Effects of Rapid Maxillary Expansion on Serum Insulin-Like Growth Factor I and Apnea Hypopnea Index Scores in a Prepubertal Population with Maxillary Constriction

By

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Abstract

Introduction: Insulin-like Growth Factor 1 (IGF-1) is a potent mitogenic hormone that is critical for normal growth and metabolism. In some cases, children with pediatric obstructive sleep apnea (POSA) may demonstrate lower serum IGF-1 levels and growth. A narrow maxilla is one morphological feature reported in a subgroup of POSA individuals. Surgical removal of enlarged adenoids and palatine tonsils in POSA individuals has been shown to increase IGF-1, normalize apnea-hypopnea index (AHI) scores, and allow catch-up growth while non-surgical maxillary expansion treatment has so far shown to improve AHI scores, at least short-term. No studies to date have assessed the effect of maxillary expansion on serum IGF-1 levels.

Objective: To determine the effect of non-surgical maxillary expansion on serum IGF-1 and AHI score in non-syndromic, prepubertal children with maxillary transverse deficiency compared to untreated controls over a period of approximately 12 months.

Methods: Sixty-five prepubertal children (ages 7 – 12) from the graduate orthodontic clinics of the Universities of Alberta and Insubria were admitted and randomly assigned to either a treatment group (intra-oral fixed maxillary expander followed by a fixed retainer) or a delayed treatment follow-up control group. Blood samples, home sleep apnea tests, body mass index score, dental models, and hand-wrist radiographs were acquired at the start and end of the trial. Multivariate and univariate statistical testing was performed to assess for interactions, covariates, and to compare means between groups

Conclusions: The control group experienced a significant increase in serum IGF-1 but the treatment group experienced a significantly larger increase over the trial period. Severity of maxillary deficiency and skeletal age significantly interacted with time to explain the variance of both outcomes. There were significant interactions between treatment group and time. Subgroup analysis showed the University of Insubria cohort had a significant but lower increase in IGF-1 levels for the treatment group and no change for the control group, and the average maxillary deficiency was significantly less for the University of Alberta cohort. AHI scores significantly decreased in the treatment group but increased slightly but significantly in the control group.

Preface

This thesis is an original work by David Vu. The research project, of which this thesis is part, received research ethics approval from the University of Alberta Research Ethics Board, under the name "Evaluation of oxygen saturation and serum levels of growth hormone and bone growth mediators after rapid maxillary expansion in growing patients" with ID No. 00061536, March 1, 2016. No part of this thesis has been previously published.

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CHAPTER 1

General Introduction

Chapter 1 – General Introduction

Section 1.1: Statement of the problem

Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) form an axis that is imperative for growth and maturation, as well as metabolic regulation in humans (Frago and Chowen, 2005). Serious disturbances to this axis can lead to detrimental effects such as growth retardation in children due to GH deficiency, or acromegaly in adults due to GH excess (Wit et al., 2019, Brooke and Drake, 2007, Melmed, 2009). In the literature, pediatric obstructive sleep apnea (POSA) has been suggested to be a potential risk factor for overall growth retardation and it is recommended by the American Academy of Pediatrics that POSA should be screened for and managed appropriately (Marcus et al., 2012, Bonuck et al., 2009).

There are certain craniofacial characteristics that may be associated with pediatric obstructive sleep apnea, and one such feature is maxillary constriction (Pizzatto and Flores-Mir, 2017). Some authors believe that correction of the constricted maxilla may contribute to resolution of associated nasal turbulence and reduced airflow (Guilleminault et al., 2008), and consequently correction of mouth breathing patterns that may alter craniofacial growth (Camacho et al., 2017). Maxillary constriction in children is routinely treated orthopedically by means of palatal/maxillary expansion with a variety of appliances and protocols (Lagravere et al., 2010; Liu et al., 2017).

Although there are clinicians who support the use of rapid maxillary expansion (RME), when indicated, in children as an alternative to manage POSA (Villa et al., 2011, Pirelli et al., 2010, Miano et al., 2009), there is no definitive reported physiological mechanism of how POSA reacts to such treatment at the cellular level. Recent systematic reviews and meta-analyses suggested that there are deficiencies in the quantity and quality of original studies evaluating the effects of RME in children with POSA (Camacho et al., 2017, Huynh et al., 2016), but have reported an

overall short-term significant reduction in the apnea-hypopnea index (AHI) values. Due to the weak level of evidence, the decision to attempt management of POSA by RME is still equivocal. There is a scarcity of studies aimed specifically to assess changes at cellular level (i.e., in serum biological markers) that would help in elucidating possible mechanisms that may lend or detract support for treatment of POSA with RME.

GH and IGF-1 exist in circulation in bound and unbound forms (Amit et al., 2000, Aneke-Nash et al., 2016). In medicine, both are used as biomarkers to aid diagnosis of disorders of growth – conditions of GH insufficiency or excess (Bidlingmaier and Freda, 2010, Wit et al., 2019, Higham et al., 2007). Hence, it may be prudent to study any potential systemic effects represented as changes in serum biomarkers in response to RME in children. This information may provide us with an improved understanding of some the actual physiological changes produced during RME treatment. The purpose of this randomized controlled trial is to evaluate changes in the following serum biomarkers: insulin-like growth factor 1 (IGF-1, and calcidiol (vitamin D3), and as well as sleep outcomes assessed through the apnea-hypopnea index (AHI) before and after RME compared to delayed treatment in a population of non-syndromic prepubertal children with maxillary constriction otherwise in good health.

Section 1.2: Study Objectives

The primary objective of this thesis is to determine if there are any differences in the serum concentrations of IGF-1 and AHI scores while controlling for the covariates of chronological age, sex, BMI, and amount of transverse discrepancy over a 12-month period between prepubertal healthy children with maxillary constriction, randomized to a treatment (RME) or control group.

The secondary objective is to determine if there are any differences in the serum concentrations of IGF-1, alkaline phosphatase, calcidiol, and AHI scores while controlling for the covariates of chronological age, sex, BMI, and amount of transverse discrepancy over a 12-month period between the treatment and control groups of the University of Alberta cohort.

The tertiary objective is to determine if there are any differences in the serum concentrations of IGF-1, and AHI scores while controlling for the covariates of skeletal age, sex, BMI, and amount of transverse discrepancy over a 12-month period between the treatment and control groups of the University of Insubria cohort.

The quaternary objective is to determine if there are any differences in the serum concentrations of IGF-1 and AHI scores while controlling for the covariates of chronological age, sex, BMI, and transverse discrepancy of children in treatment groups between the Canadian and Italian cohorts. In all four scenarios, time will be studied both as a fixed independent factor and controlled by evaluating only the mean changes in outcome variables over the 12-month trial duration.

CHAPTER 2

Review of the Growth Hormone/Insulin-Like Growth Factor-1 axis, Pediatric Obstructive Sleep Apnea, and Other Important Domains

Section 2.1.1: Systematic Review of the Literature – Introduction

The growth hormone (GH) and insulin-like factor 1 (IGF-1) axis is established as being critical for in-utero and neonatal growth and development.   Additionally, it is involved in several metabolic processes in humans (Frago and Chowen, 2005). There are several main components to the GH/IGF-1 axis. Serious disturbances of this axis causing deficiencies in GH or IGF-1 can lead to detrimental effects such as growth retardation and may ultimately result in unattained height gain. Pediatric obstructive sleep apnea (POSA) has been implicated in the literature as one potential etiological factor for growth retardation.

Obstructive sleep apnea (OSA) is a multifactorial disease. Some craniofacial features may be associated with POSA; one such example is a narrow upper jaw, also known as maxillary constriction (Pizzatto and Flores-Mir, 2017). Maxillary constriction in children is typically treated orthopedically by a technique known as palatal/maxillary expansion which uses an expansion appliance to apply force to separate the intermaxillary suture to induce bony increase in the maxillary transverse dimension (Lagravere et al., 2010; Liu et al., 2017).

Although there are clinicians who specifically support rapid maxillary expansion (RME) in children as a means to manage POSA (Miano et al., 2009, Pirelli et al., 2010, Villa et al., 2011, Marino et al., 2012), there is no definitive reported disease mechanism in response to treatment at the cellular level. There are limited human studies on this topic, and as such, using RME as a first line treatment in POSA, does not have overt support from some prominent medical and dental associations (Behrents et al., 2019, AAPD, 2016, ASO 2019), while others such as the British Orthodontic Society do not have any published guidelines on orthodontic treatment for POSA. Having stated this, in a subgroup of POSA individuals RME maybe a reasonable first approach. The identification of this cluster is still elusive.

There have been studies on the effect of RME in pediatric subjects with OSA by assessing change in AHI scores. Within the last five years there have been three systematic reviews with meta-analyses (Huynh et al., 2016, Camacho et al., 2017, Vale et al., 2017), one systematic

review (Vidya and Sumathi, 2015), one qualitative review with meta-analysis (Machado-Junior et al., 2016), and one stand-alone meta-analysis (Sanchez-Sucar et al., 2019) performed on this subject.

These review articles follow different protocols including use of tools to assess methodological quality of included studies and their associated risk of bias. Some are qualitative in nature and skip these steps altogether yet perform a meta-analysis (Machado-Junior et al., 2017). Some meta-analyses overestimate the effects of RME by incorporating studies that use duplicate data (Vale et al., 2017, Machado-Junior et al., 2017).

Most of these reviews and meta-analyses found that there were few primary clinical studies and that most of these studies had high risk of bias. The sample sizes of included studies were small and there was severe methodological and clinical heterogeneity noted between the studies. All authors reported with varying degrees of confidence that the AHI does appear to significantly improve following RME in children identified as having OSA pre-treatment, at least in the short term. One review goes as far as concluding that RME in children with OSA is an effective treatment modality for POSA (Machado-Junior et al., 2017).

Table 2.1 provides a summary of the review articles and meta-analyses. It is evident that the review articles and meta-analyses exhibit great overlap in included original articles. It would appear that within the last five years there is little new contributions to the literature with regards to the effect of RME on the outcome of change in AHI in pediatric populations with OSA.

Table 2.1: Summary of Relevant Systematic Reviews and Meta-Analyses				
Authors, Year	Type	Tools	Included Articles	Findings & Conclusions
Vidya & Felicita, 2015	R	None	Villa et al., 2011 Guilleminault et al., 2011 Miano et al., 2009 De Moura et al., 2008 Cistulli et al., 1998	Reported results from each article. RME may be efficient and the standard treatment for POSA in children with Down syndrome and young adults with narrow maxillary arch with malocclusions leading to OSAS. RME can be used solely for OSAS for those with altered morphology due to reasons such as habitual mouth breathing.
Huynh et al., 2016	SR & MA	ARRIVE for quality assessment	Pirelli et al., 2004 Villa et al., 2007 Guilleminault et al., 2011 Marino et al., 2012 Pirelli et al., 2012	Use of RME in situ results in a significant improvement in mean difference in AHI of 6.19 [5.81,6.57] in the short term in combined subject pool of 116. Considerable heterogeneity between studies (I^2=98%). Selection criteria did not account for potential confounders (i.e. adenoid/tonsil size or previous adenotonsillectomy surgery. Authors caution interpretation of orthodontic treatment results in pediatric OSA.
Machado-Junior et al., 2016	R & MA	None	Caprioglio et al., 2013 Guilleminault et al., 2008 Guilleminault et al., 2011 Miano et al., 2009 Pirelli et al., 2004 Pirelli et al., 2005 Pirelli et al., 2012 Villa et al., 2007 Villa et al., 2011 Villa et al., 2013	Meta-analysis performed indicated a significant improvement in the mean difference in AHI before and after RME as -6.86 [-7.18, -6.54]. Heterogeneity was significantly high (I^2=98%). Authors concluded that "rapid maxillary expansion in children with OSAS appears to be another effective treatment for this syndrome".
Camacho et al., 2017	SR & MA	NICE too for case series for quality. REVMAN for heterogeneity and inconsistency between studies	Rose & Scheddl, 2006 Villa et al., 2007 Villa et al., 2011 Villa et al., 2014 Hosselet et al., 2009 Miano et al., 2009 Pirelli et al., 2010 Pirelli et al., 2012 Guilleminault et al., 2011 Guilleminault et al, 2013 Goncalves et al., 2012 Kim 2014 Fatsuca et al., 2015 Taddei et al., 2015	The meta-analysis included children with Marfan syndrome and those having prior adenotonsillectomy surgery. However, the authors subcategorized the study subjects by presence or absence of tonsils (previous surgery), mixed absent or small tonsils, small tonsils only, large tonsils only, or mixed small or large tonsils. The children with small, large, or mixed small or large tonsils (no previous surgery) had significant reductions in AHI of -73%, -61%, and -63%, respectively, but mean post-RME AHI was still indicative of OSA (AHI > 1). A high risk of publication bias with regards to RME and AHI was reported. Authors conclude that improvement in AHI was consistently seen in children undergoing RME, especially over short terms of study less than 3 years follow up.
Vale et al., 2017	SR & MA	CASP for qualitative assessment	Pirelli et al., 2005 Pirelli et al., 2010 Villa et al., 2007 Villa et al., 2011 Miano et al., 2009	RME significantly reduced AHI values with the standardized mean decrease of AHI by 3.24[0.34,6.15]. Combined subject pool of 137 children. Heterogeneity was high (I^2=98%). RME has a significant effect on OSAS and improves AHI in children, but there is a lack of quantity and quality of articles that address this topic.
Sanchez-Sucar et al., 2019	MA	CONSORT for quality assessment	Hosselet et al., 2009 Miano et al., 2009 Pirelli et al., 2010 Guilleminault et al., 2011 Caprioglio et al., 2013 Fatsuca et al., 2015 Rabasco et al., 2014 Villa et al., 2014 Villa et al., 2015	Three of 9 articles had low quality and 6 had moderate quality. Publication bias is reported. Their random effects model had a significant reduction in mean difference in AHI of 5.79[9.06,2.50] with high heterogeneity (I^2=98%). There is a lack of high-quality studies. Authors concluded RME does appear to be effective for treating mild to moderate sleep apnea-hypopnea syndromes in children.

Abbreviations used:

ARRIVE: modified criteria by the animal research: reporting in vivo experiences guidelines for human experimental studies.

NICE: National Institute of Health and Care Excellence. REVMAN: Review Manager, Cochrane. CASP: Critical Appraisal Skills Programme. CONSORT: Consolidated Standards of Reporting Trials. R: qualitative review. SR: Systematic Review. MA: Meta-analysis.

Section 2.1.2: Systematic Review of the Literature - Methods

As stated before, physiological mechanisms related to how RME in children can improve pediatric OSA but are not well understood yet. It has been suggested that RME allows for increase in airway volume by increasing the dimensions of the nasal cavity and nasopharyngeal region while allowing for an improvement in tongue position which secondarily increases in the available space for improved air movement (Sanchez-Sucar et al., 2019).

The increase in the volume of the intranasal cavity may also allow for an improvement in nasal airflow, and the increased tongue space may allow for normalized growth of the mandible (Camacho et al., 2017). Other authors have suggested that in patients with deviation of the nasal septum, an increase in the maxillary transverse dimension may allow for increase in nasal cavity size that may contribute to the repositioning of the deviated nasal septum. The result is reduced nasal airflow resistance and possible reduced OSAS severity (Pirelli et al., 2004).

However, there is no reports on how RME may influence the body systemically through biological markers to rectify any disruptions to the GH/IGF-1 system that may have impeded growth due to POSA. A systematic search of the literature was performed to evaluate the how RME may affect the GH/IGF-1 system and the common available serum biomarkers calcium, phosphate, alkaline phosphatase, and calcidiol. The clinical question was set as "In growing individuals with a diagnosis of POSA and maxillary constriction, does RME compared to no treatment modify selected biological markers?" and an appropriate search strategy developed.

Eligibility Criteria

The PICOS format was used to establish our clinical question:

Population	Children under 18 years old with concurrent maxillary transverse deficiency eligible for maxillary expansion; non-syndromic

Intervention	Rapid maxillary expansion with any type of expansion appliance and anchorage setup (tooth-borne, bone-borne, or tooth and bone-borne)
Comparison	No treatment
Outcome	Before and after changes in the following serum biomarkers:

- Growth hormone (GH)
- Insulin-like growth factor 1 (IGF-1)
- Calcium
- Phosphate
- Calcidiol (25(OH)D)

Studies	No language restrictions. Any study design except for:

- Commentaries
- Case reports
- Studies with duplicate data
- Reviews and meta-analyses

Information Sources

The following databases were searched: MEDLINE, EMBASE, PubMed, Web of Science (all databases), and LILACS. A systematic search was employed with the help of a health sciences librarian.

Search Strategy

The databases were searched from inception to February 23, 2020. The search was updated on February 6, 20201. There were no language restrictions. The search strategies were tailored to each database and is summarized below in Table 2.2. Duplicates were removed with RefWorks (COS, Proquest).

| Table 2.2: Search strategies by database ||
Database	Search Strategy
MEDLINE EMBASE‡	1 (rapid-maxillary-expan* or rapid-palatal-expan*).mp. 2 (growth-hormone or somatotropin).mp. 3 (insulin-like-growth-factor or IGF-I or IGF-1 or somatomedin*).mp. 4 Calcium.mp. 5 Phosphat*.mp. 6 (vitamin-D3 or calciferol).mp. 7 1 and (2 or 3 or 4 or 5 or 6) 8 Limit 7 to humans 8‡ Limit 7 to (human and (child <unspecified age> or preschool child <1 to 6 years> or school child <7 to 12 years> or adolescent <13 to 17 years>)) ‡EMBASE search same as MEDLINE for steps 1 through 7.
PubMed	#1 (rapid maxillary expansion OR rapid palatal expansion OR palatal expansion technique) #2 ((growth hormone OR somatotropin) OR (insulin-like growth factor OR IGF-I OR IGF-1 OR somatomedin) OR calcium OR phosphate OR (Vitamin D3 OR calciferol)) #3 #1 AND #2 #4 (#1 AND #2) Filters: Humans
Web of Science	#1 TS=("rapid maxillary expan* or "rapid palatal expan*") #2 TS=("growth hormone" or somatotropin) #3 TS=("insulin like growth factor" or IGF-I or IGF-1 or somatomedin*) #4 TS=(calcium or phosphate or "vitamin D3" or calciferol) #5 #1 AND (#2 or #3 or #4) #6 #5 AND TS=(child* or adoles* or young) NOT TS=(adult) #7 #6 NOT TS=(animal or rat or rabbit or canine or dog or mouse)
LILACS	(rapid and maxillary and expansion) OR (rapid and palatal and expansion) [Words] and (growth and hormone) or (somatotropin) or (insulin and like and growth and factor) OR IGF-I or IGF-1 or somatomedin or calcium or phosphate or (vitamin and D3) or calciferol [Words]

Study Selection

The records were screened by title and abstract content for relevance by two residents (DV & SY). Any articles not meeting inclusion criteria were removed.

Data Collection Process

Selected articles would be then assessed and relevant data items (see below) be abstracted by two reviewers.

Data Items

Data from the final selected studies include: 1) demographic information of the study population (size, mean age, sex distribution), 2) details about maxillary expansion (appliance, expansion protocol, mean amount of expansion), 3) time points used for assessment, 4) levels biomarkers: GH, IGF-1, calcium, phosphate, and vitamin D3 before and after expansion.

Risk of Bias in individual studies and across studies.

Applicable Joanna Briggs Critical Appraisal Tools (e.g., Checklist for Quasi-Experimental Studies (non-randomized experimental studies) was planned for use with no modification to assess the risk of bias for individual studies (Tufanaru et al., 2017). The GRADE approach was used to assess the quality of evidence per outcome across studies.

Summary measures

The primary outcomes considered were:

1. Does maxillary expansion in growing patients alter the serum levels of growth hormone?
2. Does maxillary expansion in growth patients alter the serum levels of IGF-1?

The secondary outcome considered were:

1. Does maxillary expansion in growing patients alter the serum levels of calcium, phosphate, or vitamin D3?

Synthesis of Results

Only studies of homogenous methodology were to be combined. No relevant additional analyses were performed.

Section 2.1.3: Systematic Review of the Literature – Results

MEDLINE and EMBASE produced the same 2 articles, PubMed returned the most at 9 articles, Web of Science found 2 and LILACS returned none. All the identified articles found were irrelevant, and none satisfied more than half of the requirements. The results of the systematic search confirmed that with regards to use of RME in our population of interest, no research has

been attempted to understand the effects on serum GH, IGF-1, or the other biomarkers calcium, phosphate, or calciferol. Given the lack of consensus about whether RME should be used routinely to treat POSA (Behrents et al., 2019), and about the mechanistic details of how this is accomplished, investigation into the presence or absence of the changes in serum biomarkers is justified to further our understanding in this area.
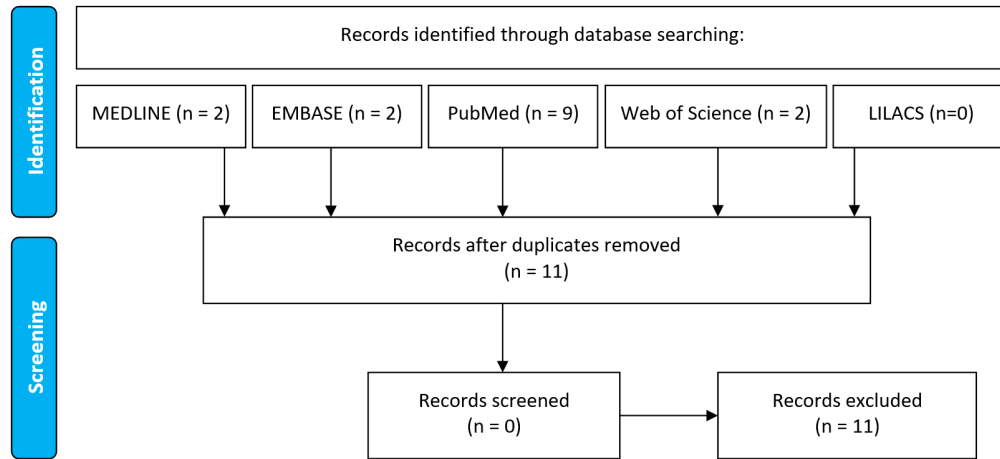
Figure 2.1: Flow chart of the article selection process. Adapted from the PRISMA 2009 Flowchart.

Section 2.2: Narrative Review of the Literature Regarding Relevant Domains

The domains involved in this study are extremely expansive in their own right and are not reported as being independent on one another. To better suggest the associations between these domains, a summary will be provided about normal and abnormal growth through the lens of GH/IGF-1, POSA, and treatment by RME. This next section is divided as follows:

1. Overview of the GH/IGF-1 system
   a. General properties and regulatory mechanisms
   b. Involvement in metabolism
   c. Involvement in normal growth in children and adolescents
   d. Abnormal growth due to disturbances to the GH/IGF-1 system

2. Pediatric Obstructive Sleep Apnea

    a. Epidemiology

    b. Phenotypes

    c. Disease mechanisms and comorbid contributory conditions

    d. Management

3. Rapid maxillary expansion

    a. Orthopedic effects

    b. Cellular effects

    c. Proposed benefits for pediatric OSA patients

4. Biochemical Assays

    a. GH and IGF-1

    b. Vitamin D and calcium

    c. Phosphate and alkaline phosphatase

5. Determination of Skeletal Age

Section 2.2.1: Growth Hormone (GH)

The GH/IGF-1 axis is vital to organism survival, prenatal and postnatal growth and development. This system is also involved in metabolism of carbohydrates, fat, and proteins as well as ion regulation in the kidney. There have been recent suggestions that proper levels of IGF-1 are required for neuronal survival and for memory and cognition functions. Growth hormone (GH), formerly known as somatotropin (Howrie, 1987) is a cytokine peptide that is produced by the somatotroph of the anterior pituitary gland.

The human pituitary GH gene (GH1) is located on the long arm of chromosome 17 (17q23). Secretion of GH is in response to various regulatory mechanisms; most importantly being the control via the hypothalamus. Growth hormone releasing hormone (GHRH) produced by neurons in the hypothalamic arcuate nucleus and ventromedial hypothalamic area induce

production and secretion of GH by the pituitary while somatostatin (SS) from neurons of the periventricular nucleus (PeN) inhibit GH release. GH is secreted in pulses and the interplay between GHRH and SS dictate the net release of GH (Frago and Chowen, 2005). Other upregulators of GH release include ghrelin, leptin, estradiol (E2), insulin, and neuropeptide Y (NPY) while down regulators include GH, IGF-1, and free fatty acids (FFA). Low levels of glucocorticoids and supraphysiological (pharmacological) levels of glucocorticoids also inhibit GH release (Kamenicky et al., 2014).

GH has a set of complementary receptors; GH receptor (GHR) and GH binding protein (GHBP), both of which have homologous binding domains. GHR is a transmembrane protein expressed in most all cells that are targets of GH, while GHBP is a circulatory protein that binds GH, stabilization it in the blood (Fernandez and LeRoith, 2005). When GH binds to its GHR at a target cell, downstream signaling cascades are initiated. There are two prominent signaling pathways: the extracellular signal-regulated kinases (ERK)1/2, and the Janus kinase 2 (JAK2)/ signal transducer and activator of transcription 5 (STAT5) pathways (Frago and Chowen, 2005). For optimal activity, GH binds to two adjacent GH receptors, which results in phosphorylation of insulin receptor substrates (IRS) 1, 2, and 3. With regards to growth, the GHR activation ultimately results in either direct growth effects on the target tissues such as bone (Fernandez and LeRoit, 2005), or indirectly through another potent mitogenic peptide call insulin-like factor 1 (IGF-1) by way of first increasing the level of IGF-1 in circulation.

Section 2.2.2: Insulin-Like Growth Factor I (IGF-I)

Originally named somatomedin (Ramadhin et al., 2014), IGF-1 is a growth factor that that has potent effects on cellular growth and differentiation with some ability to regulate metabolic activity. The human IGF-1 gene is located on chromosome 12. IGF-1 belongs to a family of peptides that also includes IGF-2 and insulin. IGF shares 50% structural homology to insulin. IGF-1 is ubiquitous and expressed in most all tissues, although the liver produces about 75% of the circulating amount. The kidney, spleen, fat, muscle, and bone are also important producers

of IGF-1 (Trejo et al., 2005). The production of IGF-1 is a response to increased release of GH. GH stimulated production of IGF-1 requires at least 4 hours before it can be detected in the blood (Campos-Barros et al., 2005). The half-life of IGF-1 is about 10 to 16 hours (Livingston, 2013); the binding proteins such as IGFBP-3 and ALS provide protection against degradation, which contributes to the longer half-life (Frago and Chowen, 2005).

IGF peptides have a complementary set of transmembrane receptors: IGF-1 receptor (IGF-IR), IGF-2 receptor (IGF-IIR), and the insulin receptor (IR). IGF-IR is widespread in the tissues and has 70% homology to the IR. IGF-IR is able to bind IGF-1, IGF-2, and insulin in concentrations above the physiological range (Frago and Chowen, 2005). IGF-IIR is critical during prenatal growth but serves only as a means to bind IGF-2 in circulation postnatally. Like GH, there are circulating binding proteins for the IGF family, which will be discussed shortly. Binding of IGF-IR activates downstream substrates including the Src homology and collagen family of proteins (SHC), and the insulin receptor substrate (IRS) family. These factors facilitate downstream signaling of IGF-1 peptides. There are four IRS proteins, named IRS1 through 4 with differential tissue expression and function. IRS1 and IRS2 are ubiquitously expressed and are involved in pathways critical for the size of the organism (Trejo et al., 2005).

IGF-1 is a potent promoter of cellular growth in all tissue types. IGF-1 is unique in that it can have endocrine, paracrine, and autocrine effects. The original somatomedin hypothesis (IGF-1 was originally named somatomedin) essentially stipulates that GH stimulates growth through increases in circulatory IGF-1 produced by the liver solely. This has evolved due to evidence that IGF-1 also has autocrine and paracrine abilities, of which skeletal tissue is a prime example. Upon activation by GH, the osteoblasts produce IGF-1 which promotes growth on this local cell population thereby demonstrating autocrine and paracrine function (Frago and Chowen, 2005, Tristos and Klibanski, 2016). IGF-1 has the ability to stimulate RANKL synthesis which activates osteoclasts while GH stimulates production of osteoprotegerin (OPG) which is a decoy ligand for RANK, for which binding of RANKL will result in activation of osteoclasts. This represents fine control of bone growth and bone turnover by the GH/IGF-1 axis (Sartoro et al., 1993).

