Early childhood growth trajectories and periodontal health among 8-10 year-old Quebec children at risk of obesity

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Dedication

To Guruji, Maa, Panna

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LIST OF ABBREVIATIONS

TNF- α	Tumor necrosis factor- α
GCF	Gingival crevicular fluid
QUALITY	Quebec Adipose Lifestyle Investigation in Youth
ELISA	Enzyme-linked immunosorbent assay
LCMM	Latent Class Mixed Model
IL-6	Interleukin-6
CDC	Centers for Disease Control and Prevention
WHO	World Health Organization
FTT	Failure to thrive

ABSTRACT

Background: Anthropometric measures including birth weight and body mass index have been associated with adult periodontal disease. However, there is limited evidence regarding the role of childhood growth in subsequent periodontal inflammation.

Objectives: We estimate the extent to which childhood growth trajectories between age 0 to 2 years are associated with indicators of periodontal health among 8-10 year old children participating in the QUALITY Cohort.

Methods: We used baseline data from an ongoing prospective study, the QUALITY (Quebec Adipose Lifestyle Investigation in Youth) cohort investigating the natural history of obesity among 8-10-year-old Caucasian children living in Quebec, Canada. This analysis included 244 boys and 186 girls for whom data were available on anthropometric measures and periodontal health, namely concentration levels of TNF α -GCF and gingival bleeding on probing. Anthropometric measures at birth and up to 2 years of age were collected retrospectively from the Quebec health booklets. GCF samples were collected from the gingival sulcus using a paper strip and the concentration of TNF α -GCF was determined by enzyme-linked immunosorbent assay and the presence of gingival bleeding was measured in buccal and lingual surfaces of the Community Periodontal Index (CPI) .Growth analysis of weight-for-length z-score by2 age was done using the 'lcmm' (Latent Class Mixed Model) package in R and the indicators of periodontal health were regressed on the resultant growth trajectories using a regression analysis adjusting for body mass index (BMI) of the child and mother, parental income, education status of mother, maternal age, child's age, breastfed.

Results: The mean age of the children was 9.1 years (SD=0.9) and majority of them were males (n= 244, 56.7%). Most children did not have excess weight (62%) and the average gestational age was around 40 weeks. The mean age of the children's mother was 30.27 (SD=4.7) years and 18% of them had history of gestational diabetes. The mean family income was 42,727 CAD\$. The median TNF α -GCF was 215 (IQR: 33, 517), and the median proportion of gingival bleeding sites

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was 75 (50, 83.33). We identified a three-class quadratic solution in the data analysis and defined as follows: Growth of the children that started out at a lower than average weight for length at birth but grew rapidly were named as Class 1; children who had a higher than average weight for length at birth but had a dip in growth were named as Class 2; while Class 3 denoted expected growth in a child who was born at the average weight for length and initially rose rapidly and then became steady. In the adjusted linear model, having a class 1 or class 2 type trajectory, compared to class 3, showed no association with concentration levels of TNF α -GCF on average [0.49 (95% Cl:-10.80, 11.79) or 0.18(95% Cl: -10.29, 10.64)], respectively. Also, another adjusted linear model having a class 1 or class 2 type trajectory, compared to class 3, showed no association with proportion of sites with gingival bleeding on probing [2.11 % (95% Cl :-4.75, 8.98) or 1.13 % (95% Cl: -5.23, 7.49)] respectively(table 3).

Conclusions: The expected growth trajectory showed no association, neither with TNF α -GCF levels nor with proportion of sites with gingival bleeding.

RÉSUMÉ

Contexte : Les mesures anthropométriques, notamment le poids à la naissance et l'indice de masse corporelle, ont été associées à la maladie parodontale de l'adulte. Cependant, il existe peu de preuves concernant le rôle de la croissance de l'enfant dans l'inflammation parodontale ultérieure.

Objectifs : Nous estimons la mesure dans laquelle les trajectoires de croissance des enfants entre 0 et 2 ans sont associées aux indicateurs de santé parodontale chez les enfants de 8 à 10 ans participant à la cohorte QUALITY.

Méthodes : Nous avons utilisé les données de base d'une étude prospective en cours, la cohorte QUALITY (Quebec Adipose Lifestyle Investigation in Youth) qui étudie l'histoire naturelle de l'obésité chez les enfants caucasiens de 8 à 10 ans vivant au Québec, Canada. Cette analyse a porté sur 244 garçons et 186 filles pour lesquels on disposait de données sur les mesures anthropométriques et la santé parodontale, à savoir les niveaux de concentration de TNF α -GCF et les saignements gingivaux au sondage. Les mesures anthropométriques à la naissance et jusqu'à l'âge de 2 ans ont été recueillies rétrospectivement à partir des livrets de santé du Québec. Des échantillons de GCF ont été prélevés dans le sillon gingival à l'aide d'une bande de papier et la concentration de TNF α -GCF a été déterminée par dosage immunoenzymatique et la présence de saignements gingivaux a été mesurée sur les surfaces buccales et linguales de l'indice parodontal communautaire (IPC). L'analyse de la croissance du z-score poids pour longueur par âge a été effectuée à l'aide de l'ensemble "lcmm" (Latent Class Mixed Model) en R et les indicateurs de santé parodontale ont été régressés sur les trajectoires de croissance résultantes à l'aide d'une analyse de régression ajustée pour l'indice de masse corporelle (IMC) de l'enfant et de la mère, le revenu des parents, le niveau d'éducation de la mère, l'âge de la mère, l'âge de l'enfant, l'allaitement.

Résultats : L'âge moyen des enfants était de 9,1 ans (ET=0,9) et la majorité d'entre eux étaient de sexe masculin (n= 244, 56,7%). La plupart des enfants n'avaient pas de surpoids (62 %) et

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l'âge moyen de la grossesse était d'environ 40 semaines. L'âge moyen de la mère des enfants était de 30,27 ans (ET=4,7) et 18 % d'entre eux avaient des antécédents de diabète gestationnel. Le revenu familial moyen était de 42 727 CAD\$. La médiane TNF α -GCF était de 215 (IQR : 33, 517), et la proportion médiane des sites de saignement gingival était de 75 (50, 83,33). Nous avons identifié une solution quadratique à trois classes dans l'analyse des données et nous l'avons définie comme suit : La croissance des enfants dont le poids à la naissance était inférieur à la moyenne mais qui ont grandi rapidement a été désignée comme la classe 1 ; les enfants dont le poids à la naissance était supérieur à la moyenne mais qui ont connu une baisse de croissance ont été désignés comme la classe 2 ; tandis que la classe 3 correspond à la croissance attendue d'un enfant dont le poids à la naissance est égal à la moyenne et qui a grandi rapidement puis s'est stabilisé. Dans le modèle linéaire ajusté, le fait d'avoir une trajectoire de type classe 1 ou classe 2, par rapport à la classe 3, ne montre aucune association avec les niveaux de concentration de TNF α -GCF en moyenne [0,49 (IC 95% : -10,80, 11,79) ou 0,18 (IC 95% : -10,29, 10,64)], respectivement. De même, un autre modèle linéaire ajusté ayant une trajectoire de type classe 1 ou classe 2, comparé à la classe 3, n'a montré aucune association avec la proportion de sites présentant des saignements gingivaux au sondage [2,11 % (95 % IC : -4,75, 8,98) ou 1,13 % (95 % IC : -5,23, 7,49)] respectivement (tableau 3).

Conclusions : La trajectoire de croissance prévue n'a montré aucune association ni avec les niveaux de TNFα-GCF ni avec la proportion de sites présentant des saignements gingivaux.

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PREFACE

I start the thesis by providing a brief introduction of the topic, followed by the review of literature in the field. Based on this knowledge, we present the objectives which are followed by the methodology used in the thesis. Chapter 5 presents the results and finally the last chapter in this thesis discusses the methodological considerations and conclusion of the study.

1) Introduction

Periodontal diseases are chronic dental biofilm induced multi-bacterial inflammatory diseases propagated by responses of the host immune system, leading to gingival inflammation and subsequently destruction of the bone that surrounds and supports the teeth (1). Despite the gains in oral health in many populations all over the world, the burden of periodontal diseases remains high. These conditions are estimated to affect around 3.5 billion people worldwide, being the 6th most prevalent disease across the world (1).

Overwhelming evidence exists for a bidirectional relationship between periodontal diseases and other chronic systemic conditions (e.g., obesity, diabetes and cardiovascular diseases) in the adult population. However, little is known of these associations in early life (2-10).

The literature also consistently suggests that diabetes and cardiovascular diseases appear to originate early in life, and childhood factors are predictive of disease later in life (9, 10). Moreover, growth patterns throughout infancy have been linked to risk of these diseases in both young children and adults (10-16).

TNF- α is one of several cytokines secreted by macrophages and monocytes in response to bacteria (lipopolysaccharides) and plays an extensive role in periodontal disease mediated bone loss by stimulating the formation of bone-resorbing cells (osteoclasts) (16, 17). It has been suggested that this cytokine is a marker for the earliest stages of episodes of periodontal disease activity (17, 18).

Infancy is considered to be one of the most critical phases of life as it is regarded as a period of rapid physical growth along with cognitive and emotional development (19). Infancy accounts from birth up to 2 years of life, which is crucial for building the foundation for future health status (19). Hence, studying different patterns of growth plays an essential role in programming long term health (19, 20).

The biological processes involved in the development of obesity, diabetes and cardiovascular diseases begin much early in life (21). Indeed, numerous studies have shown that low birth weight followed by 'catch-up growth' is strongly associated with obesity later in life (22-28). Moreover, growth patterns throughout infancy, specifically small birth size for gestational age and rapid postnatal weight gain, have been linked to risk of cardiovascular disease in both children and young adults (15, 28-30).

As stated above, periodontal diseases have a complex linkage to various systemic conditions (31). However, most studies on associations between periodontal diseases and systemic conditions have been conducted in adults. This is surprising considering that these are chronic diseases, many of which have long latency periods before they manifest themselves as overt pathologies (<u>https://www.cdc.gov/program/performance/fy2000plan/2000vii.htm</u>). Moreover, there is a robust suggestion that the biological processes involved in these chronic diseases begin much early in childhood (32, 33) (18).

While our group has previously shown an association of obesity and metabolic syndrome with concentration levels of TNF- α in the gingival crevicular fluid (GCF) (34, 35), a marker of oral inflammation that has been suggested as a precursor of adult periodontal disease, little is understood about the association between growth patterns and periodontal diseases (18). Therefore, we aimed to investigate the association between childhood growth trajectories from age 0-2 years and indicators of periodontal health in 8-10 year old Quebec children.

2) Literature Review

This review is presented in 4 sections. I start with the definition, pathogenesis, epidemiology of the periodontal disease and measurement of periodontal disease. In section two, I discuss growth patterns in children; section three presents the evidence for the association between growth patterns and chronic inflammatory diseases. Finally, the last section summarizes the above.

2.1 Definition of Periodontal diseases

Since the beginning of human existence, oral health has been quintessential to overall health. Without a shadow of a doubt, periodontal disease adds to the global burden of a disease epidemic and forms a significant part of the writings of almost all the ancient texts (1). The term "periodontal disease" refers to the inflammatory disorders of the periodontium, notably gingival and periodontal tissues. Periodontal diseases are generally classified into gingivitis and periodontitis (36).

2.1.1 Gingivitis

Gingivitis is defined as being an inflammation of the gingiva/gum characterized without visible loss of clinical attachment (37). The main cause of gingivitis is bacterial plaque. The accumulation of plaque on dental surface commences the destruction of gingival tissue which is reversible in nature (36). The choice of treatment lies in the reduction or elimination of its etiological factors, mainly plaque (31, 38). Generally, gingivitis is classified into two groups : 1. gingivitis induced by plaque and, 2. gingivitis not induced by plaque. The first is the most common form amongst children and adolescents (31, 39).

2.1.2 Periodontitis

Periodontitis is defined as a multifactorial infectious disease of the tooth-supporting tissues (periodontium) which primarily includes progressive destruction of periodontal ligament and the alveolar bone (36). The distinctive clinical aspect of periodontitis involves presence of loss of periodontal attachment that is not always accompanied by clinical signs of inflammation such as change of color, form or texture, and bleeding upon probing (31). Bleeding on probing has been

very well documented in literature as a reliable clinical parameter in monitoring gingival tissues' health or inflammation when examined at regular intervals in subsequent visits to the dentist (39-41). Diverse classifications of periodontitis amongst children and adolescents have been proposed in the past (39, 42-46). Nonetheless, the exact definition continues to be a controversial subject in periodontology; an enormous research effort has been made in recent years to simplify and provide the revised forms to familiarize the clinicians with the current classifications. This would allow them with a proper diagnosis of the patient along with an appropriate treatment plan in a timely fashion. Critical factors examined for classification are age, the disease's distribution at different sites in the mouth specifically the molar and incisor teeth, loss of teeth, and the level of progression of the disease (31). Nevertheless, worldwide prevalence of aggressive periodontitis, which is the most often viewed form amongst children and adolescents, is low (31, 42).

2.2 Pathogenesis of Periodontal disease

The development of gingival inflammation requires the presence of bacteria that reside within a rich mixture of proteins and carbohydrates called "dental biofilm". The presence of bacteria causes pathological changes in the surrounding tissues through innate and adaptive immune responses (47, 48).

The innate response involves gingival epithelial cells, neutrophils, and monocytes/macrophages. Migration of neutrophils to the site of inflammation occurs in response to the direct chemotactic effect of bacteria, leading to increased numbers of neutrophils, to infiltration of macrophages and mast cells as well as to an activation of complement proteins and an increase in gingival crevice flow, which marks the established stage of the lesion. "This can be considered as a period of transition from innate to acquired immune response" (47, 49).

These cells detect pathogens associated with bacterial molecular patterns. This detection leads to a cascade of inflammatory responses, triggering activation of host response and release of several cytokines, namely interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α) and interferon

γ. Up-regulation of the innate response by these cytokines is an essential step as it stimulates monocytes to leave the system and infiltrate surrounding tissue (49).

Neutrophils are then replaced by adaptive immune cells- lymphocytes T and lymphocytes B (48). T cells are responsible for cell-mediated immunity while B-cells for humoral immunity " as they bear immunoglobulin molecules on their surface, which function as antigen receptors "(47).T-cells are further classified into subgroups, namely; cytotoxic T cells (CD8+ T cells) and T helper cells (CD4+ T cells). Further classification of the T helper cells is done based on the cytokines they produce, Th1 and Th2. Current studies have identified two other regulatory CD4+ T-cells: Th17 and Tregs (regulatory cells). The former has been recognized in chronic periodontitis sites and is known in the production of a cytokine, namely interleukin (IL)-17, which may be produced in periodontal lesions. The latter (Tregs), CD4+ and CD25-expressing T-cells, are known to play a protective role in periodontal tissue damage and produce cytokines (IL-10, Transforming growth factor-beta, and T-lymphocyte-associated molecule 4) that decrease periodontal disease progression (47, 49). In summary, the initiation and progression of periodontal diseases (gingivitis and periodontitis) is determined by the interactions between bacteria and the immune - inflammatory response of the host (31).

2.3 Epidemiology of Periodontal Disease

The complexity underpinning the nature of periodontal diseases should be taken under careful consideration before undertaking any epidemiological study. The ever-evolving concepts of epidemiology have led researchers to dive deep into understanding the principles of epidemiologic research, into studying the periodontal disease (50). The issues related to studying the nature of periodontal disease correspond to the basic principles of science, which constitute exact observation, accurate interpretation, rational explanation, and scientific construction (51).

The intricacies involved while opting to conduct an epidemiological study revolve around three fundamental aspects namely,

1) deciding on what level the unit of observation of periodontal disease is to be held, and the unit

- of analysis on which data on all relevant variables are analyzed
- 2) choice of the index used to measure periodontal disease, and
- 3) the precision of any measurement with as minimal error as possible (50).

Although many researchers consider individuals to be the prime unit of study, there is an ongoing debate over selecting appropriate units for analysis in the case of periodontitis. This is because it is taken into account that it is a derived variable including various sites within each individual. Despite the very fact that several indices are utilized in measuring the severity of the disease, there is still no unanimity on the bounds to be considered (50).

3) Systematic error: an error in the design or conduct of a study, which might pose another challenge in evaluating the prevalence of a disease including periodontal diseases (52). When conducting an epidemiologic survey of periodontal health, the first concern is that the measurement of the number of sites may result in a systematic error (53). Such situations are often overcome by dentists by choosing a sample of the most significant active sites (50).

2.3.1 Descriptive epidemiology

In this section, we will discuss the prevalence of gingival and periodontal disease among children. Although an ongoing debate remains on the precise definition of periodontitis, the recent criterion for classifying periodontitis is essentially based on the degree of severity of the disease and far less on age.

Ginigivitis

Most commonly occurring form of periodontal infection in children is Chronic Gingivitis (46). A pilot study conducted by Dr Staffan Kaping (1973) showed that propensity to develop gingivitis differed among children and adults in case of abandonment of all oral hygiene procedures. Based on this study, Matsson undertook an identical investigation to rule out such a difference comparing the event of gingivitis amongst pre-school children and adults using objective

methods. A 21-day experimental gingivitis study was conducted amongst six children aged 4 to 5 years to six dental students aged 23 to 29 years, which showed a marked difference between pre-school children and adults within the propensity to develop gingivitis. Children showed lower tendency to develop gingivitis than adults. Also, a lesser amount of gingival exudate and a lower percentage of bleeding sites were seen after 21 days without removing plaque . A small increase in gingival crevicular leukocytes in response to the buildup of plaque than adults was also noted. The hypothesis of the study stated a difference in vascular response between children and adults (46, 54).

Matsson and Goldberg administered another similar research (54); the results showed that the high gingivitis scores were lesser in number within the 4–6-years aged group than within the older children and adults with a given plaque score (54). The best degree of gingival inflammation was noted within the 14–16 years aged group and adults within the least levels of plaque accumulation. The results indicated that the gingival reactivity increased gradually from infancy to adult age (55). Another Swedish study reported an age related gradient of prevalence of gingivitis: 36% in 3-year-olds, 64% in 5-year-olds, 97% in 10- year-olds, 74% in 15-year-olds and 97% in 20-year-olds respectively (56, 57). Also, data from the National Institute of Health (NIH-2006) showed that about 75% of adults had gingivitis in the United States (58).

Periodontitis

Globally, age standardised incidence and prevalence of periodontal disease is seen to be stable until the year 1990. A comparison between the years 1990 and 2010 showed a prevalence of about 11.2% and incidence at 696 cases per 100 000 person-years in 1990 while a prevalence of 10.8% and an incidence of 701 cases per 100 000 person-years was accounted in 2010 (1).

Severe periodontitis was the sixth-most prevalent health condition in the year 2010 which affected around 10.8% of people worldwide. Aggressive periodontitis, which is a severe form of periodontal condition, affected about 2% of youth during puberty (1).

Data from NIH-2006 stated that the prevalence of more advanced forms of periodontitis accounts for 30% (moderate disease) and 10% (advanced disease) of the adult population within the US (58). Also, a recent Centers for Disease Control (CDC) report revealed that 47.2% of adults aged 30 years or older have some form of periodontal disease. "Data from the 2009 and 2010 National Health and Nutrition Examination Survey (NHANES) cycle", conducted amongst 3,742 adults revealed that "over 47% of the sample, representing 64.7 million adults, had periodontitis, distributed as 8.7%, 30.0%, and 8.5% with mild, moderate, and severe periodontitis, respectively" (59).

