POPULATION PATTERNS OF HAIR ZINC, DIETARY AND SOCIO-DEMOGRAPHIC DETERMINANTS

by

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ABSTRACT

Marginal zinc deficiency (MZD) exists in children of industrialized societies and can impair growth and development. Presently there are no data available on its global prevalence. It is believed that MZD is one of the most common hidden deficiencies throughout the world. This is partly because of the lack of sensitivity and specificity of serum zinc, the most commonly used biomarker of zinc status, to detect MZD. This deficiency in children is always accompanied by a decrease in hair zinc. Although in research settings hair zinc is a recognized biomarker of MZD in children, health practitioners do not presently use it.

These cross-sectional studies were designed to examine the hair zinc status of preschoolers in Vancouver. They also aimed at exploring some dietary and non-dietary factors associated with hair zinc status in an attempt to construct and validate a screening tool for detection of MZD.

Our first study indicated a mean hair zinc of 75±30 μg/g, with 46% below the cutoff (<70μg/g) for a group (n=87) of low-income preschoolers (**Chapter II**). Among these children we observed negative associations between the hair zinc and consumption of dairy (R^2 =0.09, P=0.01) and milk (R^2 =0.08, P=0.01), being described as "often sick" (R^2 =0.55, P=0.00) and "eating unhealthy" (R^2 =0.16 P=0.00), and prolonged breast-feeding (R^2 =0.11, P=0.01).

Our citywide survey (n=719) indicated a mean hair zinc of 116±43 μg/g with 17% below the cutoff (**Chapter III**). Logistic regression analysis indicated sex, age, maternal education, the number of adults at home, consumption frequency of milk, "scores of activity level", "being described as frequently sick" and "taking supplements containing

iron" as the significant predictors of hair zinc status. However, the final model had 16% sensitivity while having 98.3% specificity, indicating its lack of usefulness as a screening tool.

Our study provides important information on the hair zinc status of Vancouver preschoolers. Although we did not accomplish our primary goal of constructing and validating a screening tool, we did identify some factors in children and their environment associated with hair zinc, which may help in better understanding of hair zinc as a biomarker of MZD.

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PREFACE

This thesis has been prepared using a manuscript-format described by the Faculty of Graduate Studies of the University of British Columbia University. Therefore chapters II and III are elaborated forms of the manuscripts to be submitted to relevant scientific journals.

Although chapters are arranged in chronological order of the data collection, each chapter stands on its own and can be read in any order. The relevant forms and documents of each study are presented as appendices.

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"Keep going Mom, keep going, you are almost there!"

and my daughter, Avishan, who, over the years, sang with me every morning in the car as we drove to daycare, elementary, and then high school.

"Inch by inch

row by row

We are gonna make this garden grow

all it takes is work and hope"

Thank you all!

In remembrance of Gholam-Reza Ghorbani
his life and his legacy

My years in the doctoral program were a long journey that produced above and beyond this thesis. Difficult life trials were blissfully followed by great triumphs and invaluable life lessons.

As the absence of light creates darkness and the absence of heat creates cold, lack of responsibility and respect in educational interactions create darkness and cold in which no effective education can possibly occur!

This work is dedicated to all great educators who understand the enormity of their responsibility and the significance of their roles in training students and shaping their lives.

Those who put caring and respect as essential and ever-present elements in their interactions,

and in doing so, they set us free

Since "in knowledge we find freedom"!

CO-AUTHORSHIP STATEMENT

Chapters II and III are being developed as manuscripts. For each manuscript, I have identified research questions, conceived of the study design, recruited all participants, completed all data collection and managed, planned and conducted data analyses. The co-authors will be those who make a significant contribution to either the study design and/or implementation, stimulation of the discussion of the results, or editorial process of the manuscripts.

CHAPTER I

INTRODUCTION

I.1. BACKGROUND

It is evident that good health in the adult is predicated on good health in the child. Many of the key determinants governing health have their roots in the biological and social experiences that span childhood. A healthy child population is not only one of the indicators of a society's present health, but it may also be an indicator of that society's future well being and productivity.

The health of a child derives from the influence of genes, 'health behavior', ecology, social and societal characteristics, and medical care (Brownell et al, 2002). Nutrition as a part of 'health behavior' is one of the key elements to sound childhood health.

Nutritional inadequacy can result in deficiencies of both macro- and micronutrients. Some of these deficiencies are known to have an adverse effect on growth and development. The preschool period is a crucial stage of intense and ongoing growth and development. For this reason a preschooler is highly vulnerable to the impact of these deficiencies. Zinc is one such essential micronutrient.

In its marginal form, zinc deficiency has been documented in many industrialized societies (Hambidge et al, 1972, Walravens et al, 1983, Nakamura et al, 1993, Buzina et al, 1980 and Chakar et al, 1993), including Canada (Smit Vanderkooy & Gibson, 1987 and Gibson et al, 1989-b). The nature of the diets associated with marginal zinc deficiency (MZD) suggests that it may be more common than is thought among some vulnerable age groups in industrialized countries. Durung this stage, zinc deficiency can easily go undiagnosed.

Like many efficiencies in their early stages, marginal zinc deficiency has virtually no specific clinical symptoms. At the biochemical level, however, though it does not result in any measurable change in the main biomarker of zinc status i.e. the serum zinc, it does leave some clues that can be a helpful tool for its proper diagnosis. Marginal zinc deficiency, in children, is almost always accompanied by a decline in the hair zinc level.

Hair zinc is believed to reflect the body's store of this element (Gibson, 1989). Evidence does support the view that hair zinc concentration can be an index of MZD in children, at least in developed countries where widespread protein-energy-malnutrition does not exist. The suitability and usefulness of hair zinc in the assessment of zinc status and the detection of deficiency at an early stage has been demonstrated by the growth response of affected children (low hair zinc children) to zinc supplementation. In addition, being a convenient and non-invasive test, it has this additional advantage in assessing MZD in the pediatric age group.

As a part of pediatric health promotion, management of MZD can be approached through a population health approach. In this approach knowledge of the determinants of this deficiency is pivotal. These can include any factor within a broad range from environmental and health associated socio-demographic factors to interpersonal characteristics of the affected individuals and populations. Awareness of the determinants of a disease or condition is a pre-requisite to any successful intervention and can be gained from the gathered evidence of epidemiological studies and population surveys and large-scale studies.

Studies of preschoolers have documented associations between low hair zinc and some dietary factors such as the intake of bioavailable zinc and the intake of enhancers

and inhibitors of zinc absorption (Smit Vanderkooy & Gibson, 1987). It has been further documented that certain socioeconomic factors may be associated with MZD/low hair zinc (Hambidge et al, 1976, Walravens et al, 1983 and Chakar et al, 1993).

This study was the first to examine the hair zinc status of a large sample of Canadian preschoolers. It was also the first attempt to develop and validate a screening questionnaire for detection of low hair zinc/MZD. The information collected through this thesis will enhance our understanding of the risk factors associated with low hair zinc status in the preschool population, which could in turn serve in the identification of priority groups and at-risk sectors of the population.

Prior to presenting the study and its findings, the relevant literature will be reviewed in an attempt to present the current state of knowledge and those areas where there is still a knowledge gap. The literature review will begin with a brief introduction to the population health approach as a framework for the study. Subsequently marginal zinc deficiency, its prevalence, diagnosis, signs and symptoms will be reviewed. Hair zinc as a cardinal biomarker of this deficiency will then be examined. The literature review will end by highlighting some of the gaps in our present knowledge.

I.2. LITERATURE REVIEW

I.2.I. Population health

The concept of population health in Canada began in the 1970s. Perhaps the point of genesis of population health in Canada was the federal government's White Paper, proposing that changes in lifestyles or social and physical environments would likely lead to more improvement in health than would be achieved by spending more money on existing health care delivery systems (Government of Canada, 1974).

Population health is defined as the health, well-being, and functioning of a clearly defined population (Kindig & Stoddart 2003). The defining line can be based on locality, biological criteria such as age, social criteria such as socio-economic status, or cultural criteria. Population health is an approach to health that aims to improve the health of the entire population and to reduce health inequities among population groups. In this approach population is not just a group of individuals, but also the trends and themes among them. Therefore, "... the outcome and benefit of such an approach extends beyond improved population health outcomes to include a sustainable and integrated health system, increased national growth and productivity and strengthened social structure" (Public Health Agency of Canada, 2002). The population health approach to health has some central elements on which it is founded and the knowledge of these elements is essential to its understanding.

One of the main pillars of population health is a fundamental belief that there are factors outside individuals and the health care system that can affect health significantly. Therefore, the entire range of individual and collective factors and the interaction among these factors are to be considered if one intends to address health in a comprehensive and wholesome manner. These factors, referred to as "determinants of health", are the key elements of the population health approach.

Another important notion in population health is that the earlier in the causal stream action is taken, the greater is the potential for population health gain. Therefore, "primary prevention" is the preferred point of intervention. To embark on this "primary prevention" task, a thorough knowledge of all the factors involved in, leading to and

influencing the morbidity of the disease is essential. In short, primary prevention is a socio-environmental approach, confronting the root cause of disease.

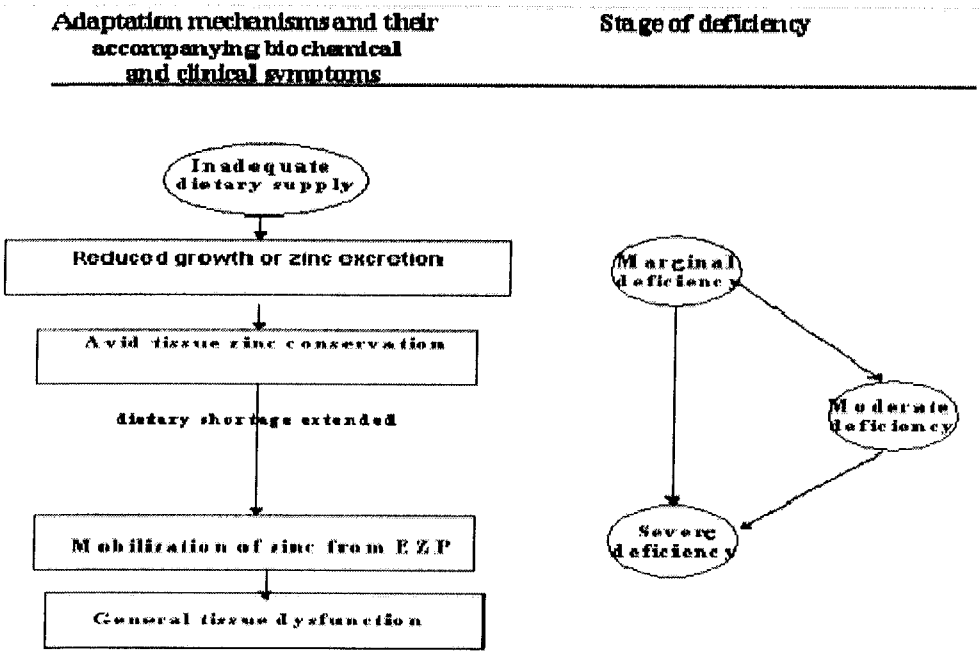
The primary prevention approach can be adopted within many intervention frameworks identified for population health improvement."Improved child development" has been acknowledged as one of these frameworks and has gained a great deal of attention from both the research and policy maker communities within the last decade. A national Children's Agenda was agreed to in the late 1990s in Canada. Since then some impressive steps have been taken towards the development of comprehensive strategies for optimum early childhood development across Canada, among them the recent initiative of the Early Development Index (EDI), which, in British Columbia, measures kindergarten children's readiness for school in 5 areas of child development that have a long-term impact on health, well-being and school success (Hertzman et al, 2004). The findings of this province-wide study have resulted in the development of BC Child Atlas (HELP, 2007). BC Child Atlas is an invaluable guide for further planning for population health promotion within the framework of child development and via a primary prevention approach.