Additionally, IGF-1 stimulates cellular uptake of glucose into muscle and fat cells, offering a form of glycemic control. GH mediates much of its growth effects through IGF-1. Metabolic effects of GH can be categorized as 1) insulin-like and include controlling lean mass by promoting protein synthesis, reducing lipogenesis and increasing lipolysis, and 2) anti-insulin-like effects, such as the stimulation of hepatic gluconeogenesis and resulting increased blood glucose levels but inhibition of gene expression of the glucose transport protein (GLUT1) which is important for modulating glucose metabolism (Frago and Chowen, 2005). Further, the GH/IGF-1 axis has been reported to regulate kidney function including fine tubular retention of water, sodium, and phosphate and partially contributing to glomerular filtration rate (Kamenicky et al., 2014).

Section 2.2.3: IGF-1 Binding Proteins (IGFBPs)

In addition to the transmembrane IGF receptors, there are six types of IGF binding proteins (IGFBP) that form binary or tertiary complexes with IGF in circulation. IGFBPs serve different functions from extending the half-life of IGFs, to regulating expression of IGF-1, to facilitating delivery of IGF-1 to target tissues. IGFBP-1 is antagonistic to the metabolic effects of IGF-1 and there is a reported inverse relationship between IGFBP-1 and IGF-1. During periods of fasting, low insulin levels induces activation of IGFBP-1 transcription in hepatocytes with a resultant decrease in free IGF-1 levels.

IGFBP-1 is upregulated by stress hormones, pro-inflammatory cytokines and hypoxia. In obese humans, the level of IGFBP-1 is observed to be elevated but progression to type II diabetes mellitus, sees the levels normalize (Silha and Murphy, 2005). IGFBP-2 is highly expressed in CNS tissues, lymph, bronchoalveolar lavage, prostate and seminal fluid. Prenatal levels are high but decreases after birth until puberty after which is slowly increases again. IGFBP-2 is not reported to have significant effects on pubertal growth.

Of the 6 types, IGFBP-3 is reportedly the most abundant IGFBP in circulation and plays a critical role in regulating IGF-1 transportation into the tissues with the unique ability to be transported into the cell nucleus. In the fetus, IGF-1 forms a binary complex with IGFBP-3, which is able to freely leave circulation. However, postnatally IGF-1 forms a ternary complex with an additional protein: the acid labial subunit (ALS) – this ternary complex is confined to circulation compared to binary complexes which can leave the capillaries. Both these two proteins are synthesized in the liver and expression is increased in response to hepatic production of IGF-1. Serum IGFBP-3 levels are constant and a decreased IGFBP-3 level is accompanied by an increase in IGF-1 clearing from circulation (Silha and Murphy, 2005; Campos-Barros et al., 2005). Postnatally even into adulthood, 80 – 85% of serum IGFs are in ternary complexes (Dunfield, 2007).

IGFBP-4 regulation can be increased or decreased by IGF-1 depending on the cell type. This binding protein is most abundant in bone. Parathyroid hormone (PTH) and vitamin D_3 stimulate local osseous production of IGFBP-4. IGF-1 inhibits production of IGFBP-4. It has been shown that systemic administration of IGFBP-4 may increase bone formation in mice, but local injection into bone inhibits bone formation. IGFBP-5 shares homology with IGFBP-3 and 58% of this protein forms a ternary complex with IGF-1 and ALS with a proposed function of protecting IGF-1 from degradation. IGFBP-5 levels increase during puberty and decline with age. IGFBP-6 has the strongest affinity for IGF-2 and is not involved in mediating any growth-related actions for IGF-1. These IGFBPs can be glycosylated, which may increase proteolytic susceptibility and subsequent decreased affinity for IGFs and release of IGF-1 from circulation (Silha and Murphy, 2005).

Section 2.2.4: Prenatal and Postnatal Growth

During prenatal life, IGF-1 and IGF-2 and their cellular receptors are essential in growth and development. Knockout mutations in mice of one or more of the IGF system yields defects such as extremely low birth size and weight (40 – 60% smaller) to a nearly total lethal phenotype due to respiratory problems. It is important to note that the loss of IGF-1 or IGF-2 is compensated

by the remaining functioning IGF gene. For example, IGF-2 may still bind to both the insulin receptor (IR) and IGF-IR and substitute for IGF-1 growth promoting actions. GH is not needed during the prenatal period; however, it is detectable in the fetal anterior pituitary as early as 6 weeks (Trejo et al., 2005).

In humans, total height is accumulated at different periods. Prenatal growth experiences the greatest growth rate followed by a period of steady growth during childhood. It is only again during the pubertal growth period that the individual gains an increased growth rate that contributes to about 18% of the total adult height. Puberty is the result of reactivation of a previously inactive hypothalamic-pituitary-gonadal axis (Guaraldi et al, 2016), and is a transition from childhood to adult maturity represented by increased growth velocity, weight gain, and emergence of secondary sexual features (Ong et al, 2009). The rate of growth and the duration of puberty (Saggesse et al., 1999), as well as the age of onset of puberty (Biro and Kiess, 2016) are important influences of total height gain during this period. Although the growth rate is temporarily accelerated, it is limited by the increasing rate of skeletal maturation. Genome-wide association studies have suggested that a certain allele for the LIN28B gene is associated with an earlier pubertal age of onset, and that although these affected individuals were taller than their peers during childhood, their growth ceased earlier and they ended up relatively shorter and heavier as adults (Ong et al, 2009). A more extreme example is precocious puberty. It is a condition where secondary sexual characteristics appear before age 8 in girls and 9 in boys. The unfortunate consequence of this rapid progression of skeletal maturation is shorter stature – age of pubertal onset is inversely correlated with loss of height potential (Guaraldi et al 2016).

Section 2.2.5: Pubertal Growth

The pubertal concentration of GH rises to about twice that of the prepubertal concentration levels mostly due to the increased GH amplitude of secretory pulses. Baseline levels of GH concentration is often below the detection limit of assays. The average frequency of nocturnal

GH pulses (significantly greater GH secretion) is about 3.0 to 3.5 pulses per 12 hours, and the mean daytime pulse frequency is about 2.0 to 2.8 pulses per 12 hours (Rose et al, 1991). The withdrawal of somatostatin (SS) is responsible for regulating the timing of the pulses while the amplitude of GH secretory pulses is related to increased GHRH secretion.

It has been reported that male mice have elevated amplitudes with a 3-hour periodicity of pulses with low interpulse baseline GH levels compared to females that have a more irregular lower amplitude pulses but elevated GH baseline. Sexual dimorphism is less distinct in humans (Frago and Chowen, 2005). The increase in GH sensitizes the sex organs and promotes maturation resulting in increasing production of sex hormones and further increased production of GH. Estradiol (E2) levels increases before the onset of puberty. E2 is produced in males by periphery aromatization of testosterone (Saggesse et al., 1999).

The increased levels of sex hormones promote linear growth with a direct effect on bone growth and indirectly through increased GH secretion. It has been demonstrated that the administration of testosterone increases growth velocity in normal children, and in children with growth hormone deficiency (GHD) or constitutional delay. Administration of low dose estradiol increases growth velocity but excessively high doses of E2 causes rapid bone maturation and reduced final height in GHD children. It has been reported that individuals with estrogen insensitivity are taller and grow into their third decade of life (e.g., males with inactive estrogen receptors or females with impaired estrogen production). Thus, puberty is both a growth-promoting and growth limiting process that is sensitive to the levels of different hormones. Furthermore, there is a concurrent increase in IGF-1, ALS, and IGFBP-3 levels that reach peak levels during puberty and then steadily decline afterwards (Trejo et al., 2005). The IGF-1/IGFBP-3 molar ratio increases with a resultant effect of increased free IGF-1 (Saggesse et al., 1999).

Section 2.2.6: Growth Hormone Deficiency (GHD)

Total stature increase is inversely related to the age at which puberty started and to the total amount of prepubertal growth.   In humans, normal males experience about 5.2 years of total pubertal growth from the onset to final height, and 4.8 years on average for females. Growth hormone deficiency (GHD) in children can manifest as isolated GHD where puberty occurs spontaneously or GHD children requiring pubertal induction due to gonadotropin deficiency by means of exogenous administration of sex steroids to induce puberty.

Individuals with isolated GHD experience on average 3.6 and 3.0 years of pubertal growth for males and females, respectively. Thus, the shortening of puberty may reflect a late entry into puberty (Saggesse et al., 1999, Ranke et al., 1997). The levels of IGFBP-3 and IGF-1 also parallels trends with GH deficiency (Silha and Murphy, 2005). For these children, two strategies may be used to maximize growth potential. The first is to increase the GH dose at puberty up to triple that of prepubertal levels, while the second is employed for those with isolated GHD by suppressing pubertal maturation by inducing gonadal suppression to attempt to prolong prepubertal growth (Saggesse et al., 1999).

Idiopathic GH deficiency has a prevalence of about 1 in 3480 individuals, and about 5% to 30% of GHD patients have a first degree relative who is affected. 20% of sporadic cases of GHD are a result of environmental factors or hypothalamic defects. GHD or GH resistant humans and mice show significantly reduced postnatal growth in the presence of normal tissue development, indicating that GH is not essential for cellular differentiation (Campos-Barros et al., 2005). Children with GHD have on average smaller bone size, decrease bone mineral density, and are shorter. Untreated children with GHD or GHD secondary to hypopituitarism reach a final height standard deviation that is 4 to 6 standard deviation scores below normal. In these individuals, treatment improves final height, but the mean midparental height is never achieved (Tritos and Klibansky, 2016).   It is also reported that GHD in children and adults is associated with

increased subcutaneous and visceral adipose tissue with a low sodium and water body content (Kamenicky et al., 2014).

The insulin tolerance test is the gold standard for diagnosing GHD. Other tests have been developed for the purposes of measuring GH levels, including GH stimulation by arginine, clonidine, L-dopa, glucagon, and GHRH. There are several methods of diagnosing GHD, and include: 1) auxological characteristics, 2) GH level is less than 3 µg/L in the insulin tolerance test, 3) when peak GH level is less than 5 ng/ml confirmed by means of 2 stimulation tests (GHRH plus galanin and L-dopa plus propranolol) (Trejo et al., 2005, Dunfield, 2007), or when the IGF-I level is 2 standard deviation scores below normal (Wit et al., 2019). Currently there are no international guidelines for the diagnosis of GHD through serum biomarkers GH or IGF-1, and admittedly there is large variability between the different assay methods used for measuring the biomarkers that varies country to country (Wit et al., 2019).

Section 2.2.7: Summary of GH/IGF-1 axis, Growth and GH Deficiency,

One can appreciate the complexities of these biological processes and how interrelated biological signaling is, even in the context of a normal individual. This is further compounded in disease states. Table 2.3 summarizes regulators of synthetization and secretion if GH and IGF. Table 2.4 summarizes some key effects on metabolism of GH and IGF-1. There is potential for overlap and interaction of the biological processes related to RME with mechanisms of OSA. Further study of these cytokines and growth factors and using them as biomarkers is necessary to increase the current understanding of the mechanisms of pediatric OSA.

Table 2.3: Regulators to GH and IGF-1 synthetization and secretion			
Growth Hormone		**Insulin-Like Growth Factor 1**	
Stimulation	Inhibition	Stimulation	Inhibition
GHRH	SS	GH	Glucocorticoids
Ghrelin	GH	Parathyroid hormone	Cortisol
Leptin	IGF-1	Triiodide	IGFBP-1
Sex hormones,	FFAs	Estradiol	Inflammatory
Estradiol	Low/high	Insulin	cytokines (IL-6)
Insulin	glucocorticoids		
Neuropeptide Y	Malnutrition		

* Growth hormone releasing hormone

Table 2.4: Effects of GH and IGF-1 on metabolism			
	Fat	Carbohydrate	Protein
GH	↓lipogenesis ↓fat deposition ↑lipolysis ↑FFA ↑glycerol ↑fat mobilization	↑glucose production (gluconeogenesis) X glucose uptake X GLUT1 gene expression (↓glucose transport)	↑IGF-1 ↑OPG ↑bone growth ↑bone turnover
IGF-1		↑cellular glucose uptake (esp. muscle & fat cells)	↑bone growth ↑RANKL ↑osteoclast activation

Section 2.2.8: Obstructive Sleep Apnea (OSA)

Obstructive sleep apnea (OSA) is one type of sleep disordered breathing (SDB). An apnea or apneic episode is an event where breathing ceases temporarily for at least 10 seconds, and in the case of OSA, occurs during sleep. There are different etiologies that may cause the apneic events and include those of the obstructive kind where elements of the upper airway contribute to luminal blockage or collapse, or those of the central kind where the respiratory muscles do not receive neural input and hence apnea occurs (Semelka et al. 2016).

OSA is a medical condition that has gained much attention due to strong associations with morbidity and mortality including associations with high rates of unintentional work-related accidents and motor vehicle collisions. OSA is also associated with a plethora of health problems such as hypertension, congestive heart failure, coronary artery disease, stroke, diabetes, and depression. The quality-of-life scores have been shown to be lower in those with OSA. It should be appreciated that this is a highly complex and multifaceted disease (Shelgikar, 2018).

Sleep disordered breathing encompasses a spectrum of conditions which include central sleep apnea, upper airway resistance syndrome, hypoventilation syndrome, and obstructive sleep apnea. Upper airway resistance syndrome, or UARS, is characterized by sleep fragmentation and signs of daytime behavioral issues that are similar to OSA, but do not meet current diagnostic criteria for OSA (Chervin et al. 2000, Semelka et al. 2016).

Obesity hypoventilation syndrome is defined by four criteria (Chung et al. 2016):
1) Body-mass index (BMI) over 30 kg/m^2
2) Presence of SDB
3) Daytime hypercapnia and hypoxemia (partial pressure of CO_2, $PaCO_2$ > 45 mm Hg and PaO_2 < 70 mm Hg)
4) Absence of other causes of hypoventilation

OSA is a severe condition and is defined by the American Academy of Sleep Medicine as "a sleep-related breathing disorder that involves a decrease or complete halt in airflow despite an ongoing effort to breathe. It occurs when the muscles lose tonus during sleep, causing soft tissue in the back of the throat to collapse and block the upper airway. This leads to partial reductions (hyponeas) and complete pauses (apneas) in breathing that last at least 10 seconds during sleep". OSA has a multitude of recognized signs and symptoms that are observed in children and adults. They include but are not restricted to cognitive impairment, excessive

daytime sleepiness, sleep fragmentation, waking with gasping for air, mouth breathing, and growth retardation in children (Chervin et al. 2000, Semelka et al. 2016).

OSA can occur concurrently with other conditions including obesity hypoventilation (Pierce and Brown, 2015, Sivam et al, 2018) syndrome and central sleep apnea (CSA). There is another condition known as treatment-emergent central sleep apnea (TECSA) where CSA may occur after the elimination of obstructive elements in OSA (Liu et al, 2017). The prevalence of TECSA is estimated to be about 8%. Most cases of TECSA resolve after 1 to 3 months of continued continuous positive airway pressure therapy, but about 0.9 to 3.2% of affected individuals have persistent CSA that is refractory to therapy (Nigam et al, 2018). It involves complex mechanisms around the balance of partial pressures of CO_2 and O_2 that affect the respiratory central pattern generator which are beyond the scope of this study (Naughton, 2010, Orr et el, 2017). Table 2.5 provides a more complete list of associated signs and symptoms of OSA.

Table 2.5: Signs and Symptoms of Obstructive Sleep Apnea	
Cognitive impairment	Mouth breathing
Excessive daytime sleepiness	Nocturnal sweating
Fatigue	Nocturia, enuresis (in children)
Insomnia	Sleep bruxism
Restlessness	Nasal congestion
Loud snoring	Mood disorders
Waking up gasping for air, choking	Growth retardation (in children)

Section 2.2.9: Epidemiology of Obstructive Sleep Apnea

When discussing the prevalence of OSA, the severity of the disease, age range, and ethnicity are factors that can greatly affect the estimate. Current studies estimate the prevalence of adult OSA to be between 2% to 14%. Using recent National Health and Nutrition Examination Survey (NHANES) data, 10% of the male Americans aged 30 to 49 years have moderate to severe OSA, compared to 3% of females (Peppard et al. 2013). Individuals of Japanese descent generally have lower prevalence rates of OSA than those of African, Caucasian, or Hispanic descent, and African Americans have higher OSA prevalence than Caucasian Americans.

However, the prevalence also increases with the amount of adipose tissue mass (BMI), age, and in individuals requiring bariatric surgery (about 70%). Postmenopausal women not undergoing hormone replacement therapy have the same prevalence as males, in that both groups are three-times likely to have OSA than non-obese premenopausal women (Peppard et al. 2013; Semelka et al. 2016). It is accepted that both an aging population and growing rate of obesity contribute to the increasing rate of OSA seen in developed populations. In children, the prevalence of OSA is estimated to be 1% to 4% with certain groups disproportionately more affected such as children with respiratory diseases (55% - 63% in asthmatics, severity dependent), obese children, those of African American descent, and those with family history of OSA (Pizzatto and Flores-Mir, 2017).

The risk factors for OSA are listed in Table 2.6 (AAO, 2019). The commercial motor vehicle driver (e.g., trucker) may not be an exclusive occupation with an associated higher risk of OSA, it just so happens that the study population was coincidentally comprised of mostly truckers.

Table 2.6: Risk Factors for Obstructive Sleep Apnea	
Increasing age (40 – 70 years old)	Certain craniofacial morphologies with or without concurrent genetic syndrome
Obesity (BMI > 35 kg/m^2)	
Male	Postmenopausal women not taking hormone replacement therapy
Family history of OSA	
Preoperative patient for bariatric surgery	

Apneic events during sleep trigger sympathetic activation of the nervous system and arousal from sleep. Sympathetic activity increases in OSA patients occur mostly during stage II and REM sleep with average activity levels that are 133% and 141% that of normal, resting sympathetic activity when awake. Peak sympathetic activity occurs at the end of each apneic event and can be as high as 299% of baseline in stage II sleep (Somers et al., 1995). OSA patients have been reported to have substantial elevations of systemic blood pressure and depressions of oxyhemoglobin saturation that are associated with the apneic events. Some researchers measured the oxygen saturation in a small sample of OSA patients in two sleep

scenarios: one with oxygen supplementation and one without. They found that the mean oxygen saturation to be 94% in during supplementation and 79 – 82% when not supplemented (Ringers et al., 1990). This indicates poor blood oxygenation.

Studies in mice showed that effective vascular gas exchange in the brain depends on the oxygen content of the blood. When oxygen levels are reduced, there is acute hypoxia which is compensated by the body through increased blood flow to the brain to restore overall oxygen delivery. However, persistent hypoxia will lead to polycythemia that suggests that the initial compensatory mechanism is inadequate to sustain normal oxygenation. The hypoxic mice exhibited lower body and brain weights with a 25% increase in brain cortex volumes compared to controls. Furthermore, the capillary density, lengths, diameters, increased in the hypoxic mice with visually more tortuous and dilated capillaries. Thus, differential blood flow changes and angiogenesis are adaptive means that the body employs in the presence of chronic hypoxia. Humans native to high altitudes have been reported to have reduction in blood flow in certain parts of the brain (Boero et al., 1999).

Untreated OSA will eventually lead to detrimental outcomes such as cardiovascular issues including hypertension and cardiac enlargement, myocardial infarction and stroke (Somers et al., 1995), and neurocognitive impairment and behavioral abnormalities such as aggression, inattentiveness, hyperactivity and poor academic performance. Chronic episodes of hypoxia can induce a state of chronic inflammation with alteration in functioning of the immune system. Hypoxia has been reported to amplify innate immune cell activity while suppressing the adaptive immune system (Eltzschig and Carmeliet, 2011). Additionally, increased expression of proinflammatory cytokines can result in development of fibrosis, such as in renal tissues (Fine and Norman, 2008).

Section 2.2.10: Obstructive Sleep Apnea Phenotypes

There are certain craniofacial features that have been found to be associated with OSA, notably the presence of retrognathic jaw(s), and the long and narrow facial type commonly described as the "adenoid face" with associated narrow dental arches and steep mandibular plane angle. Genetic syndromes with associated craniofacial anomalies can also increase the risk for OSA and include Pierre Robin Sequence, Syndromic Craniosynostosis, and Down Syndrome. A complete list of morphologies associated with OSA is listed in Table 2.7. It is worth mentioning through extensive transdisciplinary review of research publications and sharing of opinions from leaders in OSA research and from a variety of medical and dental fields, it is recognized that although these associations exist, the strength of the relationships between craniofacial morphological features and the development of OSA is still not well established (AAO 2019). Alternatively, these craniofacial morphologies may predispose a child or adult to develop OSA, but we cannot solely determine OSA risk through the presence of these features.

A significant step forward would be the correct identification of specific characteristics of individuals that respond well to concurrent management of POSA through orthodontic approaches compared to those that would not.

Table 2.7: Craniofacial Morphologies and Genetic Syndromes associated with increased risk of OSA	
Craniofacial Morphologies	Genetic Syndromes
Retrognathia (retruded jaw or jaws)	Pierre Robin Sequence
Long and narrow face (dolicocephalic face)	
Narrow and deep palate	Syndromic Craniosynostosis
Steep mandibular plane angle	
Anterior open bite	Down syndrome
Midface deficiency	
Low hyoid position	

Section 2.2.11: Pediatric Obstructive Sleep Apnea

In the pediatric population, another significant consequence of untreated OSA potentially is growth failure. There are multiple aspects that can contribute to sleep-related upper airway obstruction including abnormal motor control and muscle tone, structural integrity of the upper airway architecture, and upper airway constriction. Reportedly the most common cause of POSA in children is adenotonsillar hypertrophy (Gozal et al., 2008), which occurs in part due to differential growth of various tissue types. The facial bones grow slower than the lymphoid tissues during childhood. The relatively larger lymphoid tissues then undergo involution during puberty until normal adult size is reached. However, there is a substantial proportion of children who have abnormally enlarged tonsils with adenotonsillar hypertrophy (ATH) prevalence estimated to be from 19% to 58% in children ages 6 months to 14 years old. These lymphoid tissues are susceptible to repeated infection, inflammation and chronic enlargement (Pizzatto and Flores-Mir, 2018).

POSA is recognized as a serious disease and the American Academy of Pediatrics has recommended that any child or adolescent should be screened for the disease if snoring is present and follow up with polysomnography (PSG) in the presence of snoring and other signs and symptoms of OSAS (Marcus et al., 2012). OSA has multifactorial etiologies and these factors may exist alone or in conjunction with others compounding the complexity of the disease. This is reflected in the literature, as there are several proposed mechanisms of how pediatric OSA may be responsible for growth failure. These include the involvement of chronic inflammation, alteration of feeding habits, and increased energy expenditure from increased intercostal muscle activity, and frequent activation of the sympathetic system leading to arousals during sleep (Bonuk et al., 2009). The net outcome is disruption of the GH/IGF-1 axis during a critical period of growth.

Section 2.2.12: Pathophysiology of Obstructive Sleep Apnea

Due to the disturbance in airflow, the blood oxygen content is compromised with an ensuing state of acute hypoxia. Repeated events ultimately lead to a state of chronic hypoxia where the activity of a transcription factor called *Hypoxia Inducible Factor* (HIF) is increased after 4 to 8 hours of the initial hypoxic exposure. Normally HIF is tagged by a pair of prolyl hydroxylases (PHDs) that send HIF for proteosomic destruction. In hypoxia, HIF no longer is tagged for destruction, allowing HIF to enter the cellular nucleus and to increase the expression of genes responsible for hypoxic conditions and inflammation. One such important downstream target is NF-κB, which regulates inflammation, tissue homeostasis, and the immune response. Other responses due to HIF activation of hypoxic genes include increasing: 1) neutrophil lifespan, 2) monocyte chemoattractant protein 1 (MCP-1) which results in increased numbers of macrophages, 3) angiogenesis by way of adipocyte produced leptin and vascular endothelial growth factor (VEGF), and 4) reduction of macrophage mobility in adipose tissue, preventing their departure. The ultimate result is chronic inflammation. An interesting tangent is that tumour tissues prefer a hypoxic environment and it has been reported that there is increased HIF activation in tumour cells to promote increased vascularization (Eltzschig and Carmeliet, 2011).

The effects of hypoxia are compounded in obese individuals due to increased deposits of adipose tissue. Fat tissue is not highly vascularized in normal conditions. Due to redistribution of blood flow to vital organs in chronic hypoxia, this further reduces blood flow to adipose tissues, resulting in potential ischemia of hypoxic adipose tissue. The death of adipocytes attracts monocytes and macrophages with a concurrent increase in expression of macrophage migration inhibition factor (MIF) in response to hypoxia and increased presence of glucocorticoids. The MIF reduces mobility of the macrophages, effectively trapping these immune cells that become chronic sources of pro-inflammatory cytokines (Ye, 2009).

Due to pro-inflammatory effects of HIF and NF-κB, there is increased expression of TNF-α which reduces adiponectin production. Adiponectin is reported as having an important role for free fatty acid (FFA) metabolism and insulin sensitivity, although the exact mechanism requires further study (Ye, 2009). Obesity is reported to be the strongest risk factor for development of OSA. Obese individuals with OSA have significantly greater total tongue volume, with altered location of adipose deposits. Retroglossally located fat was significantly greater in obese individuals with OSA compared to matched obese controls, and there was a positive correlation found between apnea-hypopnea index (AHI) and tongue volume and tongue fat. The tongue is an important pharyngeal dilator muscle and the altered content and location of tongue fat may adversely affect muscle function, reducing abilities to contract as a pharyngeal dilator and increasing the risk of airway occlusion (Kim et al., 2014). Overall, this creates an environment conducive for chronic inflammation.

As discussed previously, growth is mediated through the vital GH/IGF-1 system. Chronic inflammation secondary to hypoxia induces upregulation of pro-inflammatory cytokines including interleukin-6 (IL-6) and cortisol. Elevations of these biological agents decrease the expression of IGF-1 directly or indirectly such as cortisol induced reduction of thyroxine secretion. The disturbance of the GH/IGF-1 axis by way of chronic inflammation therefore is one plausible mechanism for growth retardation in the presence of pediatric OSA (De Benedetti et al., 1997, Clemmons et al., 1989).

Section 2.2.13: Adenotonsillar Hypertrophy induced OSA – a model for growth retardation

Adenotonsillar hypertrophy (ATH) is the most common anatomic contributor to POSA. In children with ATH, adenotonsillectomy, also known as tonsillectomy and adenoidectomy (T&A) is recommended as the first-line treatment option. There are more than 500,000 T&A surgeries performed annually for pediatric OSA in the United States (Marcus et al., 2012). In a 2015 Cochrane systematic review comparing T&A to non-surgical management of pediatric

obstructive sleep-disordered breathing, only 3 studies were included for a total of 562 children. Only one article had low risk of bias. The authors found moderate quality of evidence that early T&A compared to no treatment improves the outcome of quality of life or symptoms as assessed by the OSA-18 questionnaire with a mean reduction in score of -17.7 [-21.2, -14.2, 95% CI] in non-syndromic children with mild to moderate OSAS. There is high quality of evidence that early T&A compared to no treatment reduces the AHI in these same children with a mean reduction of -4.3 [-5.7 to -2.9, 95% CI] events per hour in the same population of children (Venekamp et al., 2015) assessed at 7 months post-treatment (short-term). Some of the cases in the non-treated sample did also showcase a reduction of AHI.

Some researchers found that in pediatric OSA patients, levels of metabolic serum biomarkers such as GH and IGF-1 as well as inflammatory biomarkers change in response to T&A surgery compared to baseline or untreated controls with demonstration of catch-up growth with improvement of airway patency (Nieminen et al., 2002). Another group of researchers compared IGF-1 and IGFBP-3 levels in a small sample of children aged 4 – 8 years with OSAS due to ATH before and after T&A surgery and found that the mean IGF-1 level increased by 35%, and the mean IGFBP-3 level increased by 12% (Yilmaz et al., 2002).

A multicenter, single-blinded, randomized controlled trial was carried out with 460 children aged 5 to 9 years old with diagnosed mild to moderate OSA who were otherwise healthy with adenotonsillar hypertrophy (ATH) were randomly assigned to either early tonsillectomy and adenoidectomy (eTA) or watchful waiting with supportive care (WWSC) for a period of 7 months. This study had low risk of bias according to the Cochrane systematic view (Venekamp et al, 2015). The outcomes assessed were changes in PSG measurements and attention and executive functioning between the two groups, with a secondary objective of evaluating the outcomes between the different subgroups of the study population (Marcus et al., 2013). At the end of 7 months, the researchers found normalization of PSG measurements occurred in 79% of the children in the eTA group, and spontaneously in 46% of the children in the WWSC

group. No significant difference in attention and executive function was found between both groups.

