male, 9 years old

"…this video had made me feel to exercise and made me feel that maybe we should help out our bones and keep them healthy. I think more videos should be made so other people can feel the same feeling that I had and also be curious."

- Female, 10 years old

"…the video made me realize how important exercising actually is. The fact that your bones can break when you're older made me realize that exercising at a young age is important. Teaching children and even kids my age would help them know that not doing exercise can impact them later in life."

- Female, 13 years old

Collectively, the feedback provided by participants as indicated by their consensus statement, retention scores, and exit interviews supports the educational utility of this animation while promoting physical activity for bone health.

5.10 Implications

Exercise adaptations on muscle and bone growth in children and adults continue to be attributed to the mechanical sensitivity of these tissues, particularly with high impact exercise. This study, however, provides new insight amongst the emerging literature that supports a systemic component to the responsiveness of muscle and bone to low-impact exercise. The role of maturity in exercise-induced responses is also highlighted in this study, specifically in the response of IL-6 as well as proliferation of myoblasts and osteoblasts, all of which were higher in women than prepubertal girls. Overall, this study shows that systemic regulation may play a bigger role in

the exercise-induced adaptations of muscle and bone in adults than in children, who may require a higher systemic threshold to induce muscle and bone growth. The findings of this thesis can be used to determine an exercise intensity that will elicit a systemic response in a healthy population, which can be used as a baseline to leverage exercise protocols for clinical populations of prepubertal children and adults.

A major challenge for researchers is with transferring the knowledge generated by their research into meaningful information for the general public. In this study, we were able to demonstrate that an animation video can facilitate understanding of the effects and benefits of exercise on bone health to children. Furthermore, we were able to demonstrate the utility of Q-methodology in identifying multiple viewpoints, which provides insight on the practical application of this method for assessing the impact of interventions on viewpoints and linked outcomes of other samples for both educators and researchers alike.

5.11 Limitations

It is important to highlight that this thesis is not without limitations. Our observations of the systemic response to exercise are specific to the mechanisms that occur within 1-hour of recovery, as well as the findings described *in vitro.* That is, we are able to measure factors that are mobilized

or secreted into the systemic environment 1-hour post-exercise and link these systemic changes to the response of muscle and bone *in vitro*. However, the effects of exercise on proteomic synthesis may take longer to detect in comparison to measuring mobilized factors acutely after exercise (Eliakim et al., 2000). Therefore, assessing proteomic synthesis at additional timepoints may provide more insight on downstream effects of an acute bout of exercise on the systemic regulation of muscle and bone, which may also provide support to other studies that observe changes after hours or days of an exercise bout. Nevertheless, it is important to acknowledge that the reason why did not include additional time points was to accommodate the ethical considerations of blood sampling from children and facilitate recruitment.

Another limitation in this study was that participants were not matched according to fitness or physical activity level. Studies show that these factors may influence systemic responses to exercise, which may translate into the growth effects as observed in muscle and bone. This was confirmed by controlling for fitness, which appeared to negate the effects of development on the proliferation of osteoblasts and myoblasts *in vitro.* However both girls and women were screened for their self-reported physical activity prior to participating in this study to ensure that they were physically active according to the Canadian Physical Activity Guidelines.

Another primary limitation in this thesis was the use of murine cell lines to explain the systemic effects of exercise on muscle and bone growth in humans. Although there are many similarities between phenotypical responses and genomic characteristics of murine and human cell lines, variations may exist that subject these cell lines to differences in growth rate and maturation (Cheng et al. 2004). Moreover, C2C12 and MC3T3E1 may be responsive to select systemic factors (IL-6, IGF-1), however it is challenging to determine whether these cells are responsive to all mediators in human serum. Therefore, the findings of this thesis should be verified with human myoblasts and osteoblasts. Nevertheless, the use of these cell lines allows us to study the effects of exercise from rest using human serum, which serves an important comparator in this thesis and allows for conclusions to be made based on the findings. Despite limitations, the use of C2C12 and MC3T3E1 allows us to bypass the ethical considerations of collecting pediatric biopsies such that we may be able to assess the effects of exercise on muscle and bone growth *in vitro.*

Due to the nature of factor analysis, which is specific to the sample to which it is applied, it is inappropriate to generalize the conclusions drawn from Chapter 4 to similar populations. That is, the implementation of this Q-methodology-based study is likely to generate in varying concourse statements, viewpoints and feedback subjected to the animation video

across different samples. Nevertheless, the goal of Q-methodology is to identify different typologies within a specific cohort, not their relative distribution, and therefore is not a major limitation (Brewer-Deluce et al. 2019).

Finally, that attitudes and understanding towards exercise was only assessed once after watching the video presents another limitation. Studies show that short term knowledge retention is more superior than long-term retention (Baker-Ward, 1993). Therefore, following up with the participants after a longer duration from watching the video would improve the evaluation of any changes in attitudes and understanding as a result of the intervention.

5.12 Novelty of findings

The general objectives of this thesis were to investigate the systemic effects of exercise in prepubertal girls and women on muscle and bone growth *in vitro* and develop a knowledge translation tool that would convey the findings of this thesis to the community. The integration of wet lab sciences and knowledge translation for a graduate dissertation is rare and time-consuming, however it facilitates the opportunity of contextualization of results that equip the graduate researcher with science communication skills, which are key to researchers, scientists, and educators alike. Indeed,

the interdisciplinary approaches of Chapters 2,3, and 4 yield multiple novelties that are worth highlighting in this thesis:

- **Chapter 2:** This study compared the effects of an acute bout of moderate intensity exercise on systemic regulators of muscle and bone in prepubertal girls and women. The findings of this study suggest that prepubertal girls and women respond similarly to exercise at this intensity, with inflammatory regulators presenting a higher sensitivity to level of maturity (e.g. IL-6). To the author's knowledge, this was the first study to compare prepubertal girls and women (who are not on oral contraceptives) in exercise conditions and provide resting and exercise values for circulating CX3CL1 and FGF-2 levels in healthy, prepubertal girls.

- **Chapter 3:** This study compared the effects of an acute bout of moderate intensity exercise (Chapter 2) on muscle and bone growth *in vitro.* The findings of this study suggest that an acute bout of exercise does not yield anabolic effects on proliferation and differentiation in prepubertal girls and women and promotes a catabolic response in bone as observed with a decrease in mineralization. The higher value of proliferation observed in

myoblasts and osteoblasts treated with serum collected from women indicates that systemic differences related to maturation translate into differences in muscle and bone development between children and adults. To the authors knowledge, this was the first study to implement an osteoblast model to investigate the effects of exercise in humans.

- **Chapter 4:** This study explored children's perceptions on the educational utility of an animated video about exercise and bone health, while assessing effects of the animation on their learning. The findings of this study support that an animation video facilitates children's perceived understanding of scientific processes involved in exercise, and the extent of this understanding may vary depending on the learning viewpoints of children. Furthermore, this study provides insight on how the use of animation videos can be enhanced to advance the understanding and engagement of children irrespective of their learning viewpoints. To the authors knowledge, this was the first study to construct an animation video about the systemic effects of exercise on musculoskeletal health.

5.13 Future research directions

The concurrent use of systemic profiling with *in vitro* application reveals interesting findings about the effects of exercise on muscle and bone growth. The anabolic effect of exercise as suggested by the systemic response was not conserved in the proliferation and differentiation of myoblasts and osteoblasts. This suggests that the stimulus of an acute bout of moderate intensity exercise was not potent enough to induce muscle and bone growth in this model. Therefore, when assessing the effects of low-impact exercise on muscle and bone growth, it is important to consider multiple types of low-impact exercise that may drive substantially different systemic responses, with particular attention paid to frequent bouts (i.e. training) or bouts of higher intensity. The inclusion of these types of low-impact exercises in future studies may facilitate the identification of key regulators in the systemic environment that influence muscle and bone responses to exercise. These findings may also narrow down the types of low-impact exercises that are optimal for muscle and bone growth in children and adults.

One of the prominent issues in muscle and bone development is identifying the window of opportunity for assessing the effects of exercise in children. The comparison of pre-pubertal girls and women in this study was specified to determine the effects of maturity on this exercise response on

muscle and bone growth. However, as we observed, prepubertal girls and women shared similar exercise responses systemically and *in vitr*o. Although prepubertal girls experience hormonal adaptations that facilitate their development, these changes are regarded relatively stable comparing to other stages of puberty, namely early adolescence (Casazza et al. 2010). Indeed, the adolescent population experience the highest rate of bone mineral accrual and lean height velocity, both of which are largely attributed to dynamic fluctuations in growth and sex-related hormones. Consequently, adolescents may exhibit an increased sensitivity to stimuli (e.g. exercise), which may possibly drive a greater anabolic response than observed in prepubertal girls and women. Therefore, future studies should aim to characterize the effects of exercise on muscle and bone growth during early and late stages of adolescence to determine the role of maturity on these effects. Addressing this hypothesis will also provide insight on the importance of physical activity for this age group, as it is during the period of adolescence where females typically become less active than their male counterparts.

The primary objective of using Q-methodology in reference to the effects of the animation video was to assess learning perceptions and knowledge retention. A long-term objective of this video would be to assess whether watching a video about exercise may also influence children's

behaviours. Indeed, indicators of interest can imply that participants are likely to adopt these behaviours (Sullivan et al. 2007). Although we were able to explore participants attitudes towards to exercise in this study, we cannot confirm that these attitudes are associated with behaviours linked to physical activity. Therefore, future studies should focus on implementing animations with the addition of follow-up assessments on behaviours linked to physical activity.

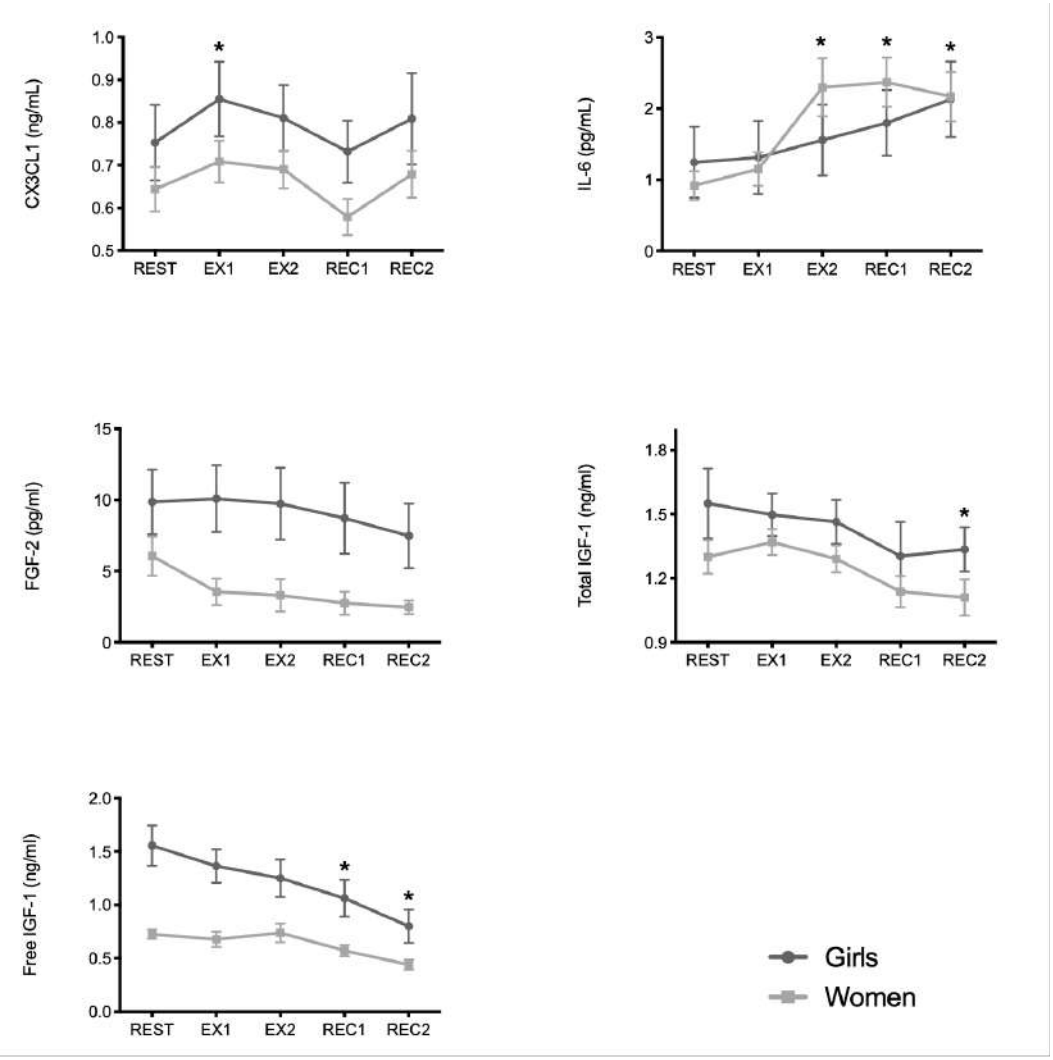

Figure 5.1: Responses of systemic regulators of muscle and bone in prepubertal girls and women. EX1: midpoint of exercise, EX2: end of exercise, REC1: midpoint of recovery, REC2: end of recovery. Values are displayed as mean ± SE. (*) denotes significant interaction between prepubertal girls and women from baseline. Significance was denoted at $p<0.05$.

Myoblast proliferation					
REST	-0.208	-0.075	-0.173	-0.255	-0.255
EX1	-0.237	0.099	-0.197	-0.240	-0.178
EX2	0.385	-0.091	0.921	-0.174	-0.290
REC1	-0.140	-0.222	0.061	0.394	-0.190
REC2	-0.311	-0.259	0.083	-0.392	-0.318
Osteoblast proliferation					
REST	-0.075	-0144	-0.207	-0.136	-0.260
EX1	-0.213	-0.285	-0.017	-0.218	-0.408*
EX2	0.868	-0.303	0.497	-0.138	-0.479*
REC1	0.008	-0.326	-0.138	0.241	-0.426*
REC2	-0.177	-0.231	0.324	0.025	-0.310
Myonuclei fusion index					
REST	-0.273	-0.158	-0.248	-0.337	-0.523
EX1	-0.301	-0.102	0.103	0.095	-0.063
EX2	0.906	0.295	0.262	0.095	-0.186
REC1	0.117	0.067	0.115	-0.285	-0.189
REC2	-0.369	-0.041	0.059	-0.237	-0.349
Mineralization					
REST	-0.171	-0.035	0.201	0.102	0.918
EX1	0.207	0.135	-0.019	-0.011	-0.171
EX2	0.313	0.008	-0.019	0.010	0.073
REC1	0.102	-0.152	0.095	0.229	-0.197
REC2	0.013	-0.305	0.060	0.124	0.198

Table 5.1: Correlations between systemic regulators and myoblast and osteoblast outcomes *in vitro*. Significance was denoted by (*) $p<0.05$ and (**) $p<0.001$.