Apart from environmental factors such as community resources, early child development can also be affected by nutrition. Nutritional inadequacies in childhood can give rise to macronutrient and micronutrient deficiencies. Although the former are not frequent among the affluent and industrialized nations, the latter are a valid pediatric health concern of these nations. Among the micronutrient deficiencies of concern in North America is marginal zinc deficiency (MZD). In the following sections this

deficiency, its prevalence, signs and symptoms and potential biomarkers will be reviewed.

I.2.II. Marginal zinc deficiency (MZD)

Zinc deficiency has been observed across a wide spectrum from marginal and mild to advanced and severe (Prasad, 1985). Clinical signs of marginal zinc deficiency in childhood include impaired growth, poor appetite and diminished taste acuity (Gibson, 1989), none of which is specific to zinc deficiency *per se*. These symptoms, however, are usually accompanied by a decline in hair zinc in the case of MZD. Marginal zinc deficiency can best be detected through a positive growth response in supplementation trials (Prasad, 1988). **Figure I.I** is a schematic presentation of the three stages of zinc deficiency and their accompanying clinical and biochemical signs and symptoms.



EZP: Exchangeable Zinc Pool

In marginally zinc deficient individuals, the deficiency can advance to a severe stage without going through a moderate zinc deficiency if the zinc need increases drastically (e.g. in case of injury, burn and trauma)

Figure I.I. Steps in development of zinc deficiency (constructed based on King, 1990, and Prasad, 1988)

As **Figure I.I** depicts, marginal zinc deficiency is a start point that can progress to the more serious stages of deficiency i.e. moderate and severe zinc deficiency.

A moderate level of zinc deficiency has been reported in a variety of conditions as a secondary/conditional deficiency due to chronic diseases like cystic fibrosis and pancreatic insufficiency. Clinical manifestations of a moderate level of zinc deficiency include growth retardation, rough skin, poor appetite, hypogonadism in males, delayed wound healing, lethargy, abnormal neurosensory changes and compromised cell-mediated immune dysfunction (Prasad, 1988).

Chronic or severe deficiency is often accompanied by severe malnutrition and will exhibit clear signs of lowered immunity, halted or delayed puberty, stunting, dermatitis, alopecia, diarrhea, emotional disorder, and weight loss (Prasad, 1988). With this level of deficiency one often sees a pronounced decline in serum/plasma zinc. Reduction in hair zinc may or may not be present depending upon the absence or presence of protein energy malnutrition (PEM) (Gibson, 1989).

I.2. II.A. The prevalence

Marginal zinc deficiency was first observed in American preschoolers of low-income families in Colorado (Hambidge et al, 1972). Though at present there is no precise information as to the number of people affected by dietary zinc deficiency, from the Food and Agricultural Organization, FAO, and its national food balance data, the Stockholm Conference, 2000 reported an estimated 48% of the global population at risk of zinc deficiency (Stockholm conference, 2000). While severe deficiency appears to be predominant among underdeveloped and developing countries (Hambidge & Krebs, 2001), MZD is seen in developing and even developed countries such as the U.S.

(Hambidge et al, 1972 and Walravens et al, 1983), Canada (Smit Vanderkooy & Gibson,1987 and Gibson et al, 1989-b), Japan (Nakamura et al, 1993), and France (Chakar et al, 1993).

In North America, the first study to report the existence of marginal growth-limiting zinc deficiency found low hair zinc levels in children to be associated with impaired taste acuity, poor appetite and low growth percentiles (Hambidge et al, 1972). Studies in the 1970s and 1980s confirmed this deficiency in apparently healthy infants, toddlers, and preschoolers even after routine fortification (in the US during the 1970s) of infant formulas with zinc (Hambidge et al, 1972, Hambidge et al, 1976, and Walravens et al, 1983). Some researchers from Eastern Canada (Smit Vanderkooy & Gibson, 1987 and Gibson et al, 1989-b) have also described a growth-limiting marginal zinc deficiency in preschoolers of Ontario. Smit Vanderkooy & Gibson (1987) examined food consumption patterns and nutrient intakes of children aged 4-5 years in relation to their zinc status and suspected the existence of a marginal zinc deficiency manifested as low hair zinc (<70μg/g) and slowed physical growth. The occurrence of low hair zinc among these 107 study subjects was estimated at 21.3% for boys and 4.7% for girls. The subsequent study of this research team confirmed this deficiency among some Ontario children through the growth response of a selected (height $\leq 15^{th}$ and mid-parent height $>25^{th}$ percentiles) group of 5-7 year old boys to a zinc supplementation program (Gibson et al, 1989-b). No further study has been conducted on zinc status of this age group in Canada.

I.2.II.B. Signs and symptoms

Marginal nutritional zinc deficiency is clinically characterized by the slowing of physical growth, poor appetite, and diminished taste acuity (hypogeusia) (Gibson, 1989).

These clinical signs are non-specific and often go unnoticed. For instance, loss of appetite can be the ramification of many other physiological, psychological and emotional problems. Similarly hypogeusia,

impaired taste acuity, can be the result of a number of neurological or connective tissue disorders.

Slowing of physical growth, although often an accompanying sign of MZD, cannot be used as a reliable diagnostic tool by itself for various reasons. Firstly, the slowed physical growth at the marginal level of deficiency is not always a visually apparent and easily detectable sign. A health care professional has to obtain the anthropometric data and convert these to height-for-age, weight-for-age, and weight-for-height Z scores in order to test their normalcy, using the appropriate reference tables (Centers for Disease Control and Prevention, 2002). Secondly, as mentioned earlier, slowed physical growth is not specific to zinc deficiency and can be the consequence of other micro-nutrients deficiencies such as iron, iodine and vitamin A. Thirdly, slowed physical growth does not always manifest itself in measurable changes in Z scores or other anthropometric indices and this feature of MZD can therefore remain un-detectable for some children. Height achieved is the result of genetic endowment and macro as well as micronutrient availability during the growth period. Slowed growth may result in anthropometric measurements that are still within the expected values of child's age group, simply because the child had a larger genetic allowance. Therefore, it is reasonable to conclude that none of the clinical features are specific and as a result none can be the diagnostic tool by itself.

As for the biochemical features of marginal zinc deficiency, the situation is somewhat different. One such feature is diminished hair zinc content. Animal studies reveal that the uptake of zinc by hair is slow (Deeming and Weber, 1977) and may be impaired preferentially if the amount of zinc absorbed is decreased. Therefore, it is possible for hair zinc to be markedly depressed while more vital tissues continue to receive an adequate supply, with few, if any, other symptoms of zinc deficiency apparent (Hambidge, 1980). This physiology points to a superior sensitivity, as well as a specificity, of hair zinc as an index of body zinc status. Evidence suggests that hair zinc concentration could be an indicator of chronic marginal zinc deficiency during childhood when the confounding effect of severe protein-energy malnutrition is absent (Hambidge et al, 1972, Smit Vanderkooy & Gibson, 1987 and Gibson et al, 1989-b). Hair zinc, therefore, cannot be used in cases of very severe malnutrition and/or severe zinc deficiency when the rate of growth of the hair shaft is diminished. Under these circumstances the hair zinc level may be normal or even higher than normal (Hambidge, 1980 and Gibson, 1989). For these reasons, diminished hair zinc in children, in the absence of PEM, has been viewed as the cardinal feature of marginal zinc deficiency (Gibson, 1989).

Serum zinc, the most widely used biomarker of zinc deficiency is not a sensitive tool for the diagnosis of MZD. At this level of deficiency the homeostatic mechanisms of the body are fully functional and active and prevent any drop in the serum zinc. Below is a brief review of these 2 biomarkers and the degree of their usefulness in the diagnosis of marginal zinc deficiency.

I.2. II.B.a.Serum/plasma zinc

Serum/plasma zinc is the most common marker of zinc status. Zinc in plasma is in extremely low concentrations and is tightly regulated and maintained within a normal range, despite a wide range of change in the dietary zinc intake (Hambidge, 1980). This makes the plasma zinc an insensitive marker of dietary zinc deficiency. Despite the tight regulation/protection of this marker against dietary fluctuations, it is also easily affected by many other factors such as diurnal rhythm, stresses, infection, fasting/non-fasting, and plasma protein levels (Guillard et al, 1979). Therefore, it is reasonable to conclude that serum zinc is neither specific nor sensitive as a biomarker of zinc status during the early stage of zinc deficiency.

Despite this lack, a growing body of evidence supports its usefulness as an indicator of population zinc status (Bahl, Chaudhuri, & Pathak, 1994 and Brown, Peerson, & Allen, 1998). The risk of zinc deficiency in a population can be estimated by comparing serum zinc data of that population with statistically defined cutoffs from a presumably healthy population (Hotz et al, 2003). In addition, measurable differences in serum zinc concentration have been reported to occur in various populations due to changes in dietary zinc intakes and clinical conditions associated with zinc deficiency (Hunt, Mathys, & Johnson, 1998, and Srikumar et al, 1992). Therefore, serum zinc can elucidate how a population zinc status (mean serum zinc, distribution, percentiles...) does compare with reference to a healthy population. It can also act as a reference for a given population itself and help to monitor the changes in zinc nutriture experienced over time.

Low serum zinc concentrations have been reported in Canada in young women with low serum ferritin values (Donovan & Gibson, 1995), USA (Yokoi, Alcock, &

Sandstead, 1994), and New Zealand, (Gibson, Heath, & Ferguson, 2002). Because the dietary factors associated with the etiology of iron deficiency also induce zinc deficiency, it has been reasoned that the prevalence of sub-optimal zinc status is likely to be comparable to that of iron deficiency (Gibson, Heath, & Ferguson 2002). This has been supported by the co-morbidity of zinc and iron deficiency among many populations of underdeveloped societies, among them Middle Eastern adolescents. The zinc content of the diet of these individuals (with zero to very little animal meat) was a few times greater than the recommended nutrient intake (RNI) at that time (~30mg/day), but due to high levels of zinc and iron inhibitors in their diet they failed to meet their needs and were severely zinc and iron deficient (Prasad, 1985). Whether or not this co-morbidity of iron and zinc deficiency applies to developed societies such as ours where large-scale iron-fortification of cereals and grain products are in place, remains to be explored. No studies to date have been carried out to evaluate the impact of fortification of cereals with iron on the iron status of Canadians. However, in theory, the likelihood of being iron adequate while zinc deficient in a diet with a low intake of meat, in light of the iron fortification of cereals, could be entertained.

The serum zinc level appears to be a reflection of dietary zinc only in extreme cases. It drops profoundly in persons with severe zinc deficiency when the intake is so low that homeostasis cannot be established without the use of zinc from the exchangeable zinc pool, EZP, (of which plasma/serum is a component) (King, 1990). In view of the degree of protection conferred upon this zinc compartment in the human body (serum/plasma), it is not difficult to see why it is considered a very poor and misleading measure of marginal zinc deficiency (King, 1990 and Gibson, 1989).

I.2.II.B.b. Hair zinc level

Trace elements get incorporated in the hair during its development and are derived from the matrix and connective tissue papillae (with blood and lymph vessels) and sebaceous glands. As the extruded hair approaches the skin surface, its outer layer becomes hardened and relatively impermeable; therefore, isolating the hair from the body's continuing metabolic activities (Hopps, 1977). For this reason zinc in the hair is not affected by the factors that affect serum zinc such as circadian rhythm. It is also not subject to a rigid regulation in response to fluctuations in dietary zinc. Once the metal becomes incorporated into the hair during hair growth, it is no longer in equilibrium with the body and is thus not affected by on-going metabolic events (Assarian & Oberleas, 1977).