Adenotonsillectomy addresses only one component of a multifactorial problem, and there are some factors that increase the likelihood of persistent POSA after T&A, namely high BMI and being of African-American or Hispanic descent (Amin et al, 2008, Mitchell and Kelly, 2007). Incomplete resolution of POSA after T&A occurs in 13% to 79% of cases depending on the AHI cut-off used (Amin et al, 2008, Arima et al, 2019, Mitchell and Kelly, 2007, Tauman et al, 2006). Those researchers consistently found that the recurrence of sleep disordered breathing was associated with obesity. In one study on Japanese children with POSA treated with T&A, 25.8% of obese subjects had post-op AHI > 5 compared to 13% of non-obese subjects (Arima et al., 2019). Not surprisingly, a large proportion of children referred for T&A are either overweight or obese.

Section 2.2.14: Polysomnography

Obstructive sleep apnea (OSA) has been implicated in numerous medical conditions, notable problems of the cardiovascular system; it increases the risk of cardiovascular mortality and all-cause mortality (Marcus et al 2012). There is further associated economic costs that stem from the loss of productivity from the disease. A cross-sectional study of Americans with OSA found that employed adults with OSA had earnings that were about one third lower on average than individuals without OSA even after adjusting for the social and economic status of both cohorts (Ehsan and Ingram, 2016).

OSA is estimated to affect 1 to 5% of children and up to 30% of adults (Kundel and Shah, 2017). The relevance of the disease to overall health is represented by the intense interest in the field of sleep medicine. Sleep studies, also known as polysomnography (PSG) is a critical element for the diagnosis of sleep disordered breathing. The American Academy of Sleep Medicine (AASM) promotes itself as the "leading professional society that is dedicated to the promotion of sleep

health, and endeavors to advance sleep health policy that improves the health and well-being of the general public" (Rosen et al., 2017).

The gold standard for sleep studies is a PSG done in a sleep clinic with at least 7 channels of recording and attended by a technician. Due to costs and convenience, home sleep apnea tests (HSATs) have become more popular (Freedman and Bannockburn 2015). The AASM have published and updated specific criteria for the different levels of PSG, sleep stages, scoring of PSG results, and position statements on use of HSATs in the adult and pediatric population (Kirk et al., 2017, Rosen et al., 2017). The AASM developed a new manual for sleep scoring that replaced the extensively used Rechtschaffen and Kales (R & K) manual.

The 2007 AASM Scoring Manual defines distinct sleep stages that include wakefulness (Stage W), Stages N1, N2, and N3, and rapid eye movement sleep (REM). The night is divided in 30 second periods, or epochs, and is assigned a single stage based on the presence or absence of features. Stage N3 is slow wave sleep where growth hormone is maximally released. PSGs are classified into 4 levels based on essentially the total number and type of physiological variables that are recorded (channels).

Level 1 is the gold standard and requires an attending sleep technician and has at least 7 channels of recording and include electroencephalography (EEG), electrooculography (EOG), electromyography of 2 areas (EMG, submental and anterior tibialis), airflow, respiratory effort, electrocardiography (ECG), and blood oxygenation (O_2 saturation via pulse oximetry).

Level 2 is a comprehensive portable PSG and has similar channels as level 1 but has no attending technician. A heart rate monitor can replace the ECG.

Level 3 is a modified portable PSG and has heart rate monitoring, blood oxygenation, and at least 2 channels for ventilation which usually are airflow and respiratory effort.

Level 4 has at least one channel of recording (pulse oximetry) but does not meet the criteria for level 3 (Jafari and Mohsenin, 2010). An example of a level 4 device is shown in figure 2.2.

Table 2.8 provides a summary of the 4 levels of PSG.

Table 2.8 Summary of Sleep Monitoring Systems				
	Level 1: In-clinic PSG	Level 2: Comprehensive portable PSG	Level 3: modified portable PSG	Level 4: Dual or single channel recording
Parameter count (channels)	≥ 7	≥ 7	≥ 4	1 – 2
Types of channels	EEG, EOG, EMG, ECG, airflow, respiratory effort, oximetry	EEG, EOG, EMG, ECG, airflow, respiratory effort, oximetry	ECG, airflow, respiratory effort, oximetry	Oximetry, ± airflow
Attending sleep technician	Yes, usually in sleep centre	No	No	No

In 2017, the AASM released position statements on the use of HSATs for the diagnosis of OSA in adult and pediatric populations. They have separate scoring rules for pediatric sleep studies and provided the option of allowing the scoring of a hypopnea if an event is associated with an arousal instead of just a 3% oxygen desaturation event. This requires that the PSG whether in clinic or portable, must be able to discern between sleep and wakefulness states. The ideal HSAT device according to the AASM, would fall into level 2 sleep testing (Virk et al., 2017). Level 3 and level 4 are unable to determine the total sleep time and use proprietary algorithms to determine the number of events averaged across the total recording time.

This leads potentially to underdiagnosis of OSA or underestimation of the severity of the disease. This is even more relevant in light that lower apnea-hypopnea index values (AHI) for cutoffs are used to diagnose pediatric OSA (POSA). The AASM International Classification of Sleep Disorders, 3rd edition (ICSD-3) defines POSA as having an AHI value of ≥ 1, or a pattern of obstructive hypoventilation defined as at least 25% of the total *sleep* time with hypercapnia (partial pressure CO_2/$PaCO_2$ > 50 mm Hg) in association with snoring, flattening of the nasal pressure waveform, or paradoxical respiratory efforts (AASM ICSD-3, 2014).

Although level 1 PSG is the standard for sleep testing, it has been reported that the diagnostic sensitivity of a single night of in-lab PSG to detect an AHI of > 5 ranges from 75 to 88%. Further, at minimum a second PSG is required to determine the optimal settings in patients receiving positive airway pressure (PAP) treatment. The US government saves an approximate $1,140 USD per Medicare patient if level 3 HSAT is employed instead of level 1 (Freedman and Bannockburn, 2015). Barriers exist that limit access to PSGs, and can be divided into 1) economic, 2) accessibility, and 3) social reasons. The direct and indirect costs (e.g., loss of pay from time off) may prohibit some patients from accessing this service, while others are limited temporally or geographically due to limited sleep centres, and others are dissuaded by the perceived inconvenience (Kirk et al. 2017). Despite inherent limitations, the barriers to access to portable monitors (PMs) for HSAT are less than in-lab PSG and serve as an attractive alternative.

Figure 2.2: Front (left) and top (right) views of a level 4 portable sleep monitor (Embletta MPR, Natus Neurology, Middleton, USA. This particular device has channels to record airflow via nasal cannula, pulse oximetry, and a microphone.

A 2001 study used level 2 HSAT to estimate the prevalence of sleep disordered breathing in preadolescent children in a population of 157 children aged 6 to 12 years old. They reported obtaining technically acceptable sleep studies in 91% of the children on the first night, and 97% were acceptable after a second night of testing was performed (Goodwin et al., 2001). HSAT

devices have undergone rigorous validation testing. In terms of AHI, good correlation between HSAT and PSG were reported. However, HSAT achieved higher sensitivity and specificity only when higher diagnostic cutoffs for the disease were used. A recent systematic review and meta-analysis found that the mean difference in AHI or RDI (respiratory disturbance index) between portable monitors and PSG ranged from -14.8 to 10.6 events per hour, and at an AHI cutoff of $\geq$ 5, the sensitivities of level 4 PMs were 0.68, 0.77 to 0.93, and 0.96 to 1.00 for single, dual, and quadruple channel PMs respectively (Abrahamyan et al., 2018). A Cochrane meta-analysis compared level 1 to level 3 sleep studies and found that level 3 portable monitors showed good concordance with level 1 PSG for adult patients with a high pretest probability of moderate to severe OSA (El Shayeb et al., 2014).

What was gleaned from these reviews was that level 3 and 4 HSATs were acceptable for use in select patient populations – those who had high pretest probabilities of moderate to severe OSA. With regards to adults, the essence of AASM's stance is that HSAT may be considered as an alternative to in-lab PSG for the diagnosis of OSA in adults without comorbidities with signs and symptoms suggestive of increased risk of moderate to severe OSA, and that a clinical assessment is required in addition to the data from the HSAT (Rosen et al., 2017). However, HSAT is not recommended for diagnosing OSA in children, citing the lack of insufficient evidence comparing HSATs to PSGs in children, and that level 3 and 4 PM devices do not have the ability to identify hypopneas associated with cortical arousals (Virk et al., 2017). In both populations, the AASM recommended that HSAT not be used to screen an asymptomatic population (Kundel et al., 2017).

The AASM further counter use of HSATs in children with reasons including how children may be cognitively and emotionally immature and are less predictable than adults and may not tolerate the numerous sensors that must be worn for the duration of the sleep study, or the aptitude of the parents or caregiver in placing the sensors. While they provide valid evidence to support their position statements, it must be recognized that the barriers to assess of level 1 PSG exist and may affect a substantial proportion of the children with undiagnosed POSA. It has been

validated that with increasing disease severity, the sensitivity and specificity of HSATs increase (Kirk et al., 2017). In this author's opinion, the use of HSAT in children with current signs of POSA with or without symptoms who otherwise do not have access to in-lab PSG, is justified. It is better to identify some children with moderate or severe OSA than to stand idle and not make any attempt for diagnosis at all.

Section 2.2.15: Management of Pediatric Obstructive Sleep Apnea

The American Academy of Pediatrics recommended in 2012 that pediatric OSA refractory to T&A or without initial ATH should be treated with continuous positive airway pressure (CPAP), or recommendation of lifestyle changes by way of weight loss, and, finally, use of intranasal corticosteroids (Marcus et al., 2012). However, due to the not uncommon observance of maxillary constriction in pediatric populations with OSA, some clinicians have recommended rapid maxillary/palatal expansion (RME/RPE) as a means to manage pediatric OSA. It should be noted that a constricted maxilla in presence of a malocclusion would need treatment regardless for orthodontic reasons to establish proper skeletal and dental relationships (Pirelli et al., 2004, Villa et al., 2011).

A recent systematic review (Camacho et al., 2017) evaluated the effects of RME on mean oxygen saturation, lowest oxygen saturation, and AHI values – all parameters that are commonly assessed in the treatment of OSA. The authors found that oxygen saturation improved from 87.0 ± 9.1% to 96.0 ± 2.7% (mean ± standard deviation); however, the findings are reported from studies with low to moderate quality. Overall, the AHI improved after RME in children with OSA by about 70% (AHI improved from 8.9 ± 7.0 to 2.7 ± 3.3 events per hour) but without complete elimination of signs and symptoms.   Another study followed a small sample of individuals who were treated with RME as children for OSA after 12 years. These children lacked ATH and did not receive T&A surgery. All presented with a constricted maxilla and none were overweight. Immediately after RME PSG values normalized, but interestingly in this sample the results stayed stable 12 years later (Pirelli et al., 2015).

Section 2.2.16: Rapid Maxillary/Palatal Expansion

The maxilla is composed of two hemimaxillae that articulate with the complementing member through the midpalatal suture. The maxilla is attached and stabilized to the neurocranium via the circummaxillary suture system consisting of the frontozygomatic, frontomaxillary, frontonasal, nasomaxillary, zygomaticomaxillary, temporozygomatic, and pterygopalatine sutures. The suture system consists of two bony sides lined with osteogenic cells (resembling the periosteum) with a fibrous tissue system consisting of mostly collagen and elastic fibres linking the two sides. As skeletal maturity advances, the bony surfaces at the sutures become more interlocked and tortuous with ensuing increase in numbers, thickness, and strength of the sutural fibres (Carlson and Buschang, 2011).

The maxilla and mandible should be well proportioned and positioned relative to each other and to the neurocranium for dentofacial esthetics and dental occlusion that are closer to ideals. However, this is quite often not the case as varying degrees of skeletal discrepancies in occur in all 3 dimensions and affect one or both jaws. Maxillary constriction is characterized by a maxilla that is transversely narrowed relative to the complementing mandible. This condition may be represented by a unilateral or bilateral posterior crossbite with or without a functional shift. The estimated prevalence of children with posterior crossbites in the general population is reported to be about 5.9% to 16%; however, the ages of the examined children vary from preschool age up to about 11 years old (Thilander et al., 1984, Pinto et al., 2001, Melsen et al., 1979).

Rapid maxillary/palatal expansion (RME) is a well-known technique use in orthodontics to primarily improve the width of a narrow maxilla. It has been reported that RME was first used in 1860 in a 14-year-old girl by EH Angell but became more mainstream in the mid-20th century (Haas AJ, 1961). The goal of RME is to apply appropriately heavy forces to cause mechanical strain across the midpalatal suture to induce separation of the maxillary halves.

The success of RME depends on the degree of patency of the midpalatal suture. A more mature suture will require more force (around 10 to 20 pounds equivalent) to separate the hemimaxillae. Conversely, in young children where the suture is relatively immature, the same amount of force for an adolescent would be considered excessive. A plethora of appliances exists for maxillary expansion, and an appliance that is capable of applying a desired force should be selected for each specific case should be selected. These appliances are collectively termed *expanders* and function by compressing their inherent flexible elements (e.g., a Quad-Helix appliance), or require manual turning of a jackscrew mechanism (e.g., Hyrax or Haas-type expanders). The jackscrew increases its transverse dimension by 1.0 mm every 4 or 5 turns (depending on the manufacturer), which in an ideal scenario would allow equivalent maxillary skeletal expansion. The rate of rapid maxillary expansion is agreed to be from 0.5 mm to 1.0 mm of expansion per day (Proffit et al., 2013). Figure 2.3 illustrates the use of a Hyrax-type expander in an adolescent patient with maxillary constriction.

Figure 2.3: Before (left) and after (right) rapid maxillary expansion with a Hyrax-type expander of an adolescent patient. In this particular (non-study) patient, braces were applied to other teeth after adequate transverse correction was obtained.

In young children the required forces needed to separate the sutures are much less and traditional tooth-borne expander such as Hyrax is adequate. Successful orthopedic maxillary expansion has been shown to increase the width of the nasal floor in addition to correcting maxillary arch constriction (Lagravere et al., 2010; Liu et al., 2017).

RME can be considered a form of distraction osteogenesis as it uses mechanical strain to enhance the biological machinery for the creation of new bone and a net increase in bone volume. The biological processes involved with fracture healing, in the form of a fracture callus has been reported to be different than in distraction osteogenesis with differential expression of cytokines. In fracture repair, there are four stages: 1) inflammation, 2) formation of cartilage and the periosteal response, 3) cartilage resorption and primary bone formation, and 4) secondary bone formation and remodeling. Distraction osteogenesis, however, has different phases that are divided into 1) latency, 2) distraction, and 3) consolidation.

Latency starts right after the separation of the bony segments and ends when active distraction starts around this period. The inflammatory processes have been completed and the callus formed. With application of a tensile force, the callus stretches and grows with some concurrent mineral deposition.   The bone then undergoes extensive mineralization and eventually remodeling after the final bone length is achieved in the consolidation phase. The molecular players such as pro-inflammatory cytokines and various growth factors involved in fracture repair are also involved in distraction osteogenesis, however, expression of these agents differ in amount and timing (Al-Aql et al., 2008).

Once the desired change in transverse dimension is achieved, the expanded segments are retained in place for a period of about 4 months to allow adequate bone to fill in (retaining phase). Multiple protocols exist with varying rates of expansion, but the usual rate for *rapid* maxillary expansion is about 0.25 mm per day. This rate is still within physiological tolerances for the body and allows for concurrent growth of surrounding soft tissues (Roberts, 2011).

Section 2.2.17.1: Biochemical Assays – Growth Hormone and Insulin-Like Growth Factor 1

The anterior pituitary gland secretes growth hormone (GH) in pulses against a low baseline level. Rose et al. (1991) examined the 24-hour GH serum profiles (73 total samples per child) of 132 healthy children aged 4 to 18 and found that average night-time pulse frequencies ranged

from 3.0 to 4.5 pulses per 12 hours while mean daytime pulses were 2.0 to 2.8 pulses per 12 hours. In between pulses, the baseline GH concentration was below the detection limit of the GH assay 66% ± 21% (standard deviation, SD) of the diurnal period, and 46% ± 38% of the nocturnal period. Nighttime GH concentrations were higher than daytime measurements, and girls aged 8 to 10 years had significantly elevated levels of GH compared to boys aged 8 to 11 years. Figure 2.4 is an illustrative example of a typical 24-hour serum GH profile. Highest levels of GH release are during stage N3 of sleep (Mohsenin et al., 2010).
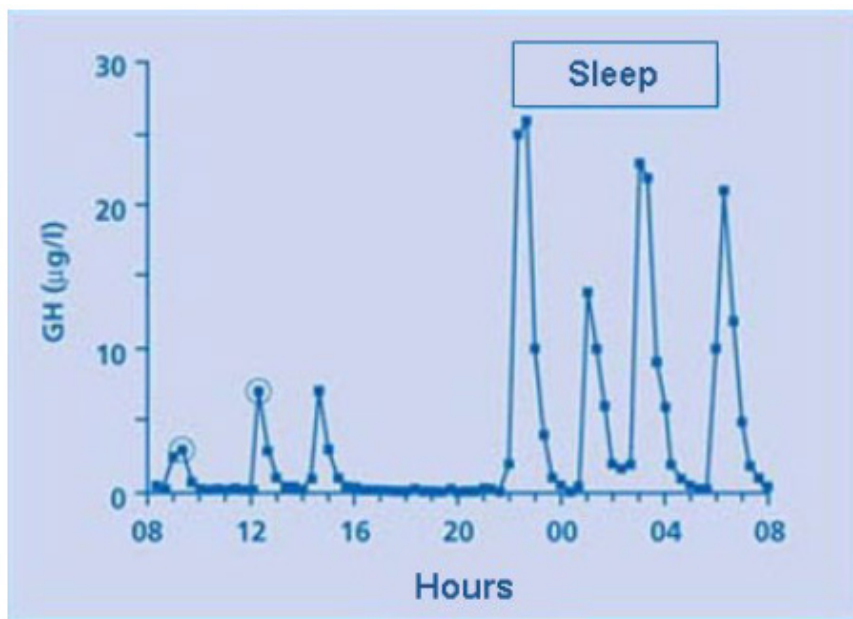
Figure 2.4: Example of a 24-hour serum growth hormone profile of a normal child. Note the barely detectable baseline levels of GH and greater amplitude of pulses at night (from Chanson and Salenave, 2008).

There are multiple isoforms of GH in circulation. The most abundant isoform is 22kDa followed by a 20kDa isoform. GH can also exist in groups as homodimers, multimers, and heterodimers. In humans, up to 50% of GH is complexed with GH binding protein (GHBP), which is a shortened form of the GH membrane bound receptor. Historically, competitive radioimmunoassays were used to measure concentrations of GH. This technique involved the use of antibodies that initially had bound radioactively labelled GH. Antibodies bind targets by recognition of specific amino acid sequences called epitopes. When mixed with a sample containing GH, the new molecules of GH would compete with the currently radiolabeled GH tracers. The greater the GH in a sample, the greater proportion of antibody sites become occupied by the unlabeled sample GH, and the weaker the radioactive signal. Antibodies against GH may be from a single

source (monoclonal) or multiple sources (polyclonal). Polyclonal antibodies result in binding to a broader spectrum of GH isoforms making it more difficult to differentiate which isoform was being measured (Chanson and Salenave, 2008). The presence of GH isoforms is a significant source of heterogeneity between assays (Bidlingmaier and Strasburger, 2007).

Sandwich immunoassays (ELISA/enzyme-linked immunosorbent assay) are now the popular choice for measuring GH and use monoclonal antibodies. The first set of antibodies specific to GH immobilizes it (these are usually fixed to the wells of the microplates), and a second set of antibodies also able to bind to GH with an additional region consisting of an attached enzyme capable of a light generating reaction. These two antibodies effectively "sandwich" the GH molecule. Substrate for the enzyme linked to the second antibodies is then added to allow the chemiluminescence reaction to occur and the signal intensity is compared to a set of standards to allow calculation of GH concentrations. ELISA is capable of rapidly processing large series of samples (Bidlingmaier and Freda, 2010).

The first standard preparations for GH were pituitary gland extracts. The first GH international reference preparation (IRP) was released in 1969, dubbed 66/217. The GH content was actually unknown and arbitrarily assigned a concentration of 2.0 units/mg. Assay kits developed by different companies did not have uniformity for standard preparation and the preferred commercial assay kits (company specific) of laboratories is inconsistent between countries. In a recent European consensus workshop, it was recommended that the World Health Organization (WHO) International Standard (IS) 98/574 be used for GH assays (Chanson and Salenave, 2008, Bidlingmaier and Freda, 2010). Published data of assay-specific cut-off values for diagnosis of certain conditions (e.g. growth hormone insufficiency or excess) for commercially available kits is also reportedly limited.

Published studies involving GH levels over the last four decades have an assortment of combinations of protocols, reference standards, as well as assay kits used by researchers or outsourced laboratories. Due to the inherent methodological heterogeneity of assessing GH

concentrations, direct comparison of results is difficult and, in many cases, not possible as no conversion formula exists for the older assays. Significant findings therefore may be limited to the particular assay that was used in that respective study. There are additional drawbacks to the use of GH to assess the status of the GH/IGF-1 axis. The half-life of GH is around 20 minutes (Brooke and Drake, 2007), and GH circulatory concentration is highly variable and is affected by age, adipose content, nutrition, stress, and sleep quality.

Despite improved detection limits of newer assays (0.2 µg/L to up to 0.002 µg/L), GH levels fluctuate considerably. The lowest GH levels (nadir) for normal individuals are indistinguishable from those with GH deficiency, and GH dynamic testing by provoking either suppression or stimulation of GH release is not indicative of the normative levels of the subject. The diagnostic criteria for GH deficiency are not uniform across nations (Inoue-Lima et al., 2019). In addition to ruling out other conditions that may cause growth failure such as hypothyroidism and Turner syndrome (GH Research Society, 2000), one set of criteria for GHD are when the peak concentration of GH is under 3 µg/L after an insulin tolerance test, and less than 5 µg/L in a second dynamic test stimulated with arginine, clonidine, glucagon, or L-dopa (Dunfield, 2007).

Evaluation of serum IGF-1 concentrations is now widely accepted as a method of indirectly assessing the GH/IGF-1 axis function. Although the level of IGF-1 is a function of GH secretion, it does not exhibit vast fluctuations like GH. Although the half-life of free, unbound IGF-1 is about 10 to 12 minutes, 99% of IGF-1 exists in a complexed state with IGF binding proteins, of which IGBBP-3 and the acid labile subunit (ALS) are majority components (Guler et al., 1989). These complexes stabilize and restrict IGF-1 in circulation and extend the half-life to about 10 to 15 hours (Livingston, 2013) effectively acting as a reserve pool for IGF-1 and therefore not responding greatly to subtle GH changes. Therefore, the circulating IGF-1 and IGFBP-3 levels are a representation of the overall status of GH secretion (Krebs et al., 2008). The free form of IGF-1 is thought to be the bioactive component (Livingstone, 2013), and during the pubertal growth spurt the IGF-1 levels increase at a faster rate than IGFBP-3 with a resulting increase in molar ratio of IGF-1 to IGFBP-3 (Delvin et al., 2006).

Using IGF-1 as a biomarker for growth also has associated problems similar to that of GH. IGF-1 levels may be altered in conditions such as malnutrition (fasting reduces IGF-1 levels by as much as 50%), hypothyroidism, hepatic disease, diabetes mellitus, and presence of sex steroids, in addition to GHD (Brooke and Drake, 2007, Shalet et al., 1998). The IGF-1 serum concentrations progressively increase in childhood up until puberty where the levels significantly increase to a maximum. In a healthy population of over 700 Danish children, IGF-1 levels peaked around 14.5 years for girls and 15.5 years for boys, coinciding with Tanner stage 3-4 for girls, and stage 4 for boys (Juul et al., 1994). Other researchers found similar results, specifically girls exhibited peak values about 1 year earlier than boys, and that the mean age for peak IGF-1 was 14 for girls and 16 years for boys. The study populations reported consisted of 600 to over 1400 healthy children (Delvin et al., 2006). Figure 2.5 shows mean IGF-1 concentrations as a function of age from two large studies attempting to establish reference curves for IGF-1 in children.
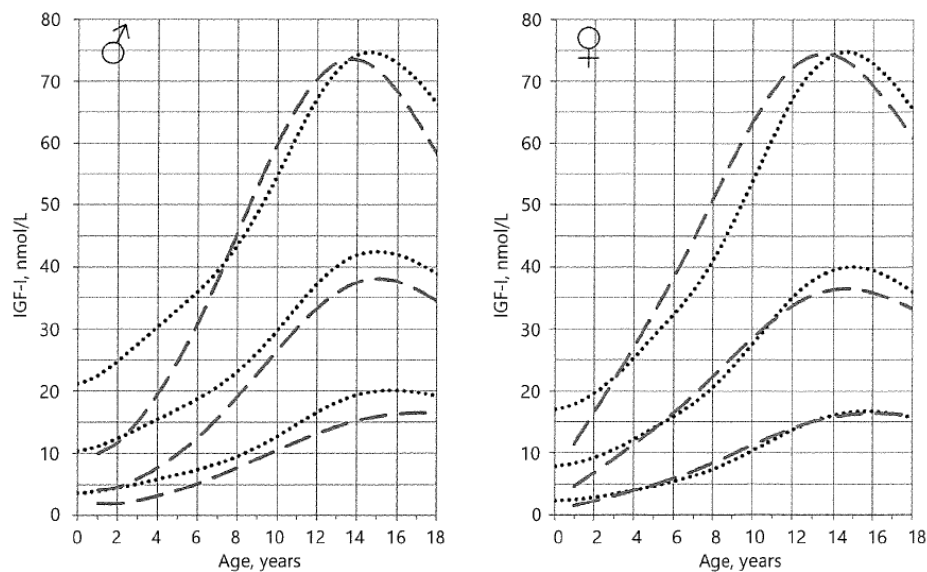
Figure 2.5: Plots of mean IGF-1 concentration as a function of chronological age. The 3 sets of lines depict the curves for -2 std deviations, mean, and +2 std deviations of IGF-1 in males and females. Dashed curves are from the Dutch harmonized study, and dotted curves from Bidlingmaier's multinational collaborative data set. Figure from Broeren et al. (2018).

Another common set of problems for IGF-1 determinations are the choice of reference preparation and available IGF-1 assay kits. It is the norm that countries may use different commercial assay kits with intra-assay coefficient of variation up to 8%, and 5 to 20% for inter-assay variations (Aneke-Nash et al., 2016, Wit et al., 2019). Some reference standards and assays kits that were used for previous studies have now been long discontinued (Krebs et al., 2008). The current endorsed standard is WHO IRR (International Reference Reagent) 02/254 for human recombinant IGF-I immunoassays (Varewijck et al., 2018).

Historically, competitive membrane binding assays and RIAs were the methods of choice but suffered from reproducibility and accuracy problems due to interference from the IGFBPs which had high affinity for IGF-1 and IGF-2. Currently non-competitive immunoassays (ELISA) have become the preferred assay type (Ramadhin et al., 2014). There are 3 commercial strategies that are used to dissociate and remove the IGFBPs from IGF-1. The first required pretreatment with acid and acetone or ethanol to allow extraction of the binding proteins followed by a second step used size-exclusion chromatography to filter out the large binding proteins. Another strategy is to saturate the sample with IGF-2, which will allow exchange of IGF-1 into solution. This method of IGF-2 saturation is more conducive for rapid turnover assays (Delvin et al., 2006).

It has been reported that IGF-I assessment of the GH/IGF-I axis is less successful in children younger than 5 years, and difficult to interpret in the state of puberty due to great intra-individual variation. However, in prepubertal children, it is generally assumed that the IGF-I SDS can be calculated for age and sex but the maximum age is admittedly arbitrary (Shalet et al., 1998, Wit et al., 2019). Due to the relative daily stability of IGF-I concentrations, examining this biomarker only requires one simple blood test instead of a 24-hour profile or dynamic testing (Brooke et al., 2007, Inoue-Lima et al., 2019).