Myoblast proliferation				
REST	-	0.547**	0.270	0.111
EX1	-	0.337	0.127	0.021
EX2	-	0.465*	0.347	0.370
REC1	-	0.617**	0.141	0.239
REC2	-	0.389	0.124	0.039
Osteoblast proliferation				
REST	0.547**	-	0.514*	0.024
EX1	0.337	-	0.368	-0.059
EX2	0.465*	-	0.352	-0.103
REC1	0.61**	-	0.175	0.154
REC2	0.389	-	0.341	0.011
Myonuclei fusion index				
REST	0.270	0.514*	-	-0.116
EX1	0.127	0.368	-	-0.230
EX2	0.347	0.352	-	0.071
REC1	0.141	0.175	-	-0.089
REC2	0.124	0.341	-	-0.252
Mineralization				
REST	0.111	0.024	-0,116	-
EX1	0.021	-0,059	-0.230	-
EX2	0.370	-0.103	0.071	-
REC1	0.239	0.154	-0.089	-
REC2	0.039	0.011	0.235	-

Table 5.2: Correlations between myoblast and osteoblast outcomes *in vitro*. Significance was denoted by (*) $p<0.05$ and (**) $p<0.001$.

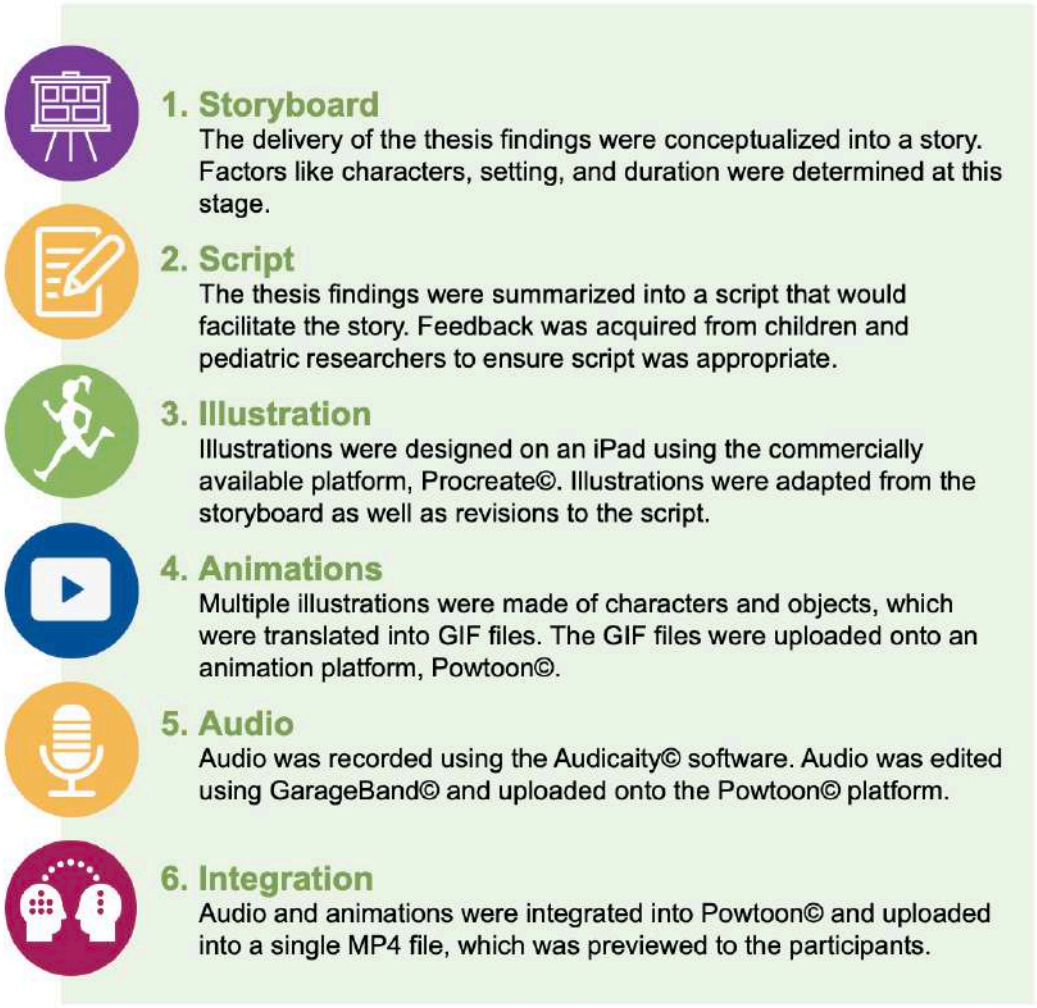

Figure 5.2: Stages of Animation Video Development

Myoblast proliferation (nm)	0.33	0.45	0.47
Osteoblast proliferation (nm)	0.41	0.60	0.64
Myonuclei fusion index (%)	19.12	18.20	19.01
Mineralization (nm)	3.87	3.09	3.30

Table 5.3: In vitro outcome measures comparing growth media conditions (10% FBS) to resting human serum.

CHAPTER 6: REFERENCES

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APPENDICES

APPENDIX A – Participant Recruitment

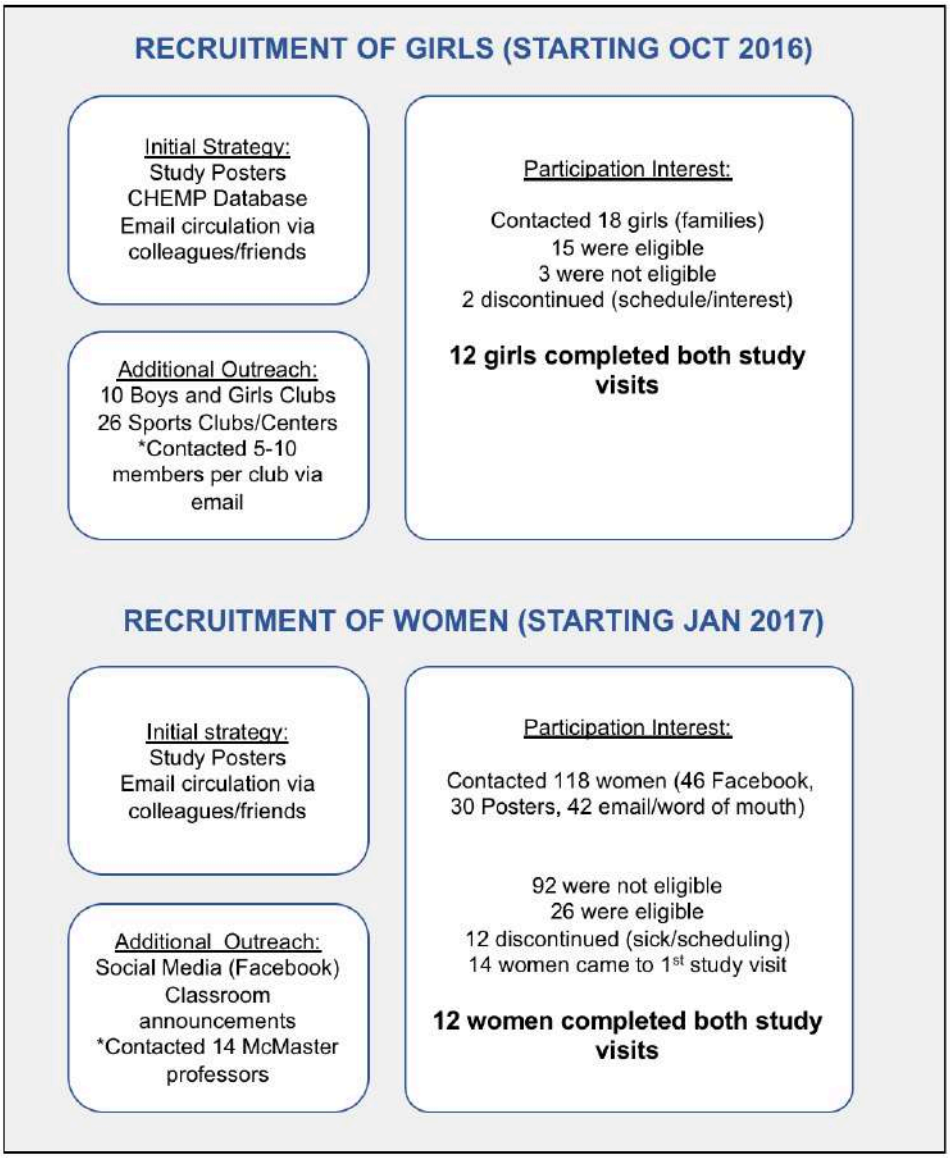


Figure A.1: Recruitment strategies for data collection in Chapter 2.

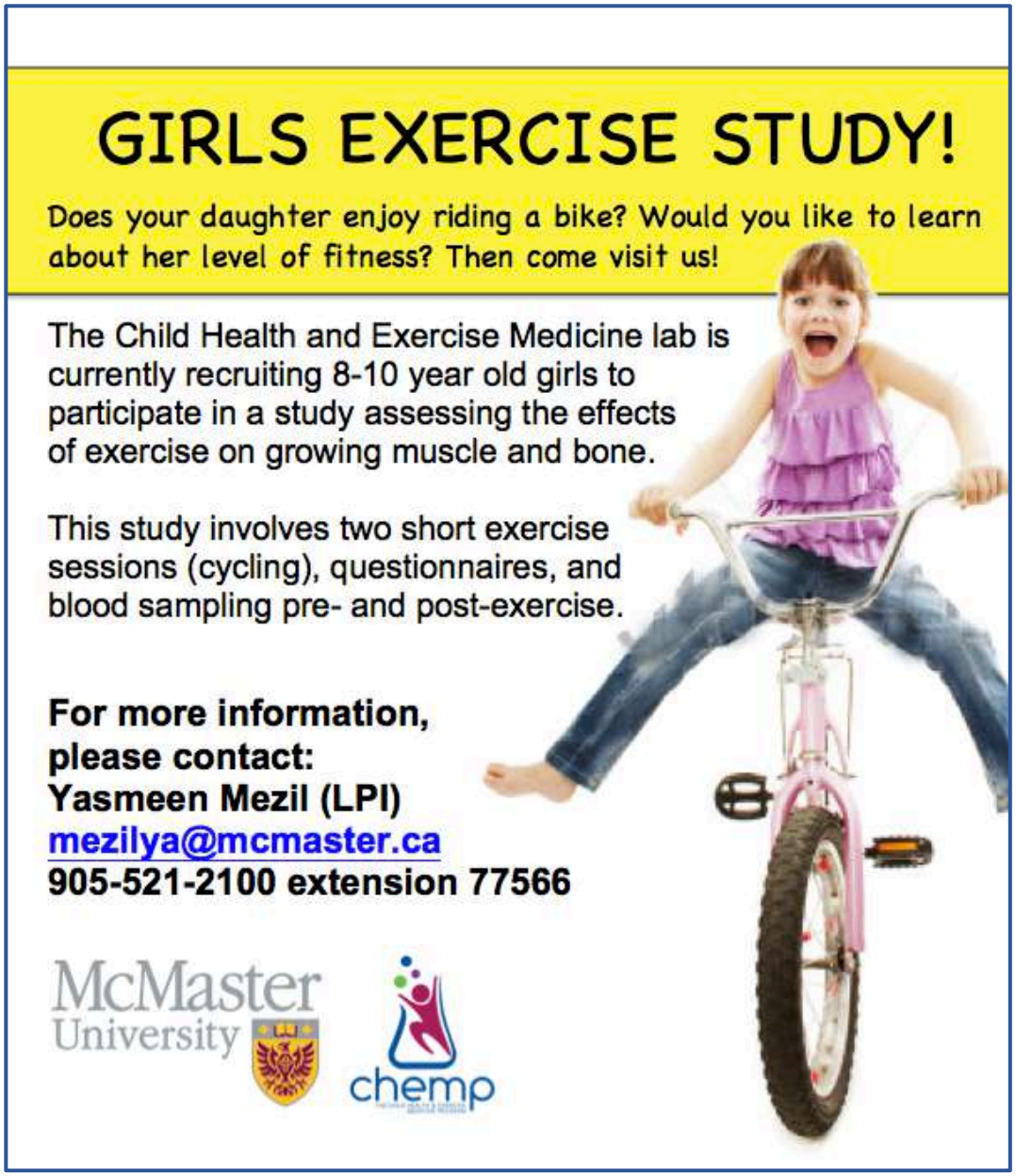


Figure A.2: Recruitment poster for prepubertal girls in Chapter 2.

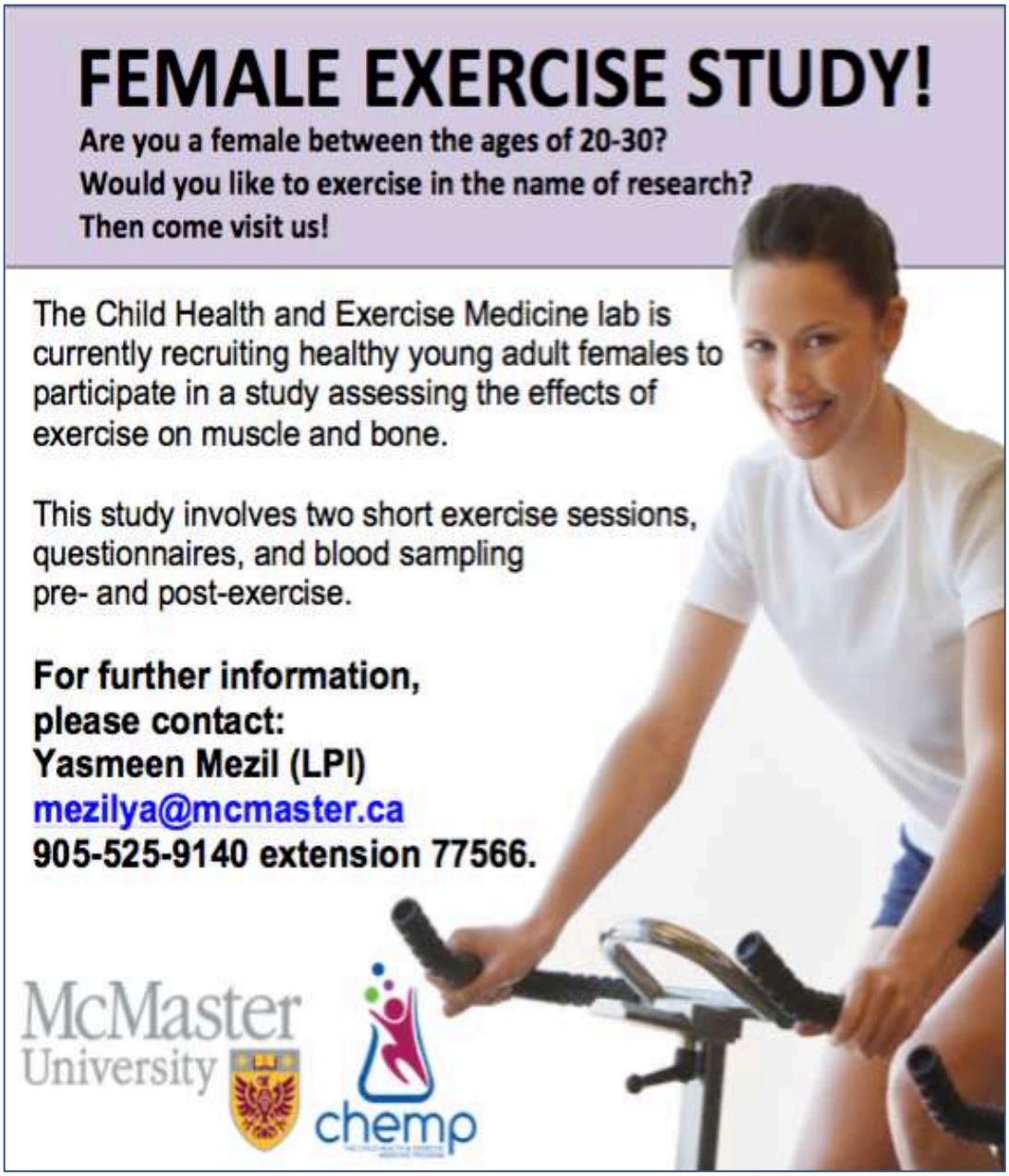


Figure A.3: Recruitment poster for women in Chapter 2.