Hair zinc reflects body stores of this element (Dorea & Paine, 1988). Indeed, many researchers have discussed the suitability of this biological sample as an index of zinc status. As an index of zinc status, hair zinc has gained popularity over the last few decades. Perhaps this, in part, is due to the ease of sample collection as well as sample storage and handling. This is a particularly important factor when sampling large populations and in young children whose pain and discomfort experienced in an invasive sample collection method such as venipuncture may defer parents from participating in studies or conducting a regular follow-up on a symptom-free child.

II.B.b.1. Advantages and Shortcomings of hair zinc as a biomarker

Hair as biopsy material offers many advantages such as ease of sample collection, handling and storage. In addition, the zinc in hair is much more concentrated than in other biological samples such as blood and urine. This higher concentration facilitates the analysis (Gibson, 1989).

On the down side though, hair mineral content may be subject adventitiously to a variety of trace elements from environmental sources. However, it has been shown that washing procedures can overcome the effects of these exogenous materials and, if not to eliminate them completely, at least to minimize them (Bate, 1966). Other environmental factors such as geography and season may also affect the hair zinc content. This creates problems in terms of interpreting and comparing the results of various studies that are done in different places or different seasons at the same location.

A **seasonal variation** in hair zinc content has been reported by some Canadian scientists (Gibson et al, 1989-a). Gibson and co-workers have studied groups of Canadian and Malawian children on a plant-based diet during 3 survey periods, and reported an inverse relationship between daytime light and hair zinc content. It appears that hair zinc has its highest levels in the seasons with the shortest daylight hours (December/January in Canada and July/August in Malawi). It can be argued that the observed seasonal changes in hair zinc may originate from the seasonal changes in the rate of growth since seasonal changes in the growth of children are well documented (Marshall, 1975 and Marshall & Swan, 1971), and a positive relationship between hair zinc concentrations and growth has been demonstrated in some studies (Smit Vanderkooy & Gibson, 1989 and Hambidge et al, 1972). However, a similar trend in the hair zinc of American adults (Greger et al, 1978) and the absence of seasonal change in the serum copper content of Canadian children (Gibson et al, 1989-a) all indicate that the rate of general growth and hair growth are not the underlying reason for the seasonal changes observed in hair zinc concentration.

Based on the above-mentioned study Gibson and coworkers (1989-b) have proposed the use of 2 different cutoffs for children's hair zinc; 1.68 μmol/g (110 μg/g) and 1.07 μmol/g (70 μg/g) for seasons with shortest and longest daylight, respectively (Gibson et al, 1989-a). It is important to note that the 70 μg/g cutoff appears to be universal. A search in pub med (http://www.ncbi.nlm.nih.gov/sites/entrez) using the key words of "hair zinc", "zinc deficiency", and "children", reveals that except for this research group (Gibson and coworkers) no other researcher has used the winter cutoff.

Geographic variations in the level of trace elements have been shown to exist. Gibson and DeWolfe (1979) have shown that hair zinc levels in full-term neonates from Cincinnati were comparable to those seen in similar subjects in Halifax, Nova Scotia, and the hair zinc levels of both of these groups of infants were higher than those found in comparable subjects from Denver (Hambidge et al, 1972). Some have proposed that this difference arises from differences in the degree of environmental pollution (Zachwieja et al, 1995). For instance, in Canada, a regional difference in the trace metal content of ground water has been documented (Gibson et al, 1987). Whatever the underlying reason, it has been argued that the hair zinc of infants from 2 different geographic locations can only be compared if their identical hair growth rate has been established (Hambidge, 1980). The normal hair growth rate for infants has been reported at 0.2 mm/day for scalp hair, which increases to 0.3-0.5 mm/day during the post infancy period (Gibson, 1989). Accurate and well-designed studies addressing the geographic variations in older children do not, as yet, exist.

Besides these environmental factors some **individual characteristics** such as hair color/pigmentation (Sky-Peck, 1990), sex (Smit Vanderkooy & Gibson 1987 and Esteban

et al, 1999), location of collected hair samples (Paschal et al, 1989) have been demonstrated to affect hair concentrations of several minerals including zinc.

In general, **sex** is believed to affect hair metals due to the presence or absence of sex-linked hair treatment activities (e.g. coloring, permanent) (Esteban et al, 1999). While this may be part of the reason for the higher hair zinc of females, the higher physiological need of males for zinc (Castillo-Duran et al, 1994) should not be overlooked. A higher physiologic requirement may lead to the provision of most of the available zinc to the tissues having zinc-dependent activities and may result in a very small amount left to be channeled to the hair follicles, where the element (Zn) will stay in a storage system that is no longer available to the body. In any event, sex differences have been observed both in terms of hair zinc content and the occurrence of low hair zinc (Smit Vanderkooy & Gibson, 1987, Hambidge et al, 1980). Nevertheless, whether these differences originate from endogenous reasons (higher needs for zinc) or exogenous ones (such as differences in grooming habits) they have not been properly investigated. In view of the fact that grooming habits are almost the same for both sexes when it comes to younger children, and also in light of the fact that sex differences reported in the 3 aforementioned studies (Smit Vanderkooy & Gibson, 1987, Gibson et al, 1989-b and Hambidge et al, 1976) were all in young children (<9 years old), the higher physiological needs may appear a more convincing explanation for the observed sex-based differences in hair zinc content. However, the existence of few studies reporting a comparable hair zinc for the 2 sexes (Sakai, Warishi, and Nikiyama, 2000), or higher hair zinc for male children (Zachiwieja et al, 1995) may indicate the involvement of some other factors besides the physiology.

The amount of **hair pigmentation** has also been argued as a factor that may affect the hair zinc content. However, well-designed studies investigating the effect of pigmentation while controlling all other factors have yet to emerge. In a study of zinc deficiency among short Iranian teenagers, a *post hoc* analysis compared the hair zinc of these teenagers (all brunets) with their American counterparts (all blonds) and revealed no significant difference (Prasad, 1991). This study, however, was a cross cultural comparison and other factors which may affect hair zinc content such as geography, ethnicity, and habitual diet, were not taken into account. At the organ level, however, Czechoslovakian investigators have documented a 5 times higher concentration of zinc in the pigment-containing organelle of hair, the melanosomes, to that of the hair shaft as a whole (Borovansky, Horckcko & Duchon, 1976).

II.B.b.2. Significance of low hair zinc in individuals and in a population

The validity of hair zinc as a diagnostic tool of MZD in children as noted above has been demonstrated by the growth response of affected children to zinc supplementation (Gibson et al, 1989-a, Walravens et al, 1983 and Hambidge et al, 1972). In some of these studies, but not all, hair zinc has also increased in response to the supplementation. The discrepancies encountered may be due to variations in dose and duration of the zinc supplementation period and confounding effects of the season on hair zinc concentrations. Periods of 6 weeks or less are too short for a response in hair zinc (Gibson, 1989).

Although the suitability and usefulness of hair zinc in the assessment of zinc status and detection of MZD in children of developed countries has been documented by zinc researchers (Gibson, 1989, Laker, 1982 and Van Wouwe & Van den Hamer 1985),

the value of hair zinc as an index of sub-optimal zinc status in adults is uncertain and controversial.

In children, hair zinc correlates with many other indices of zinc deficiency, but not with serum zinc. The lack of relationship between the hair and serum zinc concentrations reported by studies of marginal zinc deficiency is expected and does not challenge the potential value of hair zinc in determination of zinc deficiency. In fact, it serves to underline that a low hair zinc value has a significance that is different from that of hypozincemia (Hambidge 1980), seen only in cases of severe zinc deficiency.

While one needs to be very careful in interpreting results of hair zinc coming from isolated samples, an epidemiological use of samples obtained from a wide population may provide worthwhile information to the clinicians. At the population level, hair zinc seems to correlate with the intake of bioavailable zinc as shown in studies of zinc in populations with high dietary phytate intake (Gibson & Huddle, 1998), or those with little meat intake (Smit, Vanderkooy & Gibson, 1987). Whether a change in dietary patterns brings a change in hair zinc in a given population or not, has not yet been investigated.

All in all, it is believed that, if used in conjunction with other clinical and biochemical information, hair analyses may become a useful screening tool for determination of nutritional disorders including marginal zinc deficiency in individuals (Sky-Peck, 1990). At the population level, analysis of recently grown hair for zinc content provides a biomarker of overall zinc status and could be useful in the assessment of the zinc status of groups of individuals and large populations (Contiero & Folin, 1994).

II.B.b.3. Issues around hair sample collection

Hair sample collection is easy and except for some rare occasions when some cultural taboos or individual beliefs militate against individuals having their hair touched by a stranger (Personal observation, March 23, 2004), the compliance for donating a sample is generally high. As much as the ease of sample collection has contributed to the popularity of hair as a biological sample for assessment of zinc status, the significance of a proper sample collection should not be overlooked. Aside from potential cultural barriers to sample collection, the lack of a standard protocol for sample collection can also be a cause of potential problems.

At present the most reliable protocol appears to be one proposed by the International Atomic Energy Agency (IAEA) (Dippisch et al, 1999) and adopted by the Center of Disease Control (CDC) and the US Environmental Protection Agency (EPA). However, within the research setting, depending on the analytical methods used by the analyzing laboratories, wide variations of this protocol have been used.

The 3 important factors in hair sample collection are 1) Sites of sample collection, 2) the size of the sample (mg) and 3) the length of the samples. Below is a brief review of these criteria and the possible modifications that they have been subjected to based on research resources and practicalities.

1. The sites of sample collection: Interpretation of analytical data is dependent on the assumption of a normal rate of hair growth, approximately 1 cm/month. At any given time some scalp hairs are in a resting (telogen) phase (Hambidge, 1980). Therefore, it is crucial to obtain a sample from various sites on the scalp. If analyses are limited to very few hair shafts, the chance selection of hairs in the resting phase may provide misleading

information. This criterion appears to be one that every research paper has provided assurance on in their sample collection procedure. The sample should be taken in small portions from at least 3-4 different locations. The recommended areas for collection are the nape (bottom) of the neck, the top portion of the neck, and the side of the scalp.

2. Sample size: Although the protocol adopted by the CDC defines an acceptable sample size as one weighing 500-1000 mg, in practice the sample size used does not always fall within this range. At present the sample size reported in the zinc literature varies within a larger range of 20-1000 mg. Part of this variation can come from the analytical techniques used for a given study. For instance, a study conducted to compare the recovery rate and detection limit using the spectrofluorophotometric method and atomic absorption spectrophotometry method revealed that while both can detect greater than 90% of the added zinc, the former has a lower detection limit and can be used confidently with much smaller samples (Kazi et al, 2001).

3. Length of the sample: The length of the collected hair should be the 2-3cm closest to the scalp which contains the newest growth. As the hair ages and grows away from the scalp the protein structures can "unwrap" and elements from external sources can be bound to the hair structure (Mertz, 1975). Assuming a normal rate of hair growth, the zinc level in the proximal 1-2 cm of hair reflect the zinc uptake by the follicles during the period 4-8 weeks prior to the sample collection (Gibson, 1989). It has been shown that the zinc content shows wide variations throughout the hair strand (Sky-Peck, 1990). The protocol adopted by CDC recommends 2-3 cm from the scalp as the preferred sample length, the remainder being discarded.

I.2.II.C. Population health approach to MZD

As noted, diagnosis of marginal zinc deficiency is hampered by the lack of a single, specific and sensitive biochemical index of zinc status. A large number of indices have been proposed, but many have problems affecting their use and interpretation. The most reliable method for diagnosing marginal zinc deficiency at present is a positive response to zinc supplementation. But such an approach is time consuming and cumbersome. Above all, the likelihood of overloading some individuals at the higher end of zinc intake is a concern that should be given serious thought. Potential hazards resulting from excessive intake of zinc makes the general supplementation of a population an impractical solution.