Section 2.2.17.2: Biochemical Assays – Vitamin D and Calcium

Vitamin D is a component of the calcium-parathyroid hormone-vitamin D axis responsible for maintaining calcium homeostasis and metabolism. In humans, vitamin D can be obtained naturally in food sources as ergocaliferol (vitamin D_2) found predominantly in plants, fungi, oily fish and cod liver oil. Endogenous vitamin D_3 (cholecalciferol) can be produced non-enzymatically in the skin with UV-B radiation exposure with optimum wavelengths from 280 to 320 nm. Within the skin, 7-dehydrocholesterol is converted to pre-vitamin D_3 (and lumisterol and tachisterol if there is an excess of vitamin D_3) before final conversion to cholecalciferol. Although vitamin D_2 and D_3 differ slightly in structure, they do not differ in metabolism or function. It has been reported that inactivation enzymes break down vitamin D_2 faster than D_3, limiting D_2's action capability in cells (Chiang et al., 2017). When in circulation, both forms are bound to vitamin D binding protein (DBP), a type of albumin. In the liver, vitamin D_2/D_3 is converted to 25(OH)D, also known as calcidiol by CYP2R1 25-hydroxylase (Goltzman et al., 2018).

Sites of storage for vitamin D are mainly in adipose and muscle tissue as well as the liver. 25(OH)D is inactive unless the serum levels reach that of toxicity and constitutes the major form of circulating vitamin D. In the kidneys (by proximal renal tubular epithelial cells), 25(OH)D is converted to the active form of vitamin D ($1,25(OH)_2D$) also known as calcitriol. This active metabolite is involved in homeostasis of calcium levels by binding to vitamin D receptors (VDR) expressed in most body tissues to stimulate rapid duodenal and jejunal uptake of calcium and as well as at the level of the chondrocytes in the growth plates. $1,25(OH)_2D$ may act on the bone to increase the ratio of RANLK to osteoprotegerin (OPG) to enhance propagation and activity of osteoclasts. Calcitriol is also involved in its own negative feedback loop by inhibiting CYP27VB1 1-α-hyroxylase in the kidney that produces active vitamin D (Goltzman et al., 2018).

There is no consensus for criteria for vitamin D (in)sufficiency. Vitamin D levels can be affected by diet, inadequate exposure to sunlight, geography of residence (related to amount of

sunlight), drug use, and disease of the kidneys, liver, gut, or thyroid. The Endocrine society

defines deficiency when 25(OH)D serum concentration is under 20 ng/ml, and insufficiency

from 21 – 29 ng/ml, while the Institute of Medicine considers vitamin D levels to be adequate

when the level is at least 20 ng/ml. A daily intake of 1000 IU of vitamin D can raise the serum

levels by about 5 ng/ml. A concentration over 50 ng/ml has been implicated to cause potential

adverse effects (Chiang et al., 2017).

Lower levels of vitamin D have been reported in adolescents and obese individuals (BMI > 30

kg/m^2) presumably due to higher amount of vitamin D sequestered in fat stores (Smith et al.,

2018, Mortensen et al., 2019). However, others have reported that vitamin D_3 is not stored

until 25(OH)D levels reach 50 ng/ml and that a low calcidiol concentration represents a state of

substrate starvation (Chiang et al., 2017). Serum levels of 25(OH)D is accepted currently as the

best indicator of the vitamin D status due to the long half-life of 15 days and can be measured

with chemiluminescence or liquid chromatography with tandem mass spectrometry (Igra et al.,

2019, Mortensen et al., 2019).

Calcium is an element that is required for survival, mediating signaling of many cellular

processes including gene transcription, cellular proliferation, muscle contraction, and

exocytosis. On and off reactions that affect calcium dependent reactions are controlled by

entry of calcium into the cell. A 1000-fold calcium concentration gradient exists that favours

the entry of calcium into cells. Different intracellular signaling pathways are mediated by a

variety of messengers that result in either a sharp, short lived spike in intracellular calcium

concentration increase, or longer, repetitive waves. Temporally rapid calcium pulses in the

millisecond range are germane to synaptic transmission or cardiac muscle contraction while

slower processes such as gene transcription depend on successive rounds of calcium release

and diffusion. Ion pumps and active channels are responsible for resetting the calcium gradient

(Berridge et al., 2003).

In the human body, about 98% percent of calcium is stored in the skeleton in the form of hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$) while the remainder is in circulation or stored in soft tissues. Circulating calcium exists in 3 forms with about 45% bound to protein (mostly albumin and globulin), 10% complexed to negative ions such as phosphate or citrate, and 45% in an unbound/free state. The bioactive form of calcium is free and ionized (Ca^{2+}) and only this form can enter cells to activate downstream reactions. Free calcium levels can be affected by 1) fluctuations of albumin levels, 2) dehydration, and 3) changes to blood pH. Acidosis reduces protein binding while alkalosis increases binding. In the extracellular fluid calcium concentration is tightly regulated on a minute-to-minute basis to maintain homeostasis. Calcium sensing receptors (CaSR) on parathyroid cells detect changes in serum calcium and adjusts the body's response appropriately. In a hypocalcemic state, there is less CaSR activation, which tells the parathyroid cells to release more parathyroid hormone (PTH), whereas hypercalcemia will activate the CaSRs more with subsequent reduction in PTH release (Goltzman et al., 2018).

PTH is an important calcium regulatory hormone that target the kidneys and bone via PTH type 1 receptors (PTH1R) to ultimately increase calcium levels in the blood. Activation of renal PTH1Rs triggers calcium reabsorption in the ascending loop of Henle while cells of the osteoblast lineage respond by increasing production of NF-κB and RANKL. Binding of RANKL to osteoclast precursor cells and to existing osteoclasts enhance the bone resorptive activity and subsequent release of calcium and phosphate into the blood over the course of a few days. PTH also stimulates renal production of $1,25(OH)_2D$ from $25(OH)D$, which is responsible for intestinal absorption of calcium. This mechanism is reversed in hypercalcemia and collectively maintains proper serum calcium concentrations.

Normal total serum calcium levels are accepted to be about 8.0 to 10.5 mg/dL or equivalently, 2.0 to 2.6 mmol/L. Table 1.8 summarizes the ranges of total and ionized serum calcium concentrations. Determination of calcium in human fluids including serum relies on a chemical reaction between Ca^{2+} and the compound Arsenazo III to form an intense purple complex which

can be measured using spectrometry at wavelengths of 660 and 700 nm (Subih et al., 2018). Most clinical laboratories report the total serum calcium concentration. To account for effects on calcium concentration by the content of the plasma albumin, a corrected total serum calcium concentration (in units of mg/dL) can be calculated as per the following equation (Carroll and Schade, 2003) – variables within "[ ]" indicate concentration of that particular variable:

$$Corrected\ [calcium] = ([4.0 g/dL - [plasma\ albumin\ in\ g/dL] \times 0.8) + [serum\ calcium]$$

In adults, 20 to 60% of ingested calcium can be absorbed depending on the age of the individual and the quantity of consumed calcium. The duodenum and jejunum account for 90% of the body's absorption sites. Children especially during the pubertal growth spurt will absorb more dietary calcium in the range of 55 to 75%. With healthy renal function, the kidneys will filter about 10,000 mg of calcium per day and 98% of that will be reabsorbed (Goltzman et al., 2018).

Table 2.9: Serum Calcium Concentration Ranges			
	Normocalcemia	**Mild hypercalcemia**	**Moderate hypercalcemia**
Total serum [Ca]	8.0 – 10.0 mg/dL 2.0 – 2.5 mmol/L*	> 10.0 – 12.0 mg/dL > 2.5 – 3.0 mmol/L	> 12.0 – 14.0 mg/dL > 3.0 – 3.5 mmol/L**
Ionized serum [Ca^{2+}]	4.0 – 5.6 mg/dL 1.0 – 1.4 mmol/L **lower values indicate hypocalcemia**	> 5.6 – 8.0 mg/dL > 1.4 – 2.0 mmol/L	> 9.0 to 10.0 mg/dL > 2.0 to 2.5 mmol/L **higher values indicate hypercalcemic crisis**

Data from Carroll and Schade (2003).

Hypercalcemia is the consequence of disruption of the calcium balance with skewing towards net increased in calcium uptake and/or decrease calcium elimination due to increased bone resorption, increased gastrointestinal calcium absorption, and decreased renal excretion (Lafferty, 1991). Ninety percent of hypercalcemia cases are due to primary hyperparathyroidism or malignancy. Severe hypercalcemia can cause the patient to experience a wide set of symptoms described by the mnemonic "stones, bones, abdominal groans, and psychic groans", as well as having cardiovascular manifestations. The interested reader is referred to Carroll and Schade's review on managing hypercalcemia. It is therefore expected

that the levels of calcium will be within the range of normal in our study participants and also unchanged over duration of the trial.

Section 2.2.17.3: Biochemical Assays – Phosphate and Alkaline Phosphatase

The formation of mineralized tissues including bone and teeth is a complex process that requires orchestrated deposition of hydroxyapatite in designated areas in the extracellular matrix surrounding areas of forming hard tissues. Hydroxyapatite is composed of calcium and phosphate. Similar to calcium, around 85% of the body's total phosphate is found in the bones as hydroxyapatite and the bulk of inorganic phosphate (P_i) is absorbed in the intestine. Phosphate is part of the many structures, signaling compounds, and energy pathways (e.g. generation or hydrolysis of adenosine triphosphate). Pyrophosphate (PP_i) is essentially two P_i molecules connected by an ester bond (Sapir-Koren and Livshits, 2011). Intracellular and extracellular PP_i is formed by the hydrolysis of adenosine triphosphate to PP_i and adenosine monophosphate as part of mitochondrial energy metabolism (Millan, 2013, Terkeltaub, 2001).

One model of mineralization proposes that in chondrocytes and osteoblasts, intracellular matrix vesicles accumulate calcium and inorganic phosphate (P_i) during primary skeletal mineralization which are subsequently released into the extracellular matrix onto awaiting collagen fibrils which starts the precipitation of calcium and P_i. Secondary mineralization proceeds with the rupture of these vesicles and subsequent enlargement of the hydroxyapatite crystals (Whyte, 2010). An optimal ratio of inorganic phosphate to pyrophosphate is required to allow mineralization of hydroxyapatite. Tissue nonspecific alkaline phosphatase (TNSALP) is the enzyme that maintains this critical ratio by converting excess PP_i to P_i since PP_i is a potent inhibitor of mineralization.

The literature lacks studies investigating the ideal P_i/PP_i ratio or normative serum PP_i concentrations in children. An in vitro study mimicked physiological conditions (e.g. 37 degrees Celsius, pH of 7.4) and varied the concentration of PP_i and observed the resultant favoured

crystalline reaction. It was reported that a P_i/PP_i ratio of less than 3 resulted in predominant formation of calcium pyrophosphate dihydrate (implicated in gout), but a ratio of greater than 100 results in hydroxyapatite formation (Cheng and Pritzker, 1983). Others have reported that the mean concentration of PPi in healthy men and women was 3.50 µmol/L, but at 99% confidence interval, the PP_i levels can vary from 1.19 to 5.65 µmol/L (Russell et al., 1971).

In situations of PP_i excess such as in hypophosphatasia, there is associated impairment of hydroxyapatite deposition resulting in osteomalacia, while conditions with excessive calcification may be due to depression of extracellular PP_i levels (Terkeltaub, 2001). Some authors have cautioned about interpreting serum PP_i with reasons including the short turnover of serum PP_i (minutes) and how alterations in PP_i in the bone may not result in a change in plasma PP_i and vice versa (Russell et al., 1971).

In humans, there are tissue specific variants of (intestinal, placenta, and germ cell) alkaline phosphatase that have restricted expression. A non-specific isozyme, TNSALP (tissue non-specific alkaline phosphatase), is expressed in all tissues but with greatest abundance in the bone, kidneys, and liver. Although TNSALP initially presents as a membrane protein, it can be cleaved from the anchor unit and allowed into circulation as a homodimer and accounts for 95% of the total serum ALP activity. Bone TNSALP is the most abundant form in children, but in adults there is an approximately equal amount of bone and liver isoforms (Khan et al., 2019).

The Canadian Laboratory Initiative in Paediatric Reference (CALIPER) collaborative group was formed to establish normative values of difference biomarkers for healthy children and adolescents using combined data from children's hospitals across Canada. The normal values for serum ALP activity (using 4-nitrophenyl phosphate for the assay), P_i, and calcium for males and females and relevant age groups are provided in Table 2.10.

Table 2.10: Normative Values for Calcium, Phosphate, and Alkaline Phosphatase in Children							
		Females			**Males**		
Analyte	**Age**	**Lower limit [CI]**	**Upper limit [CI]**	**N =**	**Lower limit [CI]**	**Upper limit [CI]**	**N =**
Ca^{2+} mg/dL	1 to <19Y	9.2 [9.1-9.2]	10.5[10.5-10.6]	897	9.2 [9.1-9.2]	10.5[10.5-10.6]	897
P$_i$ mg/dL	5 to <13Y	4.1[4.1,42]	5.9[5.9,6.0]	352	4.1[4.1,42]	5.9[5.9,6.0]	352
	13 to <19Y	3.2[3.0,3.3]	5.5[5.4,5.7]	95	3.5[3.4,3.6]	6.2[6.0,6.3]	95
ALP unit/L	1 to <10Y	156[145,170]	369[362,391]	391	156[145,170]	369[362,391]	391
(4-nitrophenyl phosphate)	10 to <13Y	141[114,171]	460[424,476]	154	141[114,171]	460[424,476]	154

Data from Colantonion et al., 2012

Section 2.2.18: Determination of Skeletal Age

In healthy children there is a pattern of increase in size and maturation of different systems within the body as well as changing velocities of these increases. Researchers have demonstrated that chronological age is not the most accurate indicator of an individual's maturity with prime examples seen through pubertal variations in age of onset, rate of growth, and duration of growth spurt clearly seen between boys and girls and between individuals of the same sex (Juul et al., 1994, Lofqvist et al., 2001). The use of biological age to determine maturation has been proposed by numerous authors (Tanner, 1986, Fishman, 1982, Jain et al., 2017). There are different methods of determining biological age including assessment of standing height, skeletal or bone age, presence and degree of secondary sexual characteristics, and levels of biomarkers found in the body (Tanner, 1986, Masoud et al., 2008).

There are many factors that influence the final height of an individual and a single height measurement without multiple historical timepoints is of limited value in estimation of a child's biological age. Assessment of the presence and degree of sexual characteristics may not be readily accepted by patients and parents for orthodontic and dentofacial orthopedic research. As discussed previously, IGF-I levels follow a general pattern of slow increases during the prepubertal stage of life followed by a rapid rise around and during puberty which subsequently falls after adolescence, and thus is a suitable biological marker of maturation (Juul et al., 1997). Skeletal age can be determined by assessing the number of ossification centres, size, and morphology of specific bones. Assessment of the cervical vertebra on a lateral cephalometric

radiograph and of the carpal, metacarpal, and phalangeal bones on a hand-wrist radiograph are two common sites to determine skeletal age. The hand is a convenient site for assessment since it does not contain vital organs or tissues and has multiple bones that may be evaluated simultaneously for developmental changes.

A popular method for hand-wrist radiograph analysis was developed by Greulich and Pyle in the 1950s and involves comparison of a subject's hand-wrist radiograph to that of a set of male and female standards in an atlas. Each of bone (up to 30 applicable sites) is assigned an age and the average of all of the bones provides an estimate of the subject's skeletal age. It is important to note that the Greulich and Pyle standards are derived from North American children from affluent families in the 1930s, and that each standard was selected from a pool of 100 radiographs of children of the same age and sex. For each set, the radiographs were ordered from least mature to the most mature, and the authors selected the radiograph that they felt was most representative for that particular age (Greulich and Pyle, 1959). By convention the left hand is selected for the radiograph as it usually is non-dominant and presumably would have a lower prevalence of injury than the dominant right hand. Included in the assessment are the distal ends of the radius and ulna, the 8 carpal, 5 metacarpal, 17 phalangeal bones, and the adductor and flexor sesamoids of the thumb.

Maturity indicators are distinct events that occur in a sequential pattern (usually) in every normal developing child. In the hand and wrist, these events include the formation of ossification centres, the widening of the epiphyses of the phalanges, the morphology of the bones including articulating surfaces of the carpal and metacarpal bones, and fusion of the epiphyses with the respective diaphysis. In a prepubertal child of the elementary grade years, the capitate, hamate, lunate, scaphoid, trapezoid, trapezium ossification centres have formed and are growing in size and changing shape as they move spatially closer together. The pisiform ossifies later while the epiphyses of the phalanges and arm bones are already present. Once a child moves into the pubertal stage, usually the sesamoid of the thumb appears and the epiphyses of the phalanges (typically 3rd and 5th) develop "horns", undergoing a state otherwise

known as "capping". Fusion of the epiphyses and diaphyses signifies the end of adolescence and entry to early adulthood. These events occur in all normal developing children in generally the same sequence. However, the timing of the events may show variation from child to child, and it is observed that girls typically mature about 1.5 to 2 years sooner than boys (Fishman, 1982, Tanner, 1986).

While more accurate than chronological age, there are admitted drawbacks of using a skeletal age assessment method with a set of standards; in that the results are most applicable to the population from which the standards were derived. Greulich and Pyle warned that if a subject of a different ethnicity were to be compared to their standards for Caucasian North American children, the skeletal age may require some sort of correction. Further, secular trends may also influence the timing of puberty which further reduces the usefulness of these historic standards (Greulich and Pyle, 1959, Tanner, 1986).

Fishman's method of determining skeletal maturation is much simpler and focuses on 4 sites on the hand: the sesamoid, the distal end of the radius, the epiphysis of the middle phalange of the 5th finger, and all epiphyses of the 3rd finger. Together there are 11 different skeletal maturational indicators that cover the adolescent period. As the child matures, the epiphyses widen, then "cap", followed by fusion. The presence of the sesamoid is a quick indicator that the child will be entering a period of rapid growth (Fishman, 1982). Other researchers have countered that evaluating only the sesamoid is not reliable enough to estimate when the peak height velocity will occur (Helm, 1971). Fishman's method focuses on the pubertal period and is not applicable in our study since our study sample consists of prepubertal children who are supposedly at least more than one year away from puberty.

Growth related charts that involve plotting peak height velocity, total standing height, amount of growth remaining or completed, or serum IGF-1 against chronological age for a group of children, show that the onset of peak growth shows great variation. This is especially true between boys and girls (Juul et al., 1997). If the growth attained per year or growth velocity of

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the subjects were then replotted against biological age or maturational stage, the phase shift between the different subjects is minimized (Fishman, 1982, Tanner, 1986, Flores-Mir and Burgess, 2006) and growth plots become roughly synchronized. Masoud et al., (2009) investigated the correlation between mean blood-spot IGF-I concentrations and the different maturational stages using the Fishman method in 84 subjects aged 5 to 25 years and found certain trends. The average concentration of IGF-I is below 200 µg/L prepubertally but increases with increasing skeletal maturity up to SMI (skeletal maturity indicator) stage 6-8 before falling to during the deceleration and postpubertal stages.

Kanbur-Oksuz et al., (2004) assessed the levels of IGF-1 during different pubertal stages in 205 healthy Turkish children between 9 and 17 years. The skeletal ages were determined according to Greulich and Pyle while Tanner's classification was used for pubertal staging. In females, the highest mean IGF-I levels were 414.58 and 418.22 ng/ml in Tanner stages 3 and 4 and 429.65 and 416.78 ng/ml in Tanner stages 4 and 5 in males, respectively. They also found that in females, the Tanner stages 3 and 4 correlated to mean bone ages of 12.24 and 14.20 with a standard deviation of about 1.2 years; in males, subjects in Tanner stages 4 and 5 had mean bone ages of 14.02 and 15.39 years with a standard deviation up to 3.0 years. The authors concluded that although there is great variability in serum IGF-I levels during puberty, if classifying by sex and Tanner stage, the variation is reduced.

Juul et al. (1994) showed a similar pattern of differing IGF-I levels dependent on pubertal stage with Tanner classification in a sample of 833 Danish individuals of Caucasian origin aged 5 to 20 years. In females, IGF-I increased in stage I, but was unchanged in stages II to IV, but decreased in stage V, while in males the IGF-I increased in stages I and II, was unaffected in stage III and decreased in IV and V. Although peak IGF-I occurred at different stages than Kanbur-Oksuz's children, the sequence was still the same. The differences in timing may be attributable to secular factors and ethnicity of the two subject pools. Cruickshank et al. (2001) found that different ethnic groups had significantly different mean levels of serum IGF-I.

Determining the maturity of a child is important when measurements of serum IGF-I are evaluated. In prepubertal children serum IGF-I is observed to increase slowly during the childhood period and no differences are observed in sexes (Juul et al., 1994). It is only upon entry into puberty that the effects of sex and developmental maturity become pronounced. Assessment of hand-wrist radiographs of our study subjects is one method to evaluate whether a study subject is spatially not near the peripubertal stage, which can affect the serum IGF-I measurement. This is critical given the presence of only a single time point at time of admittance into the study.

CHAPTER 3

Methods

Section 3.1: Methods

The background information from the scientific literature in the previous sections forms the foundation for this clinical trial's protocols. Ethics approval was granted previously by the University of Alberta Health Research Ethics Board on March 1, 2016 (Study ID: Pro00061536). The CONSORT 2010 checklist was followed as closely as possible for reporting of methodology (Schulz et al., 2010).

Section 3.1.1: Trial Design

This study was a parallel randomized controlled trial. Complete blinding was not possible. The study participants and the clinician involved in overseeing treatment were unblinded, but the third-party blood work laboratories, the sleep physician, and those involved in assessing intermolar widths and bone age, and statistical analyses were blinded.

Section 3.1.2: Participants

Selection criteria for inclusion in the study were as follows:

- Maxillary skeletal constriction in the form of a unilateral or bilateral posterior crossbite or crossbite tendency (maxillary molars that are flared in the lateral direction (buccal) while the lower molars are tipped in the medial direction (lingual) with or without a functional shift of the lower jaw)
- Chronological age between 6 and 12 years
- Prepubertal status; absence of menarche in females and lack of voice break and facial hair in males
- No diagnosed medical conditions or taking medications or dietary supplements at the time of admittance.

The children were not screened for sleep-disordered breathing as it was not a primary criterion for this study. Follow-up studies would include this criterion as it may complement findings derived from AHI scores. Children seeking orthodontic treatment at the Graduate Orthodontic Clinics at the Universities of Alberta, Edmonton, Canada, and Insubria, Varese, Italy, screened for eligibility from November 2016 to October 2019 based on the selection criteria. The children and their families were provided with information on the study and then asked to participate. Those who provided informed consent were admitted. Ethnicities of the participants were not provided. Patients' ethnic backgrounds are heterogenous, and verification of the specific racial origins was beyond this study's scope.

Section 3.1.3: Interventions

The children were randomly allocated into two treatment groups. The control group consisted of no provision of orthodontic treatment for 12 months from the date of inclusion into the trial (trial duration) to the end of the trial. The treatment group (maxillary expansion group) received the rapid maxillary expansion with a Hyrax-type expander (10 mm Forestadent, Pforzheim, Germany) with bands placed on the maxillary first permanent molars and metal arms soldered that extended to the maxillary first premolars or deciduous first molars dependent on the presence. After performing hygiene to ensure the maxillary first molars were clean and dried, the appliance was cemented with Ultra Band-Lok Blue cement (Orthodontic Supply Canada, Fredericton, Canada). The Italian group used a Haas-Type expander with bands cemented to maxillary primary second molars and metal arms bonded to primary upper canines.

Each child in the RME group was overexpanded until the maxillary first molars' palatal cusps touched the buccal cusp tips of the mandibular first molars. This compensates for the potential relapse upon cessation of maxillary expansion and the lower molars' future uprighting. The expansion would occur at a rate of one turn (0.25 mm) per day for the Canadian cohort, and two initial turns followed by one turn (0.225 mm) per day for the Italian cohort until the desired

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amount was achieved. The parents or caregivers were instructed on the proper procedure to turn the jackscrew and were provided with a Hyrax key. Each turn of the jack-screw was equivalent to 0.225 mm/0.25 mm (Italy/Canada) of expansion. The parents were instructed to stop turning once the prescribed turns were achieved. The patient was seen at an interim appointment to assess if expansion was completed adequately. More turns were prescribed as necessary, using the same protocol.

Once the adequate expansion was achieved, a 0.08" diameter stainless steel ligature was tight into the jackscrew mechanism to prevent further movement of the screw. The treatment group children were maintained with the Hyrax appliance for four months from the last day of activation. A passive standard transpalatal arch appliance was placed banded to the maxillary first molars (with Band-Lok) to maintain the expansion during the retention phase. If the appliances became loose, they were immediately inspected and recemented.

Section 3.1.4: Outcomes

Outcome variables of interest were categorized based on cohort composition of the main and subgroups described below:

1. Pooled Italian and Canadian Cohort:
   a. Mean serum [IGF-1]
2. Italian Cohort:
   a. Mean serum [IGF-1]
   b. Mean AHI score
3. Canadian Cohort:
   a. Mean [IGF-1]

Initially, the outcome measure of AHI score was assessed for all three cohort types (pooled, Italy only, Canada only). The plan was to retain the sleep physician's services responsible for

interpreting the Italian cohort's PSGs based out of Italy to read the PSGs from the Canadian cohort to maintain AHI scores' consistency. However, the physician could not accommodate the timeline required, especially considering the impact that COVID19 had during early 2020. Statistical tests were adjusted accordingly.

Section 3.1.5: Sample Size

A priori sample size and power calculations were not done before commencing the trial. A rule of thumb for the adequate sample size for omnibus testing is to have at least 20 subjects per outcome measure to ensure the MANCOVA or ANCOVA tests are still adequately robust. Analyses where only [IGF-1] (mean or mean change) require a minimum of 20 subjects (20 x 1 outcome), while analyses involving both [IGF-1] and AHI require a minimum of 40 subjects (20 x 2) to ensure robustness.

Section 3.1.6: Randomization

Computer software (Microsoft Excel) was used to generate a random sequence for determining which group an incoming subject was to be assigned. Each participant was assigned to a group once the inclusion criteria were satisfied and written parental consent and participant assent were given, the patient was then assigned to a group. The primary investigator then checked the randomized allocation table and assigned the patient to the predetermined group.

Section 3.1.7: Blinding

Due to the treatment nature, neither the patient nor the clinical investigator could be blinded. The group assignment was kept concealed to the commercial laboratories used for bloodwork analyses, the sleep physician interpreting the polysomnographs, and the investigator involved in assigning a skeletal age to the hand-wrist radiographs and performing statistical analyses.

Section 3.1.8: Statistical Methods

Intra-rater and inter-rater reliability were performed for the differences in IMW values in the Edmonton cohort using the intraclass correlation coefficient. The intra-rater reliability trials were conducted on 14 digital scans of non-study patients, seven days apart. Interrater reliability was assessed between the author and Dr. Manuel Lagravere.

Intra-rater reliability was performed for grading of the hand-wrist radiographs for the Edmonton cohort. Greulich and Pyle's method was used with a set of ten randomly selected hand-wrist radiographs with five from the T1 time point and five from the T2 time point chosen for reliability analysis with the intraclass correlation coefficient. The order was randomized and a 7-day period between data set analysis.

For statistical analyses, Between-Subjects Factors were identified as 1) Treatment Group (control/expansion) and 2) Sex (male/female). For some analyses, the Within-Subjects Factor was identified as "Time". The following were considered potential covariates for statistical modelling: 1) T1 chronologic and skeletal age, 2) BMI, and 3) IMW difference. The initial period, BMI, and maxillary constriction were set as covariates for all sets of hypotheses using pooled data from the two university cohorts. Initial age, BMI, maxillary constriction, alkaline phosphatase (ALP) and calcidiol (25-(OH)D) for all sets of hypotheses using data from the Canadian cohort.

It was planned a priori to combine the data from the two cohorts (Italy, Canada) if they were statistically similar. Baseline (T1) variables were compared between the two university cohorts and consisted of:

- IGF-1 concentration ([IGF-1])
- BMI
- Age (chronologic and skeletal)
- Sex
- IMW difference

The multivariate normality of the samples was assessed with scatterplots and Mahalanobis distances. Potential covariates were assessed for linearity with outcome variables visually with scatterplots and were dropped if linearity was lacking. Multivariate tests were performed to control type I error rate. Provided the cohorts were similar; the data would be pooled for subsequent analyses. Bonferroni corrections were applied to follow-up tests. If the cohorts were vastly different, then analyses would be carried out on the cohorts separately.

Section 3.1.7.1: Statistical Methods for the Pooled Cohort

For the pooled cohort, it was planned that initial skeletal (bone) age, BMI, initial [IGF-1], and maxillary constriction were considered potential covariates for all sets of hypotheses. The following hypotheses were set to investigate the effect of maxillary expansion on serum [IGF-1]:

1. **H_0**: There is no difference in mean serum IGF-1 concentration between treatment and control groups.
   H_a: There is a difference in mean serum IGF-1 concentration between treatment and control groups.
2. **H_0**: There is no difference in the mean of IGF-1 concentration between T1 or T2.
   H_a: There is a difference in the mean of IGF-1 concentration between T1 or T2.
3. **H_0**: There is no interaction between factors (treatment group, sex, time) on the mean IGF-1 concentration.
   H_a: There is an interaction between at least two factors on the mean IGF-1 concentration.