2.3.2 Analytical Epidemiology: Risk factors for periodontal diseases

"Analytical epidemiology assesses hypotheses of associations between suspected risk factors and health outcomes" (122). Periodontal disease is associated with some key risk factors that are highly important namely age, sex, sociodemographic factors and health behaviours. The following section will discuss these key factors in detail to provide a better understanding of the overall view of periodontal disease.

2.3.2.1 Demographic factors

Demographic factors mainly comprise of age, ethnicity, race, gender and sex.

To assess the oral health in children in the United States, The National Institute of Dental and Craniofacial Research led by Dr Harold Loe, conducted a national survey in the year 1986-1987. Findings from this survey showed higher prevalence rates of chronic periodontitis (2.3% and 3.2% for the age groups: 13-15 and 16-19 years old, respectively) than early-onset aggressive periodontitis (0.4% and 0.8% for the age groups: 13-15 and 16-19 years old, respectively) than early-onset aggressive periodontitis (0.4% and 0.8% for the age groups: 13-15 and 16-19 years old, respectively) (60). Another study conducted amongst a group of Uganda students in the age group 12-25 years showed similar findings with high prevalence rates for chronic periodontitis: 22.3%, while only 6.5% for early-onset (aggressive) periodontitis. An age-related gradient associated with the prevalence of periodontitis was visible for a loss of attachment \geq 4mmm: 26.8% for 12-16, 29.1% for 17–19 and 35.2% for 20–25-year-olds (61). An investigation by Loe and Brown showed that

the risk of developing localized early juvenile periodontitis in children of 15 years was 2.3% higher than in children of 14 years (56, 62, 63).

Much literary research points out a higher chance of developing chronic periodontitis among men than among women, which was supported by Loe and Brown who reported in their study a higher prevalence of periodontal disease amongst males than females (63). Also, a study on 14,000 American children showed slightly higher susceptibility to periodontitis amongst males than females (60). On the contrary, study carried out amongst 12-21 years-old Chilian college students demonstrated a higher occurrence of chronic periodontitis among females than males (64). Moreover, research carried out amongst 16-year old Swiss adolescents mentioned a comparable frequency of onset of aggressive periodontitis among a group of American adults of 17 to 26 years (56, 62, 66). Another cross-sectional study showed a higher frequency of aggressive periodontitis amongs age: a ratio of 5.3/1 amongst 12-18 years, 2.4/1 amongst 19-25 years and 1.5/1 amongst 26-32 years (56, 67). The outcomes of this study support the theory which posits that an early onset of youth and an initial eruption of permanent teeth amongst girls during the circumpubertal period might contribute to the upper incidence of chronic aggressive periodontitis amongst females (56, 67).

2.3.2.2 Socioeconomic factors

Several indicators of socioeconomic position have been associated with periodontal disease including level of education, occupation, and income. A Brazilian study by Gjermo (1984) reported a 28% bone loss in 15 year-old adolescents belonging to a low socioeconomic background compared to 5.4% bone loss in 13 year-old adolescents belonging to a high socioeconomic background (68, 69). A similar study conducted among 14-year old Norwegians in 1984 also observed a higher occurrence of bone loss in adolescents from low socioeconomic backgrounds had a higher incidence of loss of clinical attachment ≥3mm that those from high socioeconomic backgrounds (64). Finally, Ontarian school-aged immigrant children belonging to low socioeconomic status

showed a higher prevalence of gingival inflammation, dental calculus, poor oral hygiene and had a greater need for periodontal remedies compared to children born in Canada with a better socioeconomic status (71).

2.3.2.3 Health behaviour

Health behaviour is a universal risk factor of periodontal disease. It comprises mainly of oral hygiene habits (toothbrushing frequency, use of dental floss), smoking and/or tobacco consumption and visit to the dentist. A study conducted in a Sri Lankan population on natural history of periodontal disease showed that attachment loss was significantly associated with dental calculus (72, 73).

It is well established that smokers have a higher risk of developing chronic periodontal disease in comparison to non-smokers (74). A large National Health and Nutrition Examination (NHANES) survey amongst U.S. adults traced periodontal disease to current smoking in 41.9% of the cases (6.4 million adults), while to former smoking in 10.9% of the cases (1.7 million) (72, 75).

2.4 Periodontal disease in children

Gingivitis of varying severity is ubiquitous in children and adolescents (46, 56, 76). Although specific surveys have indicated that loss of periodontal attachment is fairly uncommon in young people, a rise in incidence rate from age 12 to 17 in comparison to age 5 to 11 has been observed in children (46, 76). The most common periodontal disease found in children and adolescents is chronic gingivitis, which is characterized by inflammation of the gingiva without loss of connective tissue or bone. Clinically, marginal gingival tissue is exhibited by redness and swelling which is characterized by bleeding upon probing (31).

Persistent gingivitis may be the reason for knife-edged gingival margin to become rolled, and for further causing the interdental papillae to be bulbous and enlarged. If gingivitis is left untreated, it may progress to periodontitis, which is characterized by loss of supportive connective tissues, including the alveolar bone (31, 46).

Different forms of periodontal diseases exist among children (39, 42-44). Chronic periodontitis, the most prevalent form of the disease in adults, has sometimes been seen in children and adolescents (77, 78). The aggressive form of periodontitis is relatively uncommon. However, the prevalence of aggressive periodontitis has been seen within the circumpubertal duration in children and adolescents and has additionally been proven to have familial aggregation with loss of connective tissue attachment and alveolar bone. It has been bifurcated into localized and generalized forms (46, 76, 78).

Children with "localized aggressive periodontitis have interproximal attachment loss on no more than two teeth other than permanent first molars and incisors", while children with "generalized aggressive periodontitis have interproximal attachment loss on at least three teeth that are not permanent first molars or incisors" (46, 76, 78).

2.5 Measures of periodontal disease

2.5.1 Gingival crevicular fluid inflammatory markers

Nowadays, the primary method utilized in the diagnosis of periodontal diseases among children/adolescents relies chiefly on measuring the clinical periodontal parameters, including plaque index, gingival index, bleeding on probing, clinical attachment loss or probing depths; along with radiographical findings. However, the data collected from these clinical parameters reveal earlier damage to the periodontium instead of anticipating the expected condition of the periodontal tissue in the future. Therefore, it's crucial to seek out a way which will predict upcoming periodontitis (79). Progress in oral and periodontitis diagnostic research is driving towards strategies whereby periodontal diseases are frequently diagnosed and quantified via objective measures like biomarkers (80).

"The gingival crevicular fluid (GCF) is marked as a physiological fluid and inflammatory exudate originating from the gingival plexus of blood vessels within the gingival corium, subjacent to the epithelium lining of the dentogingival area" (80-82). Collection and analysis of GCF has long been a well-appreciated approach to investigate periodontitis (83). The severity of periodontal tissue

inflammation can be gauged by measuring pro-inflammatory markers such as TNF- α and IL-8 collection that have an essential function in the pathogenesis of periodontal disease activity (79). The gingival crevicular fluid has also been described as a serum exudate that carries all requisite cellular (neutrophils and plasma cells) and molecular (complement components and antibodies) elements of the immune response important to stop tissue invasion by subgingival plaque bacteria (81).

2.5.1.1 Cytokines: Biomarkers of periodontal inflammation

Cytokines are labeled as low-molecular-weight proteins that act as messenger among cells and mediate the immuno-inflammatory–associated processes (84, 85). They play a crucial role in health and disease pathologies as they act as mediators of homeostasis and immunity (88). The measurement of cytokine profiles in patients can be a useful indicator of disease involving initiation and further stages of inflammation, during which they handle the amplitude and, consequently, the duration of the response (84,85,86,88). "Cytokines have wide-ranging and overlapping functions" which when described "in simple terms states that the balance between pro-and anti-inflammatory cytokines and regulation of their receptors and signalling pathways determines the extent of periodontal tissue destruction" (86).

"Cytokines appear to interact functionally in networks in the periodontium and integrate aspects of innate and adaptive immunity" (88). However, an enormous amount of research on the cytokine network involved in periodontal inflammation progression has laid the incentive for developing cytokine-targeting therapies for periodontitis (86, 87) (88). Pro-inflammatory cytokines, namely, IL-1, IL-6, and TNF- α , have shown to possess pleiotropic effects on lymphocyte promotion additionally to tissue destruction and bone resorption (88). They have also been recognised to minimize periodontal tissue repair through several mechanisms including increased inflammatory response, stimulation of matrix metalloproteinase, improved osteoclast formation and activity, and increased fibroblast apoptosis (84, 85, 88).

2.5.1.2 TNF- α cytokines

Tumor Necrosis Factor- α (TNF- α), a pro-inflammatory cytokine referred to as cachectin, is a pleiotropic molecule that plays a significant role in inflammation, immune system improvement, apoptosis, and lipid metabolism (89). TNF- α is one of the several cytokines secreted by macrophages and monocytes in response to periodontal bacteria (lipopolysaccharides) and plays an extensive role in periodontitis mediated bone loss by stimulating the formation of bone-resorbing cells (osteoclasts) (16, 18, 89). Its actions include regulation of several inflammatory mediators (e.g., chemokines, matrix metalloproteinases (MMPs), which can degrade the connective tissues and prostaglandins), endothelial cell activation, and endothelial-leukocyte interactions, monocyte adhesion, bone remodeling, and oxidative processes (90). Thus, TNF- α seems to be crucial for the control of periodontal infection. "Nevertheless, the persistence of pathogens results in chronic inflammation with high TNF- α production and activation of pathways that culminate with destruction of soft and hard tissues" (90-92).

TNF- α additionally stimulates gingival fibroblasts to provide collagenase implicated in tissue destruction and bone resorption and it may be a marker for the earliest stages of episodes of periodontitis activity (18, 90). Graves and Cochrane (1998) in a non-human primate model showed that "the conversion from gingivitis to periodontitis is directly associated with the movement of an inflammatory infiltrate toward alveolar bone, and that this process is atleast partially dependent upon IL-1/TNF activity" (87, 93).

2.6 Growth patterns in children

2.6.1 Definition and factors affecting growth

"Normal growth is defined as a succession of height, weight, and head circumference compatible with established standards for a given population"

(https://somepomed.org/articulos/contents/mobipreview.htm?14/43/15025/abstract/1).

It is representative of overall health and nutritional status. Hence, knowing the normal growth patterns facilitates the early apprehension of pathologic deviations (e.g., low weight gain because of a metabolic disorder, short stature because of inflammatory bowel disease) preventing any

unnecessary judgement of children with acceptable normal variations in growth (94). Therefore, it is crucial that we understand the factors that govern infant development, along with experiences and environmental influences. Factors affecting early postnatal growth include familial and environmental determinants namely gestational age at delivery, birth size, parental heights, socioeconomic status, and breastfeeding (20, 95, 96).

2.6.2 Measuring growth patterns

2.6.2.1 Growth Charts: World Health Organisation (WHO) and Others

Identifying normal patterns for child growth is of paramount concern in assessment of growth (97). The most crucial questions ask how to use growth charts to assess growth across the age range (2-5 years) when there's a change in measurement method or reference chart. Using growth charts to watch and track weight is challenging in case of longitudinal studies for several reasons. However, it has been recommended that the Centers for Disease Control and Prevention (CDC) and the WHO growth charts be used until two years old, after which the CDC growth charts are suggested (97-100).

It has also been seen that charts utilized in some countries for epidemiological studies are different than those used for clinical care (98-100). WHO recommends a growth standard for birth to 5 years, which relies on data from sites in select countries, which includes children on the basis of optimal nutrition/feeding, environment and care, and excludes children with excess weight (98-100). On the contrary, CDC recommends "a growth reference, not a standard, based on the overall US population in the 1960s through the early 1990s". However, charts for both weight-for-length and BMI-for-age exist, and therefore the CDC recommends the utilization of WHO weight-for-length charts up to two years of age (98-100).

2.6.2.2 Oral Health, Nutrition and Thriving in Childhood

Although attaining and maintaining sound oral health may be a crucial component of general health and wellbeing at any age bracket (101), this is often significantly more important for a growing child. A developing child, during the rapid climb stage, requires adequate nutrition to satisfy his/her body's high metabolic demands. However, poor oral health at this stage of life can

cause inadequate food intake, which has been shown to cause a failure to thrive (FTT) in childhood (102).Different growth failure standards have been shown to identify children with varying risk profiles and yield a wide-ranging prevalence, which was demonstrated in a Danish study of birth cohorts that investigated three of the requirements for FTT: "slow weight gain conditional on birth weight, thinness based on low body mass index, and downward crossing of two or more percentiles from birth. The criterion of conditional weight gain was associated with lower birth weight, small-for-gestational-age, and deviant overall development" (102, 103).

The pattern of growth may differ in particular circumstances. A study carried among Israeli infants showed that breastfed babies followed a separate growth trajectory than bottle-fed babies. While the former displayed a faster rate of growth within the first 2 or 3 months, it reduced later in comparison to the bottle-fed babies . Despite the slower growth rate in breast-fed infants, they eventually reach an equivalent final height by the age of two years (102, 104). The WHO child growth standards (<u>http://www.who.int/childgrowth/en)</u> have supported the premise that breast-fed babies are the norm for healthy growth among infants (102).

2.6.2.3 The use of growth trajectories

At a time when obesity is rampant among children, characterizing childhood growth trajectories (weight, length/height, or body mass index/weight for length) has undeniably turned out to be indispensable for surveillance (105). Early apprehension of abnormal growth patterns and identifying the underlying cause is crucial for suitable treatment. The foremost motive to look at the abnormal growth in infants and children is to identify conditions that endanger good health and life (106). Trajectories of growth tend to play a pivotal role in life course epidemiology, often providing fundamental indicators of prenatal or childhood development, as well as an assemblage of potential determinants of adult health outcomes (107).

Clinical epidemiologists routinely use growth curves to apprehend abnormal growth trajectories. They are interested in studying both the determinants of growth and the consequences of specific growth patterns on later health and diseases. Hence, characterizing growth trajectories

helps predict future development based on past growth and might help evaluate the impact of several interventions in the future. After birth, length/height and weight monitoring is a keystone of well-child visits, a visual record for both clinicians and parents. Studying growth trajectories may well concede more accurate identification and quantification of modifiable risk factors and predict health outcomes. The characterization of these trajectories may also accommodate critical windows during which intervention may be remarkably beneficial (20, 105).

2.7 Association between growth patterns and chronic inflammatory diseases

2.7.1 Birth weight and cardiovascular origins

Infant growth is of elemental importance in terms of later health and well-being (108). It is well established that fetal life may be a critical period for early cardiovascular origins (29). Significantly, the fate of infants who demonstrate rapid postnatal growth and are born small (SGA) or large (LGA) for fetal age has shown to be intimately involved (9, 10, 29).

Birth weight appears to have severe implications for cardiometabolic health in adulthood, which is in line with findings from a Helsinki Birth Cohort Study in Finland (109)(110). Eriksson et al. reported that growth patterns in infancy and childhood have been identified as significant factors connected to the pathogenesis of these disorders (e.g., obesity, cardiovascular disease, insulin resistance, and hypertension) (9, 10, 23, 29, 30, 110). Similarly, Frontini et al., using data from a Heart Study conducted in Bogalusa, reported an association of low birth weight with adverse metabolic outcomes (including altered glucose homeostasis) from childhood to adolescence. Indeed, a constellation of risk factors in childhood tend to predict cardiovascular disease later in life (9).

2.7.2 Birth weight and diabetes

Mounting evidence exists for the complex relationship between birth weight and early life factors associated with type II Diabetes (22). There is rising agreement on the notion that obesity may additionally, at least in part, be programmed through events early in life (111, 112). Low-birth-weight infants usually show rapid catch-up growth at some stage in the first year of life, and proof increasingly highlights the importance of growth patterns for the postnatal duration (21, 112).

The concept of catch-up growth continues to be the subject of interest since years (22). A prospective cohort study conducted in Bristol suggested that "catch-up growth was predicted by factors relating to intrauterine restraint of fetal growth" (27). Also, collected data point towards strong association of low birth weight and catch-up growth with heightened risk of insulin resistance and type II diabetes mellitus (22). Moreover, numerous other studies have suggested that people who had low birth weight or showed faltered growth during infancy and childhood, but subsequently showed catch-up growth have higher susceptibility for obesity, type 2 diabetes, and cardiovascular diseases later in life (15, 24, 25, 27, 28, 30, 113). In contrast, a systematic review and a meta-analysis conducted among several countries, suggested that a high birth weight, particularly when not followed by a postnatal catch-down, was linked with subsequent obesity, a crucial risk factor for type II diabetes (26, 114).

2.8 Periodontal disease and growth patterns in children

As stated above, periodontal disease, a multifactorial condition of the tooth-supporting tissues, has a complex linkage to various systemic conditions (e.g., obesity, type II diabetes, cardiovascular disease, insulin resistance) (31). Periodontal disease is determined by the interactions between bacteria and the immune-inflammatory response of the host (31). While the prevalence of these systemic diseases is higher in adults, there is a strong suggestion that the biological processes involved in their development begin way earlier in childhood (32, 33). Likewise, it has been observed that there may be a detection of markers of oral inflammation at an early age even before the disease is clinically observable (18).

2.9 Conclusion of the literature review

Periodontal disease is a multifactorial infectious disease (31). The complexity underpinning the nature of this disease makes the advancement of measurement methods very challenging. Therefore, a standard definition of periodontal disease has not been established in the literature. Despite these limitations, several studies have reported a prevalence of gingivitis in children and adolescents (64% and 75%), depending on age (56). The prevalence of periodontitis, which is

more destructive than gingivitis, ranges between 0.8% and 4.5% in children and adolescents, respectively (56).

Interplay between bacteria, the immune-inflammatory response of the host system, and environmental risk factors is the main determinant that guides the establishment and progression of the disease (31). Potential factors that contribute to the risk of periodontal diseases include the socioeconomic standing, age, sex, health behaviours, and systemic diseases (39). Cardiovascular diseases, diabetes, and obesity are among the foremost studied systemic diseases associated with periodontal diseases. Compelling evidence shows the origin of these diseases early in life (32, 33). Moreover, the biological processes involved in the development of these conditions begin much earlier in life. It has also been seen that due to the long-lasting latent periods of periodontal disease in adults, there might be a detection of oral markers of inflammation at an early age, before the disease is clinically observable (18).

"Advances in oral and periodontal disease diagnostic research are moving towards methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers" (80). Collection and analysis of gingival crevicular fluid, which is a physiological exudate, is involved in detecting biomarkers of oral inflammation namely cytokines (80). Functional interactions among cytokines appear to be in networks within the periodontium and integrating aspects of both innate and adaptive immunity, particularly molecular and cellular pathways, are associated with disease pathogenesis (86, 87). Recent theories have suggested that TNF- α , a pro-inflammatory cytokine, plays a key role in tissue destruction in periodontal connective tissue and alveolar bone (16). The presence of high levels of TNF- α and IL-6 represent the initial action of cytokines leading to inflammatory cascade at the origin of destruction of periodontal tissue. Therefore, TNF- α can be considered a suitable biomarker for early detection of signs of periodontal disease (16, 18, 88, 90).

Normal growth represents overall health and nutritional status. Hence, knowledge of normal growth patterns is crucial to understand factors that govern infant development (94). Infancy, a

period from birth up to 2 years of life, is crucial for building the base for future health status and different patterns of growth play an essential role in programming long term health (94-96, 115). Indeed, infancy and growth patterns have been linked to the risk of chronic diseases in both children and adults (9, 10, 29). Moreover, numerous studies have shown that low birth weight followed by 'catch-up growth,' is strongly associated with obesity later in life (22). Specifically, small birth size for gestational age and rapid postnatal weight gain have been linked to risk of cardiovascular disease in both children and young adults (9, 10, 29).