APPENDIX B – Muscle-Bone Unit Study Protocol

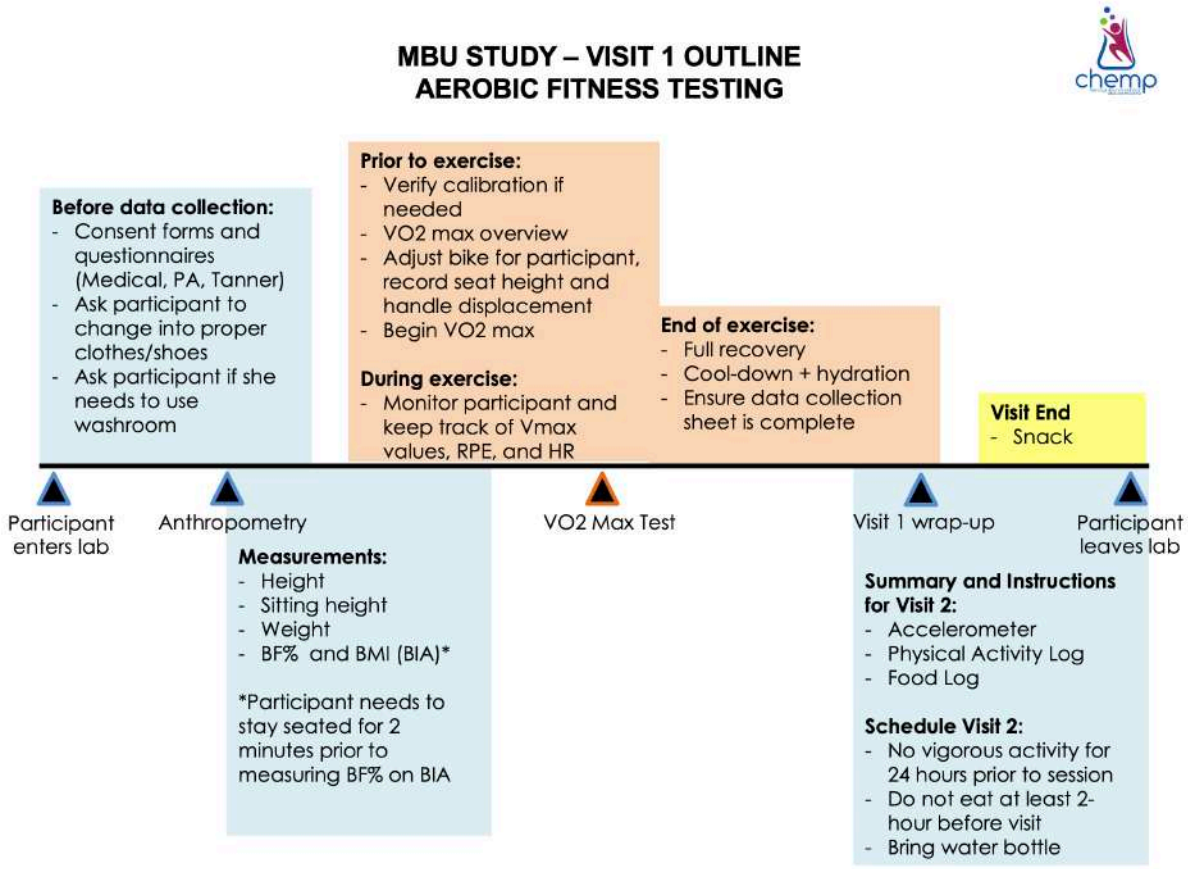


Figure B.1: Testing outline for Visit 1 (Aerobic fitness testing)

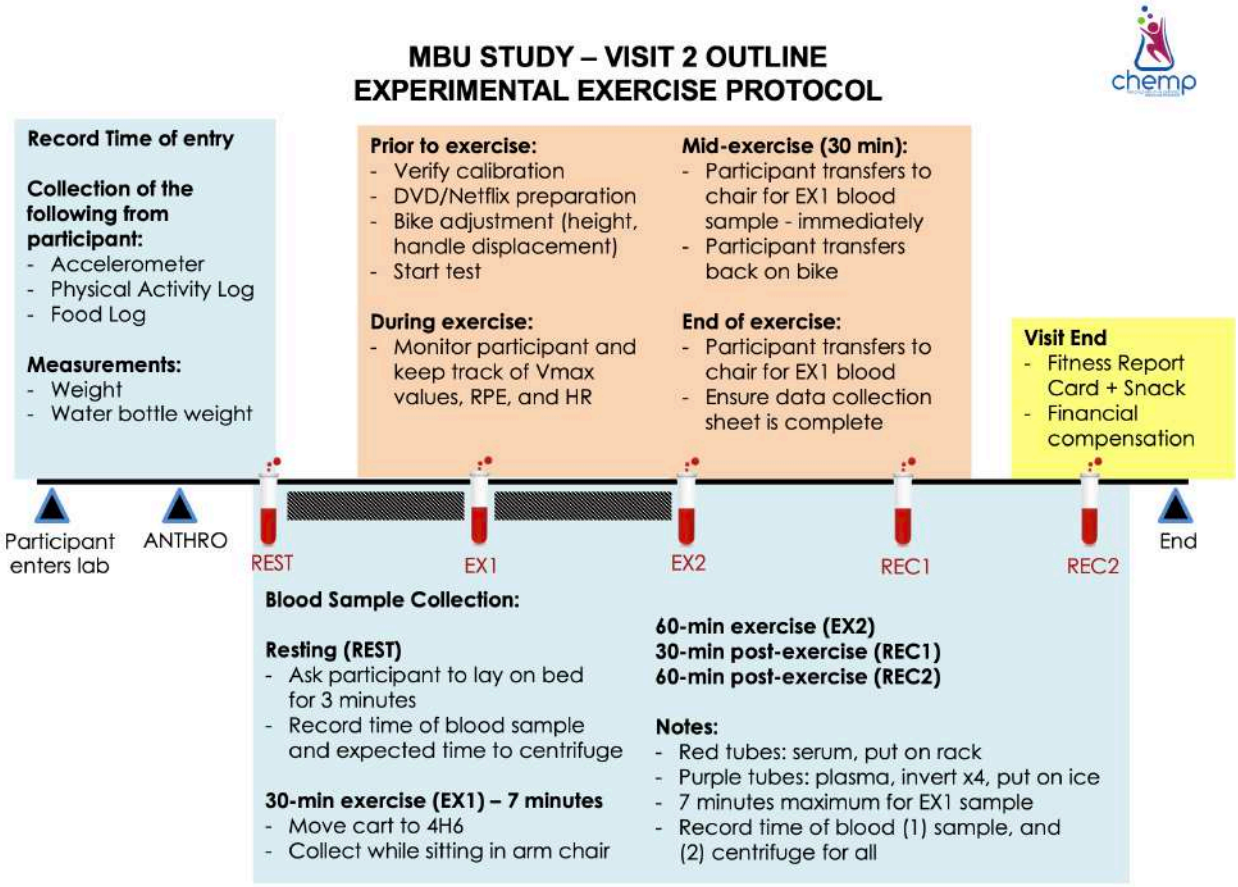


Figure B.2: Testing outline for Visit 2 (Experimental Exercise protocol)

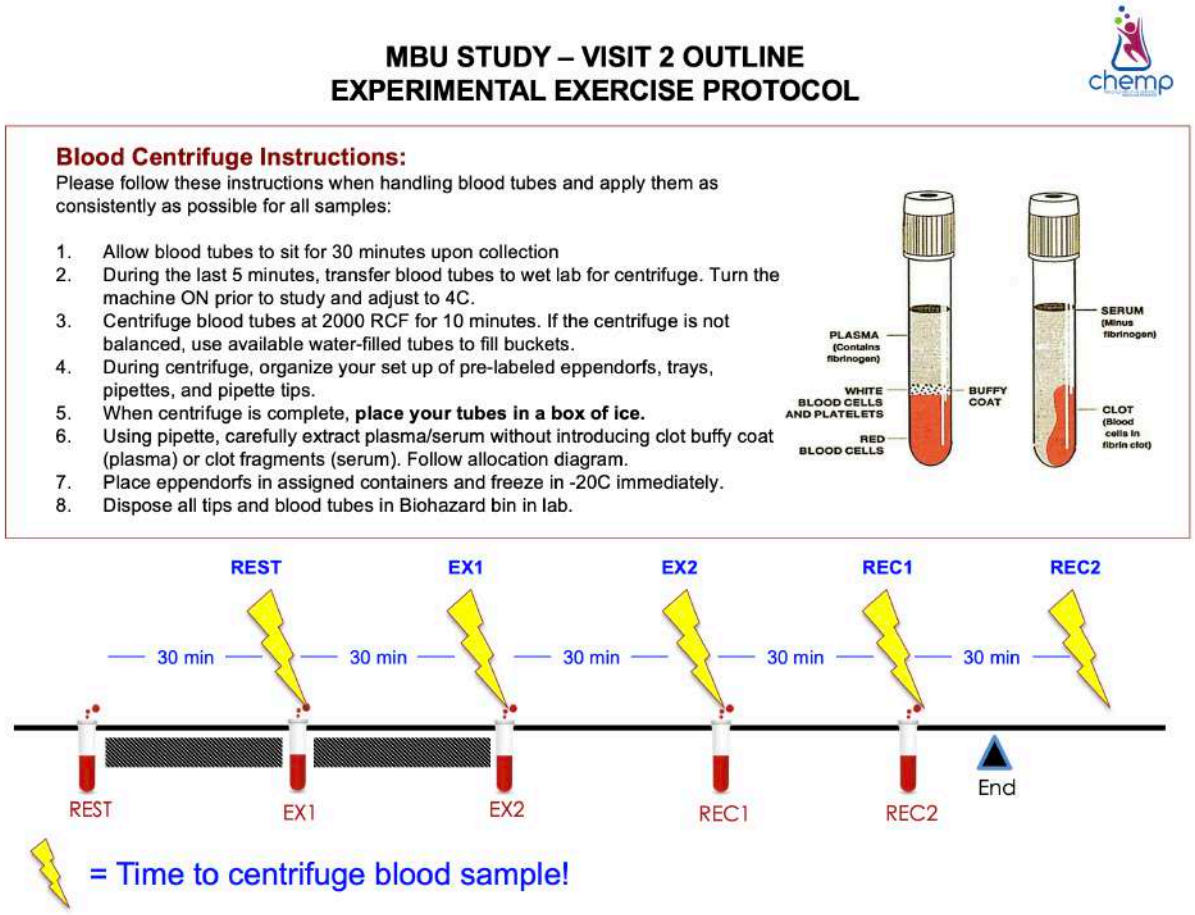


Figure B.3: Blood centrifuge instructions

APPENDIX C – Exercise Data Collection Sheets

Date: _____ I.D: _____

Muscle-Bone Unit Exercise Study
Experimental Exercise Data Collection Sheet

Participant D.O.B (mm/dd/yyyy): _____

(1) Warm-up and Exercise Log

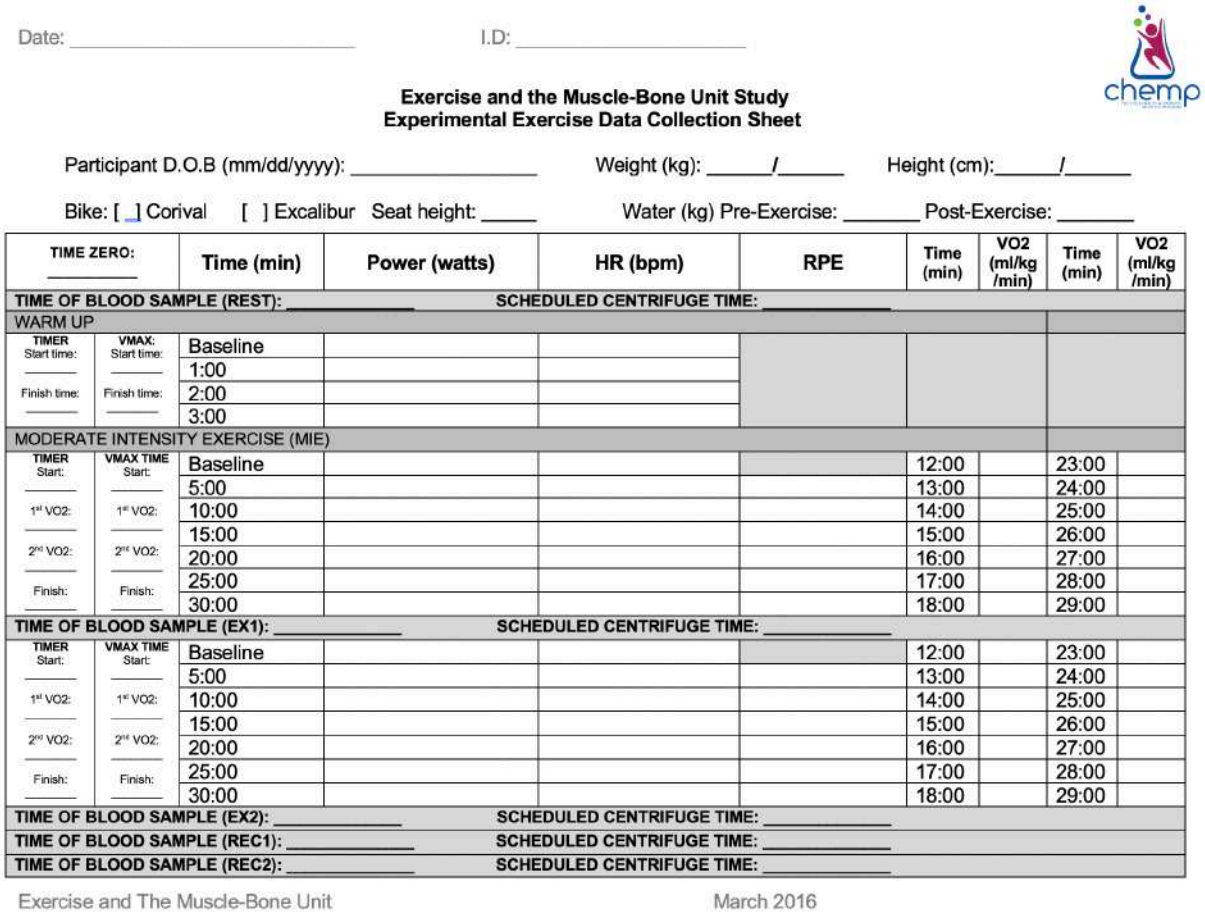
	Time (min)	Power (watts)	HR (bpm)	RPE
WARM UP				
Start time: _____	Baseline			
	1:00			
	2:00			
Finish time: _____	3:00			
	4:00			
	5:00			
MODERATE INTENSITY EXERCISE (MIE)				
1st bout	Baseline			
Start time: _____	5:00			
	10:00			
	15:00			
Finish time: _____	20:00			
	25:00			
	30:00			
BLOOD SAMPLE (EX1)				
2nd bout	Baseline			
Start time: _____	5:00			
	10:00			
	15:00			
Finish time: _____	20:00			
	25:00			
	30:00			
BLOOD SAMPLE (EX2)				

(2) Blood Collection Log

Sample	Time of sample	Time of centrifuge
REST		
Exercise 1 (EX1)		
Exercise 2 (EX2)		
Recovery 1 (REC1)		
Recovery 2 (REC2)		

Exercise and The Muscle-Bone Unit March 2016

Figure C.1: Aerobic fitness data collection sheet.