Statistical tests were set up as follows for the pooled cohort:
1. Covariates were selected based on linearity, as seen in scatterplot pairs with the outcome variables of interest.
2. Omnibus testing was performed to reduce the risk of a type I error through simultaneous comparisons and detect significant interactions. Three-way repeated measures ANCOVA (analysis of covariance) was chosen. Sex and treatment groups were

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the two between-group factors (hence "two-way") used, and time was a within-group factor

3. Follow-up testing with pairwise t-tests and Bonferroni correction.

Section 3.1.7.2: Statistical Methods for Subgroup Analysis of the Italian Cohort

Both university cohorts had different data collected and available for use when writing this manuscript. These included:

- AHI scores ready only for the Italy cohort.
- Other serum metabolites of calcidiol, calcium, phosphate, and total alkaline phosphatase were measured only for the Canadian cohort.

Separate subanalyses were planned for each cohort to investigate any potential effect of expansion treatment on AHI score in the Italy cohort and if other serum metabolites could be considered covariates for the Canadian cohort.

For the Italian cohort subanalyses, it was planned that initial chronologic and bone ages, BMI, initial [IGF-1], and maxillary constriction were considered potential covariates for all sets of hypotheses. The following hypotheses were set to investigate the effect of maxillary expansion on serum [IGF-1] and/or AHI score:

1. **H$_0$**: There is no difference in mean IGF-1 concentration or in mean AHI between treatment and control groups.
   H$_a$: There is a difference in mean IGF-1 concentration or in mean AHI between treatment and control groups.
2. **H$_0$**: There is no difference in the mean of IGF-1 concentration or AHI between T1 or T2.
   H$_a$: There is a difference in the mean of IGF-1 concentration or AHI between T1 or T2.
3. **H$_0$**: There is no interaction between factors (treatment group, sex, time) on the mean IGF-1 concentration or AHI.
   H$_a$: There is an interaction between at least two factors on the mean IGF-1 concentration or AHI.

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Statistical tests were set up as follows for the Italy cohort:

1. Covariates were selected based on linearity, as seen in scatterplot pairs with the outcome variables of interest.

2. Omnibus testing was performed to reduce the risk of a type I error through simultaneous comparisons and detect significant interactions. Three-way repeated measures MANCOVA (multivariate analysis of covariance) was chosen. Sex and treatment groups were the two between-group factors (hence "two-way") used, and time was a within-group factor

3. Follow-up testing with pairwise t-tests and Bonferroni correction.

Section 3.1.7.3: Statistical Methods for Subgroup Analysis of the Canadian Cohort

For the Canadian cohort, it was planned that initial chronologic and bone ages, BMI, initial [IGF-1], alkaline phosphatase (ALP) and calcidiol (25(OH)D), and maxillary constriction were considered potential covariates for all sets of hypotheses. The following hypotheses were set to investigate the effect of maxillary expansion on serum [IGF-1]:

1. There is no difference in mean serum IGF-1 concentration between treatment and control groups.

   H_a: There is a difference in mean serum IGF-1 concentration between treatment and control groups.

2. H_0: There is no difference in the mean of IGF-1 concentration between T1 or T2.

   H_a: There is a difference in the mean of IGF-1 concentration between T1 or T2.

3. H_0: There is no interaction between factors (treatment group, sex, time) on the mean IGF-1 concentration.

   H_a: There is an interaction between at least two factors on the mean IGF-1 concentration.

Statistical tests were set up as follows for the pooled cohort:

1. Covariates were selected based on linearity, as seen in scatterplot pairs with the outcome variables of interest.

2. Omnibus testing was performed to reduce the risk of a type I error through simultaneous comparisons and detect significant interactions. Three-way repeated measures ANCOVA (analysis of covariance) was chosen. Sex and treatment groups were the two between-group factors (hence "two-way") used, and time was a within-group factor

3. Follow-up testing with pairwise t-tests and Bonferroni correction.

Statistical analyses were performed using IBM SPSS version 21 with the significance level set at α = 0.05. Values were reported as mean [95% Confidence Interval / C.I.] unless indicated otherwise. Interactions were assessed with profile plots. Means are displayed as mean [lower bound, upper bound of 95% confidence interval]. A summary of the planned statistical tests is shown below in Table 3.1.

| Table 3.1: Summary of Statistical Methods			
Cohort	Pooled (n=60)	Italy (n=32)	Canada (n=31)
Outcome Measures	Mean [IGF-1]	Mean [IGF-1], mean AHI	Mean [IGF-1]
Between Subjects Factors	Sex, Tx Group	Sex, Tx Group	Sex, Tx Group
Within-Subjects Factor	-	Time	-
Potential Covariates	Bone age, BMI, Max constriction, initial [IGF-1]	Bone age, BMI, Max constriction, initial [IGF-1]	Bone age, BMI, Max constriction, initial [IGF-1], 25-(OH)D, ALP
Statistical Tests	3-way repeated measures ANCOVA, Follow-up t-tests	3-way repeated measures MANCOVA, Follow-up t-tests	3-way repeated measures ANCOVA, Follow-up t-tests
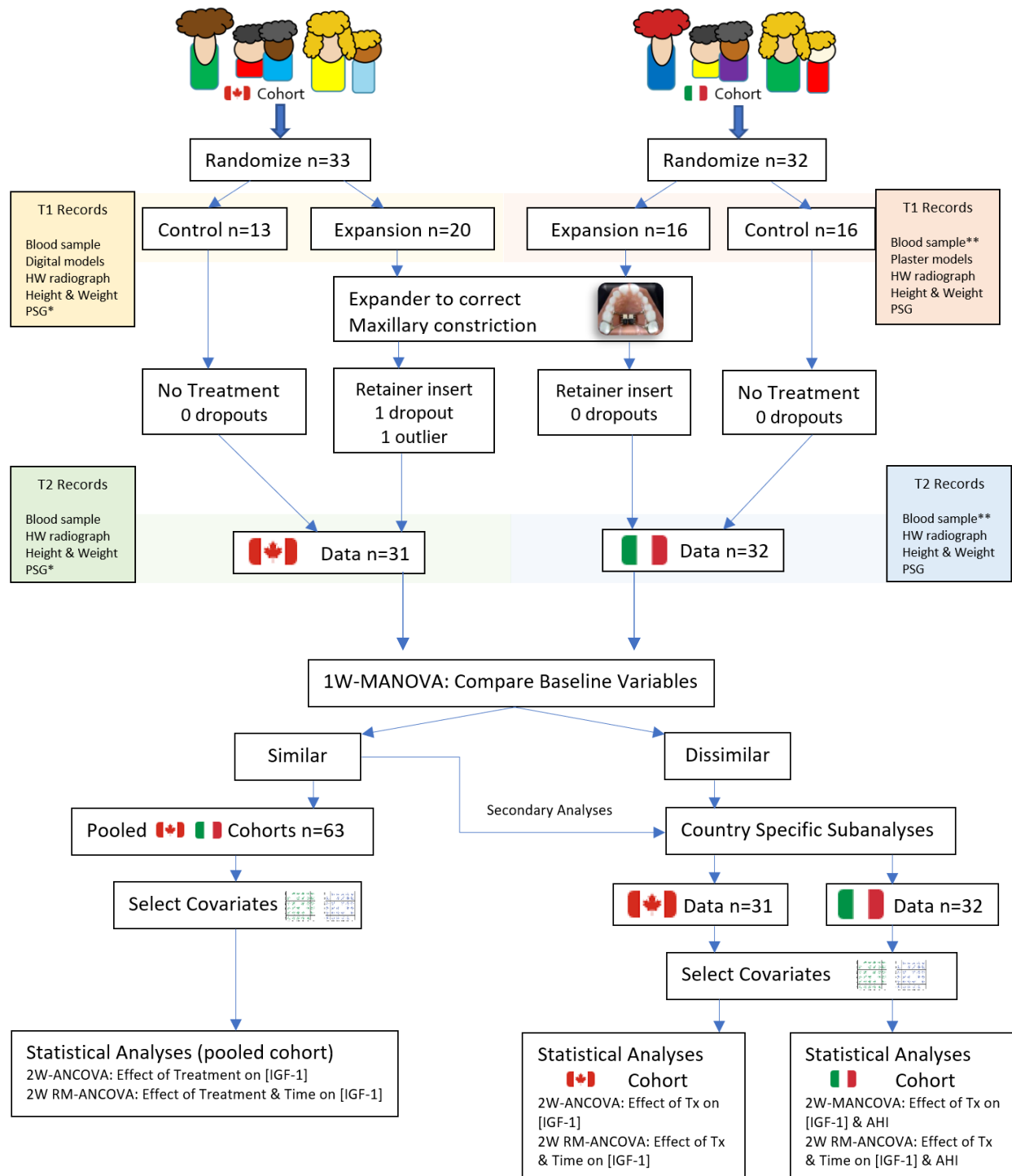
Figure 3.1 summarizes the study design. *AHI scores derived from PSGs for the Canadian cohort were not available when writing this manuscript. **Italy's cohort did not evaluate serum calcidiol, calcium, inorganic phosphate, or ALP in their bloodwork analysis.

Section 3.2: Supplemental Methodological Information

Section 3.2.1: Anthropometric Measurements

Two-time points were established. T1 was designated as the trial start, which would be when the first set of measurements were obtained. T2 was approximately 12 months after the date of acquisition of the first set of data. Attempts were made to see the patients precisely one year after T1. At T1 and T2 appointments, the following anthropometric variables were recorded:

1. Standing height to the nearest 0.1 centimeter (without shoes)
2. Weight to the nearest 0.1 kilogram (minimally clothed)
3. Calculation of BMI (kg/m^2)
4. Difference between maxillary and mandibular first permanent intermolar widths (Intermolar width) to the nearest 0.1 millimeter

The intermolar width (IMW) is a linear measurement from set landmarks on the maxillary and mandibular first permanent molars. The upper IMW is the linear distance from one mesiopalatal cusp (hereon in referred interchangeably with palatal cusp) to the contralateral molar mesiopalatal cusp (Figure 3.2). The lower IMW is the linear distance from the central pit where the lingual developmental groove intersects with the main central groove fossa (Nelson and Ash, 2010) of one lower first molar to the contralateral landmark. Units are in millimetres (mm), and the difference is calculated as the upper IMW minus the lower IMW. A negative value suggests maxillary constriction. However, the molars' arrangement (and other teeth) can sometimes be independent of the underlying skeletal discrepancy – meaning that the difference in IMWs will numerically not indicate maxillary constriction, yet further radiographic analysis will reveal the discrepancy. Due to differences in equipment availability, radiographic determination of maxillary skeletal constriction was not performed. We used the difference in IMW as a proxy for the maxillary constriction for our statistical analyses for simplicity.

The IMW values for the Italian cohort were obtained from plaster models with a digital caliper. The same measurements for the Canadian cohort were measured digitally using intraoral scans acquired from the iTero Element 2 scanner (Align Technologies). The scan started on each arch's left side and measured through the OrthoCAD 5.9.0.36 software (Cadent) as per figure 3.2.

A recent systematic review from the field of prosthodontics has found that digital scanning of teeth prepared for dental crowns displayed better marginal accuracy than conventional elastomeric impression materials. In particular, the iTero scanner had a standardized mean difference of -0.44 micrometres [-1.35, 0.47, 95% C.I.] of the marginal discrepancy between the restoration and finish line (Tabesh et al., 2020). The maximum acceptable, marginal gap is about 120 μm (Diker and Tak, 2020).
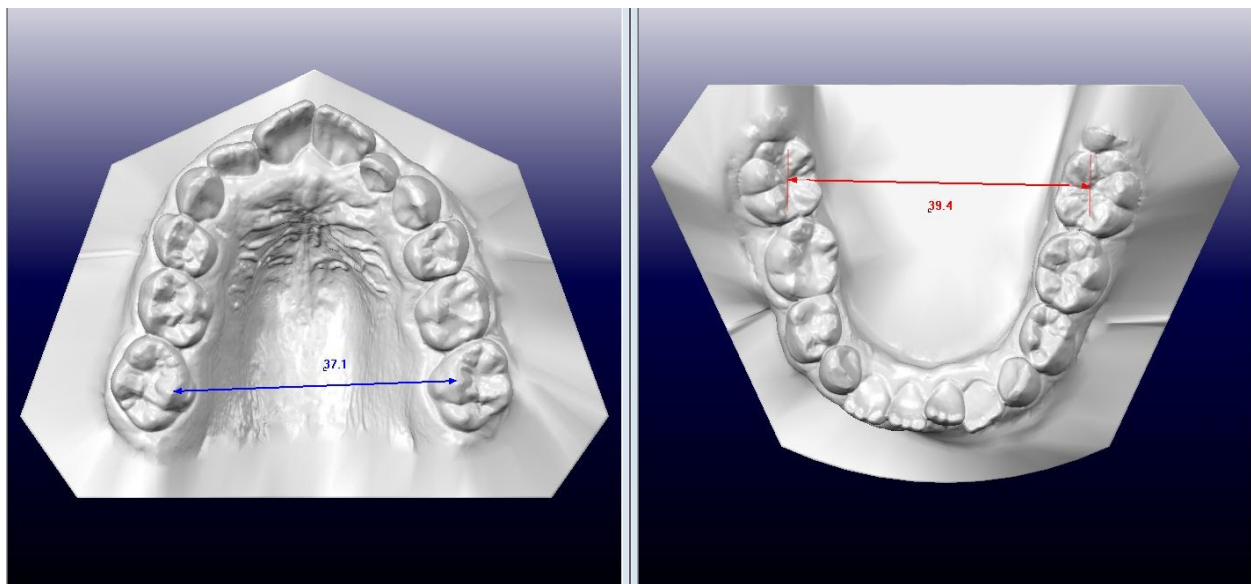

Figure 3.2: Example of measuring upper and lower intermolar widths using digital models derived from the iTero Element 2 scanner on OrthoCAD 5.9.0.36 software (Cadent).

The median trueness (how close the test measure is to a recognized reference value) and the precision of the iTero scanner was found to be about 27 and 12 μm, respectively (Park et al.

2019), while in another study, these two values were found to be 44 and 7 μm for the iTero Element 2 scanner if the manufacturer's instructions were followed (Diker and Tak, 2020).

More current studies have evaluated the difference between IMWs measured digitally and on plaster. Abizadeh et al., 2012 found the mean difference to be 0.07 mm for the lower and 0.15 mm for the upper IMW. Camardella et al. (2017) compared these two methods and found the mean difference between upper IMW to be 0.013 mm and 0.038 mm and for the lower IMW to be 0.035 mm and -0.423 mm different investigators. Some of these mean differences were statistically significant, but the differences are probably clinically insignificant; therefore, both digital and plaster methods were accepted as an equivalent for this study.

Section 3.2.2: Serum Biomarker Measurements

Similarly, for both time points, the patients were asked not to fast the night before their appointment. Blood was drawn from the antecubital vein. Blood samples were acquired and analyzed the same day by a commercial laboratory, DynaLife Medical Labs (Edmonton, Canada) for the Canadian cohort. The following serum biomarkers were quantified, and their reference values as provided by DynaLife Medical Labs are shown in Table 3.2:. Bloodwork was performed by the University of Insubria hospital laboratory (personal communication, Fastuca, 2021).

Table 3.2: DynaLife Medical Labs Reference Ranges for Serum Biomarkers						
Sex	Female			Male		
Age Range (years)	6 - 8	9 - 11	12 - 13	6 - 8	9 - 11	12 - 13
IGF-1 (ug/L)	100-306	134-454	200-535	65-325	84-455	126-535
25-OH vitamin D nmol/L	80-200			80-200		
Calcium (mmol/L)	2.20-2.80			2.20-2.80		
Inorganic Phosphate (mmol/L)	1.10-1.90			1.10-1.90		
Total alkaline phosphatase (Units/L)	130-430			130-430		

Although measured, the calcium and inorganic phosphate levels were consistent due to their critical roles required for homeostasis as manifested in the narrow normal value range and as described in the previous chapter. They were not used for statistical analyses. Serum calcidiol, calcium, inorganic phosphate, and total alkaline phosphatase were not measured as part of the Italian cohort's bloodwork.

Section 3.2.3: Handwrist Radiography

A hand-wrist radiograph of the left hand was acquired of each child at the start and the end of the trial simultaneously as other records. The hand-wrist radiograph was used to determine the skeletal or bone age using the standards and methods described in Greulich and Pyle's Atlas of Skeletal Development of the Hand and Wrist (Greulich and Pyle, 1959).
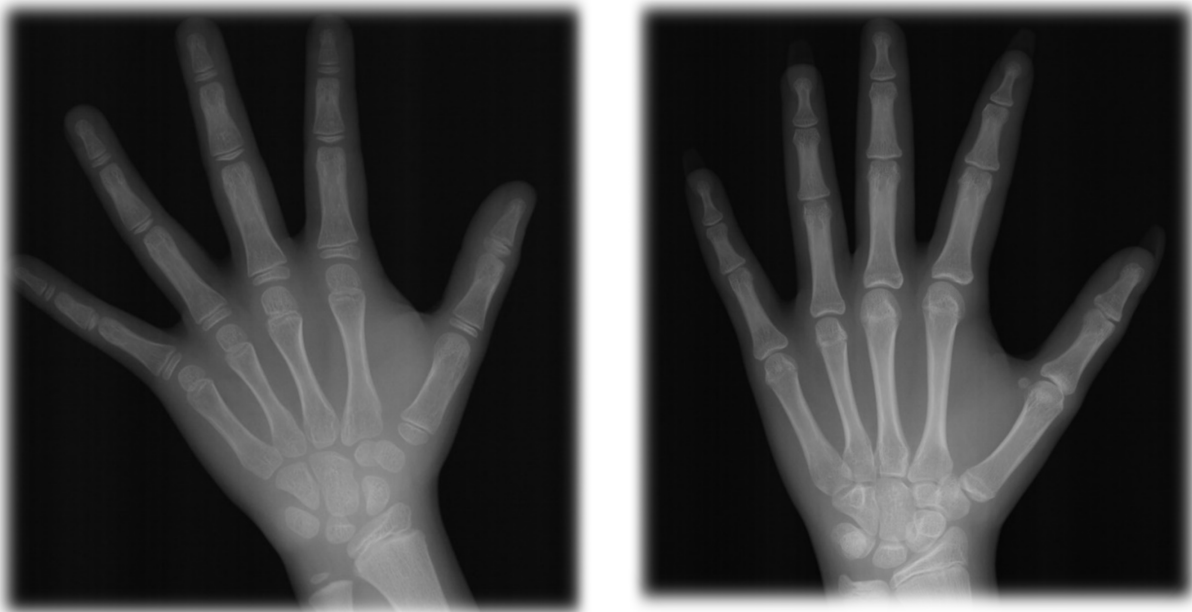

Figure 3.3: Typical hand-wrist radiograph of the left hands of study subjects.

The standard radiographs of the same sex were used for the corresponding subject. The standard of the corresponding sex and chronologic age closest to that of the subject was visually assessed for similarity to the subject's hand-wrist radiograph. The preceding and subsequent standard films were evaluated. Upon finding the closest match, the skeletal age was assigned to each carpal, metacarpal, phalangeal bone, and the distal ends of the ulna and radius. Any bone deviating much ins developmental stage was provided with a skeletal age from a different standard than visually provided the closest match. The child's overall skeletal age was obtained from the average skeletal age of all applicable bones for that particular patient.

Skeletal age determination can be used to estimate if peak growth was to occur within the next 12 months that the film was acquired. Relatively high levels of IGF-1 characterize puberty and the peripubertal periods compared to childhood levels. The skeletal ages of the Insubria cohort were provided. Still, the collaborators could not provide the associated original records or the reliability data at the time that this manuscript was written.

Section 3.2.4: Polysomnography

The patients were sent home with a level IV portable monitoring device, the Embletta MPR (Natus Neurology), for a sleep pattern study at the end of the T1 and T2 appointments. The portable monitoring device used measured oxygen saturation (via pulse oximetry) and airflow via nasal cannula. In the study, children's parents or caregivers were provided instructions and demonstrations on the placement of recording leads and Nexcare™ Sensitive Skin Tape to secure the leads. The parents were asked to perform the recording on a single night with a high likelihood of a sleep duration of 8 hours. The sleep study recordings were sent to one certified sleep physician in Italy for interpretation. The Canada cohort's sleep pattern studies were not ready at the time of writing of this manuscript, and the AHI outcome was removed for the pooled and Canada only cohort subanalyses.

CHAPTER 4

Results

Section 4.1: Recruitment and Participant Flow

Participants were admitted into the study from November 2016 onwards for the Canadian cohort. The cutoff date for inclusion in data analyses was set as November 30, 2020. All included participants had data available for both time points. Due to government mandated restrictions and the fallout from COVID19, 4 participants from the Canadian cohort had their T2 appointment deferred by four months. The trial was still ongoing at the time of writing.

Thirty-three children were randomized in the Canadian cohort, with 13 assigned to the control and 20 into the treatment group. One child in the treatment group dropped out due to the inability to travel to the University and all other children completed the trial. For the Italian cohort, 32 children were randomized; 16 into the control and 16 into the treatment group, with all children completing the trial.

Figure 4.1 summarizes the flow of participants. For the primary analysis, the final sample consisted of 63 children. Ancillary statistical analyses of each country's cohort were done with n = 31 and n = 32 for the Canadian and Italian samples, respectively.
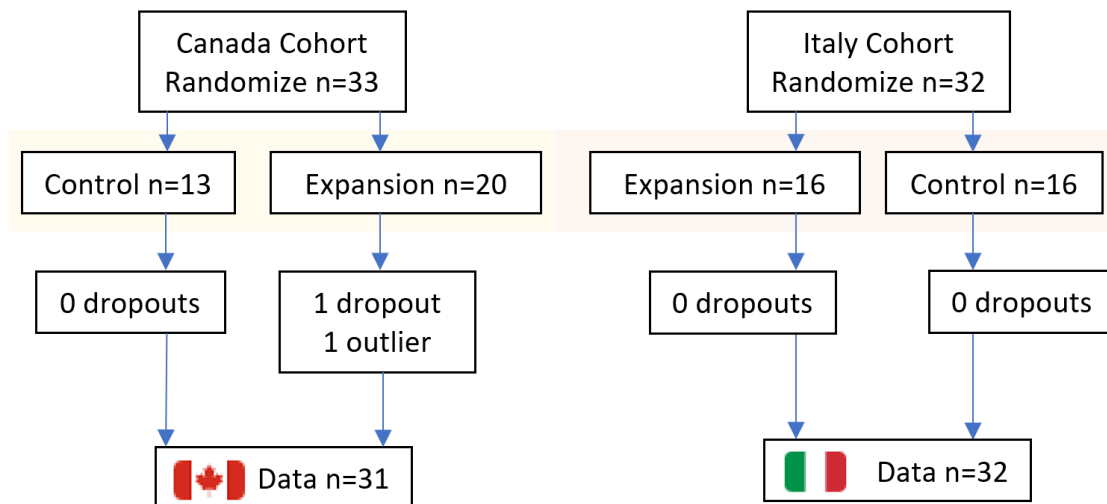

Figure 4.1. Summary of participant flow in trial. All subjects completed the trial in the Italy cohort. There was one dropout in the Canadian cohort, and one had an extreme baseline IGF-1 level and both were removed from the final analyses.

Section 4.2: Baseline Data

Two participants were removed from the Canadian sample – one dropout and one outlier. All AHI scores were also not available for the Canadian cohort. The number of participants based on sex, country, and treatment group are shown below in Table 4.1. Baseline characteristics for the pooled cohort are displayed in Tables 4.2 and 4.3. Results are shown as mean [95% confidence interval] unless otherwise indicated. Baseline features divided by country are available in Appendix Tables 4.4 to 4.6.

Table 4.1: Distribution of Male and Females by Group Type							
Group	Male (%)	Female (%)	Total	Group	Male (%)	Female (%)	Total
Canada	13 (42)	18 (58)	31	Control	15 (52)	14 (48)	29
Italy	16 (50)	16 (50)	32	Expansion	14 (41)	20 (59)	34
Total	29 (46)	34 (54)	63	Total	29 (46)	34 (54)	63

Table 4.2: Mean Values of Baseline Characteristics Between Treatment Groups			
Treatment Group	Control	Expansion	p-value
Chronologic Age (years)	9.8 [9.4, 10.2]	9.4 [8.9, 9.9]	0.170
Bone Age (years)	9.8 [9.2, 10.3]	9.3 [8.8, 9.8]	0.173
T1 BMI (Kg/m^2)	19.1 [17.8, 20.5]	17.4 [16.4, 18.5]	0.048
T1 [IGF-1] (µg/L)	169 [143, 196]	195 [172, 218]	0.134
Max deficiency (mm)	4.5 [3.5, 5.5]	3.6 [2.8, 4.5]	0.194

Table 4.3: Pairwise Comparisons of Baseline Statistics between Country Cohorts		
Baseline Variables	Mean Difference between Italy and Canada [95% CI]	p-value
Maxillary Deficiency	2.7 [1.5, 3.8] mm	< 0.001
Chronologic Age	-0.1 [-0.6, 0.7] years	0.865
Bone Age	-0.3 [-1.0, 0.4] years	0.399
BMI	0.2 [-1.3, 2.1] kg/m^2	0.613
[IGF-1]	5 [-30, 40] µg/L	0.770

Section 4.3: Reliability

Reliability analyses for skeletal age and maxillary deficiency amount were not available for the Italian cohort due to one the involved researchers moving away. For the Canadian sample, using the lower limits of agreement, the author demonstrated good reliability for determining

skeletal age and estimating maxillary deficiency with single measures intraclass correlation coefficients of 0.951 [0.816, 0.988] and 0.979 [0.936, 0.993] respectively. The author and his supervisor's interrater reliability was 0.940 [0.812, 0.981] for average measures for maxillary deficiency, indicating good agreement. See Appendix Tables 4.7 and 4.8.

Fisher's Exact Test found no significant differences in proportions of male to female between countries (p = 0.616) or treatment groups (p = 0.454). The same net result was found even if all original patients were included (data not shown).

Multivariate normality was not satisfied. A follow-up assessment of univariate normality found that one patient in the Canadian cohort had an unusually high initial IGF-1 level of 671 µg/L. A similarly aged patient's normal range would be 134 to 454 µg/L according to the assay kit used by the DynaLIFE Medical Labs (Alberta Health Services, 2020). Removal of this patient satisfied multivariate normality – all further statistical analyses excluded this patient.

MANOVA testing with follow-up pairwise comparisons found all baseline characteristics similar between Canada and Italy's cohort except for maxillary deficiency. There was overwhelming evidence that Italian sample had on average 2.7 [1.5, 3.8] mm (p < 0.001) more constriction than the Canadian children (Table 4.3). The cohorts were considered dissimilar overall and were not pooled. No statistically significant differences in baseline characteristics between the control and expansion groups were found in the Canadian cohort, and the Italian cohort except for T1 maxillary deficiency (p = 0.049) with the control group having a greater mean value of 6.1 [5.0, 7.2] mm versus the expansion group's 4.6 [3.5, 5.7] mm (Appendix Table 4.7.3).

The effects of maxillary expansion treatment, time, and sex on serum IGF-1 levels while controlling for covariates were evaluated. Interactions between the factors of "Time", treatment group ("Group"), and sex were of particular interest – in that the outcome variable mean [IGF-1] ("[ ]" represents "concentration") may vary depending on the level of the

independent variables (also known as between-subjects factors such as treatment group and sex or within-subjects factors like time).