In a large-scale zinc supplementation program a concern for children who are taking adequate zinc is therefore a valid concern. The Tolerable Upper Intake Level (UL) of zinc (combined food and supplements) has been set by the Food and Nutrition Board of the Institute of Medicine at 7mg/day and 12 mg/day for preschoolers of 1-3 and 4-8 years of age, respectively (Institute of Medicine, 2000). Due to the lack of data from children, the UL for children were extrapolated from adults. The UL has been defined as the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals. It is advisable not to exceed this level, even though exceeding it would not be harmful for all children.

In recent years some researchers have challenged this upper level. Analyses of data from the 4th Total Diet Study (TDS) of Health Canada, conducted from 1992-1999 in 8 cities: Vancouver, Whitehorse, Calgary, Winnipeg, Ottawa, Toronto, Montreal, and Halifax, has revealed that for infants and young children 2 months to 3 years of age,

mean zinc intake estimates exceeded the zinc UL, which is based on the adverse effect of zinc on copper metabolism (i.e., reduced copper status) (Cockell et al, 2003). Analyses of the dietary intake data obtained during the 1994-1996 and 1998 Continuing Survey of Food Intakes by Individuals (CSFII) have also documented an intake much higher than the recommended levels among most US preschoolers. The intake of many of these children has been estimated to be larger than their respective UL (Arsenault & Brown, 2003). Perhaps due to the bioavailability issue these high levels of dietary zinc intake do not seem to have posed a health problem for these children. However, if zinc intake continues to be at the higher end on a regular basis and through supplementation, the amount of zinc consumed by children may become excessive and subject the child to excessive intake risks.

While there have been no recent reports of zinc toxicity in Canadian or US children, and it is unlikely that zinc intake from food is high enough to have a negative effect on health status, this is not the case for supplemented zinc. Until such a time that the controversy around the new Dietary Reference Intake, DRI, of zinc is addressed and resolved in a scientific manner, it is only reasonable to comply with the current guidelines and avoid routine excess intake.

The adverse effect of the excess intake of zinc on the bio avaiability and homeostasis of other essential trace elements has been documented. It has been shown to affect adversely the balance of copper and iron. Studies have reported copper deficiency anemia and decreased serum copper and ceruloplasmain (Parasad et al, 1978), decreased erythrocyte copper-zinc superoxide (Fisher et al, 1975) and increased copper excretion (Festa et al, 1985) as undesired side effects of increased zinc intake on copper

metabolism. Zinc sulfate supplementation studies of women with low iron stores have revealed amelioration in zinc indices while inducing a cellular iron deficiency and further exacerbation of iron status (Donangel, Woodhouse, & King, 2002). Alterations in lipoprotein metabolism have also been reported with excessive zinc intake (Chandra, 1984).

The usefulness of general supplementation of a population becomes particularly questionable when the gravity of some of the potential adverse effects associated with a chronic intake of excess zinc, such as suppression of the immune response and reduction of HDL cholesterol (Chandra, 1984), are considered. This line of reasoning is not meant to discredit the therapeutic value of supplementation, but to point to the importance of identifying the right subset of the population for this therapeutic approach. Identifying the sector of population eligible for supplementation will not only reduce the cost of the program, but will also circumvent the unwanted ramifications of large-scale supplementation which would include children who are at the higher end of zinc intake. Therefore, when it comes to populations, the need to be able to identify the subset of populations that are at risk appears to be a rational pre-requisite to any widespread program such as supplementation.

To identify the at-risk sectors of population the first and most important step is to identify the risk or predisposing factors of MZD. These factors when compiled can be used to construct a questionnaire that can serve as a screening tool to detect at-risk individuals and to detect the deficiency early on. Individual studies of zinc deficiency have documented associations between various socio-demographic and/or dietary factors

and zinc deficiency/status in general. The following sections are a brief review of these factors.

I.2.III. Factors associated with zinc deficiency

Studies of zinc deficiency have documented associations between zinc deficiency and various dietary and non-dietary factors. These are discussed under 3 subheadings:

1) Dietary factors associated with zinc deficiency/status

2) Socio-demographic factors associated with zinc deficiency/status

3) Other factors associated with zinc deficiency/status

I.2.III.1. Dietary factors associated with zinc deficiency

After a meal, digestive processes release zinc from the food components. This zinc, along with endogenously secreted zinc, is present within the intraluminal compartment as free zinc. As this free zinc moves, it forms complexes with ligands and with other food components. This process of complex formation can either enhance or hinder the process of absorption. Below is a brief overview of these food components and the nature of their effect on overall zinc absorption.

Protein:

The amount of protein in a meal is positively correlated with zinc absorption. When compiling results from several studies with humans to whom various protein sources and amounts had been administered, fractional zinc absorption increased in linear fashion with increasing protein content (Sandström & Cederblad 1980). The type of protein in a meal will also affect zinc bioavailability. Animal protein has been shown to counteract the inhibitory effect of phytate on zinc absorption from single meals

(Sandström & Cederblad 1980). It has been speculated that this may be due to amino acids released from the protein that keep the zinc in solution and not a unique effect of animal protein *per se*. Either way the evidence supports the view that the presence of animal proteins affects the zinc status of humans positively. Study of the dietary intake of Canadian preschool children revealed an association between the lower consumption of animal proteins and the existence of suboptimal zinc status (Smit Vanderkooy & Gibson, 1987).

Phytate:

The finding of zinc deficiency in human subjects on a high phytate diet in the Middle East suggested that phytate could adversely affect zinc status in humans (Halsted et al, 1972). Inositol hexaphosphates (I6P) or phytate and inositolpentaphosphates (I5P) bind zinc and reduce its absorption (Lonnerdal et al, 1989). Phytate is found in varying amounts in plant products, with grains and legumes having especially high levels. The phosphate groups in inositol hexaphosphate can form strong and insoluble complexes with cations such as zinc, and because the gastrointestinal tract of higher species lacks any significant phytase activity, phytate-bound minerals will be excreted in the stool. Among the inhibitors of zinc absorption, phytate has been recognized as the most potent inhibitor and has been studied extensively. A direct association between the amount of phytate in the diet and occurrence of zinc deficiency has been documented in many populations (Prasad, 1991).

Various food-processing procedures such as soaking, fermenting and germinating, have been shown to reduce the phytate content of the food (Gibson et al, 2003). Among

them, perhaps due to the large consumption of bread in many societies, fermentation has attracted a great deal of attention.

Phytate levels decrease during baking. It is possible to control the extent of this decrease and thus create bread with higher mineral bioavaility. Normally, to reduce the phytate content of the bread to a level considered not to mal-affect mineral absorption, without adding phytase, it would be necessary to increase the activity of the naturally occurring phytase in the flour or that in the yeast.

During the traditional transformation of flour into bread, reduction in phytate content as a consequence of the activity of native phytase usually does not greatly improve mineral bioavailability. Although during traditional bread making the phytate (IP6) content of the bread is decreased considerably, inisitol pentophosphate, IP5 will still be present in the media. It has been shown that IP6 and IP5 are the strongest inhibitors of zinc absorption (Sandstorm & Sandberg, 1992) and need to be removed almost completely in order to increase absorption of this mineral. In addition, although IP4 and IP3 in isolation are shown to have no inhibitory effect on zinc absorption (Sandberg et al, 1999), when present in a mixture of inisitol phosphates, IP3 and IP4 may also contribute to a negative effect on bioavaialaility of minerals. This effect has been clearly shown for iron (Brune et al, 1992). This is accomplished through interactions with the more phosphorylated inositol phosphates (Sandberg et al, 1999). This is probably also true for zinc absorption as a strong negative correlation was found between zinc absorption and the sum of native IP3 through IP6 from cereal meals (Sandberg, 1991).

As for the reduction of phytate due to the phytase activity of the leavening agent, various bread-making procedures using different leavening agents have been designed to

improve the nutritive value of the bread. For instance, sourdough is a symbiotic culture of lactobacilli and yeasts used to leaven bread. Studies suggest that sourdough fermentation is the best way to hydrolyze phytate and to improve zinc assimilation. Sourdough bread contained only about 30% and 61% of the phytate content of the unprocessed flour and yeast bread, respectively. The traditional bread-making agents are more stable in the physiological conditions of the intestine (Fredrikson et al, 2002) and as a result more resistant to undergoing further chemical reactions.

***Fiber*:**

Fiber is often implied as having a negative effect on zinc absorption. However, this is usually due to the fact that most fiber-containing foods also contain phytate. Studies have shown that experimental reduction of the phytate content of a high fiber bread considerably increased zinc absorption to a degree similar to that from white bread (low fiber), suggesting that fiber in itself has little or no effect on zinc absorption (Nävert' Sandström & Cederblad, 1985). In agreement with this finding, studies on isolated fiber components such as cellulose have shown no significant inhibitory effect on zinc absorption (Turnlund et al, 1984).

Calcium:

The inhibitory effect of calcium on zinc absorption is not that straightforward. Some argue that it is unlikely that calcium *per se* has a negative effect on zinc absorption (Lönnerdal et al, 1984). Studies on long-term use of calcium supplements have shown no adverse effect from calcium on zinc status; Gambian women who were given 1000 mg calcium/d had plasma zinc concentrations similar to those of unsupplemented women (Yan et al, 1996).

Calcium potentiates the complex formation of zinc with phytate (Oberleas, Muhrer and O'Dell, 1966) and hinders its absorption through this avenue. It has been suggested that the ratio formula [Ca] x ([phytate]/[Zn]) can be used as a predictor of zinc bioavailability (Gibson, Smit Vanderkooy, & Thompson, 1991). In rodents the phytate zinc ratio of <6 was found to be optimum for the growth of rats fed a diet containing 1.6 percent calcium. However, this ratio had to be lowered if the dietary calcium increased (Oberleas, Muhrer, and O'Dell, 1966). Study of the dietary phytate content of Canadian preschoolers showed that dietary [Ca x (phytate/Zn)] millimolar ratios, expressed per 1000 Kcal, correlated with the zinc nutriture of the children. Children with hair zinc below the cutoff had diets with a significantly higher ratio of [Ca x (phytate/Zn)], than children who had hair zinc above the cutoff value (Gibson, Smit Vanderkooy, & Thompson, 1991).

I.2. III.2. Socio-demographic factors associated with zinc deficiency/status

***Family income*:**

In general the health-wealth relationship is a well-established and consistent phenomenon. Differences in per capita income (a measure of wealth) appear to be closely and positively related to the length and healthfulness of life. The pattern is not the simple difference between the healthy rich and unhealthy poor. It is present in a "gradient" manner and applies to each and every class within the population i.e. the health status of each class within the population appears to be better than the classes below and worse than the classes above (Hertzman, 1998). This unique and remarkably persistent influence of wealth on health is played through various avenues, nutrition being one of them.

Undernutrition or malnutrition often occurs in the context of poverty and predisposes the individual to a wide array of health problems. For instance, the best sources of dietary zinc (i.e. animal proteins) are the most expensive food items; therefore, those subsisting on low-income diets are likely to lack or have small amounts of these rich sources of zinc in their diet and as a result be at higher risk of developing marginal zinc status (Sandstead, 1973). Children with a physiologic need to have a positive zinc balance are more vulnerable in this sense. Studies have shown significantly lower (yet normal) serum and hair zinc means for children of low-income families than those of middle income in the U.S. (Hambidge et al, 1976) and France (Chakar et al, 1993). Associations between poverty and low serum zinc have also been reported among the participants of NHANES II (Pilch & Senti, 1985).

sex

Sex differences have also been discussed in the zinc literature. In general, starting from early childhood, males have been reported to have higher mean serum zinc levels than females. The difference in serum zinc between sexes grows as the child does and peaks at 20-44 years. Despite this higher serum zinc, when it comes to MZD, evidence suggests that boys may be at higher risk than the girls of the same age. (Walravens et al, 1983 and Smit Vanderkooy & Gibson 1987). The Canadian study of zinc nutriture in preschoolers of Ontario reported a significantly higher rate of occurrence of low hair zinc (< 70μg/g) for boys (21% and 5% for boys and girls, respectively, P=0.00) (Smit Vander kooy & Gibson, 1987).