Date: _____ I.D: _____

Exercise and the Muscle-Bone Unit Study
Experimental Exercise Data Collection Sheet

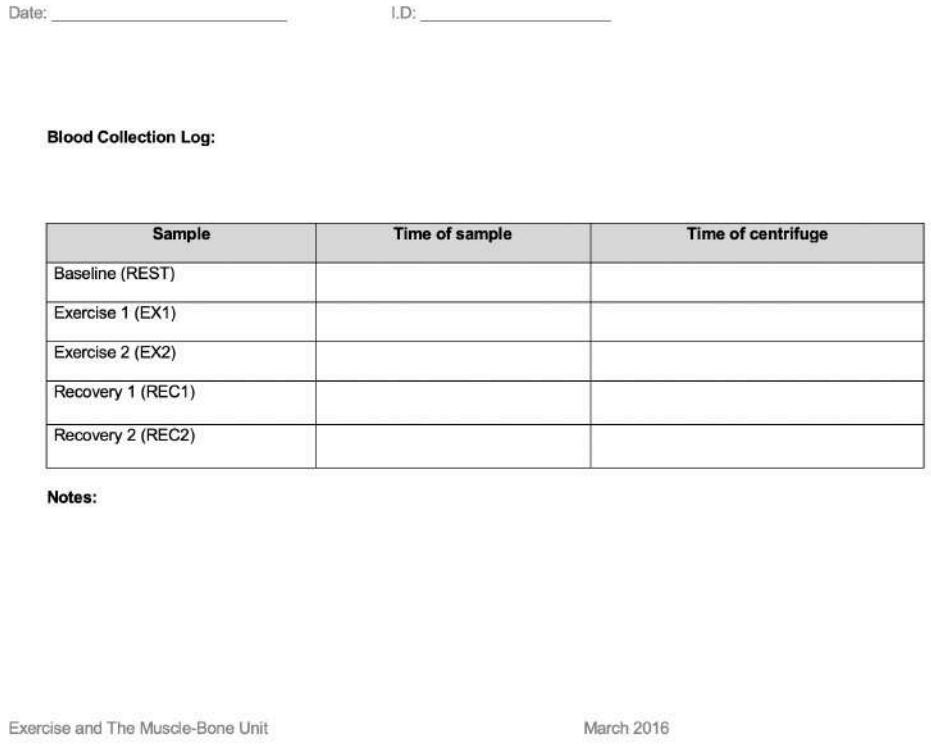
Participant D.O.B (mm/dd/yyyy): _____ Weight (kg): _____/_____ Height (cm):_____/_____

Bike: [_] Corival [ ] Excalibur Seat height: _____ Water (kg) Pre-Exercise: _____ Post-Exercise: _____

TIME ZERO: _____		Time (min)	Power (watts)	HR (bpm)	RPE	Time (min)	VO2 (ml/kg /min)	Time (min)	VO2 (ml/kg /min)
TIME OF BLOOD SAMPLE (REST): _____			SCHEDULED CENTRIFUGE TIME: _____						
WARM UP									
TIMER Start time: _____ Finish time: _____	VMAX: Start time: _____ Finish time: _____	Baseline							
		1:00							
		2:00							
		3:00							
MODERATE INTENSITY EXERCISE (MIE)									
TIMER Start: _____ 1st VO2: _____ 2nd VO2: _____ Finish: _____	VMAX TIME Start: _____ 1st VO2: _____ 2nd VO2: _____ Finish: _____	Baseline				12:00		23:00	
		5:00				13:00		24:00	
		10:00				14:00		25:00	
		15:00				15:00		26:00	
		20:00				16:00		27:00	
		25:00				17:00		28:00	
		30:00				18:00		29:00	
TIME OF BLOOD SAMPLE (EX1): _____			SCHEDULED CENTRIFUGE TIME: _____						
TIMER Start: _____ 1st VO2: _____ 2nd VO2: _____ Finish: _____	VMAX TIME Start: _____ 1st VO2: _____ 2nd VO2: _____ Finish: _____	Baseline				12:00		23:00	
		5:00				13:00		24:00	
		10:00				14:00		25:00	
		15:00				15:00		26:00	
		20:00				16:00		27:00	
		25:00				17:00		28:00	
		30:00				18:00		29:00	
TIME OF BLOOD SAMPLE (EX2): _____			SCHEDULED CENTRIFUGE TIME: _____						
TIME OF BLOOD SAMPLE (REC1): _____			SCHEDULED CENTRIFUGE TIME: _____						
TIME OF BLOOD SAMPLE (REC2): _____			SCHEDULED CENTRIFUGE TIME: _____						

Exercise and The Muscle-Bone Unit March 2016

Figure C.2: Experimental Protocol Data Collection sheet.



Date: _____ I.D: _____

Blood Collection Log:

Sample	Time of sample	Time of centrifuge
Baseline (REST)		
Exercise 1 (EX1)		
Exercise 2 (EX2)		
Recovery 1 (REC1)		
Recovery 2 (REC2)		

Notes:

Exercise and The Muscle-Bone Unit March 2016

Figure C.3: Blood log data collection sheet.

APPENDIX D – Study Visit Set-up

Figure D.1: Participants performed their aerobic testing and experimental protocols in the exercise chamber at the Child Health and Exercise Medicine Lab. A few of the prepubertal girls were tested in pairs to encourage participation, whereas all of the women completed the protocols independently.

APPENDIX E – Preliminary Optimization of Cell Proliferation

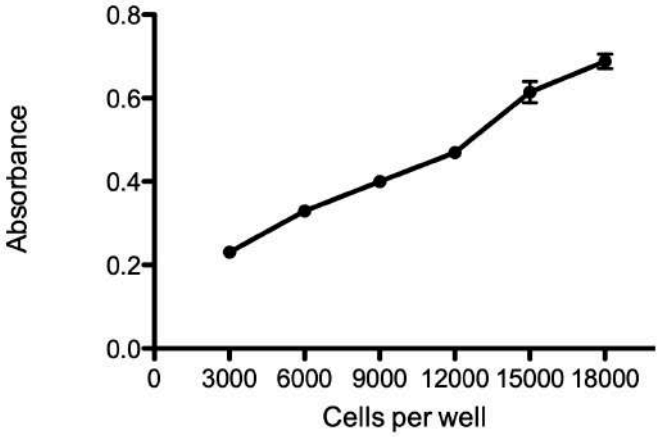


Figure E.1: MTS sensitivity with increasing osteoblast sending densities. MTS was read 2-hours post-seeding.

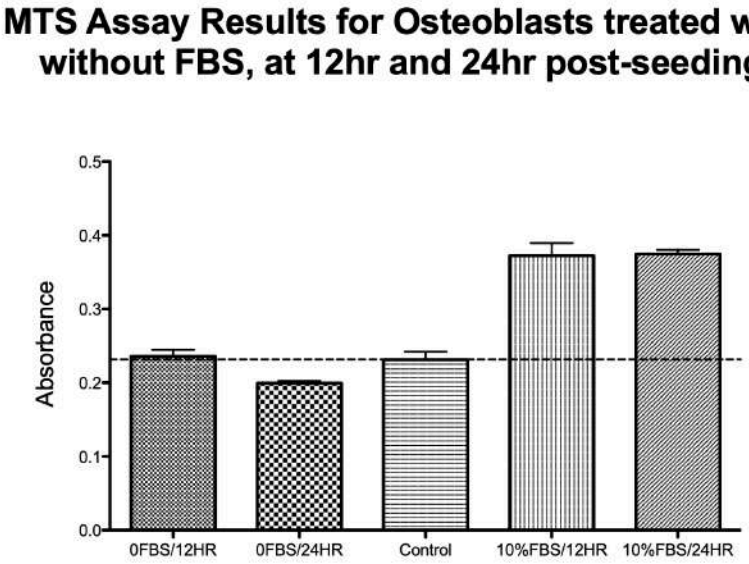


Figure E.2: The effects of serum deprivation on osteoblast proliferation. Cells were deprived of FBS for 12 hours or 24 hours, post-seeding, and compared to cells there were incubated in 10% FBS for 12 hours and 24 hours post-seeding. Control (10% FBS) was measured 2-hours post-seeding.

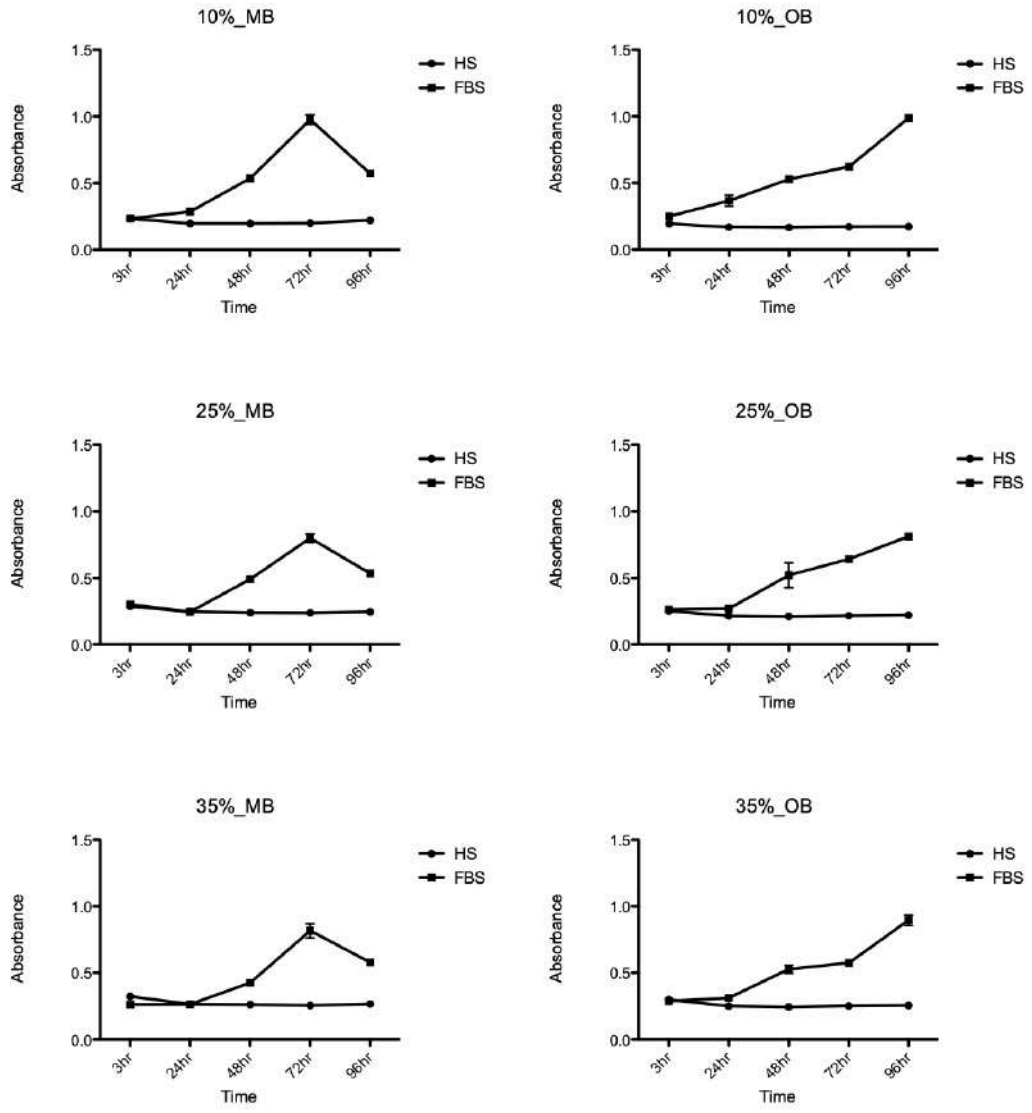


Figure E.3: Growth curves for myoblasts (MB) and osteoblasts (OB) at varying percentages of FBS and human serum (HS). HS was not heat-inactivated.

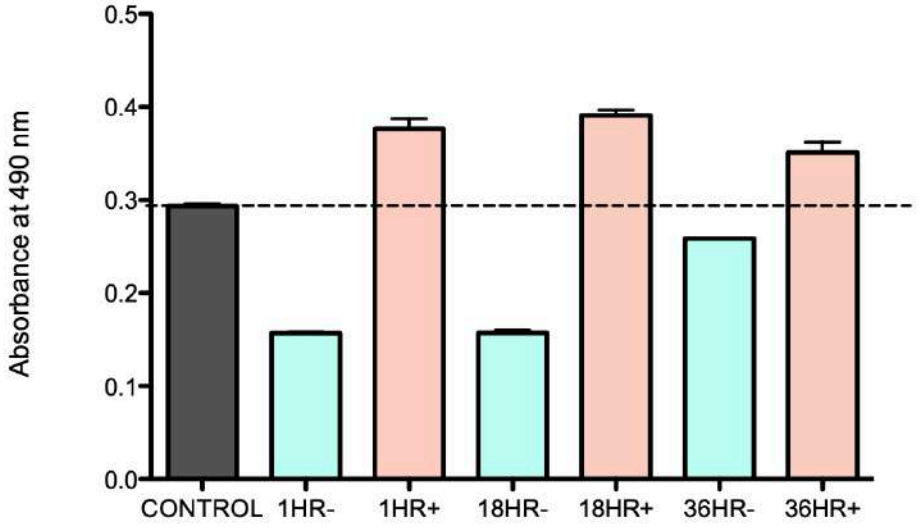


Figure E.4: Osteoblast proliferation with and without heat inactivated human serum. Osteoblasts were treated with human serum for 1, 18, or 36 hours post-seeding. Heat inactivation is denoted by (+).