The Canadian cohort did not have the AHI scores ready at the time of manuscript writing. In contrast to the Italy cohort, the Canadian participants had bloodwork that additionally measured serum calcium, calcidiol, total alkaline phosphatase and inorganic phosphate. In addition to the dissimilarity at baseline, subgroup analyses were warranted to examine the effect of treatment on AHI scores in the Italy cohort and the possibility that the additional serum metabolites measured in the Canadian cohort were covariates that influenced IGF-1 levels. Planned analyses are shown below (Figure 4.2).
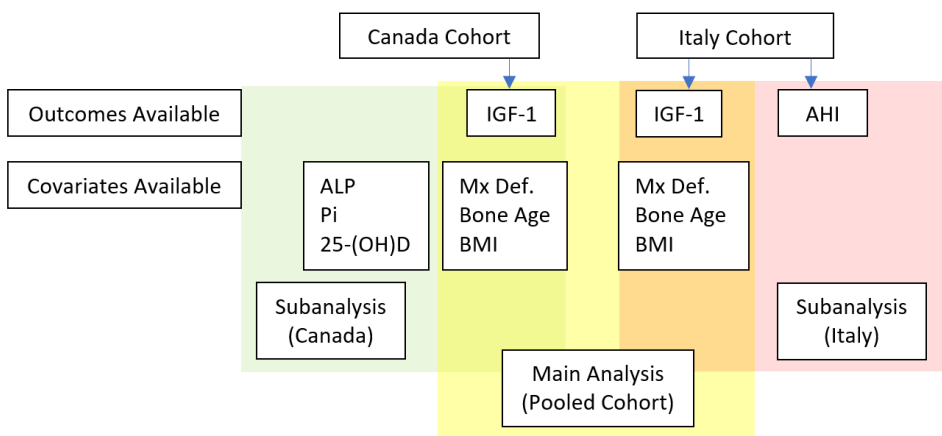
Figure 4.2: Main and subgroup analyses divided by the outcome variable(s) and covariates. Calcidiol is represented as 25-(OH)D. Pooled analyses were not performed.

Section 4.4: Effect of Treatment and Time on Mean [IGF-1], while Controlling for Covariates - Canada Cohort

The Canadian cohort had additional serum biomarkers in the blood samples analyzed than the Italian cohort which may be potential covariates that need to be controlled. Homeostasis requires that the serum levels of calcium and inorganic phosphate stay relatively constant regardless of age. Also, linearity was not displayed with the outcome variable and they were

not included in the list of candidate covariates. Total alkaline phosphatase (ALP) and calcidiol were additional measures available only for the Canadian cohort. Due to their involvement with bone metabolism, they were considered potential covariates that may contribute to the variance of serum [IGF-1]. The hypotheses for this subgroup analysis are found in Appendix Table 4.7.8.

No linearity was seen for [IGF-1] and BMI, ALP, and 25-(OH)D, while linearity for bone age and maxillary deficiency was observed, and these variables were used as covariates. Assumptions of equal covariance matrices (Box's M test p = 0.112) and equality of variances were confirmed with Levene's Test for IGF-1 levels at both time points (p > 0.05).

There was no significant three-way interaction between Time*Group*Sex (p = 0.992) on serum IGF-1. All two-way interactions were non-significant (p > 0.05). The main effects of "Time", treatment group, and sex were evaluated for effects on IGF-1. "Time" had a statistically significant main effect of for serum IGF-1 (p = 0.007, partial η^2 = 0.255) as did treatment group (p = 0.020, partial η^2 = 0.199). The significant interaction required follow-up testing.

Pairwise comparisons were run to assess [IGF-1] differences between T1 and T2 and control and treatment groups with the Bonferroni adjustment. No significant difference between the treatment group and the control group at T1 was found (mean 37 [-6, 80] µg/L p = 0.087), but a significant difference between the two groups at T2 was seen (mean 78 [17, 138] µg/L p = 0.014). The estimated mean [IGF-1] at T2 while controlling for covariates is 268 [228, 308] µg/L for the expansion group compared to 190 [146, 235] µg/L for the control group (Appendix Tables 4.7.13 to 4.7.16). The difference in means at T2 had a large effect size (Cohen's d = 0.96).

For the control group, there was a significant difference in mean [IGF-1] between T2 and T1, with a mean difference of 35 [2, 68] µg/L (p = 0.038) more for T2. For the expansion group, there was a mean difference of 75 [46, 105] µg/L (p < 0.001) greater for T2 than T1. The profile

plot in Figure 4.4 shows that in the Canadian cohort, the non-treatment control children underwent an increase in serum IGF-1 just through elapsing time; however, the children receiving expansion treatment experienced a greater increase in mean [IGF-1] while controlling for covariates. In this statistical model, the covariates were significant contributors to the variance of serum IGF-1, but not independently. Maxillary deficiency in conjunction with "Time" explained 15.6% (p = 0.042) of the [IGF-1] variance while bone age with "Time" explained 40.6% (p < 0.001) of the variance.
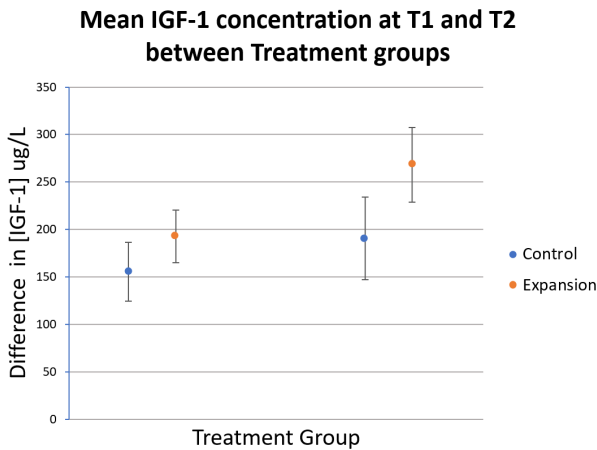
Figure 4.3: Estimated marginal means of [IGF-1] between T1 and T2, categorized by treatment groups. Bars represent 95% confidence intervals. Data from the Canadian cohort.
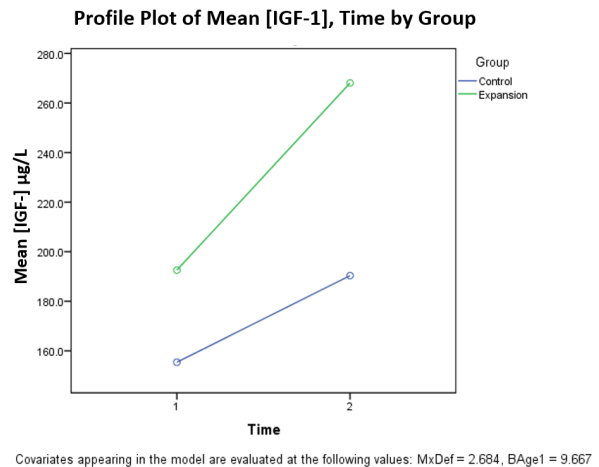
Figure 4.4 Profile plot of the mean [IGF1] of Time by Treatment Group with Control group as blue and Expansion as Green. Data from the Canadian cohort.

Section 4.5: Effect of Treatment and Time on Mean [IGF-1] and AHI while Controlling for Covariates – Italy Cohort

The hypotheses set up is found in Appendix Table 4.7.8. Bone age and maxillary deficiency were selected as covariates due to linearity. There were inequalities of covariance matrices (Box's M test p < 0.001) and of variances for [IGF-1] at T1 and T2 (p = 0.026, p = 0.015,

respectively). Statistical tests used were robust due to equal sample sizes. A three-way repeated measures MANCOVA was run to determine if there are any significant three-way interactions affecting the combined outcome variables and to control the type I error rate.

There was no significant three-way interaction between Time*Group*Sex (p = 0.434) on the combined outcome variables. There was a significant two-way interaction between Time*Group (p < 0.001, partial η^2 = 0.694) on the combined outcome variables. All other two-way interactions were non-significant (p > 0.05). Follow-up univariate interaction effects were evaluated with three-way repeated measures ANCOVA tests done to determine which outcome variable had a significant Time*Group interaction.

There were statistically significant interaction effects between Time*Group for serum IGF-1 concentration (p = 0.011, partial η^2 = 0.222) and AHI score (p < 0.001, partial η^2 = 0.660). For serum IGF-1, follow up testing was run and simple main effects were evaluated. Pairwise comparisons between IGF-1 levels at T1 and T2 with the Bonferroni adjustment found no significant differences in the control group between T1 to T2, with a mean difference in IGF-1 of -3 [-15, 8] µg/L (p = 0.563), but a significant difference between in the expansion group with a mean difference 26 [14, 37] µg/L (p < 0.001) greater at T2 (Figure 4.6).

When comparing differences in mean serum IGF-1 at different time points, no significant differences were seen between control and treatment group at both time points. In this statistical model (used for subanalyses of both outcome variables), maxillary deficiency was set to 5.3 mm and bone age at 9.4 years. At T1, the difference between expansion and control groups was 8 [-50, 66] µg/L (p = 0.780) while at T2 the difference was 31 [-32, 93] µg/L (p = 0.324). The estimated marginal means for [IGF-1] at T2 were 216 [174, 258] µg/L for the treatment group and 185 [143, 227] µg/L for the control group while controlling for covariates. In this model no covariates were significant contributors to the variance of serum IGF-1 (p > 0.05).

Pairwise comparisons between AHI score found statistically significant differences. When comparing AHI scores at T2 and T1, the control group had a significant increase in AHI score of 0.7 [0.1, 1.2] events/hour (p = 0.019) while the expansion group had a significant decrease of -2.1 [-2.6, -1.6] events/hour (p < 0.001). The T2 estimated marginal mean for AHI score for the control group was 2.9 [2.3, 3.5] events/hour compared to 1.4 [0.7, 2.0] events/hour for the expansion treatment group (Appendix Tables 4.7.17 to 4.7.21). The difference in means is suggestive of a very large effect size (Cohen's *d* = 1.20). In this model no covariates were significant contributors to the variance of AHI score (p > 0.05).
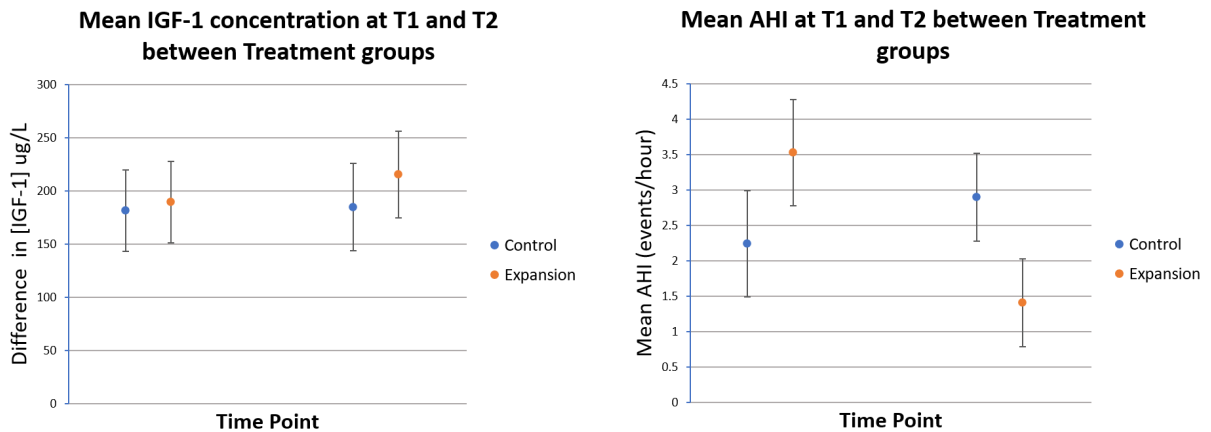
Figures 4.5: Mean [IGF-1] (left) and AHI score (right) between treatment groups. Bars represent 95% confidence intervals. Data from the Italian cohort.

The profile plots illustrate that in the control children with elapsed time but no expansion treatment, the mean [IGF-1] increases slowly while the AHI score worsens (increases). With passing time *and* with expansion treatment, the mean [IGF-1] increases significantly more and the AHI value decreases, representing an improvement.
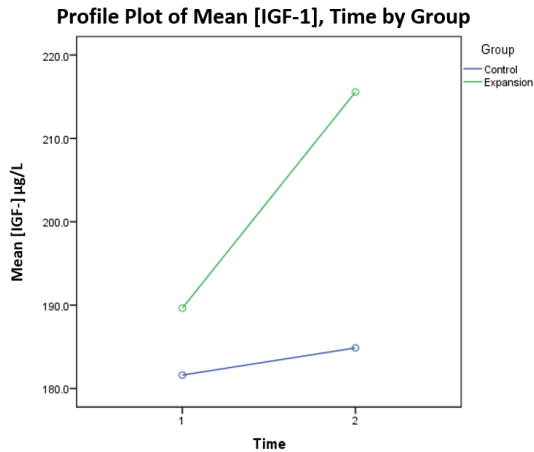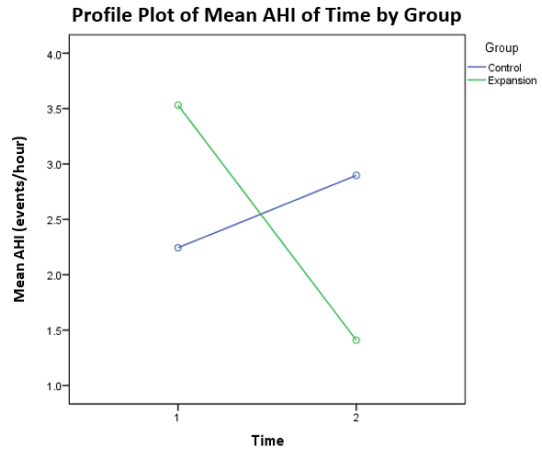
Figures 4.6: Left: Profile plot of the mean [IGF1] of Treatment Group by Time with blue as Timepoint 1 (T1) and green as Timepoint 2 (T2). Right: Profile plot of mean AHI of Time by Treatment Group with control group as blue and expansion group as green. Both plots suggest that there is a significant interaction between time and treatment. Children who undergo maxillary expansion experience a greater increase in serum IGF-1 concentration and a reduction in AHI score. Non-treatment control children experience a slower increase in serum IGF-1 concentration and an increase in AHI score. Data from the Italian cohort.

Section 4.6: Adverse Effects

Maxillary expansion is a routine orthodontic procedure to resolve the problem of a constricted maxilla in growing individuals. It is accepted that the success rate for skeletal improvement of the transverse maxillary dimension decreases after the pubertal growth spurt and becomes unsuccessful in most individuals in adulthood due to the mature intermaxillary suture. There were no adverse effects incurred to the expansion treatment children in this study due to maxillary expansion.

Maxillary transverse deficiency may sometimes lead to a functional shift of the mandible into a position of more stable dental occlusion. This may lead to facial asymmetry with the mandible resting in a more lateral and/or protruded position. It has been generally accepted that if left untreated, this persistent functional shift may lead to permanent skeletal changes later in life. There is no data provided in this study with regard to facial asymmetry or the presence of a

functional shift in the sample population. While it is conceivable that the deferred treatment

may result in prolonging an existing functional shift, it is important to realize that the sample

children are all prepubertal and have much growth potential remaining, which could be utilized

to correct the transverse dimension and improve any existing skeletal asymmetry.

The benefit of early maxillary expansion is that a major problem in one of the three dimensions

(the others being the vertical and sagittal dimensions) is resolved prior to comprehensive

orthodontic treatment if the patients are compelled to do so later on in life. Alternatively,

deferring treatment as in the control children to a later point in life reduces the overall time in

orthodontic appliances, which reduces the possibility of problems with patient compliance or

oral hygiene related issues stemming from intraoral appliances. It is possible to provide

maxillary expansion treatment simultaneously with fixed orthodontic appliances.

Section 4.7: APPENDIX

Prior to comparing the baseline features of the Italian and Canadian cohorts statistically, the

assumptions for statistical tools were checked. Multivariate normality was assessed visually with

scatterplot pairs and statistically with Mahalanobis Distances (MD). If the associated Mahalanobis

distance probability (the p-value) is less than 0.001, this indicates that multivariate normality is not

satisfied. As a rule of thumb, multivariate normality takes precedence over the individual variables'

univariate normality. Univariate normality was done as a follow-up assessment with Kolmogorov-

Smirnov testing and visually with box plots and scatterplots in the event multivariate normality

were not satisfied.

Patient 27 (Canadian Cohort) had a MD probability of $p < 0.001$ and did not satisfy multivariate

normality. Boxplots revealed it was her initial [IGF-1] that was abnormally high and considered an

outlying point. Multivariate normality was not satisfied for patient 27 (Canadian cohort) due to a

[IGF-1] of 671 µg/L, considered abnormal according to 3 different references. The normal range

provided by the respective analyzing lab (DynaMed Life) is 134 to 454 µg/L for a 12-year-old female

[Alberta Health Services, 2020]. Upon removal of patient 27, multivariate normality was satisfied. All subsequent statistical analyses excluded patient 27. See Appendix Figures 4.7.1 to 4.7.3.

Cohorts were assessed for mean differences for chronologic age, bone age, [IGF-1], BMI, and maxillary deficiency amount at an initial time point using a one-way (1 between-subject factor) MANOVA testing.   The between-subjects factor was set as cohort Country. MANOVA assumptions of independence, linearity, multivariate normality, homogeneity of covariance matrices and variances, and sample size adequacy were assessed. Multivariate normality (MD probabilities), independence (randomized design, each subject does not occupy more than one cell), linearity (scatterplot), homogeneity of covariance matrices (Box's M Test p = 0.340), and sample size adequacy (using the rule of thumb of 20 x p intended outcome variables, which in this case would be 1 or 2 based on our future planned tests) assumptions were satisfied.

Equality of variances was satisfied for all comparative outcome variables, with the smallest Levene's Test p value of 0.064. The MANOVA overall test had a p-value = 0.002 (Wilk's λ), and follow-up pairwise comparisons showed that only the maxillary deficiency amounts differed significantly between the cohorts.

Table 4.4: Descriptive Statistics by Country Cohort				
Variable	**Italy, Mean [95% C.I.]**		**Canada, Mean [95% C.I.]**	
	Control	**Expansion**	**Control**	**Expansion**
Chronologic Age (years)	9.8 [9.4, 10.3]	9.3 [8.7, 10.0]	9.8 [9.0, 10.6]	9.5 [8.7, 10.3]
Bone Age (years)	9.7 [9.2, 10.2]	9.0 [8.5, 9.6]	9.9 [8.8, 11.0]	9.5 [8.7, 10.4]
T1 BMI (Kg/m²)	19.5 [17.7, 21.3]	17.3 [15.8, 18.8]	18.6 [16.3, 21.0]	17.6 [15.9, 19.2]
T1 [IGF-1] (µg/L)	176 [133, 218]	196 [161, 230]	161 [127, 196]	195 [161, 228]
Max deficiency (mm)	6.1 [4.9, 7.3]	4.6 [3.5, 5.6]	2.5 [1.4, 3.7]	2.8 [1.5, 4.1]
Trial Duration (years)*	1.1 [1.1, 1.2]	1.3 [1.2, 1.3]	1.2 [1.1, 1.3]	1.3 [1.2, 1.4]

*Trial duration is the time elapsed from T1 to T2.

Table 4.5: Mean Values of Baseline Characteristics Between Treatment Groups – Canada Cohort			
Treatment Group	**Control**	**Expansion**	**p-value**
Chronologic Age (years)	9.8 [9.0, 10.6]	9.5 [8.8, 10.2]	0.560
Bone Age (years)	9.9 [8.9, 10.6]	9.5 [8.7, 10.4]	0.573
T1 BMI (Kg/m²)	18.6 [16.6, 20.6]	17.6 [15.9, 19.2]	0.419
T1 [IGF-1] (µg/L)	161 [125, 197]	195 [164, 225]	0.161
Max deficiency (mm)	2.5 [1.2, 3.9]	2.8 [1.6, 3.9]	0.763

- Box's Test p = 0.473. Levene's Test for all variables p > 0.05.

Table 4.6: Mean Values of Baseline Characteristics Between Treatment Groups – Italy Cohort			
Treatment Group	**Control**	**Expansion**	**p-value**
Chronologic Age (years)	9.8 [9.3, 10.3]	9.3 [8.8, 9.8]	0.126
Bone Age (years)	9.7 [9.2, 10.2]	9.0 [8.5, 9.5]	0.063
T1 BMI (Kg/m^2)	19.5 [17.9, 21.1]	17.3 [15.7, 18.9]	0.053
T1 [IGF-1] (µg/L)	177 [139, 213]	197 [159, 233]	0.442
Max deficiency (mm)	6.1 [5.0, 7.2]	4.6 [3.5, 5.7]	0.049

- Box's Test p = 0.633, Wilk's Lambda p = 0.080, partial η^2 = 0.301. Levene's Test for all variables p > 0.05.

Table 4.7: Maxillary Deficiency Reliability Measures - Canadian Cohort		
	Intrarater Reliability (Consistency)	**Interrater Reliability (Absolute)**
	Intraclass Correlation [95% CI]	Intraclass Correlation [95% CI]
Single Measures	0.979 [0.936, 0.993]	0.884 [0.686, 0.961]
Average Measures	0.989 [0.967, 0.997]	0.939 [0.814, 0.980]

Table 4.8: Skeletal Age Determination - Reliability Measures - Canadian Cohort		
	Intrarater Reliability (Consistency)	
	Intraclass Correlation [95% CI]	
Single Measures	0.951 [0.816, 0.988]	
Average Measures	0.975 [0.899, 0.994]	
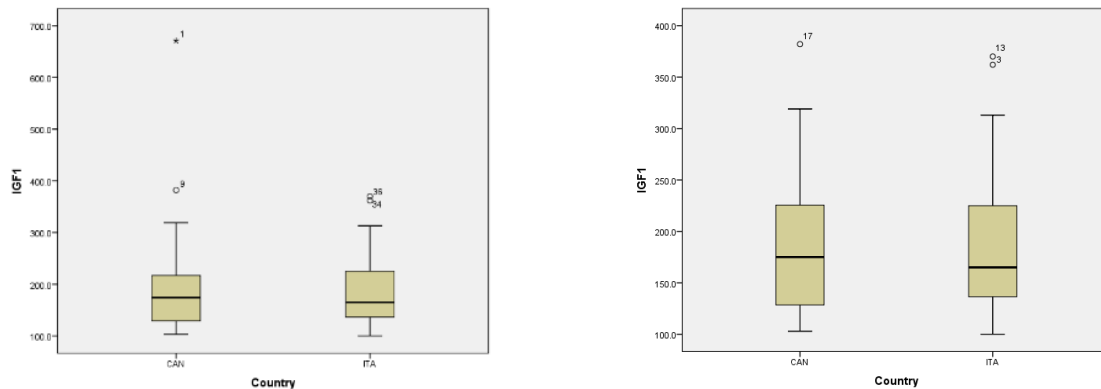
Figure 4.7: Left) is a boxplot of [IGF-1] at T1 for Italian and Canadian cohorts. The outlier (starred, #1) is patient 27 with an [IGF-1] of 647 µg/L. Right) is the plot for both cohorts after patient 27 was removed.
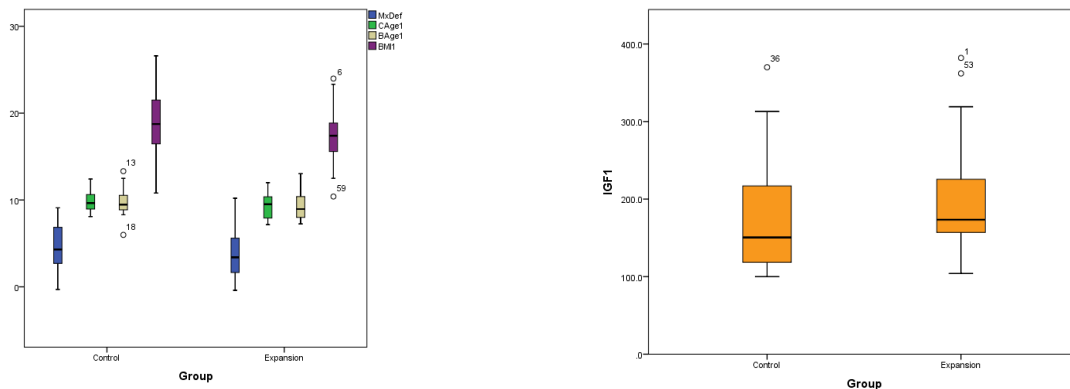
Figure 4.8: Box plots of mean maxillary deficiency, chronologic age, bone age, BMI, and IGF-1 concentration at the start of the trial.
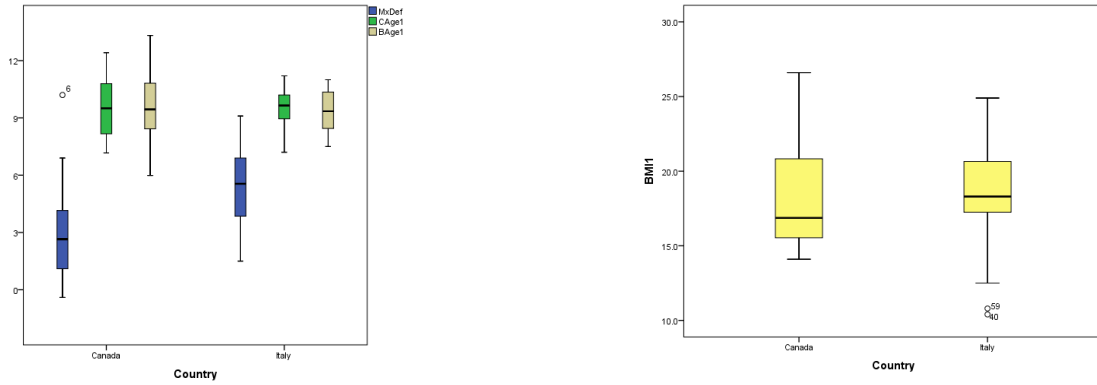
Figure 4.9: Boxplots of chronologic age, bone age, and BMI at an initial time point (T1) for both country cohorts.
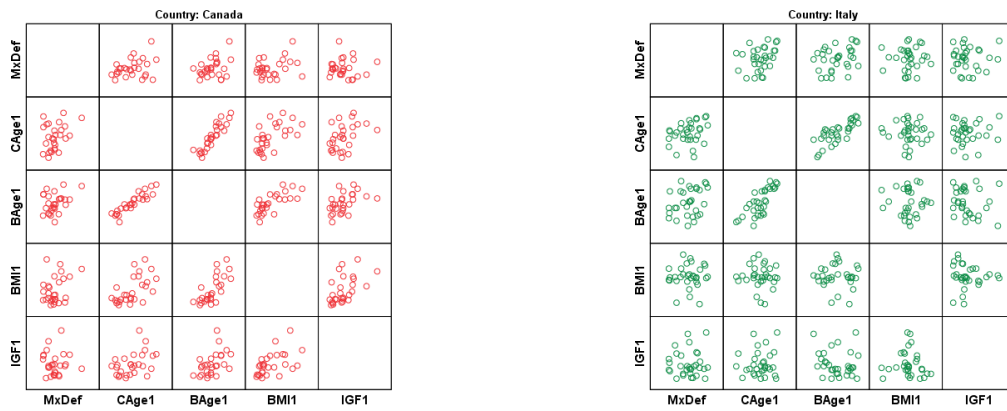
Figure 4.10: Left: Scatter plot pairs for interest variables for the Canadian cohort. Right: Scatter plot pairs for variables of interest for the Italian cohort.
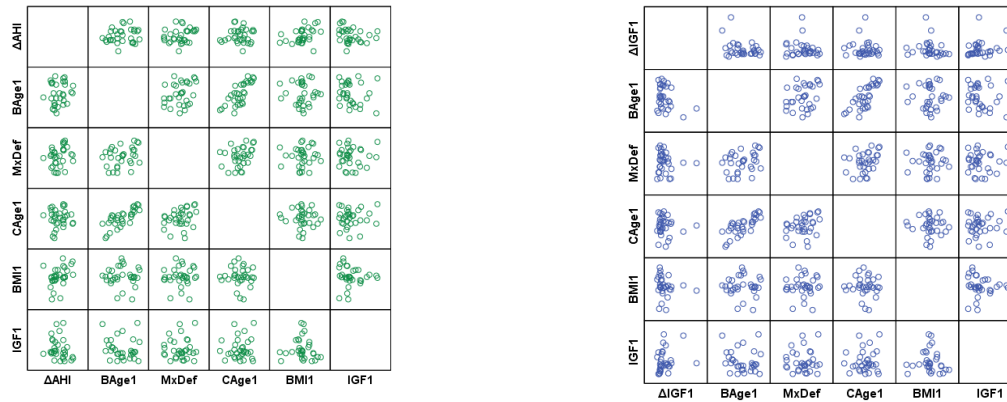
Figure 4.11: Scatterplots of the outcome variables Δ[IGF-1] and ΔAHI with potential covariate candidates showed that bone age and maxillary deficiency were roughly linear with Δ[IGF-1], while bone age, maxillary deficiency, and BMI appeared to have linear relationships with ΔAHI. Italian Cohort only.
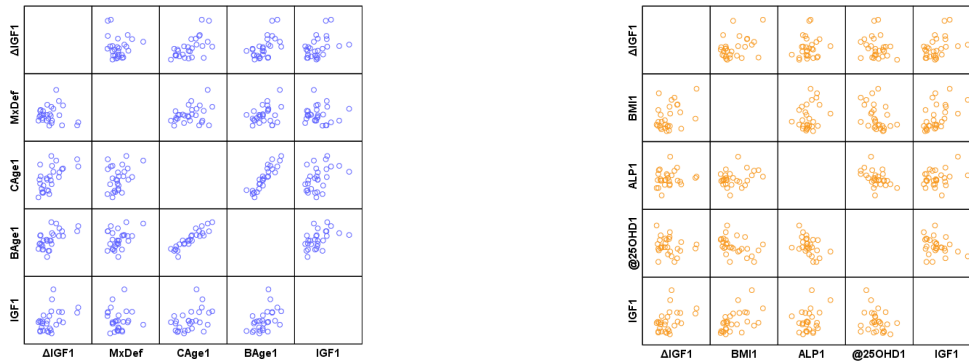
Figure 4.12: Scatterplots of Δ[IGF-1] with potential covariate candidates showed that bone age and initial [IGF-1] were roughly linear with Δ[IGF-1]. Canadian Cohort only.