Sex has also been reported as an important factor affecting the hair zinc content of both children and adults (Creason et al, 1975, Gibson & DeWolfe, 1980, and Smit

Vanderkooy & Gibson, 1987). A higher physiological requirement in males has often been speculated as the underlying reason for their lower hair zinc. Factors such as lower absorption of dietary zinc in males (Turk, 1977) and their higher obligatory losses (Hunt et al, 1992 and Lee et al 1993) have been presented as the underlying reasons for this higher need. Although available data for adults adequately support a higher physiological requirement for males, the relevant data for children is missing. For this reason the physiological requirement of male and female preschoolers (computed by extrapolation from infants) have been estimated the same for male and female preschoolers (Institute of Medicine, 2002).

***Age*:**

The preschool period comprises a large portion of childhood. Younger preschoolers (24-36 months) may still have not recovered from the unfavorable condition of in-utero period (such as mother's zinc deficiency during pregnancy), at birth (such as prematurity) or infancy (such as being breast-fed by a mother with low zinc status). Age differences in the hair zinc level of preschoolers (younger preschoolers having lower hair zinc level than the older ones) have been shown in different studies (Klevay, 1970, Hambidge et al, 1972), however, it is not yet clear whether this is a normal physiological decline similar to what is experienced in the hemoglobin level during post infancy (Irwin and Kitchener, 2001), or originating from environmental factors such as changes in nutrition.

***Maternal education*:**

Maternal education may also affect the nutriture of zinc. In general, increased maternal education can positively affect the food choices made, and methods of food

preparation and preservation positively. In addition, rising maternal education could elevate the economic status and as a result the buying power of the family. It is important to note that this positive correlation between family income and maternal education may not be true in certain population pockets. For instance, in societies such as Canada where the former education of many immigrants is not recognized, higher education and training does not necessarily translate into a better paying job. In such populations the effects of maternal education on a child's health is likely to be distorted.

Ethnicity:

Analysis of NHANES II has revealed lower serum zinc for African Americans compared to Caucasians (Pilch & Senti, 1985). Although the difference was not great, it can be indicative of a higher risk at the population level and further nutritional assessment of this group in respect to its zinc nutriture would be advisable.

I.2.III.3. Other factors associated with zinc deficiency/status

Iron supplementation

At levels of essential micronutrients present in foods, most micronutrients appear to use specific absorptive mechanisms and they don't seem to be at risk of being mal-affected by the interaction of other micronutrients. However, in aqueous solutions and/or at higher intake levels, competition between elements with similar chemical characteristics and uptake by non-regulated processes can take place.

Elements with similar physiochemical properties may interact with each other at the level of absorption. Iron and zinc share a part of their absorptive pathway. Iron absorption involves two carriers, one involved in the uptake of iron across the microvillus membrane of the enterocyte and the other in its transfer to the plasma at the basolateral

surface. The uptake phase is thought to involve divalent metal transporter-1 (DMT1). This transporter is a carrier for zinc as well. Therefore, at the point of transfer from brush border into the enterocytes, if concentration of anyone of these two metals increased the absorption of the other will be affected in a negative manner as a result of the unavailability of the carrier, DMT1.

These interactions have clearly been demonstrated in experimental absorption studies (Pedrosa & Cozzolino, 1993, and Isfaoun, 1997) and to some extent have been confirmed in supplementation studies (Hambidge et al, 1987). Some investigators (Hambidge et al, 1987) have reported a decline in serum zinc during the course of iron therapy. Although most studies have demonstrated iron and zinc interactions with amounts of supplemental iron over 30 mg/d, multivitamins containing as little iron as 18 mg/d have also been found to lower plasma zinc concentrations in pregnant teens (Dawson et al. 1989). Furthermore, alterations in plasma zinc concentrations during pregnancy may be evident following only 1 week of iron supplementation (20 mg/d) (Hambidge et al. 1987). Studies of zinc status in pregnant women immediately before iron therapy and at 1 and 4 weeks thereafter indicated a decline in plasma zinc from baseline in just 1 week. The decline remained statistically significant even after adjustment for the expected physiologic decline over the same interval of gestation. Similar effects of iron therapy on zinc status (serum zinc) have been confirmed by some (Bloxham et al, 1989 and O'Brien et al, 2000) but not all (Yip et al, 1985) investigators.

Early childhood experiences

Early experiences affecting zinc balance such as prematurity and being born from a zinc deficient mother may also affect the zinc status of young children. Some studies

suggest that prematurely born infants are at higher risk for inadequate intake of dietary zinc (Friel et al, 1985). Premature infants are not only born with a negative zinc balance, but also, due to a susceptibility to after-birth infections, have a higher zinc demand. Such children starting life with a negative zinc balance and having a high zinc demand due to the high rate of growth (a highly zinc-dependent physiologic event) may not have a chance to correct the deficit and may continue carrying it to the later stages of life.

Breast-feeding:

Breast-feeding and its duration have been shown to affect children's zinc status. The bioavailability of zinc from human milk is significantly higher than that of cow milk (Lonnerdale, Keen &Hurley, 1981) and soy-based formulas (Sandstrom, Cederblad & Lonnerdal, 1983), due partly to the presence of zinc-binding ligands facilitating zinc absorption (Duncan & Hurley, 1978). Some studies (Walravens et al, 1992), including a Canadian study (MacDonald, Gibson & Miles, 1982), have reported the most favorable zinc status in breast-fed compared to formula-fed infants, even those fed with zinc-supplemented formula.

However, there is an unusually sharp physiological drop in the zinc content of human milk after birth (Krebs & Hambidge, 1986) making this food unable to provide an infant with adequate zinc beyond 6 months. Growth-limiting zinc deficiency has been shown in infants principally fed with human milk even after the age of 4 months (Walravens et al, 1992).

Erratic eating behaviors:

Erratic appetite and seemingly problematic eating behaviors such as being a picky eater, and lack of variety in the diet are some of the features common to young children.

Being a picky eater is part of what it means to be a young child and particularly a toddler. They may eat only fruits one day, and vegetables the next, and peanut butter and jelly sandwiches for breakfast, lunch and dinner for few days at a time. These behaviors can easily affect the quantity and quality of the food consumed by children. In fact it is believed that they may serve as a means for adjusting the food intake to meet the relatively decreased demand during this stage of life. Preschoolers gain weight more slowly compared to the prior stage of life, so they need relatively less food, and perhaps nature gets this accomplished through these eating behaviors (Sears, 2006). This appears to be problematic, and often it really isn't. As expected and often transient as these behaviors are, they may result in nutritional deficiencies when they become chronic. Lack of variety in the diet and elimination of some foods or food groups can adversely affect the micronutrient balance in general, with some nutrients being at higher risk than the others. Zinc with its high requirement during this stage, is an example of such a nutrient. It is possible for zinc balance to be disrupted if these behaviors persist for a prolonged period of time.

I.3. The present knowledge gap

After recognition of MZD in 1970's, 2 studies addressed this deficiency among Canadian preschoolers. The first study (Smit Vanderkooy & Gibson, 1987) documented the existence of potential MZD, while the second (Gibson et al, 1989-b) confirmed its existence among a group of preschoolers following a zinc supplementation study. Since then, there has never been another report of zinc status in Canadian preschoolers.

Despite the lack of any further work with this age group in Canada, combined evidence from the children of other industrial societies indicate that MZD is common

among the children of developed societies (Hambidge et al, 1972, Walravens et al, 1983, Nakamura et al, 1993, Buzina et al, 1980 and Chakar et al, 1993). Many factors may contribute to this high prevalence ranging from human physiology (such as the sensitivity of the human body to the shortage of this element and the inefficiency of the human homeostasis for zinc balance) to environmental (such as the changes in the intake of certain food groups, such as red meat) to the inadequacy of the diagnostic tools.

The inadequacy of diagnostic tools is perhaps one of the main contributors to the high prevalence of MZD in developed countries. Serum zinc, the most commonly used biomarker of zinc status, is not capable of diagnosing the deficiency during its very early stages when the deficiency is only marginal. At present the only certain way of diagnosing MZD, in research as well as among practitioners, is by the growth response observed in the child following a period of zinc supplementation.

On the other hand, studies have shown a consistent decrease in hair zinc of children in whom MZD has later been confirmed through their positive growth response to a course of zinc supplementation (Hambidge et al, 1976, Walravens et al, 1983, and Gibson et al, 1989). However, hair zinc is not used or recognized by practitioners. Under these circumstances, an awareness of the risk factors of the deficiency and factors associated with it can be invaluable. These factors when compiled can be used to construct the profile of at-risk individuals and may, therefore, have some usefulness as a screening tool.

This study was designed to fill the gaps in our knowledge about the present zinc status, (as indicated by hair zinc) of healthy free-living Canadian preschoolers. It also aimed to construct and validate a screening tool for low hair zinc/MZD among children

by identifying and compiling a list of some dietary and social factors associated with hair zinc.

I.4. Purpose of the study

This was an exploratory study and its chief purpose was to examine the hair zinc status of preschoolers of Vancouver, and to construct and validate a short and easy-to-administer screening questionnaire that could be used as an upstream preventative measure to predict low hair zinc/MZD among preschoolers. In doing so, we planned to investigate both the dietary and socio-demographic population patterns of hair zinc for this age group and to understand better the eating patterns, social settings and factors in family environments that associate with the hair zinc level of the preschoolers.

I.5. Objectives

I.5.I. Objectives for chapter II

In a group of conveniently sampled low-income preschoolers from 4 inner-city neighborhoods:

1) To determine mean hair zinc levels and the prevalence of low hair zinc.

2) To explore the relationship between the 5 main socioeconomic and demographic factors (age, sex, ethnicity, family income and maternal education) and hair zinc levels, both individually as well as collectively.

3) To investigate the relationship of consumption of some food groups ("dairy", "meat, fish and poultry" and "cereals and grains") as well as some nutrition, health related variables (as perceived by the caregivers) with hair zinc levels, both univariately and after adjustment for demographic and socio-economic factors.

4) To assess the collective contribution of all of the above-mentioned factors (factors

significantly associated with hair zinc) for explaining the variability in hair zinc.

I.5.II. Objectives for chapter III

In a large representative sample of preschoolers from the city of Vancouver;

1. To assess the hair zinc status and the extent of low hair zinc in Vancouver preschoolers through a large and representative sample from the city.

2. To explore the relationship between the 5 main demographic and socioeconomic factors (age, sex, ethnicity, family income and maternal education) and hair zinc levels.

3. To examine the relationship between some additional family characteristics (family size, composition, food security and paternal education) and hair zinc levels.

4. To investigate the relationship between some eating behaviors such as the intake frequency of meat, dairy, cereals and grains, as well as variables such as "being concerned about child's eating", "adequacy of child's eating", "pickiness", and "unhealthy eating" with the hair zinc level of children.

5. To examine the relationship of parental perceptions of some health-related variables (such as overall health, frequency of a child being sick, breast-feeding pattern, and intake of supplements containing iron), and behavior related variables (such as activity level and attentional focusing scores) with the hair zinc of children.