Given the importance of growth in infancy in determining later health outcomes including obesity, diabetes and cardiovascular diseases, and of the links between periodontal diseases and these chronic conditions, we propose to investigate the association between growth in infancy and periodontal inflammation in adolescence. We argued that growth patterns may play a role in explaining the observed associations of obesity, diabetes and cardiovascular diseases with periodontal diseases in adulthood.

3) Aims and Objectives

To estimate the extent to which growth trajectories between age 0 to 2 years are associated with with indicators of periodontal health, namely concentration levels of TNF α -GCF and gingival bleeding on probing, among 8-10 year old children participating in the QUALITY Cohort.

4) Methodology

4.1 Overview of the study design

Baseline data from an ongoing prospective study, The Quebec Adipose Lifestyle Investigation in Youth (QUALITY) Cohort, were used for this project (116). The QUALITY Cohort is an on-going prospective longitudinal study that was set up to investigate the natural course of obesity and resultant disease outcomes among Quebec children. The Cohort started in the summer of 2005 and 630 families completed the baseline evaluation in 2007-08 when children were aged 8-10 years. Two follow up visits were then conducted in 2010-11 and 2015-16. For this project, we will use only baseline data (116).

4.2 Study Setting

The QUALITY Cohort study was conducted in the Canadian province of Quebec and domiciled at the clinical research units of Sainte-Justine Hospital in Montreal and Laval Hospital in Quebec City. Montreal and Quebec City are the two largest cities in Quebec with over 3.8 million and 765,000 in population, respectively.

4.3 Study population

The QUALITY cohort included 8-10 year-old children of Caucasian origin with history of parental obesity in one or both biological parents. Parental obesity was defined as a BMI > 30 kg/m2 or an elevated waist circumference (>102 cm in males and >88 cm in females). Only children of Caucasian origins were included to reduce genetic variation.

4.3.1 Inclusion Criteria

For children to be eligible to be a part of the study they had to:

• have both biological parents available and willing to participate in the study data collection (e.g., fill out the questionnaire)

- have at least one of the parents who was obese (i.e., body mass index ≥30Kg/m² or waist circumference >102cm in men and >88cm in women).
- be of Caucasian origin
- be 8-10 years old

4.3.2 Exclusion Criteria

Children were excluded if they:

- had a serious illness (cancer, inflammatory bowel syndrome, anorexia nervosa, inborn errors of metabolism, cerebral palsy, and others) that might modify the natural history of obesity and its metabolic consequences
- had any previous diagnosis of diabetes
- were taking medication like antihypertensives or steroids (except topical administration or via inhalation)
- had any underlying psychological conditions or cognitive disorders that might hamper the participation
- were following any diet restrictions(<600kcal/day)
- had any pending plans of the family to move out of the province.

4.4 Recruitment procedures

Majority of the children were recruited through their schools (32 school boards and 1304 schools of both private and public sectors) located within the radius of 75km of Montreal, Quebec City and Sherbrooke in the province of Quebec. This school-based sampling scheme distributed around 400,000 brochures that were circulated among children studying in 3rd, 4th and 5th standard. In addition to this recruitment strategy, families were approached using the study investigator's connections.

Families interested in participating in the study contacted the QUALITY Cohort personnel by phone to determine if they met the eligibility criteria detailed. Those families which passed the screening evaluation, were invited for a visit to the hospital and were further explained about the study (e.g., frequency of return visits, required physical and clinical examination, specimen collection and interviews) and height, weight and waist measures were confirmed. Families which met the eligibility criteria and accepted the study requirements were then invited to participate in the study and were asked to sign an informed consent form.

4.5 Ethics and confidentiality

The QUALITY cohort study gathered approval from the Ethics Review Boards of the 'Centre Hospitalier Universitaire' Sainte-Justine, Quebec Heart and Lung institute, McGill University, and Laval University. Consent forms were signed by the parents, while children provided assent. Results of the following tests along with their interpretations were sent to the families, and if required, a recommendation of follow-up in concern with the current standard of care: blood pressure, fasting glucose and lipids for parents and child; 2-h post load glucose, aerobic fitness, and bone mineral density for child only. Since the families recruited were from a high-risk background of metabolic abnormalities, they were given Canada's Food Guide to Healthy Eating and Physical Activity Guidelines for Children and Youth. Detailed instructions on oral health behaviours were also provided and, if necessary, they were advised to visit the dentist.

All information was handled with high confidentiality. Computerized data containing nominal information was encrypted. A confidentiality form had to be signed by all the investigators for assessing the data.

4.6 Data collection and definition of the variables

Data collection for the cohort was performed during a full day visit at the clinical research units of 'Centre Hospitalier Universitaire' Sainte-Justine or Hôpital Laval (Quebec City). It comprised biological, physiological and arthropometric measurements, four questionnaires (child's, mother's, father's, both parents' questionnaires), oral clinical examinations.

4.6.1 Questionnaires

Both the child's mother and father individually completed a questionnaire that collected information about general and oral health status, oral health behaviour, physical activities, social

and financial situation of the family, and family medical history. They also completed another questionnaire that was addressed to both parents inquiring about family history, education level, neighbourhood characteristics, parents-child relationship, child allergies and medical history, dietary supplements, dental attendance such as dental visits and fluoride exposure of the child, social life of the child, personality of the child, and financial situation (e.g., family income). Finally, a research assistant interviewed the child using a questionnaire that collected information on several factors such as the child's age, sex, education level, physical activities, psychological state, social life, and oral health behaviour such as frequency of tooth brushing. The questionnaires can be found in the appendices 1 and 2.

4.6.2 Oral examination procedures

Dental examinations of the children were conducted in a room that was exclusive for the study and was set up in advance in a standard manner. A reclining position of the dental chair was used with a standardized source of artificial light. The angle of inclination of the chair was the same for each examination. The instruments were placed on a clean cloth where the child could not see them to avoid increased stress, but where they were accessible to the examiner. A dental hygienist or a dentist (graduate students) specially trained for this project conducted the dental examination and a research assistant recorded the dental exams in a specific form designed for the study. The oral clinical examination included assessment of dental caries, periodontal health and traumatic dental injuries in this sequence. For this project, we only used indicators of periodontal health for which we describe the details below. A full description of the oral health examination can be found in the manual of procedure (Appendix 3).

4.6.2.1 Periodontal disease measurements (outcomes)

Children were examined lying on a dental chair under a standardized source of artificial light. Dental examinations included clinical (presence and absence of bleeding on probing, calculus and plaque) and inflammatory indicators of periodontal health. Two indicators of periodontal inflammation were collected in this cohort: (i) Gingival crevicular fluid (GCF) which was subsequently analysed for the cytokine TNF- α and was used as a biological marker of periodontal
inflammation (primary outcome); (ii) and the presence and absence of gingival bleeding on probing, which is an accepted clinical marker of gingival inflammation (secondary outcome). TNF- α is one of several cytokines secreted by macrophages and monocytes in response to bacteria (lipopolysaccharides) and plays an extensive role in periodontitis mediated bone loss by stimulating the formation of bone-resorbing cells (osteoclasts) (16, 17). It is a marker of the earliest stages of episodes of periodontal disease activity (17, 18).

The presence or absence of gingival bleeding was measured on the buccal and lingual surfaces of six community periodontal index (CPI) teeth, that is, teeth 16,11,26,36,31,46, using a community periodontal index probe (117, 118). For the purpose of data analysis, we used the proportion of the number of surfaces with bleeding on probing, which is calculated by dividing the number of dental surfaces with bleeding by 12 (i.e., number of surfaces at risk: buccal and lingual surface of each six CPI teeth), which is the Gingival Bleeding Index (117, 118). The variable was used as a continuous variable in the data analysis.

GCF samples were collected using periopaper strips that were inserted into the mesio-buccal sites of the respective teeth: (11, 16, 31, 36). Proper isolation was carried out using cotton rolls followed by insertion of the periopaper strips into the gingival sulcus for approximately 30 seconds. Thereafter, a periotron 6000 machine recorded periotron units that converted to the actual volume of GCF(μ I) according to a standard curve (Appendix 3). Four of the strips gathered from each child were then stored in a cryotube at -80C until laboratory analysis. Levels of GCF-TNF- α were measured using an enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions. Minimum volume of 0.2I was considered adequate for ELISA analysis. GCF-TNF alpha had an inter-assay coefficient of variation of 16% at 9.02pg/ml. Log of GCF TNF- α was used as a continuous variable.

4.6.3 Anthropometric measurement (main exposures)

4.6.3.2 Height and weight from birth to 2 years

In Quebec, parents of newborns were issued the Quebec Health Booklets to track the growth and other health-related indices of their children at each hospital visit. During these routine medical visits, health care professionals measured and recorded important anthropometric variables including gestational age, birthweight, weight and length from 0 to 24 months of age.

These variables were extracted from the booklets of the children participants on entry into the QUALITY Cohort. To be included in this project, these measurements for each child had to include a minimum of three time points during the first 24 months of life (i.e., at birth, at least once between 1 and 12 months; median: 6 entries [range: 3-10 entries]). From this, weight and length measurements from birth to 24 months were transformed to sex-specific weight-for-length z-scores (zWFL) using child growth standards from the WHO (119).

4.6.3.2 Height and weight at age 8-10 years

The child's weight and height were taken with the child dressed in light indoor clothing without shoes, using a stadiometer for height (measured at maximal inspiration) and an electronic scale for weight. The child's weight and height were measured using an electronic balance, and stadiometer recorded each of them to the nearest 0.1 kg or 0.1 cm, respectively.

This procedure was repeated twice and if each measurement differed by more than 0.2 kg for weight and 0.2 cm for height, a third measurement was taken and the average of the two closest measurements was used.

Body mass index (BMI) was calculated by dividing weight by height squared. We used the United States-Centers for Disease Control (US-CDC) growth chart reference values to calculate sex and-age-specific BMI values which were then categorized into normal weight (≤85th percentile), overweight (85th–95th percentile) or obese (≥95th percentile) (120).

4.6.4 Covariates used as potential confounders

As described in the next section, we selected our potential confounders using Directed acyclic Graphs (DAGs). The variables included in the DAG are as follows:

Sociodemographic factors: These include age of the child, parental income and education status which was obtained from self reported questionnaires.

Age of the mother at the birth of child was calculated by taking the difference of child's date of birth and mother's date of birth (Appendices 1 and 2).

Education level of parents was collected via questionnaires and then recoded into a binary variable based on university degrees achievement (yes/no).

Family income was defined as total income within the last fiscal year (before taxes and deductions) from all people who occupied the household in which the child lives most of the time and it was measured in an ordinal scale containing 12 categories (appendix 2). A continuous variable was then later created for family income, adjusted for the number of family members, as follows (121):

 $Adjusted family income = \frac{Family income (\$)}{\sqrt{number of family members}}$

Other variables include breastfeeding (ever breastfed or not), body mass index of mother and child's gestational age. In addition, information on maternal history of gestational diabetes, hypertension and smoking were collected from the questionnaries (Appendices 1 and 2).

4.7 Analytical strategy

4.7.1 Directed acyclic Graphs (DAGS)

DAGs, also termed as "Causal Diagrams", are an extensively elaborated form of traditional graphs that have been widely used in epidemiologic research (122-124). They offer a way to express the underlying biology of the subject-matter relationship and help identify any confounding effect (123-125).

DAGs help to conceptualize the research problem by allowing us to generate links between graphical causal paths and statistical associations (123-126). Although DAGs are mathematical ground, using them to draw our causal assumptions between our exposure and outcome of interest can help pick the most straightforward set of variables for our models without understanding much about the maths behind it (123-126). In other words, it allows us to take out paths between variables without taking into consideration the shape of this relationship but only considering its direction (124-126). The connections are often simple, linear, or of a more complex function (123, 124).

Directed – all variables connected by arrows

<u>Acyclic</u>- "unidirectionality" of the arrows as cause always precedes the effect thus forming a closed loop and cannot end up on the same variable where it started from.

<u>Graphs-</u> graphical representation

Key elements of a DAG include:

a) Variables (nodes, vertices)

b) Arrows (directed edges, arcs)- depicts the direction of the causal relationship (123)

A hypothetical diagram (Figure 1) depicting the DAG will allow us to choose a "minimally sufficient set of variables from a group of potential confounding variables, to be adjusted within the analysis, to urge an unbiased estimate of the entire or the direct effects of exposure on outcome "(123-125, 127).



Figure 1: Example of a Directed acyclic Graph

In our example (Figure 1), letters X and Y represent exposure and outcome, respectively. The edges going from one node to another depict the direction of the causal relationship (123, 124). Letter P represents the covariate that might affect exposure-outcome relationship while letters U1 and U2 represent unmeasured covariates (124). These would include any descendants of X (exposure). Any path which leaves X through the arrowhead entering it and ends up in Y is termed as a backdoor path (123, 128). This way we come out with 4 different pathways. Thus, all possible backdoor pathways include (XU1PU2Y, XPU2Y, XU1PY, XPY). But we notice that our path "XU1PU2Y" is hampered by the collider 'P' so that implies that we are left with three unblocked paths (Collider is the one that receives two opposing arrows into it. In this case the letter 'P' is the collider as it has two opposing arrows into it). As we are well aware that the unmeasured covariates are causally related to 'P' which is the descendant of our exposure X, hence controlling for P would lead to an induced spurious association between U1 and U2 (dotted line). This will lead to opening of a new backdoor path 'XU1U2Y' which in reality is not even there, eventually leading to biased results (124, 127). Finally, the minimal sufficient set of covariates that we adjust for will include either [U1, P] or [U2, P].

4.7.2 Variable and model selection

Figure 2 shows the Directed Acyclic Graph for the association between growth trajectories and TNF-alpha. The key variables identified that may confound this relationship are: age of the mother at the birth of the child, maternal education status, body mass index of the mother and the child, breastfed, diet, tanner age. Figure 2 shows that diet has arrows pointing to it hence it is said to be a collider which we will not adjust for else it will create a spurious association between the exposure and the outcome when there isn't one, leading to biased results (124, 127). Here, the variables- maternal education and tanner age tend to be potential modifiers. Finally, mother and child's body mass index, age of the mother at the birth of the child and breastfed have been chosen as a set of variables to be adjusted for based on the DAG (which were chosen with the help of use of DAGGITY software) (129) http://dagitty.net/dags.html#).

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Figure 2: Directed Acyclic Graph showing minimal set of confounders (in white) to be adjusted for and the remaining being the ones that are based on the background information of the study design.

4.8 Overview of analytical techniques

4.8.1 Latent Class Mixed Model Analysis

Latent Class Mixed Model (LCMM) is an analytical technique analyzing change over time of a longitudinal outcome with a Gaussian distribution and helps assess the effects of covariates on it. Latent in itself implies hidden or unobserved (130, 131). Latent class analysis is a data-driven method, that is, it is dependent on the data from which it is derived wherein the individuals are probabilistically grouped into classes or categories (class membership) based on an unobserved (latent) variable (130, 131).

The Latent Class Mixed Model:

a) It groups individuals into trajectories

b) Akaike Information Criterion (AIC) or Bayesian Information Criterion (BIC) criteria are used to compare different number of class solutions or models to see which model adequately fits the data

c) In terms of probability, which class each individual most likely belongs to i.e., which trajectory it belongs to (posterior predictive probability).

d) Thereafter, the system locates the class with the highest (130-132). Due to the unobserved segment, classes can only be estimated. Within each class, longitudinal profiles are modeled by a typical linear mixed model. Individual contributions to the likelihood are thus the weighted sum of class specific densities (131), refer to the equation below) (https://lib.ugent.be/fulltxt/RUG01/002/349/892/RUG01-002349892_2017_0001_AC.pdf).

$$f_i(y_i) = \sum_{g=1}^G \pi_g f(y_i / c_i = g)$$

With:

$$0 \le \pi_g \le 1$$
 and $\sum_{g=1}^G \pi_g = 1$

and

$$Y_{ij}/c_{i=g} = X1_{ij}\beta + X2_{ij}v_g + Z_{ij}u_{ig} + \epsilon_{ij}$$

 π_q = class-specific probabilities

 c_i = discrete latent variable that equals g if subject i belongs to latent class g

X1= vector of covariates that are associated with common fixed effects over classes β

X2= vector of covariates that are associated with class-specific fixed effects v_g

Z= vector of covariates associated with random effects

 u_{ig} = class-specific random effects, u_i / c_i = g~N ($O, \omega^2 g B$)

& with B being an unspecified variance-covariance matrix and ω_g a proportional coefficient allowing class-specific intensity of individual variability.

 ϵ_{ii} = measurement error, ~N (0, σ_{ϵ}^2)_

(https://lib.ugent.be/fulltxt/RUG01/002/349/892/RUG01-002349892_2017_0001_AC.pdf)

To estimate the parameters, the Maximum Likelihood Theory with a robust modified Marquardt iterative algorithm (133) and strict convergence criteria were used. Post-fit functions were also equipped to assess the models' goodness-of-fit, predictive accuracy, and compute output average or individual predictions from the models (131, 133).

We used the R package LCMM to perform our analysis (134). The predicted class is used as exposure and regressed on the outcome. To improve our model-fit, we used log transformation of our primary outcome, concentration levels of TNF- α in GCF in this analysis.

4.8.2 Generalized Linear Modelling

A generalized linear model (GLM) was fitted to data to model the association between growth trajectories and concentration levels of TNF- α in GCF (primary outcome) and the severity of bleeding index (secondary outcome), adjusting for confounders designated within the DAG (Figure 2).

GLM, as popularised by McCullagh and Nelder, is a way to model univariate data which follow exponential family distributions (135). The model commands give large degrees of flexibility within the selection of each of the model features (136). It is feasible to model variables with normal, gamma, Poisson, inverse Gaussian, binomial, geometric, and negative binomial distributions by selecting an appropriate link function (135, 136).

The GLM framework constitutes three components:

<u>Random Component</u>: implies to the conditional distribution of Y (response variable), e.g., in the case of a linear regression, this is a normal distribution: $N(\mu_i, \sigma^2)$ and σ is called the dispersion parameter.

Systematic Component (Right hand side of the equation): specifies explanatory

variables($X_1, X_2, ..., X_l$) in the model, which when used in case of linear regression create the linear predictor ($\beta_0 + \beta_1 x_1 53 + \beta_2 x_2 ...$)

$$\eta_i = \alpha + \beta_1 \times x_{1,i} + \beta_2 \times x_{2,i} + \dots + \beta_p \times x_{p,i}$$

<u>Link function</u>: links the linear predictor to the left hand side of an equation and link function: $g(\mu_i)$, where $\mu_i = E(Y_i)$

Hence, the equation becomes

$$g(\mu_i) = \eta_i = \alpha + \beta_1 \times x_{1,i} + \beta_2 \times x_{2,i} + \dots + \beta_p \times x_{p,i}$$

Since the link function is reversible, the equation could also become

$$\mu_i = E(Y_i) = g^{-1}(\alpha + \beta_1 \times x_{1,i} + \beta_2 \times x_{2,i} + \dots + \beta_p \times x_{p,i})$$
(55 Published on *STAT 504* (https://online.stat.psu.edu/stat504/node/216))

4.9 Sample size

The final sample size included in this project was 244 boys and 186 girls for whom data were available on anthropometric measures and indicators of periodontal health (levels of TNF- α in the GCF and gingival bleeding).

5) Results

5.1. Descriptive statistic

For this thesis work, a total of 430 children participating in the QUALITY cohort were considered for the analysis. Data on selected characteristics of the study population are presented in Table 1. The mean age of the children was 9.1 years (SD=0.9) and the majority of them were males (n= 244, 56.7%). Most children did not have excess weight (62%) and the average gestational age was around 40 weeks. The mean age of the children's mothers was 30.27 (SD=4.7) years and 18% of them had history of gestational diabetes. The mean family income was 42,727 CAD\$.