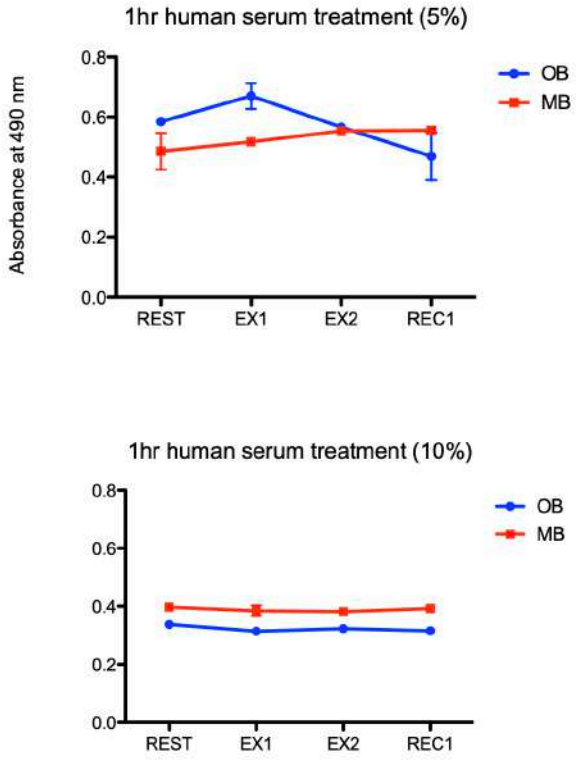
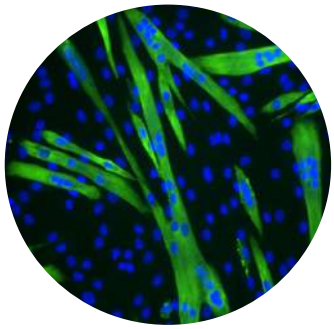


Figure E.5: Myoblast and osteoblast proliferation with 5% or 10% heat inactivated human serum.

APPENDIX F – Differentiation experiments



REST

Figure F.1: Myotube formation as indicated by embryonic myosin heavy chain expression and myonuclei fusion, shown at rest. No changes in myonuclei fusion index were observed across time points or between groups.

Total number of nuclei					
Girls	293.6 ± 15.50	291.5 ± 8.7	293.5 ± 10.1	291.21 ± 8.4	281.1 ± 42.4
Women	294.9 ± 8.9	275.41 ± 43.0	286.2 ± 19.0	290.1 ± 17.3	291.6 ± 7.2
Total number of myonuclei					
Girls	52.8 ± 9.2	58.4 ± 6.6	56.1 ± 6.9	52.9 ± 8.1	52.3 ± 10.7
Women	55.8 ± 5.7	55.1 ± 10.8	56.4 ± 6.6	56.2 ± 7.1	55.4 ± 6.2
Myonuclei fusion index					
Girls	18.2 ± 2.4	19.1 ± 2.6	19.1 ± 2.5	18.2 ± 2.9	18.6 ± 2.3
Women	19.1 ± 2.4	20.0 ± 2.4	19.7 ± 1.9	19.4 ± 2.2	19.0 ± 2.5

Table F.1 Myonuclei fusion index after myoblast treatment with serum from prepubertal girls and women. Values displayed as mean ± SD

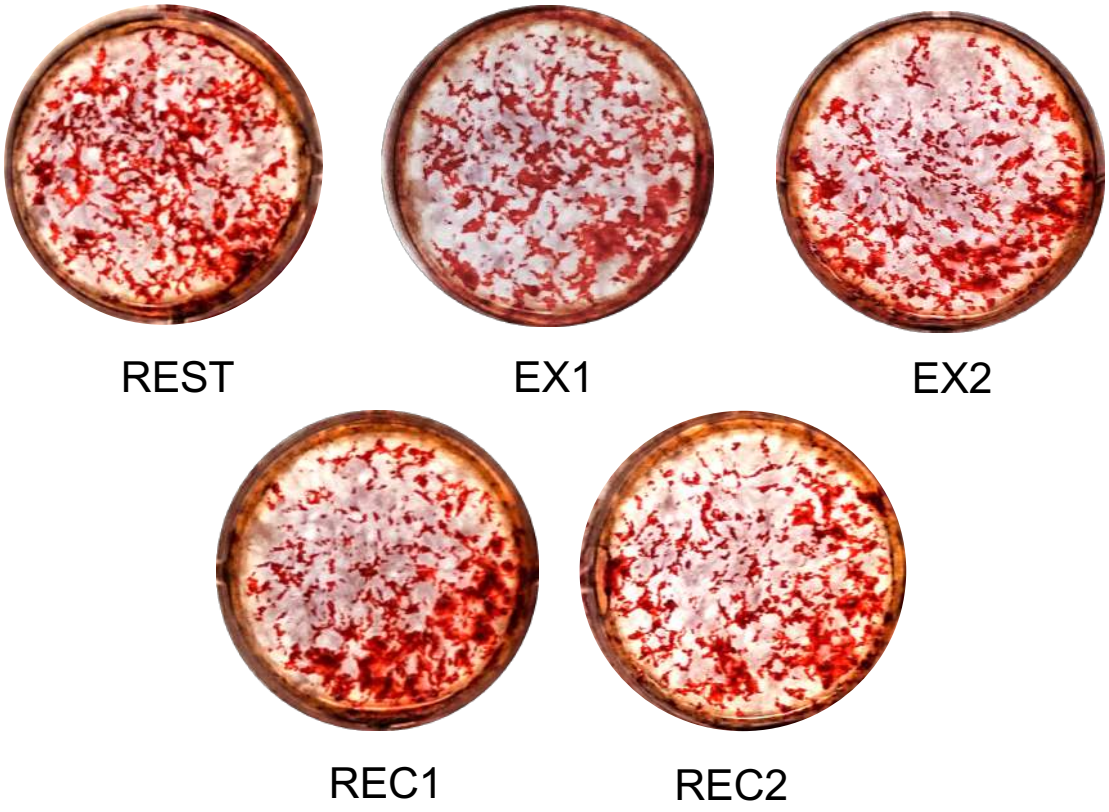


Figure F.2: Sample of wells representing bone mineralization prior to alizarin red extraction. Each well represents one time point from one child participant. Mineralization was significantly reduced at REC2 relative to REST ($p<0.05$).

Absorbance					
Girls	3.09 ± 0.39	3.00 ± 0.29	3.07 ± 0.29	3.02 ± 0.38	2.98 ± 0.35*
Women	3.30 ± 0.29	3.24 ± 0.27	3.16 ± 0.31	3.14 ± 0.16	3.01 ± 0.36*

Table F.2 Mineralisation after osteoblast treatment with serum from prepubertal girls and women. Values displayed as mean ± SD. (*) denotes significance from REST at $p<0.05$

APPENDIX G – Animation Storyboard and Script Development

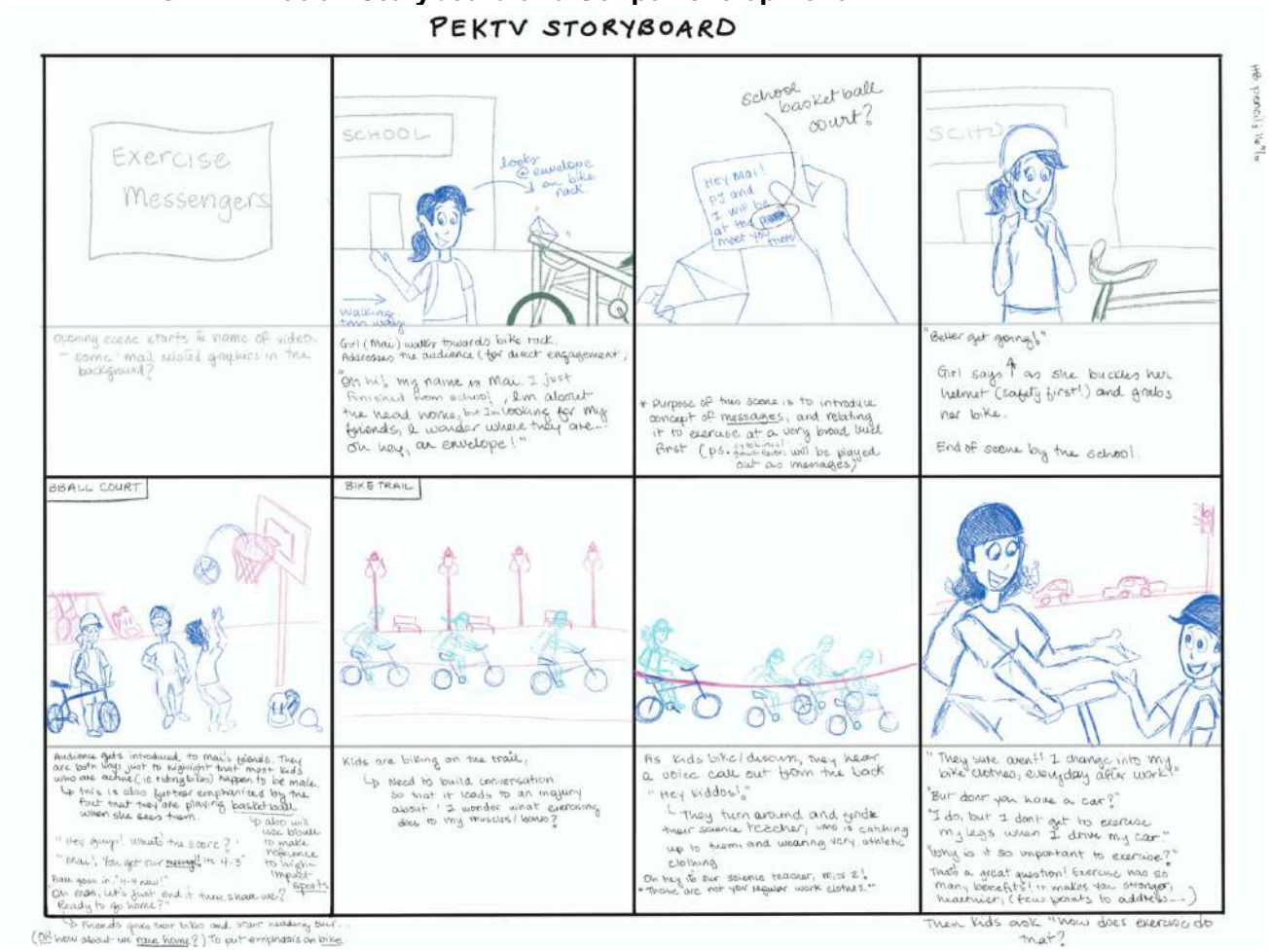


Figure G.1.1: Preliminary stages of "Exercise Messengers" storyboard.

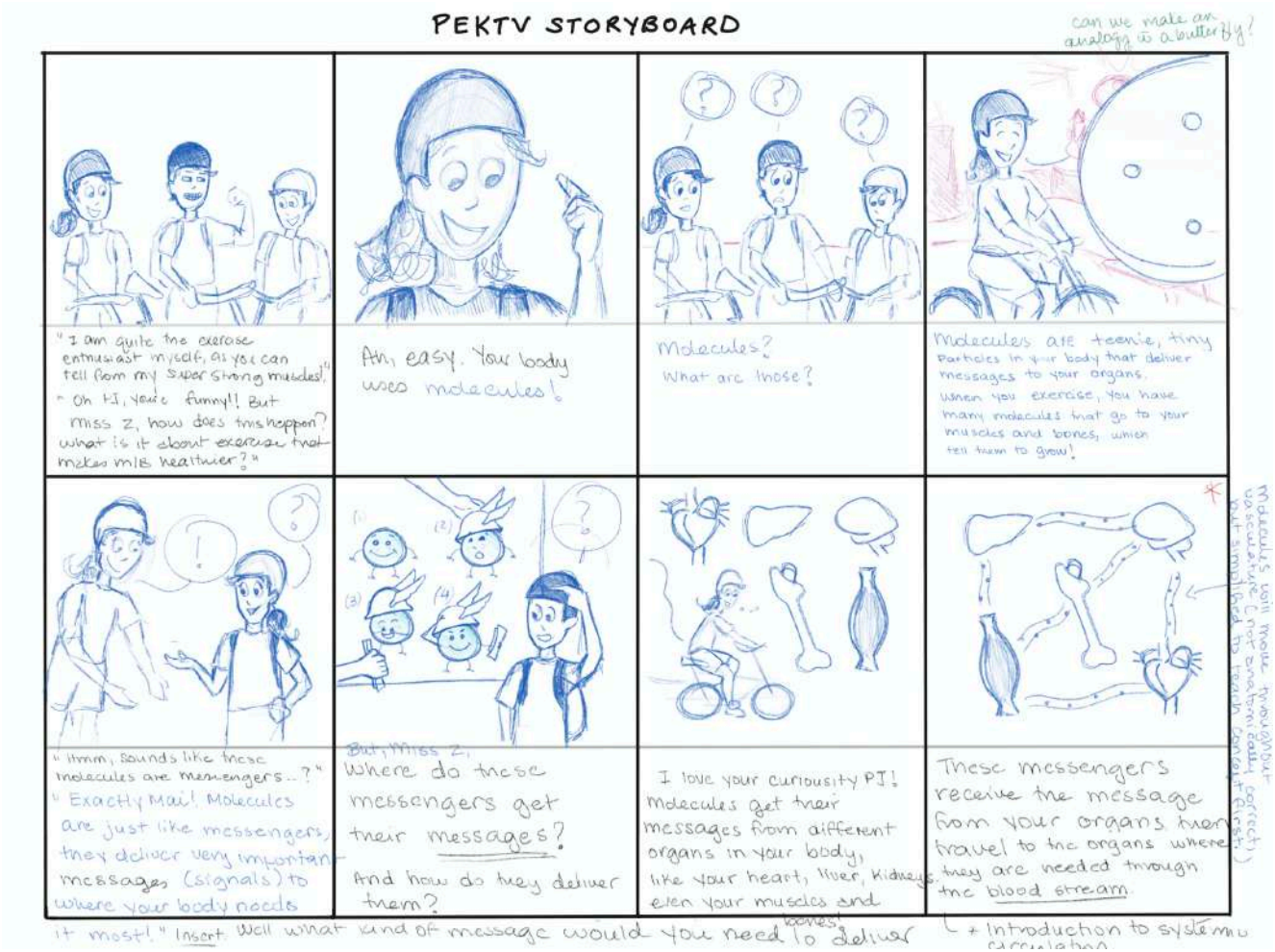


Figure G.1.2: Preliminary stages of "Exercise Messengers" storyboard.