6. To examine the relationship between the indices of growth (height-for-age Z scores and weight-for-height Z scores) and nutrition (weight-for-age Z scores) with the hair zinc of children.

7. To identify which of the variables significantly associated with hair zinc can predict the hair zinc levels.

8. To find out which of the variables significantly associated with low hair zinc status can predict hair zinc status (i.e. low hair zinc).

9. To assess the collective usefulness of the variables identified under objective 8 and the constructed questionnaire based on this model, for predicting the hair zinc status of children by exploring the classification tables and the Receiver Operating Characteristic (ROC) curve of the predictive model.

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CHAPTER II[1]

HAIR ZINC STATUS AND DETERMINANTS OF LOW HAIR ZINC AMONG A GROUP OF LOW-INCOME PRESCHOOLERS OF VANCOUVER

[1]A version of this chapter has been developed for publication, Vaghri , Z, et al.(2008). Hair zinc of a group of low income preschoolers of Vancouver.

II.1. INTRODUCTION

Studies of zinc deficiency in industrialized societies (Hambidge et al, 1972, Buzina et al, 1980, Walravens et al, 1983, Nakimura et al, 1993, and Chakar et al, 1993), including Canada (Smit Vanderkooy & Gibson, 1987 and Gibson et al, 1989-b), have reported a consistent diminution in the hair zinc level of children with marginal zinc deficiency, MZD. Marginal zinc deficiency is encountered when dietary zinc intake, over time, is marginally short of meeting the body's zinc needs. This deficiency has been described as having effects such as slight growth retardation, poor appetite, impaired taste acuity (Hambidge et al, 1972), and male oligospermia (Polysangam et al, 1997), none of which are specific clinical symptoms.

Diagnosis of MZD is also hindered by the fact that serum zinc, the most commonly used biomarker of zinc status, is neither sensitive nor specific enough to detect this deficiency. In part due to this, there is at present no data available on the prevalence of MZD. However, MZD is believed to be the most common type of zinc deficiency world-wide (Endre, Beck & Prasad, 1990). Over the last few decades, hair zinc has been discussed frequently and often used as the biomarker of this deficiency. It appears to have both the sensitivity (Hambidge, 1980) and specificity (Hopps, 1977) required as a biomarker of zinc status during the very early stages of the deficiency.

Zinc is incorporated in hair during its development and is derived from the matrix of connective tissue papillae (with blood and lymph vessels) and sebaceous glands (Hopps, 1977). The uptake of zinc by the hair is quite slow and may be hindered preferentially if the body's zinc supply is decreased. In these circumstances the more important zinc dependent organs and machineries continue to receive an adequate supply

without any symptom of zinc deficiency becoming apparent (Hambidge, 1980) while the concentration of zinc in the hair is reduced. It is this special physiology that confers sensitivity to hair zinc as a biomarker of marginal zinc deficiency.

Hair zinc as a biomarker of MZD is also specific. This important feature derives from the anatomy of hair. During the hair's growth the metal is incorporated in the hair. Once the follicle reaches the skin surface and buds out, it is no longer in equilibrium with the body (Assarian & Oberleas, 1977). As the extruded hair approaches the skin surface, its outer layer hardens and becomes relatively impermeable, thus isolating the hair and its contents from the body's continuing metabolic activities (Hopps, 1977). Due to this characteristic, hair zinc is trapped and no longer available to the body for redistribution to zinc dependent organs/processes in a time of need. Nor is it under tight homeostatic regulation as is serum zinc. All in all, hair zinc concentration is believed to be a good index of MZD in children, at least in developed countries where widespread protein-energy-malnutrition does not exist (Gibson, 1989).

Hair zinc levels in humans reflect dietary intake of this element for the 4-8 weeks preceding the sample collection (Hambidge et al, 1972). Studies of MZD in children (Smit Vanderkooy & Gibson, 1987, Gibson et al, 1989-b and Krebs, Hambidge & Walravens, 1984) suggest that in developed countries low dietary zinc intake is not the only cause of MZD. In developed countries changes in food consumption patterns can result in this deficiency, while the amount of dietary zinc (the zinc ingested through the food) is seemingly adequate. Studies in Canada (Gibson et al, 1989) and elsewhere (Hambidge et al, 1972) have shown a positive correlation between the intake of bioavailable zinc from animal protein and hair zinc status. Animal protein - red meat in

particular - is not only an abundant source of zinc (Health Canada, 1999) but also has been shown, even from a single meal, to counteract the effect of zinc absorption inhibitors (Sandström & Cederblad, 1980). In contrast, intake of some plant foods has shown a negative correlation with hair zinc (Smit Vanderkooy & Gibson, 1987). It is generally believed that changes in dietary patterns may, in fact, be the principal underlying reason for the widespread occurrence of this deficiency in developed countries (Gibson et al, 2001) and people with particular food choices/patterns may therefore be at higher risk for this deficiency.

Food choices are made under the influence of many factors. Among them income, a strong determinant of health, affects food choices in several ways. With the best sources of dietary zinc located in the most expensive foods, those subsisting on low income are at particular risk of inadequate zinc nutrition (Sandstead, 1973). Overall, socio-economic factors do leave their footprint in most nutritional deficiencies. Differences in zinc deficiency based on the socio-economic factors, whether mediated by food choices and intake or independent of them, are noteworthy.

Information available on the zinc status of Canadian preschoolers is scarce with no information at all on the zinc status of low-income preschoolers. However, it has been reported that low-income children are at higher risk for some nutritional deficiencies (Silva et al, 2001, and Skalicky et al, 2006). We, therefore, designed this study to investigate the hair zinc status of a group of low-income preschoolers from the Vancouver inner city. The specific objectives of this study were:

1) To assess the hair zinc status of a group of conveniently sampled low-income preschoolers from 4 inner city neighborhoods.

2) To explore the relationship between the 5 main socio-demographic and socioeconomic factors (age, sex, ethnicity, family income and maternal education) and hair zinc levels, both individually as well as collectively.

3) To examine the existence of any association between hair zinc and the anthropometric Z scores (as indices of growth and nutrition) in an attempt to verify any functional ramifications (effects on vertical as well as ponderal growth) of low hair zinc.

4) To investigate the relationship of consumption of the main food groups as well as some nutrition, health and behavior related variables, with hair zinc levels, both univariately and after adjustment for socio-demographic and socio-economic factors.

5) To assess the collective contribution of all of the above-mentioned factors (factors significantly associated with hair zinc) for explaining the variability in hair zinc.

II.2. METHODS AND MATERIALS

II.2.a. Study design

This study was a cross-sectional exploratory study carried out with a group of conveniently sampled low income preschoolers in Vancouver's inner city during February-March of 2004. To ensure the low-income status of the study, participants living in 4 low-income neighborhoods (Strathcona, Mount Pleasant, Grandview-Woodlands and Hastings-Sunrise) located for the most part in East Vancouver were selected for recruitment. This is a multicultural region where the income of at least 30% of the families fell below the Statistics Canada low-income cutoff in 2001 (Canadian Council on Social Development, 2001). All 4 neighborhoods have also been identified as being highly vulnerable regarding early child development (Hertzman et al, 2002). Through field visits and consultations with community workers, 2 large community

centers and/or neighborhood houses from each neighborhood, that provided a wide variety of programs for families with preschoolers, were contacted and selected to participate in the study by a) allowing us to recruit from their programs and b) hosting a nutrition clinic for the recruited subjects. Of the 8 centers contacted, 2 did not respond to our initial and follow-up contacts. Two centers refused to participate, 1 because it was already engaged in another ongoing study, and the other because its board refused for unexplained reasons. The 4 remaining centers did respond to our request and agreed to participate in the study. The study was approved by the Human Ethics Committees of the University of British Columbia (Appendix I-1) and the BC Children's and Women's Health Center. Informed consent was obtained from the parents.

II.2.b. Subject recruitment

Through the programs offered in the centers, parents/caregivers of apparently healthy 24-71 months old preschoolers were approached either individually or in groups. The study purpose and protocol were explained to those parents wishing to participate in the study. They were then requested to fill out a 9-item recruitment questionnaire (Appendix II-2). From a list provided at the bottom of the recruitment sheet they were also asked to check a preferred clinic date and a morning or afternoon appointment. In addition, ads were placed at the centers on various bulletin boards inviting families to attend the nutrition clinics (with recruitment to take place at the clinics) (Appendix II-3). A total of 250 families were thus recruited.

II.2.c. Inclusion criteria

The inclusion criteria for this study were; 1) being healthy (not diagnosed with any major or chronic illness), 2) being between 24-71 months of age, 3) residing in

Vancouver, 4) not having a sibling already recruited for the study, and 5) the primary care giver being fluent in English, Mandarin or Cantonese.

II.2.d. Nutrition Clinics

Six clinics from 9am to 5pm were held at Britannia Community Center (in Grandview Woodlands), Kiwassa Neighborhood House (in Hasting Sunrise), Mount Pleasant Neighborhood House (in Mount Pleasant) and Strathcona Community Center (in Strathcona). Britannia and Strathcona each hosted 2 clinics.

The 250 recruited families were contacted by phone and an appointment was confirmed for their preferred clinic day 2-4 days prior to the clinic day. Out of 250 calls made 207 accepted our invitation (acceptance rate 83%) and 144 attended the clinics (attendance rate 70%). The study purpose and protocol were re-explained to the parents, and another informed consent form (for participating in the clinics) was signed (Appendix II-4). Socio-demographic, socio-economic and dietary data were obtained, children's hair samples for zinc analysis were collected and their heights and weights were measured. At the time of admission, each child was given a colorful pictorial passport (Appendix II-5), which received a stamp at every station that the child attended. Upon inspection of his/her passport at the end of the clinic, to ensure that every station had been attended, all children with completed passports received a gift certificate as an incentive.

II.2.e. Socio-demographic and socio-economic questionnaire

The information on sex, age (date of birth) and ethnicity was collected through the recruitment questionnaire (appendix II-2). Due to a predominance of Caucasian and Chinese, and small and scattered presence of all other ethnic groups, we categorized the

ethnicity of our study participants into 3 categories of Caucasian, Chinese and "other ethnicities".

The data on maternal education and family income were collected through the socio-demographic and socio-economic questionnaire used in a sister study (food insecurity among inner city children) conducted side by side in our nutrition clinics. This questionnaire (Appendix II-6) collected information on maternal education as well as family size and income. Since the children were recruited from the very low-income part of the city, in order to measure the extent of poverty, the income groupings used were based on the gradation of Statistics Canada's low-income cut-off (LICO), commonly considered to be Canada's poverty line. According to this scaling, households that spend disproportionate amounts of their pre-tax income on food, clothing and shelter- 20% above the average family- are considered low income. Low-income cut-offs vary by family size as well as the population of community of residence. Income levels were calculated after collecting information on these other variables and using the appropriate t

II.2.f. Biochemical data

II.2.f.A. Collection of hair samples for zinc analysis

For this purpose, stainless steel scissors dipped in methanol, as recommended and provided by the trace element analysis department of BC Children's Hospital, BCCH, were used. A brief simulation session of sample collection was set up with the attendance of the research student, the person conducting the analyses and the person assigned to the clinic's sample collection station. After the mineral analyst's demonstration, a simulated hair sample collection was performed on one of the session's attendants using the demonstrated protocol and the critical points of the procedure were discussed. Hair

samples were cut, as close to the scalp as possible, at 3-4 locations at the back of the head. Only the first 1-2 cm proximal to the scalp was kept. The collected samples were placed in pre-labeled zip-lock bags and delivered to the "Nutrition Research Program" laboratories. There, the samples were thoroughly inspected for sample size adequacy and sample length, and their weights recorded. Subjects with an inadequate sample size or length were also recorded (These samples, although analyzed and their results reported, were later excluded from statistical analyses). The samples were stored overnight and delivered to the BCCH laboratory the following day.