Characteristics	n=430 (%)
Male, n (%)	244(56.7)
Birth zWFL (±SD)	-0.3(1.2)
Gestational age in weeks; median (IQR)	40 [39.0, 40.0]
Ever breastfed, n (%)	335(77.9)
Child age, mean (±SD)	9.1(0.9)
BMI at age 8-10	
No excess weight (BMI percentile < 85%)	267(62.1)
Overweight (BMI percentile>85%and<95%)	73(17.0)
Obese (BMI percentile >95%)	90(20.9)
Maternal pregnancy characteristics	
Mother age when child was born in yrs; mean (± SD)	30.27(4.7)
History of gestational diabetes, n (%)	78(18.1)
History of hypertension during pregnancy, n (%)	54(12.6)
Maternal smoking during pregnancy, n (%)	69(16.0)
Family income CAN\$; mean± SD	42,727.01 (18521.02)
TNF-α in pg/ml; median (IQR)	215 (33, 617)
Gingival bleeding sites in %; median (IQR)	75 (50, 83.33)

Table 1: Selected baseline characteristics of the study population (n=430)

IQR- Interquartile Range, TNF-α

The median TNF α -GCF was 215 (IQR: 33, 517), and the median proportion of gingival bleeding sites was 75 (50, 83.33).

The next step of the analysis was to create the trajectories using latent class analysis. Table 2 provides the model fit statistics for one-, two-, three-, and four- class linear and quadratic LCMM. The model that best explained the data was a quadratic model with a three class solution (AIC=7518.71; Table 2). In this model, 82% of the children were assigned to one of the three classes. The marginal and associated posterior probabilities are shown in Table 3. The three class trajectories associated with the final quadratic model are shown in Figure 3 and named as follows: Class 1: Growth of the children that started out at a lower than average WFL at birth but grew rapidly; Class 2; children who had a higher than average WFL at birth but had a dip in growth ; and Class 3 denoted expected growth in a child who was born at the average WFL and initially rose rapidly and then became steady.

	Linear Model	Quadratic Model
Classes	AIC Values	AIC Values
1 class	7758.225	7522.458
2 class	7764.050	7519.393
3 class	7753.852	7518.718
4 class	7758.096	7522.832

Table 2: AIC Values for Linear and Quadratic Models

POST PROB	CLASS1	CLASS2	CLASS3
N (%)	63(14.7)	76(17.7)	290(67.6)
PROB 1	0.8288	0.0000	0.0516
PROB 2	0.0007	0.7855	0.0945
PROB 3	0.1705	0.2145	0.8539

Table 3: Quadratic model for class 3 solution (Marginal Proportions and Mean of Posterior Probabilities in each class)



Figure 3: Class 3 Mean Predicted Trajectory. Class 1- Lower than average birth-weight-for-length followed by high ("catch-up") growth acceleration; Class 2- Higher than average birth-weight-for-length followed by low growth acceleration; and Class 3- Expected growth.



Figure 4: GCF TNF- α levels by growth trajectories



Figure 5: Proportion of gingival bleeding sites by growth trajectories

Figures 4 and 5 show the distribution of TNF- α and proportion of gingival bleeding sites by growth trajectories, respectively. The figures show that the median TNF α -GCF level is only slightly lower in members of the Class 3 trajectory as compared to Classes 1 and 2, while the median proportion of gingival bleeding sites is approximately equal across the three trajectories.

5.2 Association between growth trajectories and indicators of periodontal health

Multivariate linear regression analyses were performed to estimate the extent to which growth trajectories and indicators of periodontal health, namely concentration levels of TNF α in the GCF and gingival bleeding on probing, were related. The results of these analyses are presented in Table 4. Growth trajectories were not associated with TNF α -GCF levels nor with proportion of sites with gingival bleeding, after all relevant covariates have been adjusted for (Table 4).

Table 4: Associations between growth trajectories and measurements of periodontal health in children participating in the QUALITY cohort (n= 430)

Growth Trajectories	GCF TNFα	Proportion of gingival bleeding sites
	β(95% CI)*	β(95% CI)*
Class 3	1	1
Class 1	0.49	2.11
	(-10.80-11.79)	(-4.75, 8.98)
Class 2	0.18	1.13
	(-10.29-10.64)	(-5.23, 7.49)

6) Discussion

This section exhibits a comprehensive perspective of this research using the QUALITY Cohort study's data. Supporting the study's objectives, we restate the project's overall rationale, followed by a summary of results. Limitations, strengths and conclusions are then discussed.

6.1 Rationale

Periodontal diseases are estimated to affect around 3.5 billion people worldwide, being the 6th most prevalent disease across the world (1). Although the association between periodontal diseases and other chronic systemic conditions (e.g., obesity, diabetes, cardiovascular diseases) in the adult population is well recognized, little is known of this association in early life. This work addresses this gap in the literature. The limited literature on these associations is partly explained by the low prevalence of clinically measurable parameters of periodontitis in children. Regardless of the low prevalence of these health problems, our understanding of periodontal diseases' pathophysiology suggests that oral inflammation markers could also be detected in early age, even before the condition is clinically observable (18). A vast amount of literature emphasizes the linkage of the growth patterns throughout infancy, specifically small birth size for gestational age and rapid postnatal weight gain, to the risk of cardiovascular disease in both children and young adults (9, 10, 12, 29, 137, 138). Evidence also suggests that diabetes and cardiovascular diseases originate early in life, and factors in childhood are predictive of disease later in life (9, 29). Being born large for gestational age has also been linked with adverse cardiovascular consequences (10, 113). Since periodontitis shares a basic underlying mechanism of pathogenesis with these other chronic systemic inflammatory conditions, it is worth examining if a similar relationship exists between growth and periodontitis.

The biological mechanism underpinning the association between periodontal diseases and obesity, diabetes and cardiovascular disease still continues to be hotly debated. However, low-grade systemic inflammation is being considered a standard link between both these diseases (139). While these conditions (e.g., diabetes, obesity) may be considered as risk factors for periodontal diseases (5-8), their link with periodontal diseases is complex and possibly bi-

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directional (58, 140). Alternatively, inflammation, infection and adaptive immunity are other likely pathways for the periodontal-systemic association (35, 141, 142). Also, these diseases appear to originate early in life (21). Compelling evidence shows that low birth weight and thinness at birth followed by catch-up growth is associated with these chronic conditions later in life (23-28, 30). This notion is predicated on the Barker's hypothesis, which states that "fetus appears to respond to insults during the prenatal period through the process of "programming," which has short-term survival advantages but may have a long term disadvantage in that it is associated with cardiovascular disease, hypertension, type II diabetes, and later obesity" (95). Whilst it has been argued that fetal life is critical for early origins of cardiovascular disease, the early postnatal life is also crucial, as studying patterns of growth might provide an indication of the potential risks of later disease to which they are being exposed (110).

Evidence gathered so far urges us to study the influence of factors associated with growth and development on later outcomes within the context of the infancy period (143). Infancy is recognized as a period from birth to 2 years, characterized by high maternal investment and the infant's total dependence on the mother for survival (144). "The relationship of birthweight to subsequent growth appears to be critical in that it predicts physique at a later time and perhaps predicts the magnitude of risk factors for later(adult) disease" (95).

6.2 Summary of the Results

This project investigates the extent to which trajectories of growth from birth to 2 years are related to indicators of periodontal health in children. In our analysis, the three class trajectories associated with the final quadratic model (Figure 3) were named as follows: Class 1: Growth of the children that started out at a lower than average weight-for-length at birth but grew rapidly, Class 2: children who had a higher than average weight-for-length at birth but had a dip in growth, Class 3: expected growth in a child who was born at the average weight-for-length and initially rose rapidly and then became steady.

We used two indicators of periodontal health, TNF- α concentrations as a subclinical indicator of the periodontal health of youngsters and gingival bleeding on probing. Our results suggest no association between growth trajectory and TNF- α concentrations in GCF and gingival bleeding. The median TNF α -GCF level was found to be only slightly lower in members of the Class 3 trajectory compared to Classes 1 and 2, while the median of gingival bleeding is approximately equal across the three trajectories. The results reflected from the adjusted linear models showed that the different growth trajectories were neither associated with TNF α -GCF levels nor with the proportion of sites with gingival bleeding, after all relevant covariates had been adjusted for.

6.3 Strengths of the Study

The use of an innovative statistical analysis method forms an important strength of this thesis. We used the latent class analysis to classify growth variable into trajectories so that we could get the extent to which these trajectories of growth were related to indicators of periodontal health in children. Also, our study follows a retrospective cohort design in which our exposure was ascertained from Quebec health records and outcome was ascertained at the time when the baseline of the QUALITY Cohort study began.

6.4 Methodological Challenges

6.4.1 Internal Validity

"Internal validity is the quality controlling whether a valid assessment of cause and effect can be made within the context of the study" (145).

6.4.1.1 Selection Bias

"Selection bias occurs when a systematic error in the recruitment or retention of study participants (cases or controls in case-control studies or exposed or unexposed subjects in cohort studies) results in a tendency towards distorting the measure expressing the association between exposure and outcome" (122).

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Participation in the QUALITY Cohort study involved a total of 1320 families, out of which 634 agreed to participate in the baseline assessment with the exclusion of four families (116). Selection of the participants for the baseline visit in the QUALITY cohort involved stringent inclusion/exclusion criteria with a minimum of one obese parent accompanying the child for a full day clinic visit. As is usually the case in epidemiological studies, these are often dictated by cost or other logistic considerations, so was our study in which we did not have complete data in relation to the oral health component of the study (53). All these may have generated selection bias. However, the subjects in our study were excluded on a random basis, and therefore, there is no relation with the exposure or outcomes of interest. Hence, we do not think that the issues related to recruitment of our sample would distort our results.

6.4.1.2 Information Bias

"In epidemiologic studies, information bias results from either imperfect definitions of study variables or flawed data collection procedures which may result in misclassification of exposure and/or outcome status for a significant proportion of study participants" (122).

Information bias may have occurred during our study. However, due to the rigorous quality control procedures in the QUALITY Cohort, we believe that the risk of differential exposure information is minimal. For instance, the dental exams were conducted by a trained and calibrated health professional in a clinical setting with the children lying on a dental chair under adequate lighting. Moreover, indicators of periodontal health were collected using Florida probe which helps to minimize measurement error.

6.4.1.3 Exposure Identification Bias

The anthropometric measurements (weight and length)– our primary objective marker of exposure, was ascertained from health booklets which were entered by health care professionals (nurses and physicians); therefore, chances of measurement error are low. However, some amount of variability in measurement precision is probable since these measures were not intended to be for research purposes.

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6.4.1.4 Recall and interviewer Bias

Parent completed questionnaires and detailed interviews were conducted by the research assistant. Chances of interviewer bias might have been possible due to the unfamiliar setting of the interview for the child or recall bias wherein recalled information of past exposures might have been incorrect. However, the QUALITY Cohort study had strict quality control measures and highly trained personnel, including dentists, nurses and research assistants (122).

6.4.2 Outcome

Information was extracted from four sites for one of our outcomes, GCF TNF- α , which could have directed the results towards null. TNF- α levels were measured from four (mesio-buccal) sites within the mouth assuming that the chosen sites have maximum levels of TNF- α (50). Moreover, loss of site-specific information occured as we pooled the GCF TNF- α samples. However, we were able to acquire a mean of the measure of GCF TNF- α which was ample enough to explain our research question (53).

6.4.3 Sample size

The population selected for the baseline visit for this study included a total of 630 children, out of which data were missing on subjects' oral health; complete data could only be obtained for 430 families. Hence, the small sample size may have reduced the accuracy of the results accordingly.

6.4.4 External Validity

As mentioned previously, the QUALITY Cohort study was undertaken to investigate the natural course of obesity and resultant disease outcomes among Quebec children. Our study sample adopted the inclusion of children only of Caucasian origin, hence the study is not generalizable to the entire population. Nonetheless, since we did not intend to generate population prevalence estimates, representativeness is not an issue to our study (116, 145).

7) Conclusion and Future Research

The analysis of the present study leads to the following conclusions :

We did not observe an association between growth trajectories and indicators of periodontal health, namely, TNF- α concentrations in the GCF, and the extent of gingival bleeding on probing, among 8-10 year-old Quebec children at risk of obesity.

Future Research:

The broad implication of the present research was to acknowledge the role of early growth in infancy in determining later health outcomes by examining trajectories of growth. Although our results were inconclusive, the biological plausibility for the associations tested in this work is innovative and of relevance. The effect of early development on health outcome later in life is increasingly recognized and future studies should further investigate similar hypotheses by taking into consideration certain shortcomings of the current study. For example, indicators of periodontal health could have been collected from the whole mouth instead of selected sites. Also, future investigations could evaluate other inflammatory markers in the gingival crevicular fluid (e.g., IL-6). Moreover, future studies could use large sample sizes.

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8.1 Links:

The WHO Child Growth Standards. Available at: <u>http://www.who.int/childgrowth/en</u>

(55 Published on STAT 504 (https://online.stat.psu.edu/stat504/node/216))

Access via link (Last visit: 09/09/20) : https://lib.ugent.be/fulltxt/RUG01/002/349/892/RUG01-002349892_2017_0001_AC.pdf

Accessvialink:

https://somepomed.org/articulos/contents/mobipreview.htm?14/43/15025/abstract/1

9) Appendices

9.1 Appendix 1

CHU Sainte-Justine Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants









Familial Study on the Prevention of Cardiovascular Disease and Type 2 Diabetes in Children and Adolescents

Child Questionnaire for Visit 1 (1QE)

Child's PIN:

May 2006

Instructions

In this questionnaire, we ask you questions about your family, your school, the things you like to do, and your feelings about certain things.

There are no right or wrong answers. This is not a test.

Simply answer each question, indicating what you really think or feel.

General Information

1. How old are you?

_____ years old

- 2. You are...
 - ${}_{1}O$ a boy ${}_{2}O$ a girl
- 3. What language do you usually speak with your best friends?
 - A. $_1O$ French
 - ₂O English
 - ₃O Italian
 - ₄O Greek
 - $_{5}O$ Spanish
 - ₆O Portuguese
 - $_7$ O Other
 - B. If you answered «other» to the previous question, please specify what language you **usually** speak with your friends.
- 4. What grade are you in or what grade did you recently complete?
 - $_1$ O 1st grade
 - $_{2}O$ 2nd grade
 - $_{3}O$ 3rd grade
 - $_4$ O 4th grade
 - $_{5}O$ 5th grade
 - $_{6}O$ 6th grade

- 5. In the last month (or in the last month this school year), how did you **usually** get to school **in the morning**?
 - $_1 O$ School bus
 - $_{2}O$ Subway or city bus
 - ₃O Car
 - ₄O Bike
 - $_{5}O$ Walking
 - $_{6}O$ Other
- 6. If you usually **walked** to school **in the morning** over the last month (or in the last month this school year), how many minutes did it usually take?

____ minutes



7. If you usually **biked** to school **in the morning** over the last month (or in the last month this school year), how many minutes did it usually take?

_____ minutes

- 8. In the last month (or in the last month this school year), how did you **usually** get home from school **in the afternoon**?
 - $_1 O$ School bus
 - $_{2}O$ Subway or city bus
 - ₃O Car
 - ₄O Bike
 - ₅O Walking
 - $_{6}O$ Other
- 9. If you usually **walked** home from school in the afternoon over the last month (or in the last month this school year), how many minutes did it usually take?

____ minutes

 $_{97}$ O Not applicable

10. If you usually **biked** home from school in the afternoon over the last month (or in the last month this school year), how many minutes did it usually take?

_____ minutes

₉₇ O Not applicable
Physical Activity and Sports

- 11. Think about the physical activities that you did last week from Monday to Sunday **outside your regular gym class**. For each activity that I will mention, tell me if you did this activity **for 15 minutes or more at one time**.
 - > Please answer for each item.

		1. Not in the last week	2. Mon	3. Tues	4. Wed	5. Thur	6. Fri	7. Sat	8. Sun
Α.	Bicycling to school, bicycling to do errands or going for a bicycle ride	0	0	0	0	0	0	0	0
В.	Swimming/diving	0	0	0	0	0	0	0	0
C.	Basketball	0	0	Ο	0	0	0	0	0
D.	Softball	0	0	Ο	0	0	0	0	0
E.	Football	0	0	Ο	0	0	0	0	0
F.	Soccer	0	0	Ο	Ο	0	0	0	0
G.	Volleyball	0	0	Ο	0	0	0	0	0
H.	Racket sports (badminton, tennis)	0	0	Ο	0	0	0	0	0
١.	Ice hockey/ball hockey/street hockey/ringuette	0	0	Ο	Ο	0	0	0	0
J.	Skip rope	0	0	0	0	0	0	0	0
K.	Downhill skiing, snowboarding	0	0	Ο	0	0	0	0	0
L.	Cross-country skiing	0	0	0	0	0	0	0	0
М.	Ice skating	0	0	Ο	0	0	0	0	0
N.	Rollerblading, skateboarding	0	0	Ο	0	0	0	0	0
0.	Gymnastics (bars, beams, acrobatics, trampoline)	0	0	0	0	0	Ο	Ο	0

Continuation...

11. Think about the physical activities that you did last week from Monday to Sunday **outside your regular gym class**. For each activity that I will mention, tell me if you did this activity **for 15 minutes or more at one time**.

		1. Not in the last week	2. Mon	3. Tues	4. Wed	5. Thur	6. Fri	7. Sat	8. Sun
Ρ.	Exercise/working out (push-ups, sit-ups, jumping jacks, weight-lifting, exercise machines)	0	0	0	0	0	0	0	0
Q.	Ball-playing (dodge ball, kickball, wall-ball, catch)	0	0	0	0	0	0	0	0
R.	Track and field	0	0	Ο	0	Ο	0	0	Ο
S.	Games (chase, tag, hopscotch)	0	0	Ο	0	Ο	0	0	0
Τ.	Jazz/classical ballet	0	0	0	0	Ο	0	0	Ο
U.	Dancing (aerobic, folk, at a party)	0	0	Ο	0	Ο	0	0	Ο
V.	Outdoor play (climbing trees, hide and seek)	0	0	Ο	0	Ο	0	0	Ο
W.	Karate /Judo /Tai Chi /Kung Fu	0	0	Ο	0	Ο	0	0	Ο
Х.	Boxing, wrestling	0	0	Ο	0	Ο	Ο	0	0
Y.	Outdoor chores (mowing the lawn, raking leaves, gardening, shoveling snow)	0	0	0	0	0	0	0	0
Z.	Indoor chores (mopping, vacuuming, sweeping)	0	0	0	0	0	0	0	0
AA.	Walking	0	0	0	0	Ο	0	0	Ο
BB.	Running/Jogging	0	0	Ο	0	Ο	0	0	Ο
CC.	Other 1	0	0	Ο	0	Ο	0	0	Ο
DD.	Other 2	0	0	Ο	0	0	Ο	0	0
EE.	Other 3	0	Ο	Ο	0	Ο	Ο	Ο	Ο

- 12. Over the last year (12 months), did you belong to any of the following intramural or extramural **school** sports teams (teams that were **not** part of your regular gym class)?
 - > Please answer for each item.