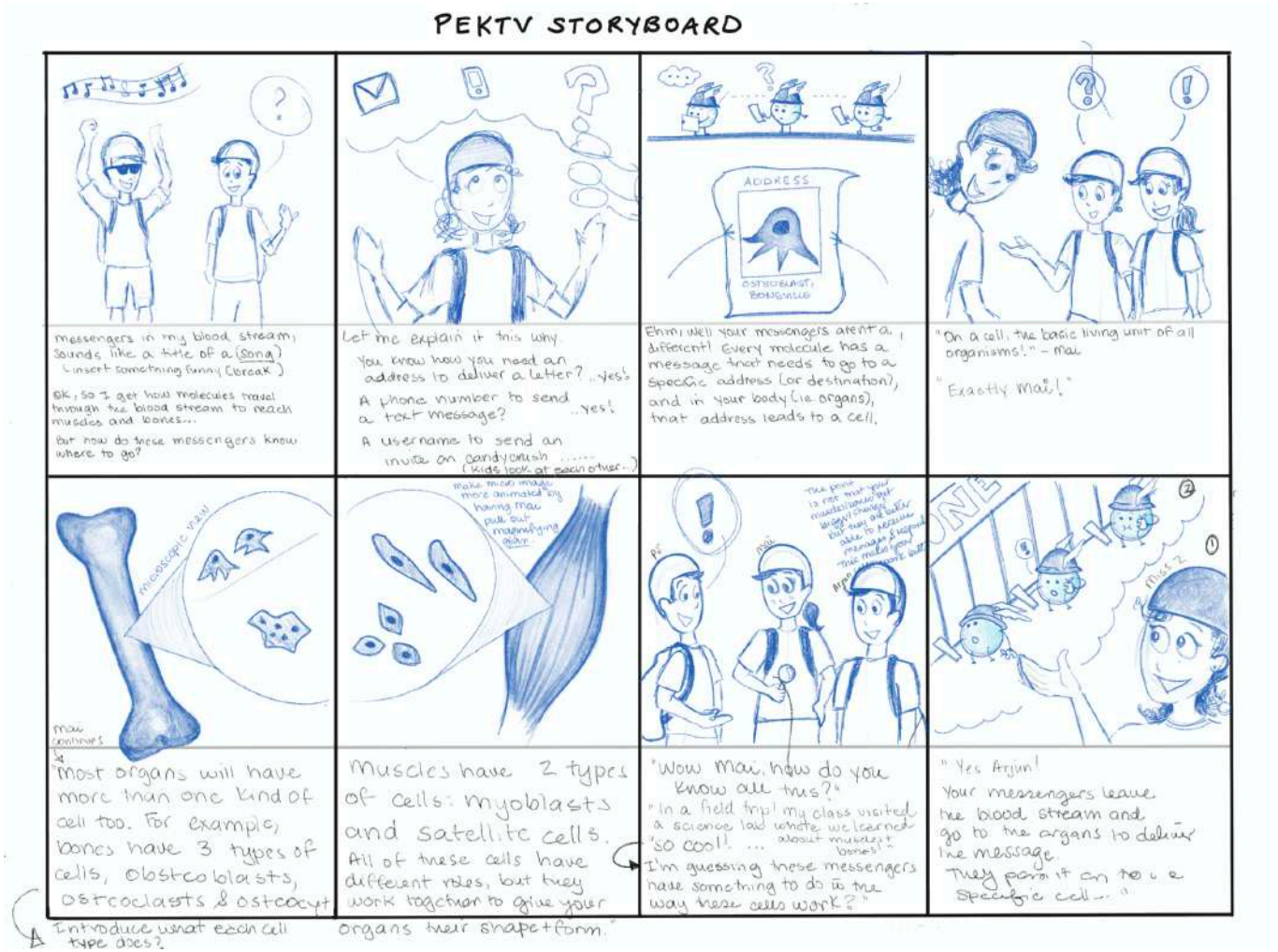


Figure G.1.3: Preliminary stages of "Exercise Messengers" storyboard.

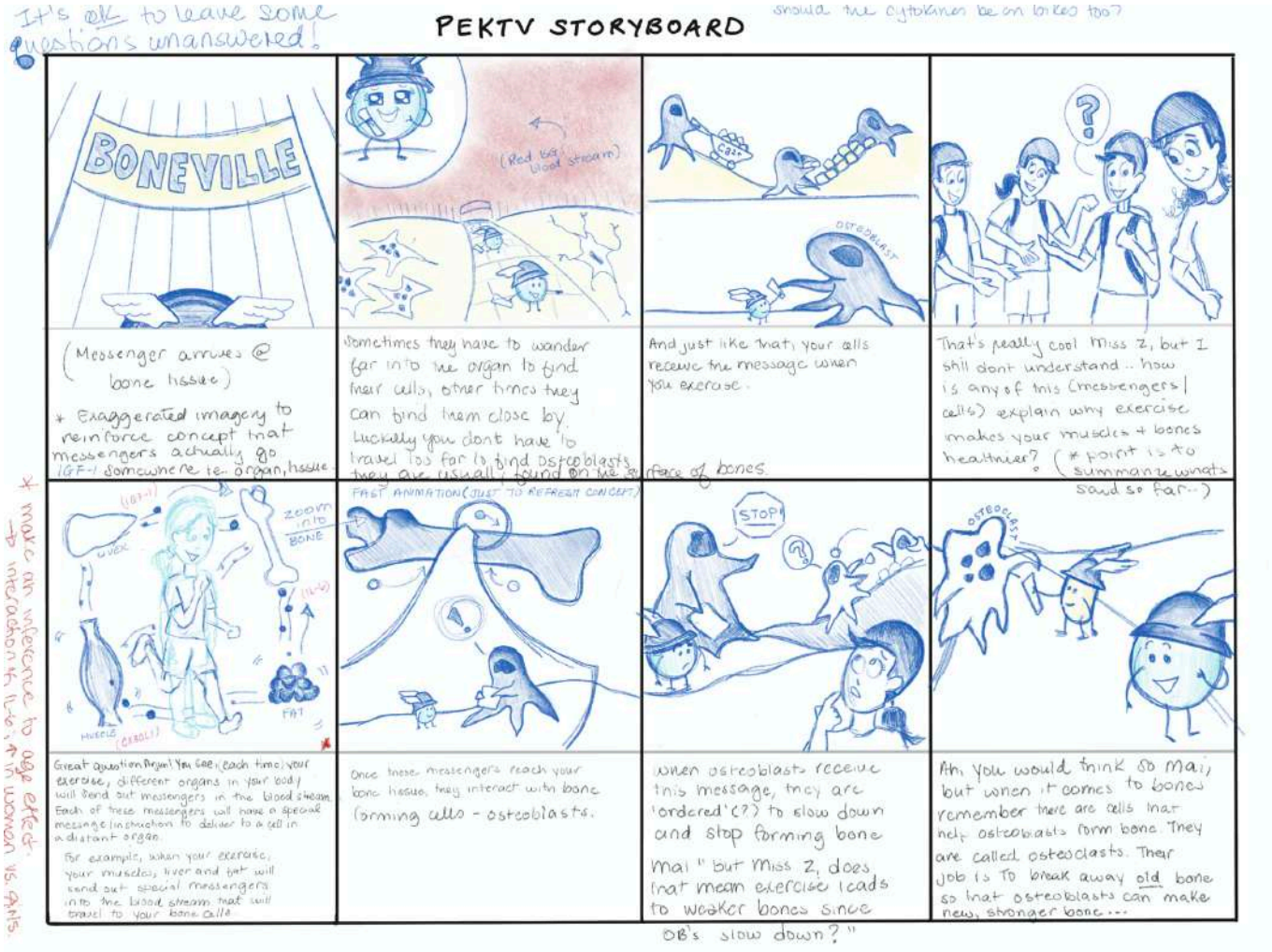


Figure G.1.4: Preliminary stages of "Exercise Messengers" storyboard.

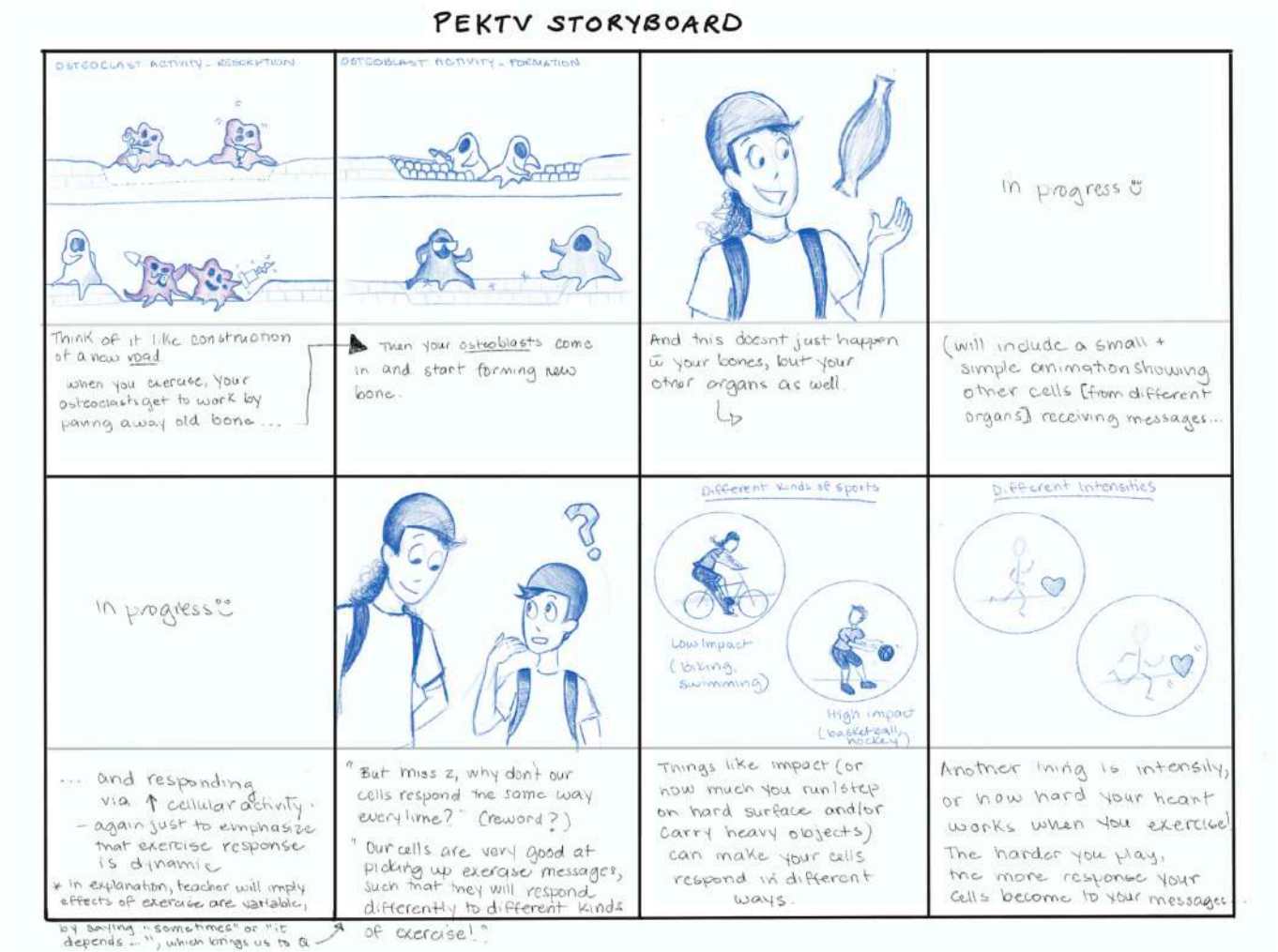


Figure G.1.5: Preliminary stages of "Exercise Messengers" storyboard.

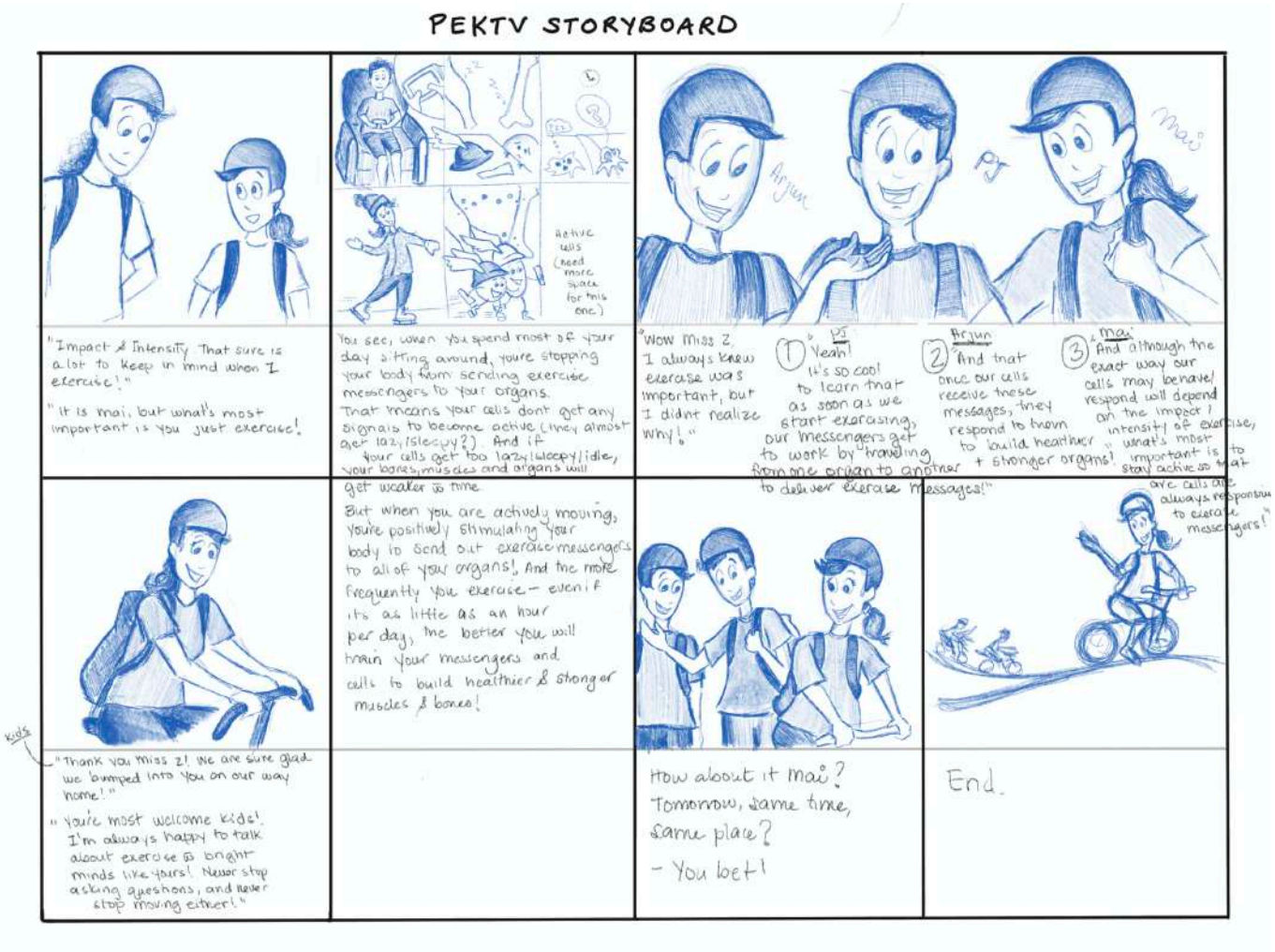


Figure G.1.6: Preliminary stages of "Exercise Messengers" storyboard.

Pediatric Exercise Knowledge Translation Video (PEKTV)
"Exercise Messengers" Script

OPENING SCENE: On a trail in the park, morning time, with the main character (Mai) biking to school. Mai addresses audience

MAI: Hi! My name is Mai, and I'm on my way to school! I just got a new bike and can't wait to show my friends. We usually bike together, they should show up any minute…

Mai is momentarily united with her friends, PJ & Arjun, who are catching up to her on their bikes.