Only 131 of the 144 children of the clinics provided a hair sample, out of which only 87 (66%) samples with acceptable weight and lengths were used in data analyses. Analyses of variances of age, sex, ethnicity, family income and maternal education between children with adequate (n=87) and inadequate (n=44) hair samples did not indicate any statistically significant differences.

II.2.f. B. Analyses of hair samples

The collected hair samples were processed (Puchyr et al, 1998), acid washed, digested and analyzed for zinc content by Inductively Coupled Plasma Mass Spectrometry, ICP-MS (Lockitch et al, 2005). The samples were run in singles (due to the inadequacy of samples to run duplicates) and standard reference materials were purchased and used during the analyses. The in-house reference (Quebec hair sample) for the Mineral Analysis Division of BC Children Hospital was also used with every run.

The most commonly used cut-off of 70 μg/g, and defined as 3 SD below the adult mean (Hambidge et al, 1972), was used for this study.

II.2.g. Anthropometrics

Trained research personnel measured the children's height and weight. Their standing heights were measured to the nearest $1/8^{th}$ of an inch with a portable direct reading stadiometer, the subjects being shoeless. Heights were converted to metric units later. Body weight was measured to the nearest 0.1 kg using a lithium electronic scale (Taylor Precision Products, L.P, Model 7300) with the scale placed on hard floor and children shoeless and wearing light indoor clothing. All the readings were done in multiples until 2 consecutive numbers were identical or not differing by more than 0.5 unit of measurement. When 2 consecutive readings were not identical, the average of the 2 readings was recorded. In addition we obtained at the clinics the self-reported heights of both biological parents (Appendix II-8).

The growth charts of the Center of Disease Control, CDC (Center of Disease Control, 2002) were used to calculate the weight for age (WAZ) z scores of the children. Because there is a significant genetic component in the attainment of height, we used the mid-parent height ([mother's height + father's height]/2) and parent-specific adjustments for stature of the children were carried out (Himes et al 1985). The resultant adjusted heights were used in the calculation of height-for-age (HAZ) and weight-for-height (WHZ) scores from the growth charts of CDC.

II.2.h. Dietary data; collection and processing

II.2.h .A. Data collection

II.2.h.A.a. Food frequency questionnaire, FFQ

The FFQ was developed combining 2 FFQs used in previous studies (Williams, 2000 and Innis et al, 1997). Adjustments were made to the portion sizes to make it

applicable to preschoolers, and some new food items were added to make it more relevant to the multiethnic makeup of the preschoolers of the study. The questionnaire underwent repeated and extensive reviews by 2 practicing dietitians in the presence of all the research staff due to be trained in its use. The final versions used in the clinics contained 177 items (Appendix II-7). Because the FFQ was not validated for data collection on children's zinc intake, these data were not used quantitatively for zinc or any of the nutrients.

We compared our mean (±SE) energy intake (1800±93 kcal for children <4 years old and 1900±107 kcal for children≥4 years old) with those reported for low income American preschoolers of the Continuing Survey of Food Intakes by Individuals data, CSFII, 1994-1998 (1481±39 for children 2-3 years old, and 1742±64 for children 4-8 years old) (Knol, Haughton & Fitzhugh, 2005). While the 2 older age groups had a comparable total calorie, those of our younger children were higher than the younger children of CSFII. We reasoned that the presence of much older children in the older age group of CSFII (2-8 years old in CSFII versus 2-6 years old in our study) had resulted in a mean high enough to be comparable with the mean of our older age group. Overall, we concluded, there was a significantly higher mean for caloric intake for children of our study compared to those of CSFII and an overestimation in our dietary tool. Such overestimation of dietary intake through a food frequency questionnaire (FFQ) is a common and recognized problem (Treiber et al, 1990 and Stein et al, 1992) that can mask the relationships between food intake and disease. To avoid this, at the time of data analyses, we entered the total energy intake of children as a covariate in all regression analyses pertaining to food intake.

Dietitians fluent in Mandarin or Cantonese administered the FFQ in these languages when needed (Canadian Council on Social development, 2001).

II.2.h.A.b. Data on other nutrition and health related variables

These data were collected through both the recruitment questionnaire and the FFQ. Nutrition and health-related data collected through the former were:

1) Two questions that assessed the parents' perception of their child's eating behavior by first asking if they are concerned about their children's eating in general, and then providing 10 options describing the common eating characteristic/problems of young children (such as picky eater and not eating enough) as identified in our meetings with community dieticians, from which they were to choose one or more that best fit. Space was also provided in this first question for concerned parents to write about any additional eating behavior/problem in their children not provided as an option.

2) Two questions asking parents first whether their children are frequently sick and then asking them to describe their general health from 3 options: "very healthy", "average health", "not so healthy".

In addition, 2 questions appended to the FFQ collected information on breast-feeding history. Each study participant had the same code on all questionnaires (the recruitment questionnaire, FFQ...). These identified the respective questionnaires and were then used in transcribing all relevant data for further analyses.

II.2.h.B. Processing of dietary data

II.2.h.B.a. Food Groups Assignment

All of the foods recorded in the FFQ were extracted to make a 'Master Food List'. Those in the master food list generally belonged to 1 of the 2 main categories of "basic

food" such as chicken or "combination food" such as shepherds' pie. All the items in the basic food category were assigned a code corresponding to the food groups they belong to based on the four food groups defined by Eating Well with Canada's Food Guide for Healthy Eating, CFGHE ('M' for meat and meat alternatives, 'F&V' for fruits and vegetables, 'D' for dairy, 'C' for cereals and grain products). For example, bagels were given a 'C' code, while cheese strings and mangos were given codes 'D' and 'F', respectively.

A number of frequently reported foods in the FFQ of children, such as popcorn, ice cream and pretzels, which, due to their excessive fat, sugar or salt content, were not considered part of the aforementioned 4 main food groups, were assigned a code 'O' standing for 'Other Food Group'. In deciding what belongs to this list, the methodology section of the recent BC nutrition survey was consulted and their list was referred to when needed (BC Nutrition Survey, 2002).

The assignment of food group codes to combination foods like commonly reported items such as pizza or macaroni and cheese was done through consultations with 2 practicing dietitians who reviewed the recipes of these foods as required. A list of combination foods together with available recipes was then given these 2 dietitians. They were asked to review the list and decide on the percentage breakdown of their ingredients. The research student made a similar list as well. The 3 lists were then reviewed and cross-matched by the research student. The items having 2 or 3 different codes were extracted and a mini-list was developed. The student met with the 2 dietitians and this mini-list of combination foods was reviewed and discussed until consensus was reached regarding the codes to be used and the percentages assigned to their components.

II.2.h.B.a.Estimating the number of servings

Once the master-sheet of the food codes was completed, the food list for each child was entered into the food analysis software, ESHA, (Esha Food Analysis Software, version 8.4.0) and printed. The printed sheets were coded manually as described and the number of servings/day for each food group was calculated. To do this, the method of Junkins and Laffey, 2003, used in the recent BC Nutrition Survey, BCNS, was adopted. For all food groups, the number of servings was calculated directly by dividing the total amount consumed (grams, ounces, milliliters etc.) by the serving size of that food group for that age. For instance, if the total bread intake of a 5 year old child was 3.5 slices /day, then since 1 slice of bread is defined as 1 serving, the total bread consumed by this child was calculated as 3.5/1=3.5 servings/day.

II.2.i. Statistical Analysis

The data were analyzed using Statistical Package for Social Science, SPSS, (version 13.0, 2005). Using this software, descriptive statistics (such as mean and standard deviation) were computed for all variables. The primary outcome variable in this study was the hair zinc level. These outcomes were explored in both continuous (hair zinc level in μg zinc /g of hair) and binary (low hair zinc i.e. hair zinc<70 μg/g and normal hair zinc) forms.

Five sets of linear regression models were used to analyze hair zinc level as a continuous outcome. First, the association between hair zinc level and each socio-demographic or socio-economic variable was assessed. Second, the association between hair zinc level and each dietary variable was assessed, both univariately and after adjustment for the socio-demographic and socio-economic variables was carried out.

Third, the association between hair zinc level and each eating behavior was assessed, both univariately and after adjustment for the socio-demographic and socio-economic variables. Fourth, the association between hair zinc level and each anthropometric Z score was assessed, both univariately and after adjustment for income, ethnicity, and maternal education was carried out. Finally, all the variables in the above-mentioned regression analyses, that revealed a significant association with hair zinc, were inserted in a forward stepwise linear regression analysis with hair zinc as the dependent variable. The forward stepwise regression analysis was carried out in order to identify the variables that could explain some of the variability in children's hair zinc. The inclusion criterion for variables to be inserted in this analysis was having a P value of ≤ 0.05 in their individual adjusted linear regression analyses with hair zinc as the outcome variable. For this regression analyses the collinearity of the variables inserted into the model was verified. Collinearity between two components of a regression model could "blow up" the standard deviation and result in elimination of one of the variables from the final model produced.

When the outcome variable was continuous and the explanatory variable of interest was categorical, differences between groups were verified using one-way analysis of variances (ANOVA) followed by Tukey's *post hoc* analyses, if applicable. When the outcome variable and the explanatory variable of interest were both in categorical form, the differences were examined using *Chi square* analysis (e.g. the difference in the occurrence of low hair zinc between boys and girls). Adjustments in *chi square* analyses were carried out when the differences between more than 2 groups were explored. For all the analyses, the level of significance was set at $P \leq 0.05$.

Although considering the power of this study (n=87) conducting multiple analyses may pose a threat to the validity of the analyses, it is important to bear in mind that the mere purpose of these analyses was to identify as many variables (variables showing significant associations with hair zinc) as possible, as components of the questionnaire that was going to be constructed in the second study.

II.3. RESULTS

II.3.a.Study population

A description of the study population is given in **Table II.I**. There were 87 children in the sample with the 2 sexes roughly equal in representation. Because CFGHE provides recommendations based on age breakdown of children<4 years old and children≥4 years old, age 4 was arbitrarily taken as the dividing line to categorize children into 2 groups of younger (<4 years old) and older (≥4 years old) preschoolers. The younger and older children, as well, were equally represented.

The families had a multi-ethnic make-up with a majority of the children having Chinese or Caucasian ethnicity. More than half of the study families (57%) were below the poverty line defined by Statistics Canada. About 36% of our study mothers did not have post secondary education.

As a population the mean (±SD) of HAZ, WAZ, and WHZ scores were within the range defined for a population with normal growth distribution. Overweight (WAZ>2), underweight (WAZ<-2), stunting (HAZ<-2) (Walravens, Krebs & Hambidge, 1983, and Gibson et al, 1989) and slowed-growth (HAZ<-1) (Gibson et al, 1989) were not observed at more than the expected baseline.

Table II.1. Description of study population (n=87).

Socio-demographic variables	Number (%)
Sex:	
Boys	43(49)
Girls	37(43)
Not recorded	7(8)
Age:	
< 4 years old	41(47)
≥ 4 years old	39(45)
Not recorded	7(8)
Ethnicity:	
Caucasian	27(31)
Chinese	35(40)
Others	21(24)
Not recorded	4(5)
Income:	
<50% LICO*	27(31)
50%- <100% LICO	23(26)
100-150% LICO	15(17)
>150%LICO	14(16)
Not recorded	8(10)
Maternal education:	
No post secondary education	31(36)
With post secondary education	48(55)
Not recorded	8(9)
Anthropometrics†:	Mean ± SD
Height for age z scores**	-0.15±1.7
Weight for age z scores	0.20±1.2
Weight for height z scores	0.27±1.3

*LICO=Low-income cutoff.