		Yes	No
Α.	School basketball team	$_1 O$	O_0
В.	School soccer team	1O	O
C.	School football team	1 O	O ₀
D.	School track and field team	1 O	O
E.	School rugby team	1O	Oo
F.	School wrestling team	1O	O ₀
G.	School swimming team	1O	O
Н.	School softball team	1O	O
١.	School cross-country ski team	1O	O
J.	School volleyball team	1O	O
К.	School gymnastics team	1O	O ₀
L.	School hockey team	1O	Oo
M.	Other school team 1	1O	O
N.	Other school team 2	1 O	O

- 13. Now think about sports teams and lessons **outside of school**. Over the last year (12 months), did you belong to a team or take lessons of...?
 - > Please answer for each item.

		Yes	No
А.	Basketball team	1O	O
В.	Soccer team	1O	O
C.	Football team	1O	O
D.	Swimming team	1O	O
E.	Baseball team	1O	O
F.	Volleyball team	1O	O
G.	Hockey team	1O	O
Н.	Ballet/dance classes	1O	O
١.	Aerobics classes	1O	O
J.	Ski lessons	1O	O
K.	Judo/Karate lessons	1O	O
L.	Other 1	1O	O_0
M.	Other 2	1O	O_0

- 14. How many hours of television (including video movies) do you usually watch in a **single day**? If the answer is zero, we will write 0.
 - A. On weekdays, I usually watch _____ hour(s) of television a day.
 - B. On weekends, I usually watch _____ hour(s) of television a day.
- 15. How many hours do you usually use the computer for fun (including the Internet), or play video or computer games (including Nintendo, Gameboy, etc.) in a **single day**? If the answer is zero, we will write 0.
 - A. On **weekdays**, I usually use the computer for fun or play video or computer games _____ hour(s) **a day**.
 - B. On **weekends**, I usually use the computer for fun or play video or computer games ______hour(s) **a day**.
- 16. How many hours do you usually spend on homework **in a single day** (including homework on the computer)? If the answer is zero, we will write 0.
 - A. On weekdays, I usually spend _____ hour(s) on homework a day.
 - B. On weekends, I usually spend _____ hour(s) on homework a day.
- 17. How much time do you usually spend talking on the phone in a **single day**? If the answer is zero, we will write 0.
 - A. On weekdays, I usually talk on the phone for _____ hour(s) a day.
 - B. On weekends, I usually talk on the phone for _____ hour(s) a day.
- 18. How much time do you usually spend reading comics, novels, or other books for fun in a **single day**? If the answer is zero, we will write 0.
 - A. On weekdays, I usually read for fun for _____ hour(s) a day.
 - B. On weekends, I usually read for fun for _____ hour(s) a day.

19. During a typical week, how often does your **mother**...?

> Please answer for each item.

_		Very often	Often	Sometimes	Rarely
A.	Do physical activity or play sports.	1 O	₂ O	O _ε	4 O
В.	Encourage you to be physically active or to play sports.	10	₂ O	О _ε	4 O
C.	Do physical activity or play sports with you.	10	₂ O	О _ε	4 O
D.	Watch you do physical activity or play sports.	10	₂ O	ЗO	4 O
E.	Tell you that physical activity and sports are good for you.	10	₂ O	О _ε	4 O
F.	Take you to a place where you can do physical activities or play sports.	10	₂ O	ЗO	4 O

20. During a typical week, how often does your **father**...?

		Very often	Often	Sometimes	Rarely
A.	Do physical activity or play sports.	1O	₂ O	О _ε	4 O
В.	Encourage you to be physically active or to play sports.	10	₂ O	ЗO	4 O
C.	Do physical activity or play sports with you.	1 O	₂ O	O _ε	4 O
D.	Watch you do physical activity or play sports.	$_{1}$ O	₂ O	ЗO	4 O
E.	Tell you that physical activity or sports are good for you.	$_{1}$ O	₂ O	ЗO	4 O
F.	Take you to a place where you can do physical activities or play sports.	10	₂ O	ЗO	4 O

21. During a typical week, how often do your **friends**...?

> Please answer for each item.

		Very often	Often	Sometimes	Rarely
Α.	Do physical activity or play sports.	1O	₂ O	О _ε	4 O
В.	Encourage you to be physically active or to play sports.	10	₂ O	ЗO	чO
C.	Do physical activity or play sports with you.	10	₂ O	Ο _ε	чO
D.	Tease you about not being good at physical activity or sports.	10	₂ O	ЗO	4 O

22. How sure are you that you can do the following things on your own time, outside of school...?

		Sure	Mostly sure	A little sure	Not at all sure
А.	Get up early, even on weekends, to exercise or do physical activity.	$_{1}$ O	₂ O	ЗO	4 O
В.	Exercise or play sports even though you feel sad or stressed.	10	₂ O	ЗO	4 O
C.	Maintain regular exercising or play sports even when your family or friends demand more time from you.	10	₂ O	Ο _ε	4 O
D.	Maintain regular exercising or play sports even when you have a lot of school work to do.	10	₂ O	Ο _ε	4 O
E.	Set aside time for regular physical activity or sports.	10	₂ O	ЗO	4 O

23. How true are each of the following statements for you....

_		Very true	Usually true	Sometimes true	False
Α.	I really enjoy being physically active.	10	₂ O	O _ε	4 O
В.	In my free time, I usually choose activities like TV or computers rather than sports.	1 O	₂ O	О _ε	4 O
C.	At home there are enough supplies and equipment (like balls, bicycles, skates) that I can use for physical activity.	1 O	₂ O	O _ε	чŌ
D.	In my house or apartment, there is space where I can do physical activity.	1O	₂ O	Ο _ε	4 O
E.	In the yard outside my house or apartment, there is space where I can do physical activity.	1 O	₂ O	O _ε	чŌ
F.	There are playgrounds, parks, or gyms close to my home.	1O	₂ O	Ο _ε	4 O
G.	It is safe to walk or bike alone in my neighbourhood during the day.	1O	₂ O	ЗO	4 O
Н.	It is safe to walk or bike alone in my neighbourhood at night.	1O	₂ O	Ο _ε	4 O
I.	It is difficult to walk or bike in my neighbourhood because of traffic.	1O	₂ O	ЗO	4 O
J.	In my neighborhood, there are a lot of kids my age who play outside.	1O	₂ O	ЗO	4 O
K.	In my neighborhood, there are a lot of kids my age who belong to sports teams.	1 O	₂ O	ЗO	4 O
L.	I am more physically active than most of my friends.	1O	₂ O	ЗO	4 O
M.	I really enjoy my gym class at school.	10	₂ O	O _ε	4 O

24. During a typical week at school (from Monday to Friday), how many days do you go to physical education (gym) class?

_____ day(s) each week

- 25. During a typical physical education (gym) class, how often are you very active (playing hard, running, jumping)...
 - $_1 O$ For all of the class.
 - $_{2}O$ For most of the class.
 - $_{3}O$ For some of the class.
 - $_4 O$ For none of the class.
- 26. During a typical week at school, what do you do most of the time at recess?
 - $_{1}O$ Sit down to talk, read, do schoolwork.
 - $_{2}O$ Walk around a little bit.
 - $_{3}O$ Run or play a little bit.
 - $_4$ O Run or play quite a bit.
 - $_{5}$ O Run or play hard most of the time.
- 27. During a typical week at school, what do you normally do during lunch time (besides eating lunch)?
 - $_{1}O$ Sit down to talk, read, do schoolwork.
 - $_{2}O$ Walk around a little bit.
 - $_{3}O$ Run or play a little bit.
 - $_4$ O Run or play quite a bit.
 - $_{5}$ O Run or play hard most of the time.
- 28. During a typical week, on how many days right after school (between the end of school and supper) do you do sports, dance, or play games in which you are very active i.e., enough that you feel hot, out of breath, or that your heart beats faster?
 - $_1 O$ None
 - $_2O$ 1 day
 - $_{3}O$ 2 or 3 days
 - $_4O$ 4 days
 - $_{5}O$ 5 days

- 29. During a typical week, on how many **evenings** do you do sports, dance, or play games in which you are **very active i.e., enough that you feel hot, out of breath, or that your heart beats faster**?
 - $_1 O$ None
 - $_2O$ 1 evening
 - $_{3}O$ 2 or 3 evenings
 - $_4$ O 4 or 5 evenings
 - $_{5}$ O 6 or 7 evenings
- 30. During a typical weekend, how many times do you do sports, dance, or play games in which you are very active i.e., enough that you feel hot, out of breath, or that your heart beats faster?
 - $_1O$ Never
 - $_2O$ 1 time
 - $_{3}O$ 2 3 times
 - $_4$ O 4 5 times
 - $_{5}O$ 6 or more times
- 31. Which one of the following describes you the best during a typical week.
 - > Let me read all 5 statements before deciding on the one that best describes you.
 - $_{1}O$ All or most of my free time is spent doing things that involve little physical effort.
 - ₂O I sometimes (1-2 times per week) do physical activities in my free time (sports, running, swimming, bicycling, aerobics, other).
 - $_{3}O$ I often (3-4 times per week) do physical activities in my free time.
 - $_4$ O I quite often (5-6 times per week) do physical activities in my free time.
 - $_{5}$ O I very often (7 or more times) do physical activities in my free time.

- 32. How true are each of the following statements for you. I often don't do physical activity because ...
 - > Please answer for each item.

	Very true	Usually true	Sometimes true	False
A. The weather is too bad.	1 ⁰	₂ O	3 О	4 O
B. I don't have enough energy.	1 ⁰	₂ O	ЗO	4 O
C. There's no place to do physical acti	vity. ₁ O	₂ O	O _ε	4 O
D. It's too boring.	10	₂ O	ЗO	4 O
E. My friends don't like to exercise.	10	₂ O	ЗO	4 O
F. My friends tease me.	1 ⁰	₂ O	O _ε	4 O
G. I'm always chosen last for teams.	1 ⁰	₂ O	O _ε	4 O
H. Physical activity messes up my app	earance. ₁ O	₂ O	O _ε	4 O
I. I am not interested in physical activ	ity. 10	₂ O	O _ε	4 O
J. I don't have enough time.	1 ⁰	₂ O	O _ε	4 O
K. I don't have anyone to do physical a with.	activities ₁ O	₂ O	Ο _ε	4 O
L. I don't enjoy physical activity.	10	₂ O	ЗO	4 O
M. I need equipment that I don't have.	10	₂ O	ЗO	4 O
N. I am not good enough.	1 ⁰	₂ O	О _ε	4 O
O. I am too overweight.	1 ⁰	₂ O	O _ε	4 O
P. I don't like to sweat.	1 ⁰	₂ O	О _ε	4 O
Q. I have too much homework.	1 ⁰	₂ O	ЗΟ	4 O
R. I don't want to get too strong or mus	scular. ₁ O	₂ O	ЗO	4 O

Cigarette Smoking and Alcohol Consumption

33. During the past 3 months, how often did you...?

	Never	A bit to try	Once or a few times a month	Once or a few times a week	Usually every day
A. Drink alcohol (beer, wine, other alcohol)	1 O	₂ O	Ο _ε	4 O	5 O

The next 9 questions are about your experience with cigarettes.

- 34. Think about your brothers and sisters (including half brothers and sisters). Together, we will write down how many brothers you have and how many of them smoke, how many sisters you have and how many them smoke. If the answer is none, we will write 0.
 - A. You have _____ brother(s)
 - B. You have _____ brother(s) who smoke cigarettes
 - C. You have _____ sisters(s
 - D. You have ______ sisters(s) who smoke cigarettes
- 35. Now, think about your friends. How many of your friends smoke cigarettes?
 - 1 O None
 - $_2$ O A few
 - $_{3}O$ About half
 - $_4O$ More than half
 - $_{5}$ O Most or all

36. Have you ever tried smoking cigarettes, even just a few puffs?

 $_{0}O$ No > Go to question 43. $_{1}O$ Yes 37. Have you ever taken cigarette smoke into your lungs for more than one puff?

 $_{0} O$ No > Go to question 40. $_{1} O$ Yes

38. How old were you when you took cigarette smoke into your lungs for more than one puff?

I was _____ years old

 $_{98}$ O I don't remember

39. The first few times you took cigarette smoke into your lungs, to what degree did you experience any of the following...?

> Please answer for each item.

	Not at all	A little	Medium	A lot
A. Coughing	1 O	₂ O	3 О	4 O
B. Burning in the throat	1 O	₂ O	3 О	4 O
C. Upset stomach	1 O	₂ O	3 О	4 O
D. Heart racing/pounding	1 O	₂ O	3 О	4 O
E. Dizziness	1 O	₂ O	3 О	4 O
F. Nausea	1 O	₂ O	3 О	4 O
G. Headache	1 O	₂ O	3 О	4 O
H. Other(s)	1 O	₂ O	3 О	4 O

- 40. Now, think about the past 7 days. Did you smoke any cigarettes in the past 7 days, even just a puff?
 - $_{0}$ O No \succ Go to question 42.

₁O Yes

41. Starting with yesterday which was <u>A.</u> (1=Monday, 2=Tuesday, 3=Wednesday, 4=Thursday, 5=Friday, 6=Saturday, 7=Sunday), follow the arrows and together we will write in the box how many cigarettes you smoked on each day. If an answer is zero, we will write 0. If you smoked less than one cigarette (for example, a few puffs or half of a cigarette), we will write 1.



> Please answer for each day.

- 42. Which one of the following best describes your usual cigarette use?
 - > Let me read all 5 statements before deciding on the one that best describes you.
 - $_1$ O I have smoked cigarettes before (even a single puff), **but not in the last 12 months**.
 - $_{2}O$ I smoked cigarettes once or more in the last 12 months.
 - $_{3}O$ I smoke cigarettes once a month or more.
 - $_4$ O I smoke cigarettes once a week or more.
 - $_{5}$ O I smoke cigarettes every day.

You and Your Health

43. In general, is your health...?

- 1 O Excellent
- $_{2}O$ Quite good
- $_{3}O$ Not very good
- 44. Have you ever tried to ...?
 - > Please answer for each item.
 - A. Lose weight.
 - 1 O Yes
 - $_{0}O$ No
 - B. Gain weight.
 - $_1O$ Yes
 - $_{\rm 0}O$ No
 - C. Maintain your weight.
 - 1 O Yes
 - $_{0}O$ No
 - D. I have never been worried about my weight.
 - 1 O Yes 0 O No
- 45. Currently, what are you doing about your weight?
 - $_1 O$ I'm trying to lose weight.
 - $_{2}O$ I'm trying to gain weight.
 - $_{3}O$ I want to maintain my weight.
 - $_4$ O I'm not doing anything about my weight.

If you are a boy \succ Go to question 48.

The following questions are for girls only.

46. Have you begun to have your periods, to menstruate?

- $_{1}$ O Yes $_{0}$ O No > Go to question 48.
- 47. How old were you when you had your first period?

_____ years old

Your Dental Health

48. How often do you brush your teeth?

- $_1 O$ Three (3) times a day or more
- $_{\scriptscriptstyle 2}O$ Twice a day
- $_{3}O$ Once a day
- $_4O$ Less than once a day

49. At what time do you usually brush your teeth?

- A. Before breakfast
 - $_1O$ Yes
 - ₀O No
- B. After breakfast
 - 1 O Yes
 - $_{\rm 0}O$ No
- C. After lunch
 - $_1O$ Yes
 - $_{\rm 0}O$ No
- D. Before supper
 - $_1O$ Yes
 - $_{\rm 0}O$ No
- E. After supper
 - 1 O Yes
 - $_{\rm 0}O$ No
- F. Before bed
 - $_1O$ Yes
 - $_{\rm 0}O$ No
- G. Any other time
 - $_1 O$ Yes
 - ₀O No

Your Diet

We will now talk about your diet.

50. Think about the **past 5 school days (or the last 5 school days this school year)**. How many days did you eat or drink something before school began in the morning? Don't count coffee, tea or water. If the answer is never, we will write 0.

____ days

51. Now think about every day of the week. In the past seven (7) days, how many times did you eat supper... If the answer is none, we will write 0.

A. Alone?

_____ times

B. With one or several members of your family?

_____ times

C. With the babysitter and no family members?

_____times

D. With your friends and no family members?

_____ times

E. You did not eat supper

_____times

52. In the past seven (7) days, how many times have you prepared supper for yourself? If the answer is none, we will write 0.

_____ times

- 53. If you have made supper for yourself in the last seven (7) days, what did it involve?
 - A. Meals already prepared by your parents at home (spaghetti, shepherd's pie, lasagna, etc.)?
 - 1 O Yes 0 O No
 - B. Store-bought meals that are frozen, canned or easy-to-prepare (such as Kraft Dinner, ravioli, dinners like Stouffer's, etc.)?
 - 1 O Yes 0 O No
 - C. Meals ordered from a restaurant (pizza, BBQ chicken, Chinese food, etc.)?
 - $_1O$ Yes
 - $_{\rm 0}O$ No
 - D. Meals you make or cook yourself (sandwiches, steak, hamburgers, etc.)?
 - 1 OYes
 - $_{\rm 0}O$ No
 - E. I did not make my own supper in the last 7 days.
 - $_1 O$ Yes
 - ₀O No

54. During supper, whether you eat alone or with someone, do you watch TV or videos?

- $_1 O$ Always
- ₂O Often
- ₃O Sometimes
- $_4O$ Never

55. During the past seven (7) days, how many times did you...?

A. Eat a meal in a restaurant?

_____ times

B. Have a snack in a restaurant?

_____times

C. Have food delivered from a restaurant to your home?

_____times

About You

56. In the last week (7 days), how often have you felt or behaved in the following ways?

		Rarely or never (less than one day)	A little or sometimes (1-2 days)	Occasionally or every now and then (3- 4 days)	Most of the time or all of the time (5-7 days)
A.	l did not feel like eating, l had no appetite.	₁ O	₂ O	3 О	4 O
В.	I felt down (had the blues) and didn't feel better, even with the help of my family or friends.	1 O	₂ O	зO	4 O
C.	I had difficulty concentrating on what I was doing.	₁ O	₂ O	3 О	4 O
D.	I felt depressed.	1 O	₂ O	3 О	4 O
E.	I felt too tired to do things.	1 O	₂ O	3 О	4 O
F.	I felt optimistic about the future.	1 O	₂ O	3 О	4 O
G.	I had trouble going to sleep or staying asleep.	₁ O	₂ O	3 О	4 O
Н.	I felt happy.	1 O	₂ O	зO	4 O
I.	I felt lonely.	1 O	₂ O	зO	4 O
J.	I enjoyed life.	1 O	₂ O	3 О	4 O
K.	I cried.	1 O	₂ O	3 О	4 O
L.	I felt like people didn't love/like me.	1 O	₂ O	3 О	4 O

- 57. During the **past 3 months**, have you been worried or stressed by any of the following...? If the situation does not apply to you, we will choose "not applicable".
 - > Please, answer for each item.

	Not at all	A little bit	Quite a bit	A whole lot	Not applicable
A. Your parents separating or divorcing	₁ O	₂ O	3 О	4 O	5 O
B. Loneliness	₁ O	₂ O	3 О	4 O	5 O
C. Breaking up with your boyfriend or girlfriend	1 O	₂ O	3 О	4 O	5 O
D. Your relationship with your father	₁ O	₂ O	3 О	4 O	5 O
E. Your relationship with your mother	₁ O	₂ O	3 О	4 O	5 O
F. Your relationship with your brother(s)/sister(s)	1 O	₂ O	зO	4 O	5 O
G. Your relationship with your friends	₁ O	₂ O	3 О	4 O	5 O
H. A health problem (such as acne or asthma)	1 O	₂ O	3 О	4 O	5 O
I. Your weight	₁ O	₂ O	3 О	4 O	5 O
J. Sex	₁ O	₂ O	3 О	4 O	5 O
K. Your new family (parents remarried)	₁ O	₂ O	3 О	4 O	5 O
L. Financial problems in your family	₁ O	₂ O	3 О	4 O	5 O
M. School work	₁ O	₂ O	3 О	4 O	5 O
N. Other(s)	₁ O	₂ O	3 О	4 O	5 O

58. Think of this ladder as representing where people stand in Canada.

At the top of the ladder are the people who are the best off: those who have the most money, the most education and the most respected jobs.