PJ & ARJUN: Hey Mai!
MAI: Hey PJ, Hey Arjun!
PJ: That's a cool bike!
MAI: Thanks PJ! I got it last week. My parents said I was getting too tall for my old one, so it was time to get a new one.
ARJUN: Funny, the same thing happened with my gym shoes! My old ones felt too small, so mom said it was time for a new pair.

This short exchange between the kids sparks a question,

PJ: My dad said all the exercise I do makes me bigger. But I don't really get why.
Mai: Maybe we can ask Miss Z?
Arjun: I bet she knows since she's a scientist!

SCENE TRANSITION TO CLASSROOM: We hear the teacher (Ms. Z) opening next scene, as she stands in front of the classroom while all 3 kids are seated.

MS. Z: Good morning class! Are you ready for a new day of curiosity and wonder!

PJ: We are! And we even came up with a question for you while we were biking to school.

MS. Z: Let's hear it!

PJ: Well we were just wondering, does exercise make us, grow bigger?

MS. Z: It most certainly can! Exercise helps our body in so many ways, it makes our muscles bigger and stronger!

MAI: I knew it had something to do with our muscles! We can't move without them!

MS. Z: That's true Mai, but don't forget about your bones. Bones hold your muscles together. In fact, if we didn't have our bones, we would all look like piles of jelly!

PJ: Oh boy, I guess bones are pretty important!

MS. Z: And very much alive!

ALL KIDS: Alive?? What do you mean Ms. Z?

Modified January 21st, 2019

Figure G.2.1: Revised "Exercise Messengers" script.

MS. Z: When you can! Some activities may not be high intensity, but it doesn't mean they are bad for you. You just need to do more of them!

(BT Sedentary example)*

MS. Z: You see, when you spend most of your day sitting or lying around, you're stopping your body from sending exercise messengers to your bones. That means your cells are not active, and with time they will lose strength, eventually turning into weak bones.

When you're growing as a kid, your bones are also growing and getting stronger and stronger. As a kid, it's your job to make sure your bones are really strong so that you have strong bones when you stop growing as an adult. If your bones aren't that strong when you're an adult, you're more likely to break them and have to wear a cast.

PJ: Ouch! That's no fun.

(BT Active example)*

MS. Z: But the more often you exercise, the better you will train your messengers and cells to build healthier and stronger bones! What's most important is that you never stop moving, whether its playing a sport or a game of tag at recess. Speaking of which, it's almost time for yours!

MAI: Wow, time sure flies when you learn about exercise science!

MS. Z: It sure does! So, who wants to share what they've learned today?

BT (Active example)

PJ: Well Ms. Z, I always thought exercise was important, but I didn't realize why! It's so cool to learn that as soon as we start exercising, our exercise messengers get to work by traveling in our arteries to deliver messages to our bones!

ARJUN: And that once our cells receive these messages, they respond to them! Sometimes that means breaking down old bone and make new, stronger bone!

MAI: And although different kinds of activities may affect our bones differently, what's most important is to stay active so that our cells respond to our exercise messengers! No matter what kind of activity that is, whether its biking, basketball, or running around in recess!

MS. Z: Excellent work class! Now that you know the effects of exercise, I'm sure you'll do your best to be active! After all, if you want strong bones, you need to play hard for it!

ARJUN: That's right! I feel like playing a game of tag, do you guys want to play?

MAI and PJ: Okay, but you're it!

PJ: Will you play with us Ms Z?

MS. Z: Count me in!! (Everyone runs out of classroom)

Modified January 21st, 2019

Figure G.2.2: Revised "Exercise Messengers" script

exercise. Before they build new bone, some of the old bone needs to get broken down first, that's what osteoclasts do!

MS. Z: The job of osteoclasts is to break the old house, I mean old bone! This happens right after exercise, this is why it's important for osteoblasts to slow down so that osteoclasts can do their job. Once osteoclasts break away the old bone, the osteoblasts form new bone on top that is stronger and healthier! And that's how your bones grow bigger after exercise!

MAI: Hmm, if only there was a way to remember what these cells do, they have similar names!

MS. Z: If you're having trouble remembering what osteoclasts and osteoblasts do, try this:

osteoClasts - with a c - crush old bone, and osteoBlasts - with a b - build new bone.

MAI: Ah, I can definitely remember that!

PJ: Wow Ms Z, I didn't realize so much happens in our bones when we exercise, or that we even have different kinds of cells in our bones! All of this makes me want to learn more about the science of exercise!

ARJUN: Does this happen with all sports Ms. Z? Will riding my bike and playing basketball change my bone cells in the same way?

MS Z: Excellent question Arjun! All sports have good effects on your bones, but they might send out different messengers that make your cells respond in different ways. The messengers that get sent out with exercise really depend on the impact and intensity of an activity.

MAI: What are those Miss Z?

(BT Impact and Intensity diagrams)*

MS Z: Well, impact is how much weight or force you put on your bones during exercise, such as the forces coming from the ground when you run or jump, or from the weight of an objects such as a basketball or hockey stick.

PJ: Like holding a basketball!

MAI: Or a hockey stick!

MS. Z: Yes, those are great examples! On the other hand, intensity is how hard you work when you exercise, so things like how fast our heart beats, how quickly you breathe and how much energy you use refers to your exercise intensity.

ARJUN: So, intensity is how hard we play?

MS. Z: Yes! The harder you play, the more benefits your cells will have.

MAI: Does that mean we should only play activities with high intensity Ms Z?

Modified January 21st, 2019

Figure G.2.3: Revised "Exercise Messengers" script

give your body energy to move, and one way that they do that is by sending exercise messengers to your bones.
ARJUN: And how do the exercise messengers get to my bones?

(BT Bloodstream, simplified)*

MS Z: They are delivered by traveling quickly through your arteries, which carry your blood to your bones and other parts of your body!

PJ: Okay, so I understand how molecules travel through the blood to reach bones… But how do exercise messengers know where to go?

MS. Z: Let me explain. You know how you need an address to deliver a letter? Or a phone number to send a text message?

KIDS: Yes!

MS. Z: Well, your exercise messengers aren't any different! Every messenger carries a specific message that needs to go to a specific address; and in your body, that address leads to a cell.

MAI: Oh I think I know where this is going! Can I take a guess Ms. Z?

Ms. Z: Go for it!

MAI: So if I'm exercising on a bike, messengers will go to the bones in my legs, and they will deliver their messages to the cells in my bones! My osteo.. cells, right?

MS. Z: Exactly Mai! Once exercise messengers arrive at your bones, they find the osteoblasts which are usually found on the surface of bones. And just like that, your osteoblasts receive the message when you exercise!

ARJUN: That's pretty cool! So what happens next Ms. Z, do our bones grow bigger?

MS. Z: Actually, they break down first.

KIDS: What?!

PJ: What do you mean they breakdown Ms. Z? Does that mean exercise is bad for our bones?

MS. Z: No, not at all! Let me explain with an example.

(BT Simplified house construction example)*
MS Z: Let's say you want to build a brand new house to replace your old house. Should you build the new house on top of your old house?

ARJUN: Probably not, the houses would break!

MS. Z: Exactly! So before you start building the new house, you need to find a way to tear down the old house. Only then can you start building. This is exactly why your osteoblasts slow down when you

Modified January 21st, 2019

Figure G.2.4: Revised "Exercise Messengers" script

MS. Z: Well just like the rest of your body, your bones are made up of cells, which are the smallest pieces of all living things! Most parts of your body have more than one kind of cell. For example, there are two main kinds of cells in your bones, they are called osteoblasts and osteoclasts.

PJ: Huh?

MS. Z: Osteoblasts and osteoclasts It's easy. Os-te-o-blasts and os-te-o-clasts!

ARJUN: Those sound like funny names! But why are there different kinds of bone cells?

MS. Z: Well that's because they do different things! Osteoblasts build bone, and osteoclasts break down small pieces of our bone! Both of these cells work together to give your bones their shape and form! And guess what? They work even better when you exercise!

MAI: That sounds cool! So how does exercise make these cells work better?

MS. Z: To learn about how that happens, we should learn about molecules first!

Kids: Molecules?

BLACKBOARD TRANSITION (BT), ie. blackboard with animated chalk drawings. BT's will be used to explain scientific concepts, which will be narrated by the characters in the background. The rest of the video will essentially be an exchange blackboard and classroom transitions.

(BT Molecules)*

MS. Z: Yes, molecules! They are teenie, tiny particles in your body that deliver messages to your bones when you exercise. You have many molecules that travel to your bones to tell them to grow!

MAI: Hmm, sounds like these molecules are messengers?

(BT Messenger 'character' is introduced)*

MS. Z: Precisely Mai, molecules are just like messengers, exercise messengers!

MAI: Do you also have exercise messengers Ms. Z?

MS. Z: I sure do! Everyone has exercise messengers. Kids and adults have different amounts of messengers because they are different ages, but they all play very similar roles in making sure our bones get healthier, stronger, and bigger after exercise.

ARJUN: But Ms. Z, where do these exercise messengers come from?

(BT Working organs + arteries)*

MS Z: I love your curiosity Arjun! Exercise messengers are stored in different parts of your body, like your muscles, liver, blood arteries, and fat! You see, when you exercise, all of these parts work very hard to

Modified January 21st, 2019

Figure G.2.5: Revised "Exercise Messengers" script

APPENDIX H – Exercise Messengers Voiceover Session

Figure H.1: The main characters of the video were brought to life by three little exercise scientists and a CHEMP Masters student.

APPENDIX I – Q-methodology Concourse Statements

PRELIMINARY CONCOURSE STATEMENTS

1. I learned something new from this video.
2. I feel that I can explain what I've learned in this video to my friends.
3. This video helped me understand what happens to my body when I exercise.
4. I understand more about bones and exercise now than I did before watching the video.
5. In order for me to understand this video, I would need to watch it again.
6. I was able to learn the meanings of new words from watching this video.
7. I think videos like this help me learn new words.
8. Videos like this are helpful for learning science.
9. I think this video and videos like this will help me do better in science class.
10. This video encourages me to ask my teacher more questions about science.
11. I prefer to learn about science using videos instead of books.
12. I would rather have my teacher explain science to me than watch a video.
13. I found it easy to pay attention to the video.
14. This video made learning science fun.
15. I found it hard to pay attention to the video.
16. This video made me want to learn more about why exercise is good for my body
17. This video makes me feel that it is my job to make my bones strong and healthy.
18. This video makes me want to learn how to play a new sport.
19. This video makes me want to explore more about exercise and bones.
20. This video made me like exercise more.
21. After watching this video, my attitudes towards exercise have not changed.
22. Thinking of bone like a house made it easier for me to understand how bone works.
23. I would like to share this video with my friends
24. I would like to share this video with my family
25. I want to go and tell my friends/parents what I learned today.
26. I think science is more fun when my friends and I learn it together.
27. I like learning science with my friends more than learning it by myself.
28. I think science can be more fun when I learn it with my friends.
29. I feel that I learned more when the students asked questions in the video.
30. I think we should watch videos like this for gym class.
31. This video makes me want to make more scientific discoveries.
32. This video encourages me to engage in exploration.
33. This video encourages me to make (scientific) discoveries.
34. I like learning science when there are videos.
35. I would like to use science in my job when I grow up.
36. This video makes me want to use science in my job when I grow up.
37. This video encourages me to do science activities at home.
38. I think more science videos like this should be made.
39. Watching this video makes me want to learn science with my friends.
40. Real life activities (like building a house) made it easier for me to understand how bone works.
41. Watching a video like this with my friends/classmates would be fun.
42. I would like to watch more videos like this to learn about science.
43. The animations made it easier for me to learn.
44. This video has changed the way I think about exercise.
45. I like this video.

Figure I.1: Preliminary list of concourse statements derived from literature, pediatric exercise researchers, educators, and knowledge translation specialists.

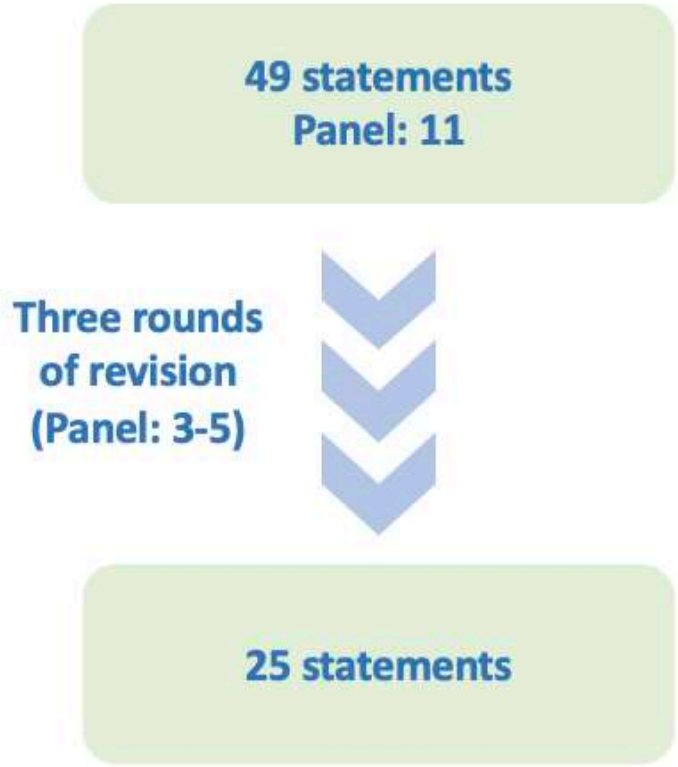


Figure I.2: Schematic of concourse revision. 49 statements were reduced to 25 statements after three rounds of revision. 25 statements were chosen to accommodate a 4x4 Q-sort matrix.