Table 4.9: Pairwise Comparisons of Baseline Statistics between Country Cohorts		
Baseline Variables	Mean Difference between Italy and Canada [95% CI]	p-value
Maxillary Deficiency	2.7 [1.5, 3.8] mm	< 0.001
Chronologic Age	-0.1 [-0.6, 0.7] years	0.865
Bone Age	-0.3 [-1.0, 0.4] years	0.399
BMI	0.2 [-1.3, 2.1] kg/m^2	0.613
[IGF-1]	5 [-30, 40] µg/L	0.770

Table 4.10: Hypotheses Setups for Sections 4.3	
Hypotheses for Section 4.3	
Set 1	H$_o$: There is no difference in the means of [IGF-1] at T1 and T2 with the covariates of bone age and maxillary deficiency between the control and expansion treatment groups.
	H$_a$: There is a difference in the means of [IGF-1] at T1 and T2 with the covariates of bone age and maxillary deficiency between the control and expansion treatment groups.
Set 2	H$_o$: There is no interaction between factors (treatment group, sex, time) on the mean [IGF-1].
	H$_a$: There is an interaction between factors (treatment group, sex, time) on the mean [IGF-1].

| Table 4.11: Hypotheses Setups for Sections 4.4	
Hypotheses for Section 4.4.1 (Italy Cohort)	
Set 1	H$_o$: There is no difference in the means of [IGF-1] or AHI between T1 and T2 with the covariates of bone age and maxillary deficiency between the control and expansion treatment groups.
	H$_a$: There is a difference in the means of [IGF-1] or AHI between T1 and T2 with the covariates of bone age and maxillary deficiency between the control and expansion treatment groups.
Set 2	H$_o$: There is no interaction between factors (treatment group, sex, time) on the mean [IGF-1] or AHI.
	H$_a$: There is an interaction between factors (treatment group, sex, time) on the mean [IGF-1] or AHI.
Hypotheses for Section 4.4.2 (Canada Cohort)	
Set 1	H$_o$: There is no difference in the mean of [IGF-1] between T1 and T2 with the covariates of bone age and maxillary deficiency between the control and expansion treatment groups.
	H$_a$: There is a difference in the mean of [IGF-1] between T1 and T2 with the covariates of bone age and maxillary deficiency between the control and expansion treatment groups.
Set 2	H$_o$: There is no interaction between factors (treatment group, sex, time) on the mean [IGF-1].
	H$_a$: There is an interaction between factors (treatment group, sex, time) on the mean [IGF-1].

Table 4.12: Combined Cohort 3-Way Repeated Measures ANCOVA						
Effect (Wilk's λ)	Value	F	Hypothesis df	Error df	p-value	Partial η^2
TIME	0.865	8.913	1	57	0.004	0.135
TIME*MxDef	0.846	10.380	1	57	0.002	0.154
TIME*BAge1	0.734	20.624	1	57	< 0.001	0.266
TIME*Group	0.885	7.413	1	57	0.009	0.115
TIME*Sex	0.981	1.085	1	57	0.302	0.019
TIME*Group*Sex	0.999	0.065	1	57	0.800	0.001

Table 4.13: Combined Cohort - Tests of Within-Subjects Effects						
Source (Sphericity Assumed)	Type III Sum of Squares	df	Mean Square	F	p-value	Partial η^2
TIME	9701.539	1	9701.539	8.913	0.004	0.135
TIME*MxDef	11298.777	1	11298.777	10.380	0.002	0.154
TIME*BAge1	22449.221	1	22449.221	20.624	0.000	0.266
TIME*Group	8068.925	1	8068.925	7.413	0.009	0.115
TIME*Sex	1180.744	1	1180.744	1.085	0.302	0.019
TIME*Group*Sex	70.461	1	70.461	0.065	0.800	0.001
Error (TIME)	62043.004	57	1088.474			

Table 4.14: Combined Cohort - Tests of Between-Subjects Effects						
Source	Type III Sum of Squares	df	Mean Square	F	p-value	Partial η^2
Intercept	2336.298	1	2336.298	0.223	0.639	0.004
MxDef	6972.269	1	6972.269	0.665	0.418	0.012
BAge1	82376.011	1	82376.011	7.853	0.007	0.121
Group	66120.708	1	66120.708	6.303	0.015	0.100
Sex	15783.727	1	15783.727	1.505	0.225	0.026
Group * Sex	20558.502	1	20558.502	1.960	0.167	0.033
Error	597912.481	57	10489.693			

Table 4.15: Combined Cohort – Pairwise Comparisons with Bonferroni Adjustment				
Comparison (Time by Group)		Difference (mean [95% CI])	Standard Error	p-value
T1	Expansion – Control	31 [-3, 65] µg/L	17.156	0.077
T2	Expansion – Control	64 [20, 109] µg/L	22.234	0.006
Comparison (Group by Time)				
Control	T2 mean – T1 mean	18 [1, 36] µg/L	8.781	0.040
Expansion	T2 mean – T1 mean	52 [35, 68] µg/L	8.227	< 0.001
Group	Time	Mean [95% CI]	Standard Error	
Control	T1	166 [141, 190] µg/L	12.370	
Control	T2	184 [152, 216] µg/L	16.032	
Expansion	T1	196 [173, 220] µg/L	11.591	
Expansion	T2	248 [218, 278] µg/L	15.022	

Table 4.16: Canada Cohort 3-Way Repeated Measures ANCOVA						
Effect (Pillai's Trace)	Value	F^b	Hypothesis df	Error df	p-value	Partial η^2
TIME	0.255	8.558	1	25	0.007	0.255
TIME*MxDef	0.156	4.608	1	25	0.042	0.156
TIME*BAge1	0.406	17.098	1	25	< 0.001	0.406
TIME*Group	0.124	3.532	1	25	0.072	0.124
TIME*Sex	0.012	0.304	1	25	0.586	0.012
TIME*Group*Sex	0.000	0.000	1	25	0.992	0.000

a. Design: Intercept + MxDef + BAge1 + Group + Sex + Group * Sex   Within Subjects Design: Time

b. Exact statistic

Table 4.17: Canada Cohort - Tests of Within-Subjects Effects						
Source (Sphericity Assumed)	Type III Sum of Squares	df	Mean Square	F	p-value	Partial η^2
TIME	13972.707	1	13972.707	8.558	0.007	0.255
TIME*MxDef	7523.791	1	7523.791	4.608	0.042	0.156
TIME*BAge1	27916.273	1	27916.273	17.098	< 0.001	0.406
TIME*Group	5766.008	1	5766.008	3.532	0.072	0.124
TIME*Sex	496.400	1	496.400	0.304	0.586	0.012
TIME*Group*Sex	0.171	1	0.171	0.000	0.992	0.000
Error (TIME)	40818.306	25	1632.732			

Table 4.18: Canada Cohort - Tests of Between-Subjects Effects						
Source	Type III Sum of Squares	df	Mean Square	F	p-value	Partial η^2
Intercept	15753.849	1	15753.849	2.112	0.159	0.078
MxDef	26475.697	1	26475.697	3.549	0.071	0.124
BAge1	172400.048	1	172400.048	23.110	< 0.001	0.480
Group	46289.221	1	46289.221	6.205	0.020	0.199
Sex	7.865	1	7.865	0.001	0.974	0.000
Group * Sex	9624.025	1	9624.025	1.290	0.267	0.049
Error	186502.398	25	7460.096			

Table 4.19: Canada Cohort – Pairwise Comparisons with Bonferroni Adjustment				
Comparison (Time by Group)		Difference (mean [95% CI])	Standard Error	p-value
T1	Expansion – Control	37 [-6, 80] µg/L	20.844	0.087
T2	Expansion – Control	78 [17, 138] µg/L	29.348	0.014
Comparison (Group by Time)				
Control	T2 mean – T1 mean	35 [2, 68] µg/L	15.965	0.038
Treatment	T2 mean – T1 mean	75 [46, 105] µg/L	14.359	< 0.001
Group	Time	Mean [95% CI]	Standard Error	
Control	T1	155 [124, 187] µg/L	15.426	
Control	T2	190 [146, 235] µg/L	21.720	
Expansion	T1	193 [164, 221] µg/L	13.875	
Expansion	T2	268 [228, 308] µg/L	19.536	

Table 4.20: Italy Cohort 3-Way Repeated Measures MANCOVA							
Effect	Pillai's Trace	Value	F^b	Hypothesis df	Error df	p-value	Partial η^2
Between Subjects	Intercept	0.245	4.058	2	25	0.030	0.245
	MxDef	0.088	1.202	2	25	0.317	0.088
	BAge1	0.075	1.017	2	25	0.376	0.075
	Group	0.020	0.252	2	25	0.779	0.020
	Sex	0.114	1.613	2	25	0.219	0.114
	Group*Sex	0.053	0.699	2	25	0.506	0.053
Within Subjects	Time	0.081	1.099	2	25	0.349	0.081
	Time*MxDef	0.009	0.110	2	25	0.897	0.009
	Time * BAge1	0.077	1.045	2	25	0.367	0.077
	Time * Group	0.694	28.311	2	25	< 0.001	0.694
	Time * Sex	0.127	1.817	2	25	0.183	0.127
	Time*Group * Sex	0.065	0.862	2	25	0.434	0.065

a. Design: Intercept + MxDef + BAge1 + Group + Sex + Group*Sex
b. Exact statistic

Table 4.21: Italy Cohort 3-Way Repeated Measures ANCOVA (Univariate, Within Subjects)							
Source (Sphericity Assumed)		Type III Sum of Squares	df	Mean Square	F	p-value	Partial η^2
TIME	IGF	468.968	1	468.968	2.137	0.156	0.076
	AHI	0.087	1	0.087	0.180	0.675	0.007
TIME*MxDef	IGF	46.703	1	46.703	0.213	0.648	0.008
	AHI	0.009	1	0.009	0.019	0.893	0.001
TIME*BAge1	IGF	324.810	1	324.810	1.480	0.235	0.054
	AHI	0.363	1	0.363	0.747	0.395	0.028
TIME*Group	IGF	1632.833	1	1632.833	7.440	0.011	0.222
	AHI	24.467	1	24.467	50.379	< 0.001	0.660
TIME*Sex	IGF	716.644	1	716.644	3.265	0.082	0.112
	AHI	0.217	1	0.217	0.447	0.510	0.017
TIME*Group*Sex	IGF	255.839	1	255.839	1.166	0.290	0.043
	AHI	0.327	1	0.327	0.673	0.419	0.025
Error (TIME)	IGF	5706.111	26	219.466			
	AHI	12.627	26	0.486			

Table 4.22: Italy Cohort 3-Way Repeated Measures ANCOVA (Univariate, Between Subjects)							
Source		Type III Sum of Squares	df	Mean Square	F	p-value	Partial η^2
Intercept	IGF	86049.688	1	86049.688	7.947	0.009	0.234
	AHI	2.998	1	2.998	1.052	0.314	0.039
MxDef	IGF	125.213	1	125.213	0.012	0.915	0.000
	AHI	7.105	1	7.105	2.494	0.126	0.088
BAge1	IGF	22682.039	1	22682.039	2.095	0.160	0.075
	AHI	0.277	1	0.277	0.097	0.757	0.004
Group	IGF	4763.249	1	4763.249	0.440	0.513	0.017
	AHI	0.128	1	0.128	0.045	0.834	0.002
Sex	IGF	35631.214	1	35631.214	3.291	0.081	0.112
	AHI	0.613	1	0.613	0.215	0.647	0.008
Group*Sex	IGF	13772.733	1	13772.733	1.272	0.270	0.047
	AHI	0.245	1	0.245	0.086	0.772	0.003
Error	IGF	281533.058	26	10828.195			
	AHI	74.073	26	2.849			

Table 4.23: Italy Cohort – Pairwise Comparisons with Bonferroni Adjustment for serum [IGF-1]				
Comparison (Time by Group)		Difference (mean [95% CI])	Standard Error	p-value
T1	Expansion – Control	8 [-50, 66] µg/L	28.422	0.780
T2	Expansion – Control	31 [-32, 93] µg/L	30.512	0.324
Comparison (Group by Time)				
Control	T2 mean – T1 mean	3 [-8, 15] µg/L	5.567	0.563
Expansion	T2 mean – T1 mean	26 [15, 37] µg/L	5.567	< 0.001
Group	Time	Mean [95% CI]	Standard Error	
Control	T1	182 [142, 221] µg/L	19.035	
Control	T2	185 [143, 227] µg/L	20.435	
Expansion	T1	190 [151, 229] µg/L	19.035	
Expansion	T2	216 [174, 258] µg/L	20.435	

Table 4.24: Italy Cohort – Pairwise Comparisons with Bonferroni Adjustment for AHI Score				
Comparison (Time by Group)		*Difference (mean [95% CI])*	*Standard Error*	*p-value*
T1	Expansion – Control	1.3 [0.1, 2.4] events/hour	0.558	0.029
T2	Expansion – Control	-1.5 [-2.4, -0.5] events/hour	0.462	0.003
Comparison (Group by Time)				
Control	T2 mean – T1 mean	0.7 [0.1, 1.2] events/hour	0.262	0.019
Expansion	T2 mean – T1 mean	-2.1 [-2.7, -1.6] events/hour	0.262	< 0.001
Group	*Time*	*Mean [95% CI]*	*Standard Error*	
Control	T1	2.2 [1.5, 3.0] events/hour	0.374	
Control	T2	2.9 [2.3, 3.5] events/hour	0.309	
Expansion	T1	3.5 [2.8, 4.3] events/hour	0.374	
Expansion	T2	1.4 [0.8, 2.0] events/hour	0.309	

CHAPTER 5

Discussion and Conclusions

Section 5.1: Study Overview

Our parallel randomized controlled trial was designed to investigate the short-term effect of maxillary expansion on AHI score and average serum IGF-1 concentrations in a population of prepubertal children with maxillary constriction. In addition to measuring bloodwork, variables such as skeletal age and body mass index (BMI) were included for assessment as potential covariates that might have a role in affecting treatment outcomes on serum [IGF-1] and AHI score. It is well known that the levels of IGF-1 steadily increase during the prepubertal childhood years until the peripubertal period is reached after which IGF-1 levels undergo substantial increases associated with the pubertal growth spurt (Juul et al., 1994).

Adenotonsillar hypertrophy (ATH) in children is associated with sleep disordered breathing and growth retardation and procedures that contribute to resolution of the obstruction and improvement of breathing, such as adenotonsillectomy (A&T) have been shown to allow affected children to experience "catch-up growth" compared to untreated control children, on average (Marcus et al., 2013). Decreases in AHI scores and increases in serum IGF-I levels in children after adenotonsillectomy or maxillary expansion have been reported (Venekamp, 2015, Yilmaz et al., 2002).

Maxillary expansion has been proposed as a less invasive alternative to alleviating upper airway restriction and improving sleep outcomes in children with concurrent maxillary skeletal transverse deficiency that is refractory to pediatric OSA treated with T&A who are unable to tolerate continuous positive airway pressure (CPAP) treatment (Camacho et al., 2017).   At the time of writing, there are no published studies that investigated the effects of maxillary expansion treatment on serum [IGF-1] in prepubertal children.

Section 5.1.1: Interpretation of Serum IGF-1 Results – Canada/University of Alberta Cohort

Despite the fact that the Canadian cohort had additional serum biomarkers of inorganic phosphate, calcium, total alkaline phosphatase and calcidiol analyzed as part of the bloodwork assessment, none of these biomarkers met the linearity requirement for use as a covariate for the three-way ANCOVA test. Serum calcium and phosphate are critical to many bodily functions and normally are constant. Fluctuations of these metabolites can cause disruption of homeostasis and lead to conditions such as hypercalcemia (Carroll and Schade, 2003).

Sex was not a significant factor influencing IGF-1 levels and did not it interact with treatment, indicating that the children were prepubertal when sexual dimorphism related to [IGF-1] is not yet apparent (Juul et al., 1994), justifying the evaluation [IGF-1] independently of sex. Children in both control and treatment groups experienced significant increases in IGF-1 compared to baseline with improvements of 23.9% for the control and 40.4% for the treatment group. Yilmaz et al. (2002) examined the change in serum IGF-1 levels in 32 children aged 4 to 8 years with airway obstructive symptoms due to adenotonsillar hypertrophy 3 to 6 months after T&A surgery. These researchers found a 35% increase in serum IGF-1 levels.

The expansion group for the Canadian cohort had a mean increase in IGF-1 that was similar to Yilmaz et al.'s T&A treatment group. The covariates interacted significantly with "Time" to influence the variance of IGF-1 with a similar contribution from maxillary deficiency but greater for initial skeletal age at 40.6%. This suggests that maxillary expansion works synergistically with increasing skeletal maturity by way of elapsing time, to increase serum IGF-1, at least short-term.

Maxillary expansion may in individuals with sleep disordered breathing that is partially due to a narrowed maxilla a tortuous nasal cavity, grant the same effect on serum IGF-1 as T&A surgery due to improvement of upper airway patency. It is possible that the effects may occur sooner

than one year after the start of the trial.   A&T treatment subjects showed improvement of sleep disordered breathing at the end of 7 months in the Marcus et al.'s (2013) randomized controlled trial.
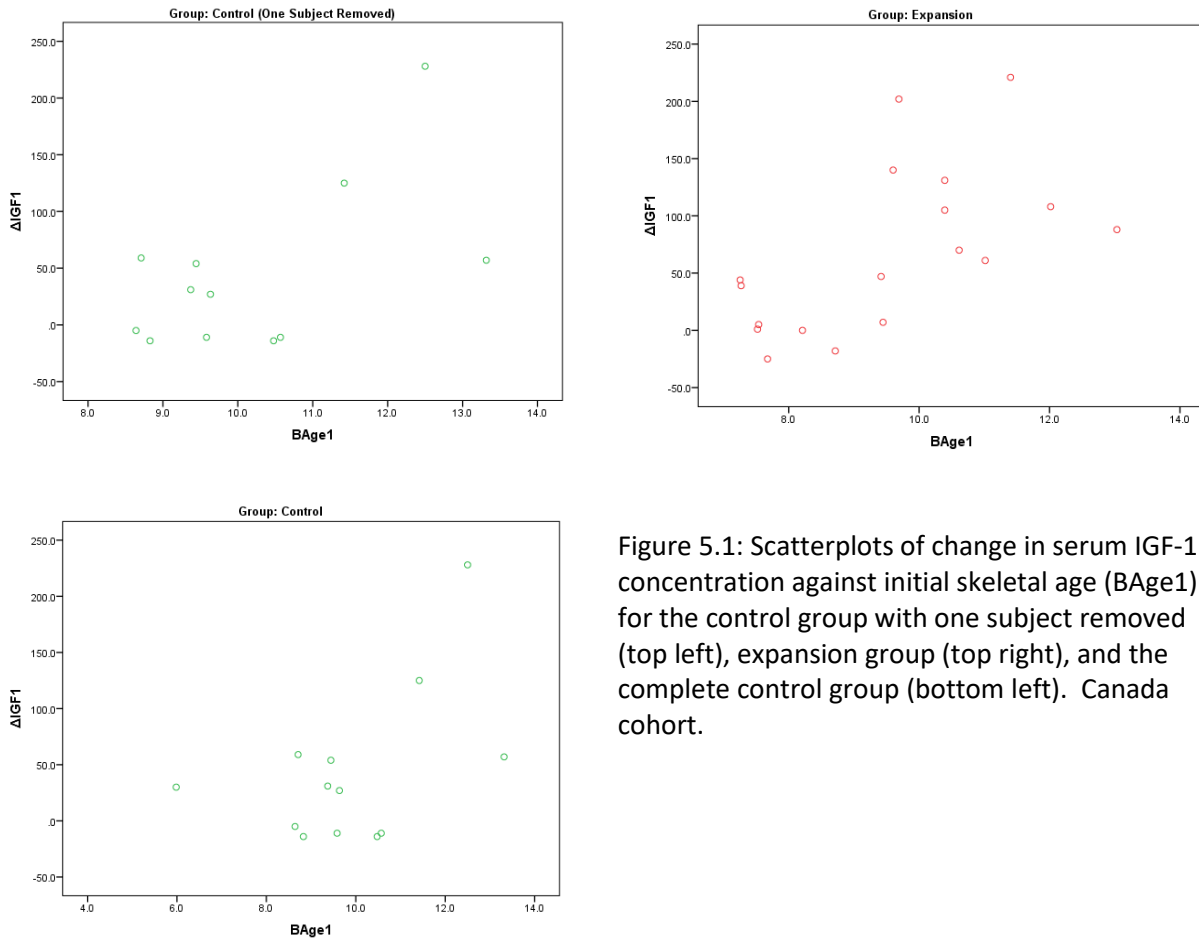

Figure 5.1: Scatterplots of change in serum IGF-1 concentration against initial skeletal age (BAge1) for the control group with one subject removed (top left), expansion group (top right), and the complete control group (bottom left). Canada cohort.

The change in ("Δ") serum IGF-1 outcome incorporates "Time" to simplify interpretation. Initial skeletal age appeared to have a linear relationship with Δ[IGF-1] for *both* treatment groups as illustrated in Figure 5.1. The trend was better seen when a control subject with bone age 6.0 years was removed. In contrast, there was an approximate positive relation between Δ[IGF-1] and maxillary deficiency in only the treatment group (Figure 5.2). It is speculated that the skeletally more mature children are closer to entering puberty, which is normally accompanied by higher and increasing levels of serum IGF-1 compared to much more skeletally immature children, which is corroborated by Kanbur-Oksuz et al. (2004). Taken together, this

suggests that in the Canadian cohort, the children have unimpaired IGF-1 regulatory mechanisms and with passing time, serum IGF-1 increases as expected.

Maxillary expansion in this subgroup only enhances these regulatory mechanisms to significantly increase IGF-1 levels above that of controls. The average baseline maxillary deficiency amount for the Canadian cohort significantly lower by 2.7 mm compared to the Italian cohort, which was the only feature that was significantly different between the two subgroups. This finding will be important in the forthcoming explanation as to why IGF-1 levels are affected differently in the Italian cohort.
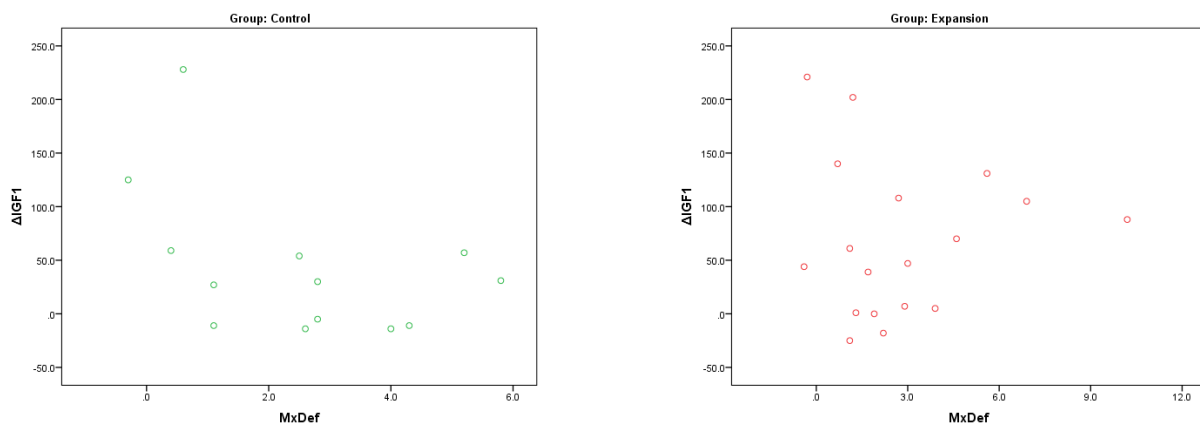

Figure 5.2: Scatterplots of change in serum IGF-1 concentration against initial maxillary deficiency (MxDef) for the control group (left) and the expansion treatment group (right). Canada cohort.

In the Canadian cohort, supplementary analyses found that BMI, calcidiol, and total alkaline phosphatase were not statistically significant contributors to the variance of the mean change in [IGF-1] and were not suitable covariates. Linearity is one requirement for use as a covariate in ANCOVA (or MANCOVA) testing. Furthermore, exploratory analyses using all these variables as covariates showed non-significance in contributing to the variance of [IGF-1].

BMI was not adjusted for age in this study which does not provide an equivalent measure of fatness when comparing across children of different ages despite it being a suitable screening tool in children with excess body fatness (Pietrobelli et al., 1998, Freedman and Sherry, 2009). However, it was used for simplicity of the model after exploratory analysis found that adjusted BMI-for-age-percentile with categorization into underweight, normoweight, overweight, and obese groups did not find any significant trends.

Trummer et al.'s (2017) study found that IGF-I levels did not improve with supplementary vitamin D in their study population. The children in the Canadian cohort had levels of all the additional serum metabolites in the range of normal. IGF-I levels are unaffected by calcidiol, and ALP in this study possibly because the values feel within the range of normal. Children with vitamin D and bone metabolism problems would not have met the inclusion criterion of "lack of diagnosed medical condition". It is important to note that none of the children in this study were diagnosed as having pediatric OSA, however, many were serendipitously found to have mild pediatric OSA from their PSGs.

Section 5.1.2.1: Interpretation of AHI Score Results – Italy/University of Insubria Cohort

Pediatric obstructive sleep apnea (POSA) is diagnosed in children having 1.0 or more AHI events per hour according to the American Academy of Sleep Medicine. It is critical to note that the level IV sleep monitors are less sensitive than the level I polysomnogram (which is the gold standard) due to the inability to differentiate between wake and sleep states so the AHI scores used in this study likely underestimate the severity of disease (Rosen et al., 2017). At T2, the treatment group AHI score was significantly lower than the control group, which experienced a significant increase in AHI which is indicative of likely worsening of the disease. Maxillary expansion resulted in a significant reduction in AHI or improvement of POSA signs and symptoms. Importantly, the final mean AHI score for the treatment group was 1.4 [0.7, 2.0] events per hour meaning that on average, POSA was still present. These results support the

findings of other researchers in that maxillary expansion does not cure pediatric OSA but only reduces the severity, which is consistent with conclusions from a recent systematic review assessing the effect of rapid maxillary expansion on AHI score (Camacho et al., 2017).

The change in AHI score observed in the Italian cohort is consistent with conclusions from other researchers. Camacho et al. (2017) reviewed the literature and found that rapid maxillary expansion resulted in average significant reductions in AHI score from 61% to 73% with the variation attributable to small, large, or mixed sized tonsils with no previous T&A surgery. They also found that after expansion treatment the mean AHI scores were still above one event per hour which indicated pediatric OSA was still present. In Venekamp et al.'s (2015) review, they found that early T&A surgery in non-syndromic children with mild to moderate OSAS compared to no treatment resulted in a greater mean AHI reduction of -4.3 [-5.7, -2.9] events per hour 7 months post-treatment.

Pirelli et al. (2015) followed 23 children who had maxillary expansion to treat pediatric OSA over a 12-year period. They found that immediately after treatment, AHI scores normalized and that results were stable 12 years later. Their study children were not overweight, lacked adenotonsillar hypertrophy, did not receive T&A surgery. They experienced a mean increase in maxillary transverse dimension of 4.3 mm and an increase in nasal pyriform aperture dimension of 1.3 mm. These dimensional increases may contribute to a reduction in upper airway resistance which is a factor in OSA. The Italian treatment group had a reduction in AHI score similar to Pirelli et al.'s (2015) subjects suggesting that the mechanism(s) responsible for the improvement are similar.