At the bottom are the people who are the worst off: who have the least money, the least education, and the least respected jobs or no job.

The higher up you are on this ladder, the closer you are to the people at the very top. The lower you are, the closer you are to the people at the very bottom.

Now, place a X on the rung where you think you and your family stand at this time.

_____th rung of the ladder



59. Finally, here is a list of feelings that you might feel because you participate in our study. For each statement, select the answer that best describes how you feel.

		Very true	Usually true	Sometimes true	False
Α.	I feel nervous or anxious.	1 O	₂ O	3 О	4 O
В.	I feel happy.	1 O	₂ O	3 О	4 O
C.	I feel sad.	1 O	₂ O	3 О	4 O
D.	I feel angry.	1 O	₂ O	3 О	4 O
E.	I'm scared that I might be sick.	1 O	₂ O	зΟ	4 O
F.	I'm scared of dying.	1 O	₂ O	3 О	4 O
G.	I'm scared that one of my parents might be sick or dying.	1 O	₂ O	3 О	4 O
Н.	I think it's cool.	1 O	₂ O	3 О	4 O
I.	I think my friends think I'm cool for doing all these tests.	1 O	₂ O	3 О	4 O
J.	I think that it's my fault if I'm sick.	1 O	₂ O	3 О	4 O
K.	I think that it's not fair.	1 O	₂ O	3 О	4 O
L.	I think that it's funny or amusing.	1 O	₂ O	3 О	4 O
M.	I feel that I'm not as good as friends my age.	1 O	₂ O	3 О	4 O
N.	Sometimes I'd prefer not to participate in the study anymore.	1 O	₂ O	3 О	4 O
О.	I'm scared that my friends might laugh at me because of all the tests I am doing.	1 O	₂ O	зO	4 O

60. Today's date

- A. Day _____ (01 to 31)
- B. Month _____ (01 to 12)
- C. Year (XXXX)
- 61. Interviewer's PIN (XXXXXXX)

9.2 Appendix 2

CHU Sainte-Justine Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants









Familial Study on the Prevention of Cardiovascular Disease and Type 2 Diabetes in Children and Adolescents

Parent Questionnaire for Visit 1 Biological Parents Section (1QB)

Child's PIN:

Instructions

To be completed by the biological parents of the child participating in the study.

Many of the questions in this questionnaire have several possible answers. Choose the answer best suited to your personal situation.

Answer to the best of your knowledge. There are no right or wrong answers.

Unless otherwise indicated, we ask that you choose only one answer for each question.

Here are a few sample questions and answers to illustrate what we mean:

Example A	Fill in the blank.
	How many people live in the household where the child usually lives?
	Include people who may be absent because of studies, travel, in hospital, etc. but who normally live in the household.
	Number of people including the child 5
Example B	Circle your answer and give details if applicable.
	Where was the child participating in the study born?
	A. Québec1
	Other Canadian province 2
	Outside Canada 3
	B. If the child was born outside Canada, please write which country.

If you have difficulty understanding any of the questions, please ask the research assistant for clarification.

Personal Information

1.	What is the date of birth of the child participating in the study?					
	Α.	Day (01 to 31)				
	В.	Month (01 to 12)				
	C.	Year 1 9				
2.	Wł	nat is the date of birth of his/her biological mother?				
	Α.	Day (01 to 31)				
	В.	Month (01 to 12)				
	C.	Year 1 9				
3.	Wł	nat is the date of birth of his/her biological father?				
	Α.	Day (01 to 31)				
	В.	Month (01 to 12)				
	C.	Year 1 9				
4.	Wł	nere was the child participating in the study born?				
	Α.	Québec 1				
		Other Canadian province 2				
		Outside Canada 3				
	В.	If the child was born outside of Canada, please write in which country he/she was born.				
5.	Wł	nere was his/her biological mother born?				
	Α.	Québec 1				
		Other Canadian province 2				
		Outside Canada 3				
	В.	If his/her biological mother was born outside of Canada, please write in which country she was born.				
6.	Wł	here was his/her biological father born?				
	Α.	Québec 1				
		Other Canadian province 2				
		Outside Canada 3				
	В.	If his/her biological father was born outside of Canada, please write in which country he was born.	as			

7. What language do you use most often when speaking with your child at home?

	Only choose one (1) answer for each parent.	1. Biological mother	2. Biological father
A.	French	1	1
	English	2	2
	Italian	3	3
	Greek	4	4
	Spanish	5	5
	Portuguese	6	6
	Other	7	7

B. If you answered «7» (you most often speak with your child in a language not listed here), please specify which language.

1. Biological mother : _____ 2. Biological father : _____

8. What is the highest level of education **completed** by the biological mother and father of the child participating in the study?

Only choose one (1) answer for each parent.	A. Biological mother	B. Biological father
No formal schooling or did not complete elementary school	1	1
Primary school	2	2
Did not complete high school (grade 7 to 11)	3	3
Graduated from high school (grade 12)	4	4
Graduated from vocational or trade school	5	5
Graduated from college (Cegep)	6	6
Graduated from university	7	7

9. Which parent(s) is completing this questionnaire?

Both the biological mother and the biological father	1
Only the biological mother	2
Only the biological father	3

Neighbourhood Characteristics

The next questions relate to the neighborhood where the child participating in the study most often lives. We are interested in neighborhood characteristics because the environment may influence lifestyle.

10. Is the neighbourhood in which the child lives ...?

Mainly residential	1
Mainly commercial	2
Mixed residential and commercial	3
Rural	4

- 11. How safe do you feel walking in your neighbourhood?
 - > Please answer for each item.

	Very safe				 Not at all safe
A. During the day	1	2	3	4	5
B. At night	1	2	3	4	5

12. Tell us whether you strongly agree, agree, disagree, or strongly disagree about the following statements when thinking of your neighbours.

		Strongly agree	Agree	Disagree	Strongly disagree
A. If there is a problem around neighbours get together to it.	d here, the deal with	1	2	3	4
B. There are adults in the neighbourhood that can be models for the children.	role	1	2	3	4
C. People around here are help their neighbours.	willing to	1	2	3	4
D. You can count on adults in neighbourhood to watch ou children are safe and don't trouble.	this t that get in	1	2	3	4
E. When I'm away from hom that my neighbours will eyes open for possible trou	ne, I know keep their ble.	1	2	3	4

- 13. How true are each of the following statements in regard to the neighbourhood where the child lives most of the time.
 - > Please answer for each item.

		Very _				Not at all true
Α.	There are no sidewalks.	1	2	3	4	5
В.	There are lots of hills.	1	2	3	4	5
C.	Dogs are well attended.	1	2	3	4	5
D.	There is a lack of equipment or facilities to incite children to be active.	1	2	3	4	5
E.	There are lots of safe places to be active.	1	2	3	4	5
F.	People walk or exercise frequently.	1	2	3	4	5
G.	Parents are very involved in watching their children participate in sports.	1	2	3	4	5
H.	There are many supervised and organised sports activities for children.	1	2	3	4	5
١.	Many people walk to the local grocery store.	1	2	3	4	5
J.	The neighbourhood is attractive.	1	2	3	4	5
K.	It is a high crime area.	1	2	3	4	5
L.	There are few local sports facilities.	1	2	3	4	5
M.	Children can play safely outdoors.	1	2	3	4	5
N.	Street lights are often burned out.	1	2	3	4	5
О.	There is heavy traffic.	1	2	3	4	5

14. On a scale from 1 to 5 (from very convenient to not at all convenient) how conveniently located are the following places/facilities with respect to the child's home?

	Very conven	Very Not at a convenient				Don't know
A. Public park or playground	1	2	3	4	5	8
B. Basketball court	1	2	3	4	5	8
C. Bike path or lane	1	2	3	4	5	8
D. Golf course	1	2	3	4	5	8
E. Mini golf	1	2	3	4	5	8
F. Playing field for soccer or football	1	2	3	4	5	8
G. Playing field for baseball	1	2	3	4	5	8
H. Tennis court	1	2	3	4	5	8
I. Sports classes	1	2	3	4	5	8
J. Public recreation center	1	2	3	4	5	8
K. Public indoor swimming pool	1	2	3	4	5	8
L. Public outdoor swimming pool	1	2	3	4	5	8
M. Walking/hiking trails	1	2	3	4	5	8
N. Dance studio/classes	1	2	3	4	5	8
O. Racquetball/squash court	1	2	3	4	5	8
P. Grocery store/shops	1	2	3	4	5	8
Q. Public gym	1	2	3	4	5	8
R. Indoor skating rink/arena	1	2	3	4	5	8
S. Outdoor skating rink	1	2	3	4	5	8
T. Fitness/exercise club	1	2	3	4	5	8

Your Child's Health

15. Does the child participating in the study **curently have or had in the last year** one or more of the following chronic health problems diagnosed or confirmed by a doctor or other health specialist? If yes, please indicate the age (in years) of your child when the diagnosis was first made.

A chronic health problem is one that has persisted for 6 months or more or that will probably last more than 6 months.

	1. No	Yes	2. If yes, age at diagnosis
A. Food allergies	0	1	
B. Respiratory problems (asthma or other)	0	1	
C. Skin problems	0	1	
D. Emotional, psychological or nervous problems	0	1	
E. Bone or joint problems	0	1	
F. Digestive system problems	0	1	
G. Thyroid problems	0	1	
H. Liver problems	0	1	
I. Kidney problems	0	1	
J. Diabetes	0	1	
K. Hypertension (high blood pressure)	0	1	
L. Cholesterol or lipid problems	0	1	
M. Overweight or obesity	0	1	

16. Compared to healthy children of the same age, is your child limited in the type or number of activities he/she can do because of a chronic physical or mental health problem?

No	0	\triangleright	Go to question 18.
Yes	1		

- 17. What is the main health problem that limits your child's activities?
- 18. In the past 2 months, did your child sustain an injury or trauma that was treated by a physician or nurse?

No 0 Yes..... 1

- 19. In the past 2 months, has your child had a serious disease or been very sick, requiring hospitalization for more than one week?
 - A. No..... 0 Yes..... 1
 - B. If yes, specify what

The next questions relate to the pregnancy and birth of the child participating in the study.

20. When pregnant with the child participating in the study, did the biological mother experience any of the following health problems?

		No	Yes	Don't know
Α.	Hypertension (high blood pressure)	0	1	8
В.	Gestational diabetes	0	1	8
C.	Was known to have diabetes before the pregnancy	0	1	8
- 21. Did the child's biological mother smoke during the pregnancy?
 - A. No..... 0 Yes..... 1 if yes ≻

During which months of the pregnancy did she smoke?

> Please answer for each item.

B. During the first trimester

No...... 0 Yes..... 1 Don't know 8

C. During the second trimester

No	0
Yes	1
Don't know	8

D. During the third trimester

No	0
Yes	1
Don't know	8

22. How frequently did the child's biological mother drink alcohol during her pregnancy?

Never	1
Less than once a month	2
Once (1) to three (3) times a month	3
Once (1) a week	4
Two (2) to three (3) times a week	5
Four (4) to six (6) times a week	6
Everyday	7
Don't know	8

23. What was the birth weight of your child?

24. What was the birth length of your child?

____ inches or _____ centimetres

Don't know 98

25. After how many weeks of gestation was your child born? If the child was born at term and you don't know exactly the number of weeks of gestation, write 40 weeks.

_____ weeks Don't know 98

26. Was this a single birth, twins, or triplets?

Single birth	1
Twins	2
Triplets	3
More than triplets	4

27. Did your child receive special medical care following his/her birth?

No	0
Yes	1
Don't know	8

28. For how many days did your child remain in the hospital after birth?

days	
Not applicable (child was not born in a hospital)	996
Don't know	998

29. Compared to other babies in general, would you say that your child's health at birth was...

Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

30. Did the mother of the child participating in the study breast-feed him/her even if only for a short time?

B. For how long?

Less than one (1) week	1
1-4 weeks	2
5-8 weeks	3
9-12 weeks	4
3-6 months	5
7-9 months	6
10-12 months	7
13-16 months	8
More than 16 months	9

Medicine Taken by Your Child

- 31. During the past two (2) weeks, did your child take any of the following medications (in pill, syrup, drops form, etc.)?
 - Please answer for each medication (A to N).
 If you have any doubts about the type of medication, consult the nurse.
 - A. Medication to reduce pain or fever

 - 2. If yes, specify which

B. Medication for a cold or allergies

- 2. If yes, specify which
- C. Vitamin(s) or mineral(s)

 - 2. If yes, specify which

	D.	Antibiotic or	anti-infection	medication
--	----	---------------	----------------	------------

1.	No	0
	Yes	1
	Don't know	8

2. If yes, specify which

E. Steroids or cortisone by mouth or injection

- 2. If yes, specify
- F. Asthma medication (such as a pump or inhaler) other than steroids or cortisone by mouth or injection

1.	No	0
	Yes	1
	Don't know	8

2. If yes, specify which

G. Medication to help him/her concentrate better (ex.: ritalin)

- 2. If yes, specify which

- H. Medication for digestive problems

 - 2. If yes, specify which

I. Medication for skin problems

- 2. If yes, specify which
- J. Medication to lower cholesterol or triglycerides (lipids in blood)

 - 2. If yes, specify which
- K. Medication for hypertension (high blood pressure)

 - 2. If yes, specify which

L. Medication for diabetes

1.	No	0
	Yes	1
	Don't know	8

2. If yes, specify which

M. Medication for weight loss, or to control appetite

- 2. If yes, specify which
- N. Any other medication

1.	No	0
	Yes	1
	Don't know	8

2. If yes, specify which

Your Child's Dental and Oral Health

32. Has your child ever been to the dentist's clinic, either for treatment, check-up, examination, or just to get used to going?

 No
 0
 ➤
 Go to question 44.

 Yes
 1

33. At what age did your child have his/her first dental visit?

34. Why did he/she go the first time? Was this because...

> Please choose only one (1) answer.

Check-up, examination, or cleaning	1
He/she was having trouble with his/her teeth	2
Recommended by the school's dental hygienist	3
He/she just went to get used to going to the dentist	4
For orthodontic treatments	5
For some other reason	6
Don't know	8

35. Has your child ever had a dental treatment of any kind (e.g. filling, tooth extraction, sealants, fluoride treatment, etc). A check-up is not considered dental treatment.

36. What kind of dental treatment(s) has your child had over the whole of his/her life so far?

	No	Yes
A. Tooth filling	0	1
B. Tooth extraction	0	1
C. A general anesthetic to have tooth/teeth taken out	0	1
D. Treatment to stop teeth decaying or going bad	0	1
E. Other reason	0	1

> Please answer for each item.

F. If other reason, please specify.

37. When was your child's last visit to the dentist?

In the last six (6) months	1
In the last year	2
Not in the last year, but in the last two (2) years	3
Longer than two (2) years	4

38. Why did he/she go to the dentist last time? Was this because...

> Please choose only one (1) answer.

Check-up, examination or cleaning	1
He/she was having trouble with his/her teeth	2
Recommended by the school dental hygienist	3
He/she just went to get used to going to the dentist	4
For orthodontic treatments	5
For some other reason	6
Don't know	8

39. Do you have any kind of private insurance (dental plan) which pays for dental care for your child?

No	0
Yes, partial reimbursement	1
Yes, total reimbursement	2
Don't know	8

40. Has your child ever had a fall or some other accident that damaged any of his/her teeth?

No	0	\triangleright	Go to question 44.
Yes	1		

41. What was this damage?

> Please answer for each item.

	No	Yes
A. Teeth chipped, cracked or broken	0	1
B. Teeth knocked loose	0	1
C. Teeth knocked out	0	1

42. Was this damage to baby (milk) teeth, to second (permanent) teeth, or to both?

Baby teeth	1
Second teeth	2
Both	3
Don't know	8

43. Did your child have to have any teeth taken out at the dentist because of this accident?

No	 . 0
Yes	 . 1

44. Has your child ever received prescription fluoride treatment, such as liquids or gels, **at the dentist office**?

Don't know	8	l	~	Go to question 46
No	0	ſ		
Yes	1			

45. How often has your child received these treatments?

Every six (6) months	1
Once (1) a year	2
Every two (2) years	3
Less often than every two (2) years	4
Don't know	8

46. Has your child ever received fluoride treatments through a school program?

- B. If yes, from what age?_____ years old
- C. Until what age?

_____ years old

47. Has your child ever taken fluoride tablets/drops, or vitamin drops/tablets with fluoride (including multivitamin with fluoride)?

A.	Don't know	8
	No	0
	Yes	1

B. If yes, from what age? (If less than one (1) year old, write 0)

_____ year old

C. Until what age? (If less than one (1) year old, write 0)

____year old

Your Child and School

48. What type of school does your child attend now (or attended during the shool year that just ended)?

Public school	1
Private school	2
Other	3

- 49. What is the name of the school that your child attends now (or attended during the school year that just ended)?
- 50. What is the postal code of the school that your child attends now (or attended during the school year that just ended)?



51. In the last two (2) years, has your child failed a grade or was he/she held back one year in school (including kindergarten)?

No .	 	 	0
Yes.	 	 	1

52. In the last two (2) years, has your child received specialized services because of difficulties experienced in school (learning disability, behavioural problems, etc.)?

No	0
Yes	1
Don't know	8

53. Other than the progression through the school system in your area, has your child changed schools?

No	0	۶	Go to question 56.
Yes	1		

54. In the last two (2) years, how many times has your child changed schools?

_____times

55. For the most recent change in schools, what was the reason for changing?

> Please choose only one (1) answer.

Family or child moved	1
Child not progressing well	2
Child not getting along well with others	3
Concerns about standards and quality of teaching at the school	4
Wanted a specific program	5
Other	6

56. With regard to how your child feels about school, how often does he/she look forward to going to school?

Almost never	1
Rarely	2
Sometimes	3
Often	4
Almost always	5

Your Child's Friends, Feelings and Behaviours

The next questions relate to your child's relationships with his/her friends, family, and other people.

57. About how many days a week does your child do things with friends?

Never	1
One (1) day a week	2
Two (2) or three (3) days a week	3
Four (4) or five (5) days a week	4
Six (6) or seven (7) days a week	5

58. About how many close friends does your child have?

None	1
One (1)	2
Two (2) or three (3)	3
Four (4) or five (5)	4
Six (6) or more	5

59. When it comes to meeting new children and making new friends, is your child...

Somewhat shy	1
About average	2
Very outgoing – makes friends easily	3

60. During the past six (6) months, how well did your child get along with other kids, such as friends or classmates (excluding brothers or sisters)?

Very well, no problems	1
Quite well, hardly any problems	2
Pretty well, occasional problems	3
Not too well, frequent problems	4
Not well at all, constant problems	5

61. During the past six (6) months, how well did your child get along **with his/her teacher(s) at school**?

Very well, no problems	1
Quite well, hardly any problems	2
Pretty well, occasional problems	3
Not too well, frequent problems	4
Not well at all, constant problems	5

62. During the past six (6) months, how well did your child get along with his/her parent(s)?

Very well, no problems	1
Quite well, hardly any problems	2
Pretty well, occasional problems	3
Not too well, frequent problems	4
Not well at all, constant problems	5

63. During the past six (6) months, how well did your child get along **with his/her brother(s)/sister(s)**?

Very well, no problems	1
Quite well, hardly any problems	2
Pretty well, occasional problems	3
Not too well, frequent problems	4
Not well at all, constant problems	5
Does not have brother(s) or sister(s)	6

Now we would like to ask you questions about how your child seems to feel or act.