APPENDIX J – Exercise Messengers Screening and data collection

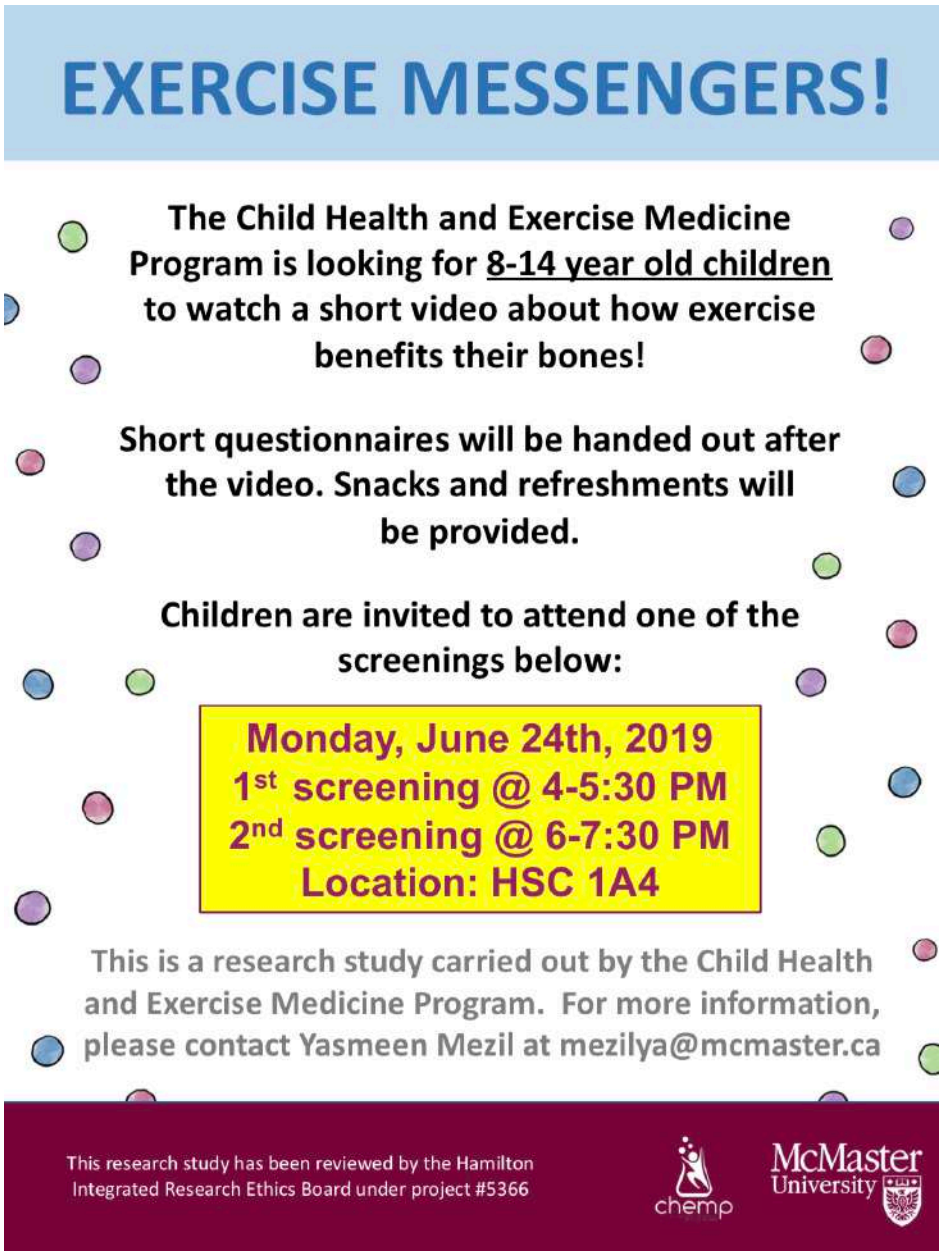


Figure J.1: Recruitment poster for the Exercise Messengers video screening.

Figure J.2: Participants sorting through concourse statements and applying them to the Q-sort table.

Figure J.3: A voluntary, artistic contribution by the participants of the Exercise Video Screening. Concluding statement: "*Exercise helps your bones, so exercise!".* The origins of "*Yoink!"* are unknown; the popular use of this term is interesting and possibly worth future investigation.

APPENDIX K – CIHR Notice of the IHDYHC Talks Award

Canadian Institutes of Health Research
160 Elgin Street, 9th Floor
Address Locator 4809A
Ottawa, Ontario K1A 0W9

Instituts de recherche en santé du Canada
160, rue Elgin, 9e étage
Indice de l'adresse 4809A
Ottawa (Ontario) K1A 0W9

Institute of Aging
Institute of Cancer Research
Institute of Circulatory and Respiratory Health
Institute of Gender and Health
Institute of Genetics
Institute of Health Services and Policy Research
Institute of Human Development and Child and Youth Health
Institute of Indigenous Peoples' Health
Institute of Infection and Immunity
Institute of Musculoskeletal Health and Arthritis
Institute of Neurosciences, Mental Health and Addiction
Institute of Nutrition, Metabolism and Diabetes
Institute of Population and Public Health

Institut du vieillissement
Institut du cancer
Institut de la santé circulatoire et respiratoire
Institut de la santé des femmes et des hommes
Institut de génétique
Institut des services et des politiques de la santé
Institut du développement et de la santé des enfants et des adolescents
Institut de la santé des Autochtones
Institut des maladies infectieuses et immunitaires
Institut de l'appareil locomoteur et de l'arthrite
Institut des neurosciences, de la santé mentale et des toxicomanies
Institut de la nutrition, du métabolisme et du diabète
Institut de la santé publique et des populations

January 9, 2020

Ms. Yasmeen Abdulkareem Mezil
25 Mccalla
St. Catharines, Ontario L2N 1A1

Dear Yasmeen Mezil:

We are pleased to inform you that your recent application to the Canadian Institutes of Health Research (CIHR) for the Institute Community Support (ICS) Prize – IHDCYH Talks has been approved for funding.

An Offer of Award and Response to an Offer of Award form are on ResearchNet. It is your responsibility to complete the Response to an Offer of Award form and return it to CIHR within 15 days of the notification date of your award.

Congratulations on your success in this competition. Should you have any questions, please do not hesitate to communicate with a Processing Officer in the Contact Center at 613-954-1968 or by e-mail at support-soutien@cihr-irsc.gc.ca.

Sincerely,

Paula Kirton

Paula Kirton
Manager, Program Design and Delivery
Research Program Portfolio

490842-201910IHT-IHT-431708-I008-201606-IHTAP

Canada

CIHR IRSC
Discoveries for life / Découvertes pour la vie

Figure K.1.1: Letter of notice from CIHR on the Institute of Human Development, Child and Youth Health Talks Competition. The original Exercise Messengers video was revised and reduced to 5-minutes (based on participant feedback) and submitted for the competition. The letter also includes feedback from two reviewers along with their ratings of the submission.

Review Type/Type d'évaluation:	Committee Member 1/Membre de comité 1
Name of Applicant/Nom du chercheur:	Mezil, Yasmeen Abdulkareem
Application No./Numéro de demande:	431708
Agency/Agence:	CIHR/IRSC
Competition/Concours:	2019-10-15 Prize - IHDCYH Talks/Prix - Entretiens de l'IDSEA
Committee/Comité:	Prize - IHDCYH Talks/Prix - Entretiens de l'IDSEA
Title/Titre:	Exercise Messengers

Assessment/Évaluation:

This was a very good video overall. This video could be very useful as a tool to inform parents about the link between exercise and bone health, which is a topic that may be unfamiliar to parents, and provides specific recommendations for improving bone health through exercise.

The main limitation that I identified was that the evidence underlying the claims in the video was not clearly integrated into the video itself, and the references that are listed in the Research Summary document are more so related to the knowledge translation evidence than to evidence supporting the health claims in the video.

Overall I found the video very accessible to a general audience. The language used was easily understood and the visuals helped me to follow the message as it was being delivered. I also appreciated how, when more complex scientific language was used (e.g. osteoblast), the word was repeated a couple of times to provide the audience with an opportunity to really digest the word and related concepts. I also found that switching between the classroom setting and the cellular/molecular settings helped keep me engaged throughout the video. I also enjoyed the analogies used to convey the topics – like comparing the messenger molecules to the mail or a text message.

Stylistically, I enjoyed the dialogue element that was used to gradually move deeper into the topic of exercise messengers and the link between exercise and bone health. I found this approach creative and innovative.

The video quality was overall very good, but the animation and sound quality was a little bit less crisp than it could have been.

2020-01-10 12:45

Figure K.1.2: Letter of notice from CIHR on the Institute of Human Development, Child and Youth Health Talks Competition.

Application Number / Numéro de demande: 431708
Name of Applicant / Nom du candidat: Mezil, Yasmeen Abdulkareem
Review Type / Type d'évaluation: Committee Member 1/Membre de comité 1
Competition: 2019-10-15 Prize - IHDCYH Talks
Concours: 2019-10-15 Prix - Entretiens de l'IDSEA
Committee: Prize - IHDCYH Talks
Comité: Prix - Entretiens de l'IDSEA

Assessment / Évaluation:

	Score / Cote	Cote Maximum Score	Multiplier / Multiplicateur	Weighted Score / Cote pondérée
Evaluation Criteria / Critères d'évaluation				
1) Impact & Relevance / Impact et pertinence	4.0	4.9	0.35	1.40
2) Accessibility / Accessibilité	4.1	4.9	0.25	1.03
3) Innovation & Creativity / Innovation et créativité	4.0	4.9	0.25	1.00
4) Video Quality / Qualité de la vidéo	3.5	4.9	0.15	0.53
5) Reach / Vote en ligne	4.0	4.9	0.0	0.00

	Score / Cote	Cote Maximum Score	Weighted Score / Cote pondérée
Total:	**19.60**	**24.5**	**3.96**

Page 1

Figure K.1.3: Letter of notice from CIHR on the Institute of Human Development, Child and Youth Health Talks Competition.

Review Type/Type d'évaluation:	Committee Member 2/Membre de comité 2
Name of Applicant/Nom du chercheur:	Mezil, Yasmeen Abdulkareem
Application No./Numéro de demande:	431708
Agency/Agence:	CIHR/IRSC
Competition/Concours:	2019-10-15 Prize - IHDCYH Talks/Prix - Entretiens de l'IDSEA
Committee/Comité:	Prize - IHDCYH Talks/Prix - Entretiens de l'IDSEA
Title/Titre:	Exercise Messengers

Assessment/Évaluation:

This is video is the last chapter of a doctoral thesis project on the effect of exercice on cytokines and bone makers in girls and women. The video displays the same message for boys and girls (and it is appropriate). Audience is clearly school-aged children. It is a remarkable and innovative last output of a doctoral thesis. Clear and well animated. The term 'exercise messenger' is an excellent term, recalling that there are stimulated by exercice. Nice animations for the actions of osteoclast, osteoblast, messengers; nice analogies to explain role of molecules, cytokines, postal address, etc. good message regarding the effect of sedentarity vs. activity.

Figure K.1.4: Letter of notice from CIHR on the Institute of Human Development, Child and Youth Health Talks Competition.

Application Number / Numéro de demande: 431708
Name of Applicant / Nom du candidat: Mezil, Yasmeen Abdulkareem
Review Type / Type d'évaluation: Committee Member 2/Membre de comité 2
Competition: 2019-10-15 Prize - IHDCYH Talks
Concours: 2019-10-15 Prix - Entretiens de l'IDSEA
Committee: Prize - IHDCYH Talks
Comité: Prix - Entretiens de l'IDSEA

Assessment / Évaluation:

	Score / Cote	Cote Maximum Score	Multiplier / Multiplicateur	Weighted Score / Cote pondérée
Evaluation Criteria / Critères d'évaluation				
1) Impact & Relevance / Impact et pertinence	4.6	4.9	0.35	1.61
2) Accessibility / Accessibilité	4.5	4.9	0.25	1.13
3) Innovation & Creativity / Innovation et créativité	4.3	4.9	0.25	1.08
4) Video Quality / Qualité de la vidéo	4.6	4.9	0.15	0.69
5) Reach / Vote en ligne	0.0	4.9	0.0	0.00

	Score / Cote	Cote Maximum Score	Weighted Score / Cote pondérée
Total:	**18.00**	**24.5**	**4.51**

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Figure K.1.5: Letter of notice from CIHR on the Institute of Human Development, Child and Youth Health Talks Competition.

APPENDIX L – 'Art and Research', a speech written and presented at part of the Gallery of Graduate Arts at Mulberry Street Coffeehouse, Hamilton, September 2018.

They say the best way to learn something is to teach it. I have a small adjustment to that: The best way to learn something is to draw it first, get up close and personal with it, draw it some more, have a passionate affair with it draw again, and then, teach it.

As a teaching assistant in the Anatomy Education Program, I learn anatomy so that I could teach it to students. I'm a very visual learner, so naturally when I study anatomy, I rely on so many visuals to understand the content. I look at specimens, textbooks, flashcards, and videos, and all so that I can fully grasp anatomical concepts. I find these tools very fascinating, but what makes me really enjoy anatomy is not my use of tools, but my ability to join it with my passion, which is art.

Art is a lens that I use to see anatomy and understand it. For example, when, I look at the brain, I don't just see an organ, I see so much more. I see a convergence of different shapes and layers. I see lines that are wavy, lines that are straight, grooves that are shallow and grooves that go deep into the unknown. What's crazy is that sometimes certain things only appear to me after I draw them out. For example, the brain has so many variations in colour and texture, some of them are so minute that they are only evident after hours of looking at brain, or several attempts to replicate it. And so when I come across these fine details of the brain, my curiosity triggers me to go and learn what these differences actually mean anatomically. So as I keep drawing, each element of the brain begins to really stand out to me, and that's how I learn anatomy.

The reason I explain this to you is that I've realized that my passion in art teaches me a lot of things. It teaches me patience, precision, and proximity, and it also gives me perspective. And its exactly for this reason that I use art as a lens to pursue my research. Before I explain how I do that, let me tell you little bit about my research.

My thesis focuses on the physiological effects of exercise on muscle and bone in girls and women. Now we know that exercise is good for you, but there is

much to learn about why that's the case, and what exactly happens at a cellular level that causes the benefits of exercise. I hypothesize that many of the benefits happen because when we exercise, our organs talk to each other. They send out specialized signals and proteins that make muscles and bones respond to exercise, making them stronger and healthier. Now the question of how this process happens is what I am trying to answer in my research, not just because I am interested in this concept, but because I would really like other people know the answer as well. As a researcher, I feel strongly about knowledge translation and finding ways to accelerate research findings to the community, as I think this should be every researchers goal. My rationale for this is that our actions are shaped by our knowledge, and they are also shaped by our passion.

So, using my passion for art, I am currently working on a knowledge translation tool so that that I can transfer the findings of my research to the public in a way that is accessible to everyone. I want my work to allow people to not only understand the importance of exercise, but to also reflect on it, discuss it, and hopefully, implement it in their lives. I believe that by incorporating art in my research, I can shape my perspective so that I see my work in both my eyes as a researcher, and the eyes of the public. This mentality that I have developed around my art as a means of knowledge transfer has really changed my outlook on my PhD. It has transformed me to being very curious and interested in my research, to also feeling strongly passionate about it, and wanting to make a difference. In fact, I can confidently say, that art has made me fall in love with my research.

Finally, my experience with pursuing art and research has taught me so much about the importance of following your passion. It not only changes your outlook on life, it also helps you deal with life. For example, every piece of art that you see today is a product of an event that was happening in person's life. These events guided these an artist to blank page, an empty canvas, a piece of thread. Behind every artistic expression was series complex thoughts and emotions that somehow led to the beautiful masterpieces you see here tonight. This is what having a passion does to do, it allows you to express yourself in the medium you hold most dear, in the good times and bad. And this doesn't only happen in art, self-expression can happen to anywhere, with any passion one holds dear. Whether it be composing sweet-sounding music, having a profound love to elephants, or baking bread to perfection. Every passion leads to something beautiful.

So, if you want your passion to bring beauty to your life, shape your perspective, and perhaps your fate, dare to follow through with it. Because in the end, everything goes full circle. My anatomical sketches and research findings both started as scribbles and lines with the same tool, the pen. To me, the pen is my fingerprint that I have chosen it to express myself, to understand my surroundings, to teach what I love, and most importantly, to live my life by my passion for art.

Thank you,

– Yasmeen