No trend between initial skeletal age and change in AHI score was observed in those with or without expansion treatment (Figures 5.3 and 5.4), implying that the change in AHI score is independent of the initial skeletal age. In the control group there is no trend between initial maxillary deficiency and change in AHI score. Spontaneous improvement and worsening of sleep disordered breathing is seen in the control group. In the treatment group, it appears that

the AHI score improves (decreases) more with greater initial severity in maxillary constriction, but it does not appear to be completely linear. These results suggest that maxillary deficiency somehow contributes to POSA, but the exact nature of the interaction is still unknown, which is in agreement with other studies (Camacho et al., 2017).
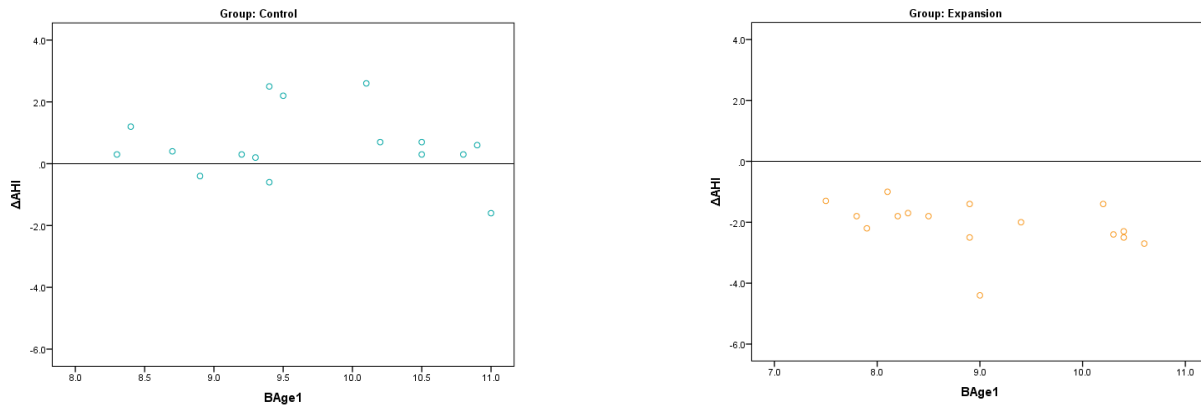

Figure 5.3: Scatterplots of change in AHI score against initial skeletal age (BAge1) for the control group (left) and the expansion treatment group (right). Italy cohort.
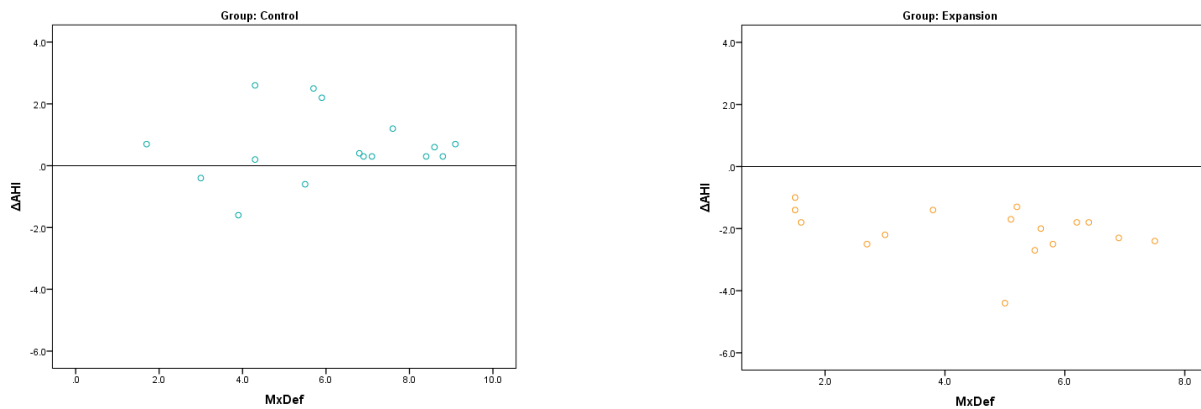

Figure 5.4: Scatterplots of change in AHI score against initial maxillary deficiency (MxDef) for the control group (left) and the expansion treatment group (right). Italy cohort.

In the CHAT trial, 46% of the children who did not undergo treatment (T&A) had normalization of PSG measurements (Marcus et al., 2013). This is different than in the Italian control group where no child had an AHI score below one event per hour at T2. In fact, the control children experienced a statistically significant increase in AHI score which indicates worsening of the sleep disordered breathing with an increase in mean AHI score at T2. This suggests that while

maxillary transverse deficiency and enlarged tonsillar tissue can lead to sleep disordered breathing, their mechanisms are different. Lymphoid tissue does exhibit more rapid growth than other parts of the face and naturally undergoes involution and reduction in size (Pizzato and Flores-Mir, 2018).   Maxillary deficiency does not spontaneously self-correct. The lack of normalization of AHI scores after expansion treatment confirms that there are other factors that contribute to this multifactorial disease.

Section 5.1.2.2: Interpretation of Serum IGF-1 Results – Italy/University of Insubria Cohort

Both treatment groups responded differently to Canadian counterparts. The Italian control group had an insignificant IGF_1 increase of 1.6% relative to baseline from T1 to T2, while the treatment group had a significant increase of 13.7% relative to baseline, which was much lower than the Canadian cohort.   The mean IGF-1 level of the expansion group at T2 did not differ significantly from the control group.

These results suggest that the regulatory mechanisms for IGF-1 are not functioning normally like the Canadian control group and expansion treatment was not able to enhance the existing mechanisms to increase IGF-1 levels.   IGF-1 secretion is regulated positively by GH release – which can be stimulated or suppressed. It is speculated that other factors may be responsible for long lasting effects that may contribute to suppression of the GH/IGF-1 axis in the Italy cohort. This will be discussed further along in the chapter.
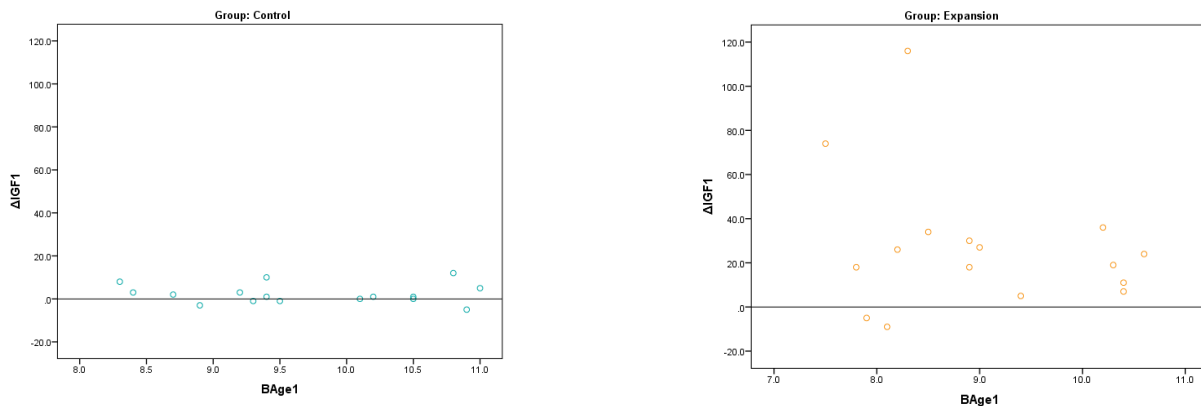

Figure 5.5: Scatterplots of change in serum IGF-1 concentration against initial skeletal age (BAge1) for the control group (left) and the expansion treatment group (right). Italy cohort.
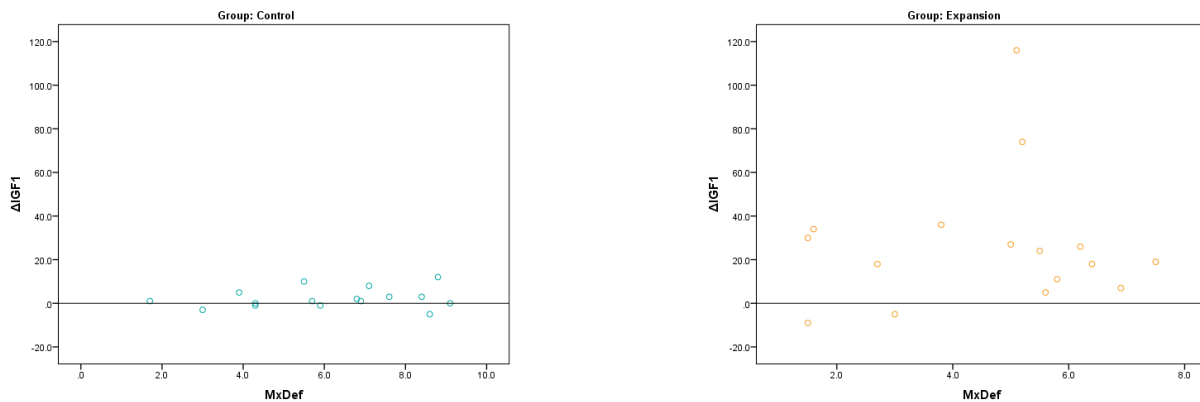
Figure 5.6: Scatterplots of change in serum IGF-1 concentration against initial maxillary deficiency (MxDef) for the control group (left) and the expansion treatment group (right). Italy cohort.

No covariates were statistically significant in explaining the variance of IGF-1. Figures 5.5 and 5.6 illustrate the lack of observable trends between either skeletal age or maxillary deficiency and change in [IGF-1] with no discernable differences between the two treatment groups. An explanation for this may be related to the outcomes of sleep testing. Maxillary expansion resulted in a significant improvement (of large effect size) in AHI score reduction after almost one year. During this same time period, despite having reduced obstructive events during sleep, the normal mechanisms involved in the downstream secretion of IGF-1 were not functioning to the same level as in the control children of the Canadian cohort. This leads to the conjecture that due to the persistent POSA, *or* due to the side effects of POSA which may be long lasting, that the normal pathways involved in IGF-1 secretion remain suppressed.

Granted the AHI scores for the Canadian cohort were not ready at the time of writing, and the presence of pediatric OSA cannot be confirmed, extrapolation from the Italian data could be made overall given the consistent trend that maxillary expansion resulted in a statistically significant increase in IGF-1 concentration. The effects of POSA may be more severe in the Italian cohort as the average baseline maxillary deficiency amount is 5.3 [4.5, 6.1] mm, which is almost double that of the Canadian cohort.

IGF-1 production is stimulated through an increase in GH levels, which is dependent on sleep quality since the bulk of GH is released in deep sleep (Van Cauter and Plat, 1996). Obstructive events during sleep lead to increase sympathetic nervous system activation during sleep thereby decreasing sleep quality which in turn affects GH release negatively. In the Canadian cohort, this may be the primary mechanism by which the maxillary expansion promotes an increase in IGF-1 levels. However, other more chronic side effects may play a larger role for the Italian cohort. One such side effect is low grade chronic inflammation due to long standing hypoxia.   The difference in responses seen between the Canada and Italy cohorts may be due to other factors that were not accounted for in this statistical model. The two clinical sites are geographically different with possibilities of differences in culture, behavior, sleep pattern, physical activity, family social economic status (SES), housing conditions including population density, and history of growth disturbances or childhood illness. Unideal in one or more of these factors may contribute to a GH/IGF-1 axis that may not be functioning at optimal levels.

Camacho et al. (2017) found that after RME, the mean oxygen saturation improved from 87% to 96%. Hypoxia contributes to chronic inflammation by removing the inhibitory mechanism for Hypoxia Inducible Factor (HIF).   Normally HIF is tagged for destruction, but if allowed to exist, this transcription factor upregulates genes responsible for preserving the continued hypoxic conditions as well as promotion of inflammation including attraction of monocytes, increasing neutrophil lifespan, angiogenesis, and inhibiting macrophage mobility which are large a source of inflammatory cytokines (Eltzschig and Carmeliet, 2011). Inflammation and upregulation of stress hormones are inhibitors of GH and IGF-1 secretion. In children with less severe maxillary deficiency, it can be conjectured that the inflammation that results from hypoxia is less severe, so the initial state is more conducive to an increase in secretion of GH and IGF-1. There are potentially less inhibitory elements such as inflammatory cytokines and high or low glucocorticoid levels.

Children with greater maxillary deficiency may have promoted a more severe proinflammatory environment which results in a less favourable starting point. Studies in mice have shown

depending on acute or chronic hypoxia, different compensatory mechanisms are engaged to sustain normal oxygenation of the brain tissues. In chronic hypoxic mice, the capillary density, lengths, and diameters are increased while the capillaries become more tortuous, which differ from acute hypoxic states where the blood flow is redirected and increased to the brain to restore overall oxygenation (Boero et al., 1999).

Upon correction of maxillary deficiency and improvement of obstructive events during sleep, the children with more severe maxillary deficiency may require more time to return to a normative state including potentially the resolution of chronic inflammation. This maybe supported with the finding that the Italian control children did not exhibit any significant changes in serum IGF-1 between T1 and T2 after a period of one year when it is expected that the IGF-1 levels should increase naturally as seen in the Canadian control group. A summary of the proposed mechanism by which maxillary expansion contributes to improvement of AHI score and increase in serum IGF-1 is shown in Figure 5.7.

The results from this study suggest that the effects on IGF-1 release by resolution of maxillary constriction in more severe cases may not be able to overcome any inhibitory effects due to other longstanding side effects of POSA, which might include chronic inflammation; however, this remains to be proven. Three theories are proposed that may explain the finding that in children with less severe maxillary deficiency, the inflammation that is due to hypoxia is less severe.

The first theory is that increasing severity in maxillary transverse deficiency may be associated with different anteroposterior (sagittal) and vertical skeletal jaw relationships. Severe maxillary deficiency is commonly associated with more severe class III (where the nasomaxillary complex is retruded) and/or skeletal relationships with increased vertical dimensions. These morphologies have been associated with increased risk of POSA (Pizzato and Flores-Mir, 2018). The reverse may be true where a less severe maxillary deficiency may indicate that the overall facial/skeletal relationship is closer to "normal".
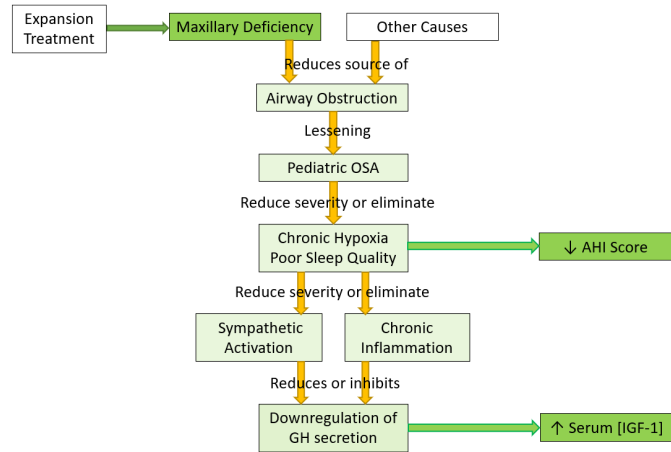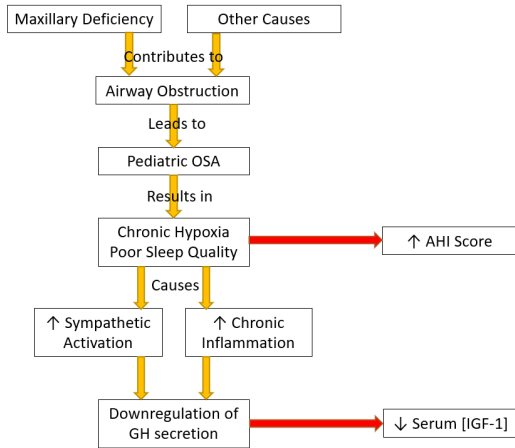
Figure 5.7: Proposed mechanism relating maxillary deficiency to serum [IGF-1] and AHI score. *Top left*: Simplified pathophysiology of pediatric OSA and resultant effects on AHI score and serum IGF-1 levels. *Top right*: Mechanism by which maxillary expansion greatly improves serum IGF-1 and AHI scores. *Bottom right*: mechanism by which maxillary expansion improves AHI but a smaller increase in serum IGF-1 is seen – primary in an individual with greater maxillary deficiency severity.
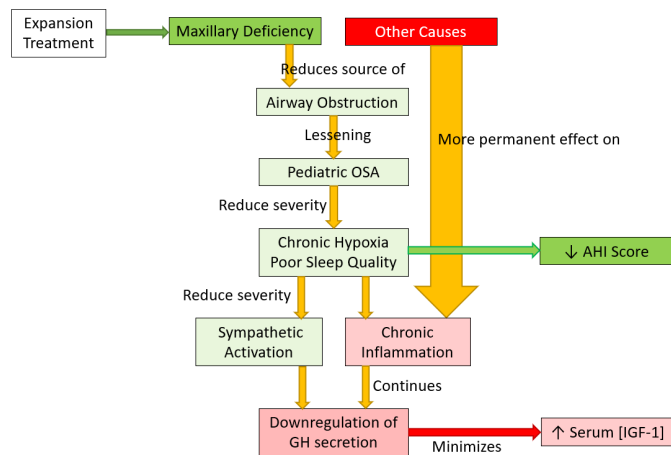
The second theory is that more severe maxillary deficiency may have concurrent greater narrowing of the upper airway, which may be associated with reduced nasal cavity volume or septal deviation defects which increase upper airflow resistance. Further, the narrow upper airway requires less soft tissue mass to cover the airway to cause a relatively greater blockage. The net result of both would be more severe and chronic hypoxia, which in turns leads to promotion and maintenance of low-grade inflammation.

The third theory is that the more severe maxillary deficiency may be associated with older chronological age and lower family SES. It is postulated that individuals and families with higher SES may seek orthodontic treatment earlier for their children knowing they have the

means to afford it, while those with less financial means may tend to defer orthodontic treatment.   The university orthodontic clinics offer treatment for a reduced fee and so on average, attract patients and families who are less affluent.

It can be seen from figure 5.8 that the Canada cohort has a wider distribution in subject chronologic age, with greater numbers 9 years and younger than the Italy cohort, which has most subjects from 9 to 11 years old. This may suggest that the Italy cohort may have on average, lower SES status than the Canadian cohort. The corollary of this is that children of higher SES families experience less health or environmental handicaps growing up.
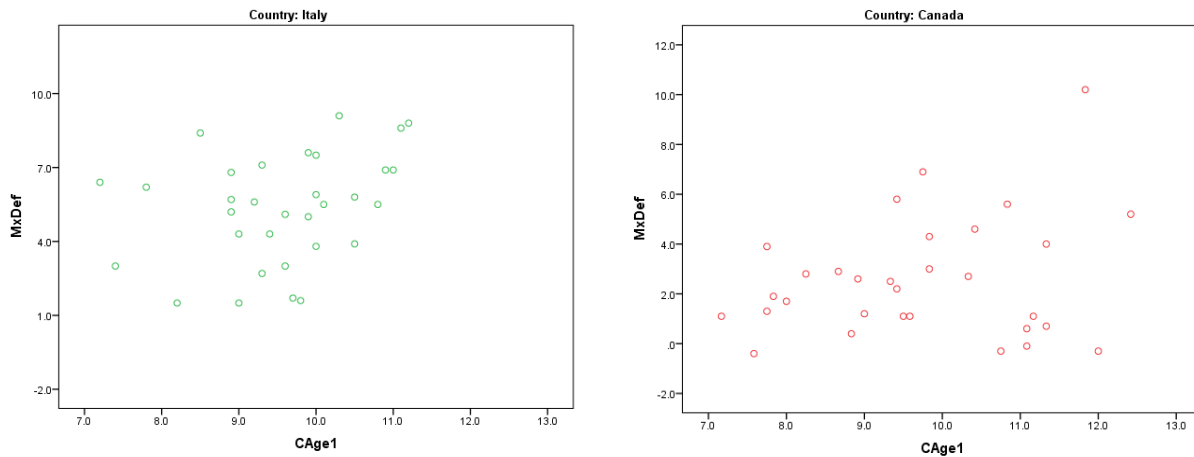
Figure 5.8: Scatterplots of maxillary deficiency (MxDef) against chronological age at start of trial (Cage1) for the Italy cohort (left) and the Canadian cohort (right).

These handicaps may be associated with greater risk for early medical conditions or environmental conditions that may have promoted acute or chronic inflammation such as inadequate housing conditions, living in highly urbanized areas with increased population density and poorer air quality. These disadvantaged circumstances may contribute to disturbances to the GH/IGF-1 system, including persistent suppression of the GH/IGF-1 axis.

The GH/IGF-1 system is sensitive to many variables, and like pediatric OSA and chronic inflammation has a multifactorial nature. The effects of chronic inflammation such as altered vasculature number or tissue architecture may require more or less time to resolve depending on severity. These other factors that result in chronic inflammation are likely contributors to the variation of serum IGF-1 levels. The results from this study show that a great percentage of the variance of the outcome variables are still unknown according to our statistical models.

Section 5.2: Trial Limitations

Although it was planned to assess the effect of treatment on the secondary outcome measure of AHI score, due to a number of factors including the COVD19 pandemic, this data was not ready at the time of writing. The results from AHI score for the Canadian cohort would have been useful in the formulation of potential biological mechanism as to how maxillary expansion may or may not influence the serum concentration. Additionally, the University of Insubria in Italy did not examine a number of serum biomarkers which collectively meant that a unified analysis could not be performed on a combined cohort. Subgroup analyses were performed to compensate for these limitations and provide preliminary insight on any trends unique to either country's cohort. A more complete conclusion can be drawn when the AHI scores for the Canadian cohort become available.

Other limitations include the unavailable reliability data for maxillary deficiency measurements and skeletal (bone) age for the Italian cohort. Heterogeneity between operators cannot be confirmed. Any discrepancy in these two covariates may result in differences in significance in explaining the variance of the outcome variables. It was assumed that there were minimal differences in technique in determining the maxillary deficiency and in assignment of skeletal ages between the author and the investigators at the University of Insubria. An alternative approach is to perform a three-way repeated measures ANOVA instead of ANCOVA with the combined cohort, thereby removing the covariates with unconfirmed reliability.

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The method of Greulich and Pyle using their atlas requires a trained eye to detect certain features in the hand and wrist bones required for assignment of a skeletal age. The standards are about 7 to 8 decades old and are based off middle class children of European descent. This may contribute to operator differences. Further, children of different racial origins may mature at different rates than these historical standards as well as the observed secular trend of children maturing earlier (Greulich and Pyle, 1959, Tanner, 1986). This trial does not have any racial data for the participating children. Canada's citizens have a diverse racial makeup. It is beyond the scope of this study to determine with certainty the exact racial makeup of any individual in the context of potentially multiple generations of interracial children. Having some genetic data would strengthen the statistical model since it has been demonstrated by other researchers that individuals from certain racial backgrounds have significantly different serum IGF-1 levels compared to those of a different racial makeup (Cruickshank et al., 2001).

Another consideration is the method used to determine maxillary transverse deficiency. Ideally, three-dimensional imaging in the form of cone-beam computed tomography (CBCT) could be used to assess the skeletal transverse dimensions directly. However, the participants from the Italy cohort did not have access to 3D imaging at the time of the trial. Using landmarks on the physical dental crowns has limitations since the teeth have the ability to assume compensatory positions to mask an underlying transverse problem. It is not uncommon to see in individuals with maxillary transverse deficiency to have maxillary molars that are flared or tipped to the cheek surface while their complementing mandibular molars are tipped towards the tongue. Some clinicians have proposed the use of a correction factor to account for these compensatory positions to provide a more accurate estimate of the true transverse mismatch (Andrews and Andrews, 2001). Such a corrective factor could have been used for in this trial, however, the original records from the Italian sample were not available to the author for analysis.

While the mean maxillary deficiency amounts were statistically different between the two countries, it was defendable to pool the data. The participants from both University clinics met

all inclusion criteria and had no reason to be excluded. This study is a parallel randomized *multicentre* controlled trial. The well-known Childhood Adenotonsillectomy Trial (CHAT) recruited 460 children from 6 clinical sites across the United States and the data was used collectively in Marcus et al.'s (2013) landmark publication in the New England Journal of Medicine. There were no baseline statistical analysis ensuring similarities between the cohorts. Supplementary analyses found that there were differences between the subgroups (Marcus et al., 2013).

Serum IGF-1 levels are considered relatively stable compared to growth hormone (GH) with a half-life of 10 to 15 hours (Livingstone, 2013), and reflect an individual's average GH level. This study found that IGF-1 increases more rapidly in this study population when combined with maxillary expansion over a one-year period but it cannot be confirmed if the increase in IGF-1 is completely linear throughout this period, and at what point during or after maxillary expansion does the change in IGF-1 levels in the treatment children diverge from the non-treatment controls.

In the Italy cohort subgroup analysis, the AHI score was found to improve with treatment at the one-year time point while other researchers have found AHI scores to improve at the 7-month post-treatment mark (Venekamp et al., 2015). Adding one or more time points for data collection would allow a rough estimation of when the significant differences start occurring and also to approximate the rate of change. Acquisition of blood sample is not free of discomfort for the child and inconvenience for the child's family and such a limit to the number of time points for blood sampling must be considered. Including one more time point immediately after maxillary expansion has been completed would allow us to determine if the increase in IGF-1 occurred during or after the expansion treatment.

Power calculation for sample size determinations should have ideally been performed prior to the start of the trial; however, it is important to note the trial involving the Canadian cohort at the University of Alberta is still ongoing. Having a larger sample size is important in the context

that there are multiple outcome measures and levels for the different between and within-subjects factors creating multiple cells for statistical analysis. At the bare minimum, there needs to be at least as many participants in each cell as there are number of outcome variables (which is two), but a rule of thumb is to have at least 20 for a robust design. There were some trends observed that had a tendency for statistical significance but due to smaller sample sizes the uncertainty may have overshadowed true differences. Having a larger sample size would allow the use of possibly more informative statistical tools such as multiple linear regression analysis where values of the outcome variables could be predicted based on a set of data.

These additional variables can be incorporated into a multiple linear regression model or a more advanced statistical model that can explain the variance of the outcome variables. These include:

- Tonsillar hypertrophy
- General sleep patterns/habits
- Sagittal, vertical facial skeletal type
- Levels of inflammatory cytokines
- Social economic status
- Physical activity
- Racial heritage

Adenotonsillar hypertrophy, a sedentary lifestyle, low social economic status and belonging to certain racial groups such as those of African American descent are factors that increase the risk of having OSA and affect responsiveness to treatment (Marcus et al., 2013, Amin et al., 2008, Dudley and Patel, 2016, Guglielmi et al., 2019). Different jaw relationships can exist in the sagittal and vertical dimensions in conjunction with a narrow transverse dimension that predispose the individual to OSA (Pizzato and Flores-Mir, 2018). Finally, confirming the presence and quantifying the levels of inflammatory cytokines would lend support or detract from the model relating resolution of maxillary deficiency to the response in serum IGF-1 concentration.

Section 5.3: Clinical Implication and Conclusions

Conclusions made from the results of this study pertain to populations of non-syndromic prepubertal children with maxillary transverse deficiency free of other overt medical conditions. A causal inference can be drawn in that maxillary expansion in this population reduces the AHI score and increases the serum IGF-1 concentration over a period of one year. Inference to population is restricted to a similar population as the one studied.

The results from this trial suggested that prepubertal children with maxillary constriction treated with maxillary expansion experience significant increases in serum IGF-1 concentrations and reduction in AHI scores compared to no treatment controls over a one-year period. Children who had less severe maxillary deficiency and who were initially skeletally more mature experienced greater increases in IGF-1 levels than those with larger maxillary deficiency and less skeletal mature.

In a population of non-syndromic prepubertal children with maxillary deficiency, those who are treated with maxillary expansion show that:

- No differences in outcome measures related to sex
- Serum vitamin D, inorganic phosphate, calcium, and total alkaline phosphatase do not contribute to the variance of IGF-1
- Initial maxillary deficiency amount and skeletal age are significant contributors to the variance of IGF-1 in mild maxillary constriction cases
- Significant increase in serum IGF-1 concentration occur compared to baseline and to untreated children who also experience a significant increase in serum IGF-1 only if the maxillary deficiency is mild
- Significant improvement in AHI score compared to baseline and to untreated children
- Lower increases in IGF-1 occur in those with more severe maxillary deficiency

The results from this preliminary data suggest that the use of maxillary expansion to correct moderate transverse maxillary deficiency early in prepubertal children diagnosed with pediatric OSA facilitates a short-term reduction of AHI score and may promote an increase in serum IGF-1 concentration. This would likely only occur if the intervention occurs before establishing a dysfunctional IGF-1 regulatory mechanism due to longstanding pediatric OSA.

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