- 64. Using one of the three (3) following answers: "never or not true, sometimes or somewhat true, or often or very true", how often would you say that your child...
 - > Please answer for each item.

		Never or not true	Sometimes or somewhat true	Often or very true
Α.	Shows sympathy to someone who has made a mistake?	1	2	3
В.	Can't sit still, is restless or hyperactive?	1	2	3
C.	Will try to help someone who has been hurt?	1	2	3
D.	Is defiant?	1	2	3
E.	Seems to be unhappy, sad, or depressed?	1	2	3
F.	Gets into many fights?	1	2	3
G.	Is easily distracted, has trouble sticking to any activity?	1	2	3
Н.	Doesn't seem to feel guilty after misbehaving?	1	2	3
I.	Is not as happy as other children?	1	2	3
J.	Fidgets?	1	2	3
K.	Can't concentrate, can't pay attention for long?	1	2	3
L.	Is too fearful or nervous?	1	2	3
М.	Punishment doesn't change his/her behaviour?	1	2	3
N.	Is impulsive, acts without thinking?	1	2	3
0.	Has temper tantrums or a hot temper?	1	2	3
Ρ.	Offers to help other children (friend, brother or sister) who are having difficulty with a task?	1	2	3
Q.	Is worried?	1	2	3
R.	Has difficulty waiting for his/her turn in games or groups?	1	2	3

		Never or not true	Sometimes or somewhat true	Often or very true
S.	When somebody accidentally hurts him/her, he/she reacts with anger and starts a fight?	1	2	3
Т.	Has angry moods?	1	2	3
U.	Comforts a child (friend, brother or sister) who is crying or upset?	1	2	3
V.	Cries a lot?	1	2	3
W.	Clings to adults or is too dependent?	1	2	3
Х.	Gives up easily?	1	2	3
Y.	Cannot settle down to do anything for more than a few moments?	1	2	3
Ζ.	Constantly seeks help?	1	2	3
AA	. Is nervous, high strung or tense?	1	2	3
BB	. Kicks, bites, hits other children?	1	2	3
СС	. Doesn't want to sleep alone?	1	2	3
DD	. Is inattentive?	1	2	3
EE	. Has trouble enjoying him/herself?	1	2	3
FF.	Helps other children (friends, brother or sister) who are feeling sick?	1	2	3
GG	Gets too upset when separated from parents?	1	2	3
НН	. Helps those who do not do as well as he/she does?	1	2	3

- 65. Has your child ever experienced any event or situation that has caused him/her a great amount of worry or unhappiness?

 - B. If yes, what was this (these) event(s) or situation(s)?

> Please answer for each item.

	No	Yes
1. Death in family (other than parents)	0	1
2. Divorce/separation of parents	0	1
3. Move	0	1
4. Stay in hospital	0	1
5. Stay in foster home	0	1
6. Other separation from parents	0	1
7. Illness/injury of child	0	1
8. Illness/injury of a family member	0	1
9. Abuse/fear of abuse	0	1
10. Change in household members	0	1
11. Alcoholism or mental health disorder in family	0	1
12. Conflict between parents	0	1
13. Parent loss of employment	0	1
14. Change in the family financial situation	0	1
15. Birth of another child	0	1
16. Other	0	1

- 1

Sibling Health

The next questions relate to the biological brothers and sisters and biological half-brothers and half-sisters of the child participating in the study.

66. Does your child have any biological brothers, sisters, half-brothers or half-sisters?

No	0	\triangleright	Go to question 71.
Yes	1		

67. How many living biological brothers, sisters, half-brothers or half-sisters does your child have?

> Please answer for each item A, B, C, and D. Write "0" if the answer is none.

- A. Number of brothers
 B. Number of sisters
 C. Number of half-brothers
 D. Number of half-sisters
- 68. How old are the biological brothers/sisters/half-brothers/half-sisters of the child participating in the study? If a child is less than 1 year old, write 0.
 - A. Age of child 1 (other than the child participating in the study) : ______ years old
 - B. Age of child 2 (other than the child participating in the study) : ______ years old
 - C. Age of child 3 (other than the child participating in the study) : ______ years old
 - D. Age of child 4 (other than the child participating in the study) : ______ years old
 - E. Age of child 5 (other than the child participating in the study) : ______ years old
- 69. Did any of your child's biological brothers or sisters or half-brothers or sisters die after birth?

No	0
Yes	1

70. Do any of your child's biological brothers or sisters or half-brothers or half-sisters actually have any of the following chronic health problems that have been diagnosed or confirmed by a doctor or other health specialist?

A chronic health problem is one that has persisted for 6 months or more or that will probably last more than 6 months.

> Please answer for each item.

	No	Yes	Don't know
A. Diabetes	0	1	8
B. Hypertension (high blood pressure)	0	1	8
C. Overweight or obesity	0	1	8
D. Elevated blood cholesterol or triglycerides (lipids in blood)	0	1	8

Your Child's Home

The next questions relate to the family with whom the participating child usually lives.

71. Does your child currently live with both of his/her biological parents?

No	0		
Yes	1	\triangleright	Please, go to question 74.

72. If the child does not currently live with both of his/her biological parents, indicate how long he/she has not been living with both. Write 0 if for less than a year.

Number of complete years	
Never lived with both his/her parents	 97

73. With which parent does the child usually live?

A.	With his/her mother only	1
	With his/her father only	2
	Most of the time with his/her mother	3
	Most of the time with his/her father	4
	Equal time with mother and father	5
	Other	6

B. If you answered that the child usually lives with a person other than his biological mother or father, please specify who this (these) person(s) is (are).

74. How many people live in the household where the child usually lives?

Include people who may be absent because of studies, travel, in hospital, etc., but who normally live in the household.

Number of people including the child

75. How many bedrooms are there in the household where the child usually lives?

Number of bedrooms			
--------------------	--	--	--

76. What type of home does your child usually live in?

> Please choose only one (1) answer.

Single detached house	1
Semi-detached or double (side-by-side) house	2
Town house or row house	3
Duplex (one above the other)	4
Low-rise apartment (less than five (5) stories)	5
High-rise apartment (5 or more stories)	6
Mobile home	7
Other	8

Again, the next questions relate to the family with whom the participating child usually lives.

- 77. At the present time, which of the following best describes your main occupational status and that of your spouse/partner?
 - INDICATE ONLY ONE (1) FOR EACH PERSON AND CHOOSE ONLY TWO (2) PERSONS. Therefore, if your child currently lives with his/her two (2) biological parents, answer for the biological mother and biological father; if your child lives most often with his/her biological mother, answer for the biological mother and her partner (if applicable); if your child lives most often with the biological father, answer for the biological father and his partner (if applicable).

	1. Biological mother	2. Biological father	3. Partner of biological mother	4. Partner of biological father
Full-time job (30 hrs. or more a week)	1	1	1	1
Part-time job (less than 30 hrs. a week)	2	2	2	2
Going to school	3	3	3	3
Homemaker	4	4	4	4
Not working for health reasons	5	5	5	5
Maternity or paternity leave	6	6	6	6
Unemployed	7	7	7	7
On welfare (social assistance)	8	8	8	8
On strike or locked out	9	9	9	9
Other (retired, sabbatical, etc.)	10	10	10	10
No partner	\triangleright	\geq	97	97

78. If you or your spouse/partner have a paid job, when do you work?

INDICATE ONLY ONE (1) FOR EACH PERSON AND CHOOSE ONLY TWO (2) PERSONS. Therefore, if your child currently lives with his/her two (2) biological parents, answer for the biological mother and biological father; if your child lives most often with his/her biological mother, answer for the biological mother and her partner (if applicable); if your child lives most often with the biological father, answer for the biological father and his partner (if applicable).

	1. Biological mother	2. Biological father	3. Partner of biological mother	4. Partner of biological father
Days	1	1	1	1
Evenings or nights	2	2	2	2
Alternating days and evenings/nights (shift work)	3	3	3	3
Don't work during the week	4	4	4	4
No partner	\triangleright	\ge	97	97

A. During the week

B. Weekend

	1. Biological mother	2. Biological father	3. Partner of biological mother	4. Partner of biological father
Days	1	1	1	1
Evenings or nights	2	2	2	2
Alternating days and evenings/nights (shift work)	3	3	3	3
Don't work during the weekend	4	4	4	4
No partner		\geq	97	97

Although many health expenses are covered by the provincial health insurance plan, there is still a relationship between health and income. We would appreciate that you answer the following question to help us study this relationship.

As with all the other information you have provided in this questionnaire, your response will remain completely CONFIDENTIAL.

79. What was your total household income for the last completed fiscal year, before taxes and deductions (i.e total income of everyone living in the same residence where your child usually lives, and who share expenses)?

Less than 10 000\$	0	1
10 000\$ - 14 999\$		2
15 000\$ - 19 999\$	0	3
20 000\$ - 29 999\$		4
30 000\$ - 39 999\$	0	5
40 000\$ - 49 999\$	0	6
50 000\$ - 59 999\$	0	7
60 000\$ - 79 999\$	0	8
80 000\$ - 99 999\$	0	9
100 000\$ - 119 999\$		0
120 000\$ - 139 999\$		1
140 000\$ and more		2

Today's date



This is the end of the first part of the parents' questionnaire. The next section should be filled out by each biological parent separately. Thank you for your collaboration and patience.

9.3 Appendix 3

Quality Cohort Study

DENTAL EXAMINERS PROCEDURES MANUAL

Belinda Nicolau

April 2005

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1 Introduction

The purpose of the oral health component of the Quality Cohort is to investigate the relative importance of the genetic, biological, environmental and psycho-social determinants of chronic oral conditions along adolescent's life course. To achieve this aim this study will assess the condition of the teeth and gum and the presence of traumatic dental injury. In addition, saliva and plaque samples will be collected to examine the constituents of saliva and oral pathogens associated with dental caries and periodontal disease. This manual describes specific procedures for conducting the oral head examination and for recording data.

2 Equipment and supplies

2.1 Dental examination area in the sites

An appropriate area to conduct examinations will be selected and prepared in advance to maximize working conditions. The location of the examination room may vary depending on the site. The exact arrangement of the room will be determined by the physical condition of the site, however certain controllable features will be standardized. The dental examinations will take place in a private room specially allocated for the study. A dental chair or adjustable bed will be provided, so that children can be examined in a reclining position. The angle of inclination of the chair and bed will be the same. The instruments will be laid out on a clean tissue out of sight of the participant (if possible), but allowing easy access to the examiner. A standardized source of artificial light will be used throughout the dental examinations. A lightweight portable examination light in a blue-white color spectrum will be used. The lamp will be set at the high power setting and dark protective glasses will be placed on the subject.

2.2 Description of equipment and supplies

Table 2.1 shows a list of equipment and supplies to be used to carry out the dental clinical examination.

2.3 Specific procedures for use of equipment and supplies

This section reviews the procedures for use of equipment and supplies at the beginning and at the end of each exam session.

- 2.3.1 Start of exam session
- ⇒ Wash hands;

- ⇒ Turn dental light on and visually check the light;
- ⇒ Prepare the room for the examination complete all infection control procedures(see section1.5)

2.3.2 End of exam session

- \Rightarrow Turn the dental light off;
- \Rightarrow Place used instruments in the Rubbermaid containers;
- \Rightarrow Clean room;

Table 2.1 List of equipment and supplies

Supply	Per site	Per month	Per child
Examination			
Bed/Chair			
Adjustable stools for examiner and recorder			
Light			
Cotton applicators			
2x2 gauze pads			
Rubbermaid container (to soak instruments)			
Paper points			
Sterile plastic tube			
Toothpick			
Instruments			
Plane surface reflecting mouth mirrors			
CPI periodontal probes			
#23 explorer			
Infection Control			
Chair covers			
Lab coat			
Latex examination gloves, powder free			
Face masks			
Safety glasses			
Restore (to soak instruments)			
Germicidal wipes, disposable			
Trash bags, biohazard			
Touchless hand soap dispenser			
Waste basket, biohazard			
Non-Dental			
Containers (to hold miscellaneous items)			
Scissors			
Scotch tape			
Paper hand towels			
Hand soap			
Hand cream			
Pens			

2.4 Examination environment

General guidelines for maintaining safety and efficiency in the dental examination environment are:

Arrange equipment so that children can move easily and safely into and out of the examination area

- ⇒ Disinfecting solutions and other liquids must be covered and out of reach of children.
- ⇒ The dental examination environment must be kept clean.
- ⇒ The instrument sterilization packets should be opened and placed in such a position so that the packet becomes the instrument tray for the children on which they are used.
- ➡ Two plastic containers with lids for used instruments must be placed out of the examination environment. Used mirrors will be placed in one plastic container and used explorers and probes in the other container. Other instruments must not be placed with the mirrors because they may scratch the mirrors.
- ⇒ The hazardous waste container lid must be closed except when depositing wastes.

2.5 Cross infection control

The dentist is responsible for ensuring proper infection control practices in the dental examination environment which are described in this section. The procedures for handling and sterilizing instruments and maintaining a safe examination environment are in compliance with regulations and recommendations of the Centers for Disease Control, U.S. Public Health Service, and the National Institute of Occupational Safety and Health.

2.5.1 Prior to the dental examination

The following must be completed prior to the start of each session:

- ⇒ Counter tops must be disinfected with an appropriate solution before arranging the instruments and supplies for daily use.
- ⇒ Disposable barriers must be placed on the following items: chair cover, light head and controls; mounted instrument tray; and the specimen collection storage container lid.
- ⇒ The examiner must wear a facemask, safety glasses with side shields, and a new pair of powderfree exam gloves for each children examination.

NOTE: If the examiner adjusts the dental stool/bed or the mask or touches any object, other than ones that have been covered or disinfected during an examination, she/he must rescrub and put on a new pair of gloves.

- ⇒ Examiners and recorders must wear neat and clean lab coats.
- ⇒ Only properly sterilized instruments are to be used for dental examinations.
- \Rightarrow The Restore holding solution should be prepared daily.

2.5.2 After each dental examination

The sequence of procedures for maintaining infection control between each examination is as follows:

⇒ Used instruments will be deposited in the used instrument containers partially filled with the appropriately diluted solution of Restore;

- ⇒ Chair covers, and instrument sterilization packets must be removed and thrown in the hazardous waste container;
- Gloves should be turned inside out as they are removed and thrown into the hazardous waste container;
- A disinfecting solution must be used on any surface that could have been contaminated during the examination;
- \Rightarrow Hands must be washed with soap and water, and then be regloved;
- A clean chair cover should be placed on the mounted instrument tray with a new instrument packet. Do not set up the new instruments until the children arrives in the room as the instruments may become contaminated if left out for a period of time;
- ⇒ When not in use, instrument containers, utility gloves, and any other supplies that come in contact with used instruments should be stored away from non contaminated items.

2.5.3 Infection control supplies

The following list summarizes infection control supplies for use in the dental examination room:

- ⇒ Light: adhesive barrier on head and controls; surface disinfectant;
- ⇒ Instrument tray: plastic chair cover; surface disinfectant;
- ⇒ Counter: surface disinfectant;
- ⇒ Instruments: used instruments should be handled carefully. After the dental examination they should be placed in plastic container and at the end of the day should be taken to dental clinics for sterilization.
- ⇒ Waste: labeled biohazard containers
- ⇒ Examiner: lab coat, mask, protective eyewear with side shields, single use gloves.

2.5.4 Instruments

All mirrors, explorers, and probes must be sterilized prior to first use and after each use. Having a sufficient number of sterilized instruments available for each examination session is the responsibility of the dental examiner.

3 Oral examination methods

The oral examination component consists of a questionnaire and clinical examination subcomponents. The dentist examiner (dentist-graduate student) and dental recorder (nurse) work as a team in conducting this examination for each study participant.

3.1 Sequence of oral examination components

Table 3. 1 lists the oral examination subcomponents in the order they are conducted. The examination procedures and methods are discussed in the following sections. The teeth will be examined in an orderly manner from one tooth or tooth space to the adjacent tooth or tooth space and following a standard order; starting from the upper right first molar to the lower right first molar. The Federation Dentaire Internationalle (FDI) two digit nomenclature will be employed. The details of each dental examination will be recorded on the dental chart that will be specifically designed for this survey. The sequence of the examination will follow the order laid out on the dental chart. The criteria for the dental examinations were chosen based on their validity, reliability and comparability with previous studies.

Oral hygiene indicators	⇒ Presence of plaque
Gingival Crevicular Fluid	
Plaque sample	
Periodontal health	⇒ Calculus
	⇒ Gingival bleeding
Tooth condition	⇒ Dental caries
Traumatic dental injury	⇒ Type of lip coverage
	⇔ TDI
	⇒ Incisal overjet

	Table3.1	Oral clinical	examination	sub-com	ponents
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3.1.1 Pre-examination procedures

The nurse will fill the dental examination form with the name and identification number of the child.

The examiner will explain the process to the child and their parents in his/her own words and include the following facts:

- ⇒ You will be looking at and lightly touching the child's teeth;
- ⇒ You will be calling numbers and letters to the technician that only have meaning for this research project;
- ⇒ Some general results will be provided when child leave the dental chair;
- ⇒ In conducting the examinations, each child will be examined in the same manner;
- ⇒ The clinical examination can be interrupted anytime if the child wishes
- Assure the child and their parents that the exam will not include treatment, X-rays, a drill, or anesthesia. The dentist will use only a mirror and dental hand instruments to examine the mouth.
- ⇒ Qualifications of the examiner. The examiner is a licensed dentist.
- ⇒ Existing dental work. The exam will not interfere with any existing dental work such as fillings, bridges, sealants, or orthodontic bands.

Disease such as AIDS (Acquired Immune Deficiency Syndrome). The Centers for Disease Control, part of the Public Health Service, has set up standard practices (universal precautions) for dentists to use to prevent the spread of diseases, viruses, and bacteria, and these procedures are strictly observed by the dentists on this study. The dentist will wear sterile gloves and a mask, and the dental instruments will be sterilized before examinations are preformed. The precautions used in the survey are the same as those maintained in dental offices.

3.2 Saliva samples

Sample of non stimulate saliva will be collected from all children. First, each child will be given a mouthwashing with distilled water then each child will be asked to spit their whole saliva in a sterile plastic tube. The tube should be immediately placed on the freezer and subsequently carried out to the laboratory.

3.3 Oral hygiene indicators

The presence or absence of plaque will be used as an indicator of children's oral hygiene habits. It will be assessed in six index teeth, 16,11,26, 36,31, 46 (Figure 1) which will be examined in the prescribed order (upper right, upper middle, upper left, lower left, lower middle and lower right). Each index tooth will be examined visually both buccally and lingually and its state coded according to one of the following categories

Code 0: No plaque

This code is used when there is no plaque visible to the naked eye.

Code 1: Presence of plaque

Gingival area covered by a visible plaque to the naked eye.

Code 9: Assessment cannot be made

Note: A probe is **not** used for this part of the examination. Consider plaque only –ignore recent debris such as small pieces of chips found in an otherwise clean mouth immediately following a lunch break.

3.4 Gingival crevicular fluid (GCF)

Gingival crevicular fluid (GCF) will be collected from four sites - the mesial and buccal surface of the first permanent molar (16b,16m) on the right side of the upper jaw and the mesial and lingual surfaces of the first permanent molar on the left side of the lower jaw (36m and 36l)- using paper strips (Periopaper, IDE Interstate, Amityville, NY). First, the sample sites will be isolated with cotton rolls and dried with a gentle stream of air to prevent saliva contamination (Alpagot et al, 1996), then a sterile Periopaper strip will be inserted 1-2mm into the gingival sulcus for 30 seconds. For the sites 16b and 36l, the Periopaper strips will be inserted into the gingival sulcus in the middle of the surfaces. The GCF volume (ul) will be

measured with a Periotron 8000 (IDE Interstate) . The periopaper will be placed in 120 µl assay buffer containing 0.9% NaCl, 0,01 M EDTA, 0.3% bovine-globulin, 0.005% Triton-X-100, 0.05% sodium azide, 0.0225 M NaH₂PO₄, 0.0245 M NaH₂PO₄, pH 6.8 and will be kept frozen at -70C.

3.5 Plaque samples

Dental plaque samples (supra- and sub-gingival) will be collected from children to evaluate risk indicators of dental caries and periodontal disease. Plaque samples will be collected after the assessment of oral hygiene. The plaque samples will be obtained from four sites: the mesial and buccal surface of the first permanent molar (16b,16m) on the right side of the upper jaw and the mesial and buccal surfaces of the first permanent molar on the left side of the lower jaw (36m and 36l). These sites will be selected on the basis that the frequency and level of bacteria colonization shows a decreasing gradient from molars to incisors (Lindquist and Emilson,1990) and to represent surfaces close to each other (same tooth) as well as far away from each other (different quadrants, upper–lower jaws). If one of the molars (16,36) is missing examine the molars on the opposite quadrants, that is, the mesial and buccal surface of the first permanent molar (26b,26m) on the left side of the upper jaw and the mesial and buccal surfaces of the first permanent molar (26b,26m) on the right side of the upper jaw and the mesial and buccal surfaces of the first permanent molar on the right side of the lower jaw (46m and 46l). If first molars in the opposite quadrants are missing collect the data in the molars present in the mouth.

Plaque samples will be obtained from four sites using the tips of sterile toothpicks. First, sample sites should be isolated with cotton rolls and then a sterile toothpick should be inserted into and passed along the gingival sulcus. The toothpicks are then stored in microcentrifuge tubes containing 500 ul of PBS at 4C. These tubes will be subsequently transferred to Dr. Simon Tran's laboratory (McGill University) and will be kept in a -70 C freezer until analysis.

3.6 Periodontal health status

The periodontal examination should be carried out in the prescribed order (upper right, upper middle, upper left, lower left, lower middle and lower right) two times; once to determine the presence or absence of calculus and once for the assessment of the gum condition. All fully erupted permanent teeth will be examined periodontal indicators will be assessed in six index teeth, 16,11,26,36,31,46, (Figure 1) using the CPI probe (WHO, 1997). This is a specially designed lightweight probe with a 0.5-mm ball tip, bearing a black band between 3.5 and 5.5 mm from the ball tip and rings at 8.5 and 11.5 mm from the ball tip. The index teeth represent six sextants in the mouth. If one, or more of the molars are missing the assessment should be abandoned for that particular segment, and a score of 9 recorded. If the upper right central incisor is missing, examine the upper left central incisor. If the lower left central incisor is absent examine the lower right central incisor. If either upper central incisors or
both lower central incisors are absent, the assessment should be abandoned for that particular segment, and a score of 9 recorded.



Fig.1 CPI Index teeth (WHO, 1997)

3.6.1 Calculus

The index tooth should be probed using the probe as a "sensing" instrument to detect sub-gingival calculus and gingival bleeding. The sensing force used should be no more than 20 grams. For sensing sub-gingival calculus, the lightest possible force that will allow movement of the probe ball tip along the tooth surface should be used. When the probe is inserted, the ball tip should follow the anatomical configuration of the surface of the tooth root. The probe tip should be inserted gently into the gingival sulcus or pocket and the total extent of the sulcus or pocket explored. For example, the probe is placed in the pocket at the disto-buccal surface of the first molar, keeping the probe parallel to the long axis of the tooth. The probe is then moved gently, with short upward and downward movements, along the buccal sulcus or pocket to the mesial surface of the first molar. A similar procedure is carried out for the lingual surfaces, starting disto-lingually to the first molar. In each child, presence of calculus and gingival bleeding from 2 sites (buccal and lingual) will be recorded.

Code 0: No calculus

Code 1: Presence of gingival calculus

This code should be used when there is a presence of sub or supra gingival calculus.

Code 9: Assessment cannot be made

3.6.2 Gingival bleeding on probing

Code 0: No bleeding from the gingival sulcus

Code 1: Bleeding from the gingival sulcus

This code should be used when bleeding observed, directly or by using a mouth mirror, after probing.

Code 9: Assessment cannot be made

3.7 Tooth condition

The criteria used to assess tooth condition will be those from the Children' Dental Health in the United Kingdom (Pendry et al., 2003). The examination of the tooth should be conducted with a plane month mirror and a #23 explorer. The instrument <u>should not</u> be used for probing into fissures or early lesions, but it may be used for the following:

⇒ Removing debris from around key areas if necessary.

⇒ Detecting and examining sealants

⇒ Placing into open crown margins or defects at the margin of restorations to estimate their dimension, but this should not be done with force.

The teeth and surfaces will be examined in the following order: Upper right, upper left, lower left, lower right (i.e. clockwise as the examiner look at the subject from in front). Where visibility is obscured, debris or moisture should be removed from individual sites with gauze, cotton wool rolls or #23 explorer. Compressed air should **NOT** be used in the interests of comparability. X-rays will not be taken.

In the first instance the tooth will be identified and ringed. If a primary tooth is missing, record the state of the permanent successor. In cases where both the primary tooth and its permanent successor are present further details will be recorded for the permanent tooth only. Permanent teeth may be absent for a number of reasons in which case code all surfaces as follows:

Code 8: Unerupted (or congenitally missing)

Code 6: Extracted due to caries

Code 7: Extracted for orthodontic reasons

Code T: Missing due to trauma

In most cases the reason for the absence of a permanent tooth will be obvious and the appropriate code may be called and recorded at once. Sometimes questioning the child will be necessary, for example – "Did you have those teeth taken out to make room for the others?" "Was that front tooth knocked out?"

Note 1: A tooth is deemed to be Present if any part of it is visible.

All tooth surfaces with code 8,6,7,T should be scored with the same code.

Tooth surfaces

If a tooth is present, each surface will be examined, coded and called in the following order: distal – occlusal – mesial – buccal – lingual. (In the cases of anterior teeth 'occlusal' surface is, of course, omitted.)

Obscured surfaces (e.g. by an orthodontic band) will be assumed to be sound unless there is clear evidence to the contrary.

Note 1: where doubt exists in the differentiation between the categories, the less severe category should always be called.

Note 2: Individuals using orthodontic appliances will not be examined.

The surface coding is as follows:

Code 0: Sound Tooth

Code 0 (Zero) is used for all surfaces that are present and have no caries experience. A surface is recorded as "sound" if it shows no evidence of treated or untreated dental caries in dentine. In the case of partly-erupted teeth, where some surfaces may not be visible, these will be considered as sound and recorded under this category.

Code 1 : Visual caries (non cavitated dentine caries)

The surface has caries present into dentine which is visible to the observer, but which is not obviously cavitated. This usually manifests as shadowing under an occlusal surface or marginal ridge.

Code 2: Cavitated dentine caries

The surface has a carious lesion into dentine which has caused the lesion to cavitate. Record 2C only if there is a cavity (but not 3 below). (Hard "arrested" caries into dentine is included in this category.) Lesions or cavities containing a temporary dressing, or cavities from which a restoration has been lost, will be coded in the appropriate category of decayed.

Code 3: Decay with pulpal involvement

Surfaces are regarded as falling into this category if, in the opinion of the examiner, there is a carious cavity that involves the pulp, necessitating an extraction or pulp treatment.

Code 4: Filled and recurrent decay (no visual cavitation)

A surface that has a carious lesion and a restoration (whether or not the lesion is in physical association with the restoration) will fall into this category if there is visible dentine caries but no cavitation (similar to code 2V).

Code 14: Filled and recurrent decay (cavitation present)

A surface that has a carious lesion and a restoration (whether or not the lesion is in physical association with the restoration) will fall into this category if there are visible caries with cavitation to dentine (similar to code 2C). Unless the lesion is so extensive as to be classified as "decay with pulpal involvement", in which case the filling would be ignored and the surface classified Code 3.

Code 5: Filled with no dentinal decay

Surfaces containing a satisfactory permanent restoration (excluding crowns and bridge abutments) of any material will be coded under this category.

Code 15: Filled, needs replacing (not carious into dentine)

A filled surface is regarded as falling into this category if, in the opinion of the examiner after inspection, it is chipped or cracked and needs replacing, but there is no "caries into dentine" present on the same surface.

Code 10: Traumatized surface

Surfaces affected by trauma, including those that are restored, will be coded in this category.

Code 11: Obviously sealed surfaces

The probe will be used to assist in the detection of sealants. (Care should be taken to differentiate sealed surfaces from those restored with tooth colored filling materials used in prepared cavities which have defined margins. These should be regarded as fillings and are coded 4V, 4C, 5 or R.) Sealant codes should only be used if the surface contains obvious evidence of a sealant (including cases with partial loss of sealant), is otherwise sound and does not also contain an amalgam or other filling.

Code 12: Crown/advanced restorative procedures

This code is used for all surfaces which have been permanently crowned or which have received permanent items of advanced restorative care in the form of a veneer or a restoration constituting a bridge abutment. This is irrespective of the materials employed (and should include stainless steel crowns) or of the reasons leading to the placement of the crown/veneer/bridge. (Note: missing teeth replaced by a bridge are coded T, 6 or 8 as for other absent teeth (congenitally missing teeth are coded 8).

Code 9: Not Recorded

Any erupted permanent tooth that cannot be examined for any reason (e.g. because of orthodontics bands, severe hypoplasia, calculus etc.)

3.8 Traumatic dental injuries (TDI)

The clinical assessment for TDI is described in this section

The dental examination for traumatic dental injuries will include only upper and lower permanent incisors. The great majority of injuries occur on front teeth and the prevalence including all teeth is very similar to the one including only incisors. The criteria used to record traumatic dental injuries will be those from the Children' Dental Health in the United Kingdom (Pendry et al., 2003). Two anatomical features – size of the overjet and type of lip coverage – will be assessed due to their highly significant association with TDI.

3.8.1 Assessment of type of lip coverage

The type of lip coverage should be assessed while the subject is not conscious of being examined. This may be done while greeting the subject and introducing yourself. Alternatively, you may ask the subject to lick their lips, swallow and relax their mouth, and then make the observation. The type of lip coverage is recorded as adequate or inadequate.

Code 1: Adequate

It is recorded if the lips completely cover the upper incisor at rest.

Code 2: Inadequate

It is recorded if the lips do not completely cover the upper incisor at rest.

3.8.2 Assessment of traumatic dental injuries

The assessment includes both treated and untreated injuries. All permanent incisors and associated buccal and lingual or palatal soft tissue will be examined in sequence from upper right to lower right. The teeth should be dried before dental examination using cotton gauze.

Code 0: No TDI

No evidence of treated or untreated TDI.

Code 1: Treated TDI

There is a range of types of treatment provided due to a TDI. All should be recorded as treated TDI. The most common types of treatment provided are: acid etch restoration, restoration located in the palatal/lingual surface of the crown suggesting root canal treatment and replacement of a missing teeth by denture or bridge element (pontic).

Note 1: Composite restorations may be difficult to recognize and can be easily missed if the restoration is of good quality. It is crucial to use the CPI probe to detect any loss in continuity in the labial and lingual/palatal surfaces to identify whether or not the tooth was restored. Repeating this procedure in an adjacent sound tooth may help to sense the difference. In addition, the mirror may be used to reflect light through the tooth from inside the oral cavity. The difference in translucence helps to identify a tooth-colored restoration placed on injured incisors.

Note 2: Replanted teeth are also difficult to recognize. Discoloration and abnormal position in the tooth socket may suggest the tooth has been replanted. The examiner must ask the subject about a history of avulsion of the tooth due to a harmful event.

Code 2: Enamel fractures

It is characterized by a loss of a small portion of the crown, including only the enamel. A fracture is considered to be limited to the enamel if it is small and of homogenous color, when observed from the incisal angle with the aid of a mouth mirror.

Note: Attrition can be easily mistaken by enamel fracture in epidemiological surveys. In order to differentiate enamel fractures from attrition, the subject should be asked to go into lateral jaw excursion and protrusion.

Code 3: Enamel and dentine fractures

It is characterized by a loss of a portion of the crown, including enamel and dentine without pulp exposure. If the exposed central area of the fracture looks darker and more yellowish than the surrounding enamel and there is no evidence of pulp involvement, a dentine fracture should be recorded.

Code 4: Pulp involvement

There are four signs of pulp involvement that can be easily identified in a survey. They are crown or root fractures with pulp exposure, sinus tract, swelling and discoloration.

⇒ Fracture with pulp exposure is characterized by a loss of a portion of the crown or/and root, including a visible direct contact of the pulp horns or pulp chamber with the oral cavity due to a tooth fracture.

Note: A probe should never be inserted into the depth of a cavity to confirm the presence of a suspected pulp exposure.

 \Rightarrow Sinus tract is characterized by a visible sinus tract in the labial or lingual vestibule due to a TDI. The examiner must check whether the sinus tract was due to caries (presence of treated or untreated caries lesion), and also ask the subject whether they have a history of a harmful event involving the front teeth/mouth.

⇒ Swelling is characterized by the presence of swelling in the labial or lingual vestibule due to a TDI. The examiner must check whether swelling was due to caries (presence of treated or untreated caries lesion), and also ask the subject whether they have a history of a harmful event involving the front teeth/mouth.

⇒ Discoloration of the crown is characterized by a homogenous discoloration of the tooth due to TDI as compared with an adjacent permanent sound tooth. It may range from yellow to dark grey when compared to other teeth. The examiner must check whether discoloration was due to caries (presence of treated or untreated caries lesion), and also ask the subject whether they have a history of a harmful event involving the front teeth/mouth.

Note: Often subjects do not recall a history of a harmful event. Therefore, in the absence of any evidence that pulp involvement was due to caries, sinus tract, swelling and discoloration should be recorded as pulp involvement due to TDI.

Code 5: Missing teeth due to TDI

It is characterized by the absence of the tooth due to a complete avulsion. The examiner must ask the subject if the avulsion was due to a harmful incident involving the front teeth/mouth or if the tooth has been extracted due to caries or orthodontic reasons, as well as whether the tooth is unerupted or absent congenitally.

Code 9: Excluded tooth

Signs of TDI cannot be assessed, i.e.: presence of appliances, all permanent incisors missing due to caries.

3.8.3 Assessment of incisal overjet

The CPI probe will be used to measure the size of the incisal overjet because it is practical. Measurement of the horizontal relation of the incisors is made with the teeth in centric occlusion. The first step is to identify the most prominent labio-incisal edge of upper incisors. Next, the distance from the labial-incisal edge of the most prominent upper incisor to the labial surface of the corresponding lower incisor is measured with the CPI probe parallel to the occlusal plane. The black line on the CPI probe is used to demarcate between overjets less than 6mm and more than 6mm.

Code 1: Less than 6 mm

It is recorded if the largest maxillary overjet is equal to or lower than 6 mm (The most prominent labioincisal edge of upper incisors is within the black band of the CPI probe).

Code 2: Greater than 6 mm

It is recorded if the largest maxillary overjet is greater than 6 mm (The black band of the CPI probe is not visible).

Note 1: If in doubt, the examiner should record greater than 6 mm (Code 2).

Note 2: If the incisors occlude edge to edge or are in lingual crossbite, code 1 should be recorded.

Note: After the clinical exam for TDI the examiner should ask the child or their parents, the following question:

"Have you (child's name) ever had an injury to your (his/her) front teeth?"

If the child/parents indicate that one or more injuries have occurred, code 1 in the first question questionnaire attached to the clinical form and ask the 3 other questions (Appendix 4).

Note1: The question should be asked regardless of the results of the clinical examination

4 Data Quality

As stated in the main study protocol, collecting data of high quality is critical to the success of this project. Each individual involved in this project is the first and best guarantor of the quality of the data being collected. Data quality is affected by every step of the dental survey including nonexam procedures leading to the examination, and nonexam procedures following the examination. The quality of data in this study will be controlled by (1) a training period for the dental teams with calibration of dental examiners prior to the beginning of the data collection (2) periodic monitoring and recalibration of dental examiners, and (3) periodic retraining of dental teams.

4.1 Training and calibration

Training is divided into three phases as follows:

1) The instructional phase in which examination team members are familiarized with research examination procedures and criteria for research assessments.

2) The standardization phase in which they are trained to use standard procedures and apply standard criteria for the oral health assessments.

3) The calibration phase in which the degree of correlation among the examiners and the standard examiner is measured.

4.1.1 Instruction

The instructional phase of the training sequence will be conducted by an oral health epidemiologist with experience in oral health assessment and survey procedures and with support and assistance from the

standard examiner. The standard examiner will be a specially trained dentist with experience in conducting oral health surveys. The expert trainers will present lectures on criteria for each of the oral health assessments to be used in the survey to the survey's examiners. Lectures are accompanied by slides depicting a wide variety of possible observations and illustrating application of assessment criteria to those observations. The lecture-slide presentations on each assessment are followed by instructions on data recording and editing for that assessment. Although the instructional phase consists primarily of lectures and slide presentations, some demonstrations of examination technique and equipment use will be included.

4.1.2 Standardization

The second phase of training will be devoted to standardization. During this phase of training, the standard examiner reviews examination procedures and techniques and the criteria for each assessment, stressing the importance of consistency and uniformity among the examiners and the standard examiner in performing the examination and in applying the criteria to observations. Rationale for differences between a research examination and a diagnostic examination will be discussed and professional ethics of research examinations reviewed. A demonstration of the examination by the standard examiner and practice examinations by the examiners being trained are among the salient features of this phase. Standardization of techniques used by all examiners will be achieved by using replicate examinations with detailed discussion of observations.

4.1.3 Calibration

The reliability of the assessments is measured by determining the degree to which examiners can produce uniform and consistent results when performing independent replicate examinations without discussion. In this phase of training, the standard examiner and all examiners in training perform components of the examination on a specified number of children. Data from the calibration sessions will be analyzed to measure correlation between each examiner and the standard examiner. If correlations between each of the examiners and the standard examiner are not within acceptable ranges, additional training sessions will be scheduled.

4.2 Complete replicates

During the data collection, 5 percent of the children will be re-schedule for a second complete dental examination to monitor intra-examiner reliability. Data from the first examination will be compared to data from the second examination to determine the level of uniformity over time for each examiner. If data show that an examiner is not within acceptable limits of uniformity and consistency in performing the dental assessments, he/she will be retrained prior to the regularly scheduled retraining session.

4.3 Expert replication and monitoring field operations

During the field operations, examiners and recorders should periodically review their

training manuals to prevent deviation, or "drift" from the standards achieved during the training period. Particular attention should be devoted to uniform adherence to the criteria for making correct decisions about observations. Strict compliance with infection control procedures is another important consideration for dental teams. In order to help the dental teams maintain their standards, the research coordinator will make periodic visits to field sites to observe the performance of the personnel and offer feedback on the results of their examinations. The standard examiner will visit each team twice a year to observe field operations and to replicate a few dental examinations during each visit. The purpose of these so called "expert replications" is to determine whether the examiners are maintaining the examination standards achieved during training, and to measure the degree of deviation, if any, from those standards. If correlation between the standard examiner and the field examiner is not within acceptable limits, retraining will be conducted on site.