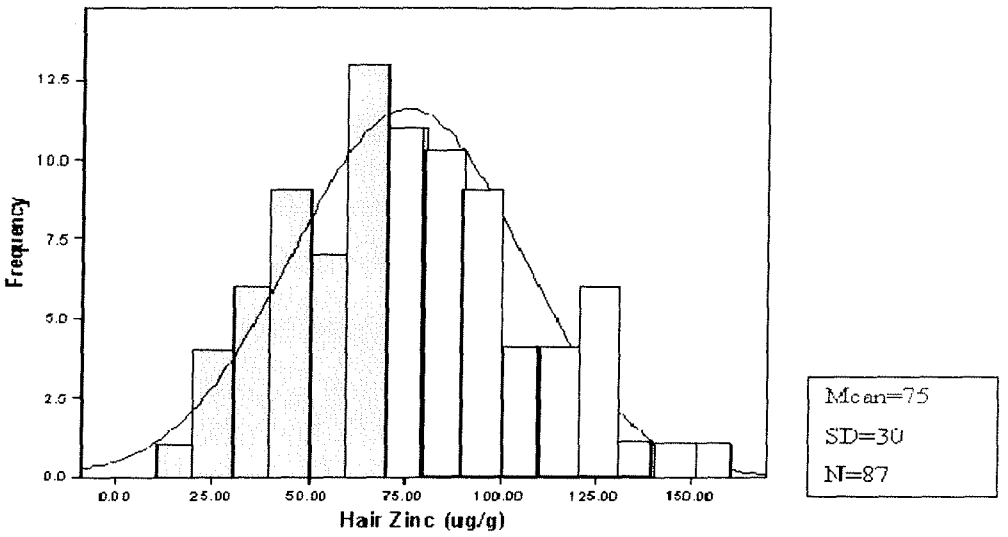
† For anthropometrics the sample sizes were 58, 61 and 69, respectively.

** Three subjects with HAZ scores falling outside the criterion of –3.5< Z scores<3.5 (Fallon and Spada, 1997) were considered outliers and were removed from the database.

II.3.b. Hair zinc status of the study population

Hair zinc of the survey population was normally distributed with a mean (±SD) of 75±30 μg/g, while their hair zinc varied within a wide range of 10-160 μg/g (**Figure II.I**).

The study children also had a high incidence of low hair zinc levels (hair zinc<70 μg/g), as illustrated by the shaded area of **Figure II.I.** The fraction of children with hair zinc below the cutoff was 43% (n=40) of the study population.



The shaded area demonstrates the children with hair zinc below the cutoff of 70 μg/g.

Figure II.I. Frequency distribution and descriptive statistics for the hair zinc level of the study population.

II. 3.c. Associations of the 5 main socio-demographic and socio-economic variables with hair zinc

Table II.II summarizes the hair zinc level together with the occurrence of low hair zinc based on the 5 main socio-demographic and socio-economic variables (sex, age, ethnicity, income, and maternal education). As shown, there was no significant difference in the hair zinc of the children based on sex, age, ethnicity, family income and maternal education. The occurrence of low hair zinc, however, was significantly higher among younger preschoolers (children<4 years old). The mean hair zinc level for younger preschoolers was also slightly lower than the older group, although this difference did not reach statistical significance.

To explore any relationship that socio-demographic and socio-economic factors may have with hair zinc, we ran the linear regression analyses of age, sex, family income, maternal education, and ethnicity with hair zinc as the dependent variable **(Table II.III).** There were no statistically significant associations between hair zinc levels and anyone of these variables. When all 5 variables were inserted in a linear regression model simultaneously, the model produced lacked statistical significance (R^2 =0.08, P=0.28).

Table II.II. Socio-demographic and socio-economic description of study population and hair zinc levels and the occurrence of low hair zinc based on these variables (n=87).

Socio-demographic variables	Number (%))	Hair Zinc (μg/g) Mean ±SD	F	p	Low hair zinc* # (%)†	χ^2	p
Sex							
Boys	43(49)	80±31	2.5	0.12	16(30)	0.96	0.33
Girls	37(43)	69±31			19(50)		
Missing	*7(8)*						
Age							
< 4 years	41(47)	73±34	0.28	0.60	22(55)	3.8	0.04
≥4 years	39(45)	76±24			13(33)		
Missing	*7(8)*						
Ethnicity							
Caucasian	27(31)	74±30			13(52)		
Chinese	35(40)	69±28	1.9	0.15	17(50)	3.5	0.17
Others	21(24)	85±32			5(26)		
Missing	*4(5)*						
Income							
≤LICO**	50(57)	73±29	0.26	0.61	23(46)	0.16	0.69
>LICO	29(33)	77±31			12(41)		
Missing	*8(10)*						
Maternal education							
No post secondary education	31(36)	76±28	0.13	0.73	12(41)	0.31	0.58
With post secondary education	48(55)	73±31			23(48)		
Missing	*8(9)*						

*Low hair zinc=Hair zinc <70 μg/g.

†Percentages of children of that category who had hair zinc data available.

**LICO=Low income cut off.

Table II.III. Relationship between hair zinc and demographic factors (n=87).

Variable	B[†] (95% CI)	R^{2*}	*p*
Age (months)	0.1(-0.5, 0.6)	0.00	0.84
Sex	-10(-23, 2.7)	0.03	0.12
Ethnicity		0.02	0.25
Caucasian	-12(-29, 6)		
Chinese	-16(-33, 0.4)		
Others	--------		
Maternal education	-2.5(-17, 12)	0.00	0.72
Family income		0.00	0.61
<50% LICO[$]	-41(-24, 16)		
50%- <100% LICO	-0.9(-21, 19)		
100-150% LICO	-1.7(-21, 24)		
>150%LICO	---------		

[†]Bs are the unstandardized regression coefficients.

[*]Values indicate R^2 of individual variables with hair zinc (unadjusted regression analyses)

When all 5 variables were inserted in the regression model, simultaneously; R2 =0.08,P=0.28).

II.3.d. Associations of some dietary factors with hair zinc

Table II.IV displays the mean (±SD) of the number of servings of the main food groups defined by CFGHE and some of their subgroups. For all main food groups the population mean met the number of servings recommended by CFGHE for preschoolers (For <4 years old the recommendations are; 1,2,3, and 4 serving/d "Meat and Alternatives", "Dairy", "Cereals and Grains", and "Fruits and Vegetables", respectively. For ≥4 years old the recommendations are; 1, 2, 4, and 5 servings/d of "Meat and Alternatives", "Dairy", "Cereals and Grains" and "Fruits and Vegetables", respectively (Health Canada, 2007).

The "whole grain" and "fruits and vegetables" were the 2 most poorly met food groups with 91% (n=75) and 54% (n=40) of the children not meeting their recommended number of servings, respectively (**Table II.IV**). The dairy group, on the other hand, was one for which most of the children met their recommendations. Only 18% (n=13) failed to meet the recommendations for dairy, while 53% (n=39) of children exceeded the recommendation of CFGHE by more than one serving per day. This high consumption of dairy mainly stemmed from the high milk consumption, which reached as high as 6 servings of milk per day for some children.

There were no significant differences between the mean intakes of boys and girls in any of the food groups (Appendix II-9). Also the percentage of girls and boys exceeding recommendations for dairy consumption were not significantly different (52% and 53%, respectively, (χ^2 =0.001, df=1, p=0.97).

Table II.IV also shows the results of regression analyses of hair zinc and intake of the various food groups. Total daily energy was entered as a covariate in regression analyses of all food groups.

As shown, there was an inverse association between the daily consumption of dairy, as well as milk consumption, with the children's hair zinc. These associations remained significant even after adjustment for age, sex, family income, maternal education and ethnicity.

Table II.IV. Average (±SD) of daily intake of different food groups (servings/day), and regression analyses (both unadjusted and adjusted) of these intakes with hair zinc (μg /g) of the study population (n=74).

	Intake	Unadjusted			Adjusted†	
Food group	Serving/day (Mean± SD)	B* (95% CI)	R^2	P	R^{2**}	P
Meat and alternatives	2.9 ± 1.7 18(24%)††	3.1(-7.9, 1.7)	0.02	0.20	0.09	0.28
Dairy	3.9 ± 2.2 13(18%)	-4.5(-7.9, -1.1)	0.09	0.01	0.16	0.02
Milk	2.1±1.6 -----***	-5.2(-9.2, -1.2)	0.08	0.01	0.26	0.01
Fruits & vegetables	7.2 ± 5.3 40(54%)	-0.1(-1.5, 1.7)	0.01	0.93	0.08	0.48
Cereals and Grains	6.9 ± 3.6 22(30%)	-1.1(-3.4, 1.1)	0.01	0.31	0.08	0.59
Whole grain	1.4 ±2.0 75(91%)‡	-3.3(-7.1, 0.4)	0.04	0.08	0.10	0.25
Non-whole grain	5.5 ± 2.8	0.0(-2.7, 2.7)	0.00	0.98	0.08	0.80

*Bs are the unstandardized regression coefficients.

† Adjusted regression for all variables has been carried out using, age, sex, ethnicity, income, maternal education and total calories as covariates.

**R^2 indicates the overall R^2 of the model produced with that variable and the 5 main socio-demographic variables (age, sex, ethnicity, maternal education and family income) in it. The R^2 for socio-demographic variables alone was 0.08.

†† Number (%) indicates the number and percentage of children whose intake (of that food group) did not meet the recommendations of CFGHE.

***There are no recommendations for milk in CFGHE.

‡ Number (%) under whole grain indicates the number and proportion of children whose intake of whole grain did not meet the recommendation of CFGHE, which recommends 50% of cereal and grains consumed should come from the whole grain products (Health Canada, 2007).

II.3.e. Associations of some health and behavior related variables with hair zinc

As **Table II.V**. displays, prolonged breast-feeding was associated with low hair zinc while breast-feeding *per se* was associated with increased hair zinc. Both these associations remained significant even after the adjustments for socio-demographic and socio-economic variables were carried out. Being described by the caregiver as either "often sick" or "eats unhealthy," were negatively associated with hair zinc. These associations were independent of the socio-demographic and socio-economic factors. Neither being described as "not eating enough" nor "being in a single parent household" revealed any significant association with the children's hair zinc.

Regression analyses indicated no significant associations between hair zinc and HAZ and WHZ scores. Weight-for-age Z scores on the other hand revealed a positive association with hair zinc. However, when the regression was adjusted for socio-demographic factors, the association was not significant anymore. When the children were also categorized based on sex and/or hair zinc status (separately, then together) there was no significant association between hair zinc and any one of the anthropometrics Z scores of boys, girls, all children with low hair zinc, all children with normal hair zinc, low hair zinc boys or low hair zinc girls (data not shown).

Among these preschoolers, prolonged "exclusive" breastfeeding was common and 52% of breast fed children were reported to have been breast fed exclusively for >6 months (**Figure. II.II**). We do not have any explanation for this high rate of prolonged "exclusive" breast feeding among this group of low-income children. However, in light of the fact that most of our Chinese caregivers filled out FFQ through an interpreter, we suspect that the concept of "exclusive breast feeding" may not have been communicated

to the caregivers properly and caregivers may have reported the duration of breast feeding rather than that of exclusive breast feeding.

Figure II.II. also displays that children fed for >6 months had hair zinc lower than those fed for ≤6 months (70.2±28 and 89.7±28, respectively, p= 0.02) and comparable to those never breast-fed (70.2±28 and 50.3±22, respectively, p=0.30), as indicated by one-way analysis of variance, ANOVA followed by *Tukey's post hoc* analyses.

Table II.V. Relationship between hair zinc and some nutrition and health related variables among study population.

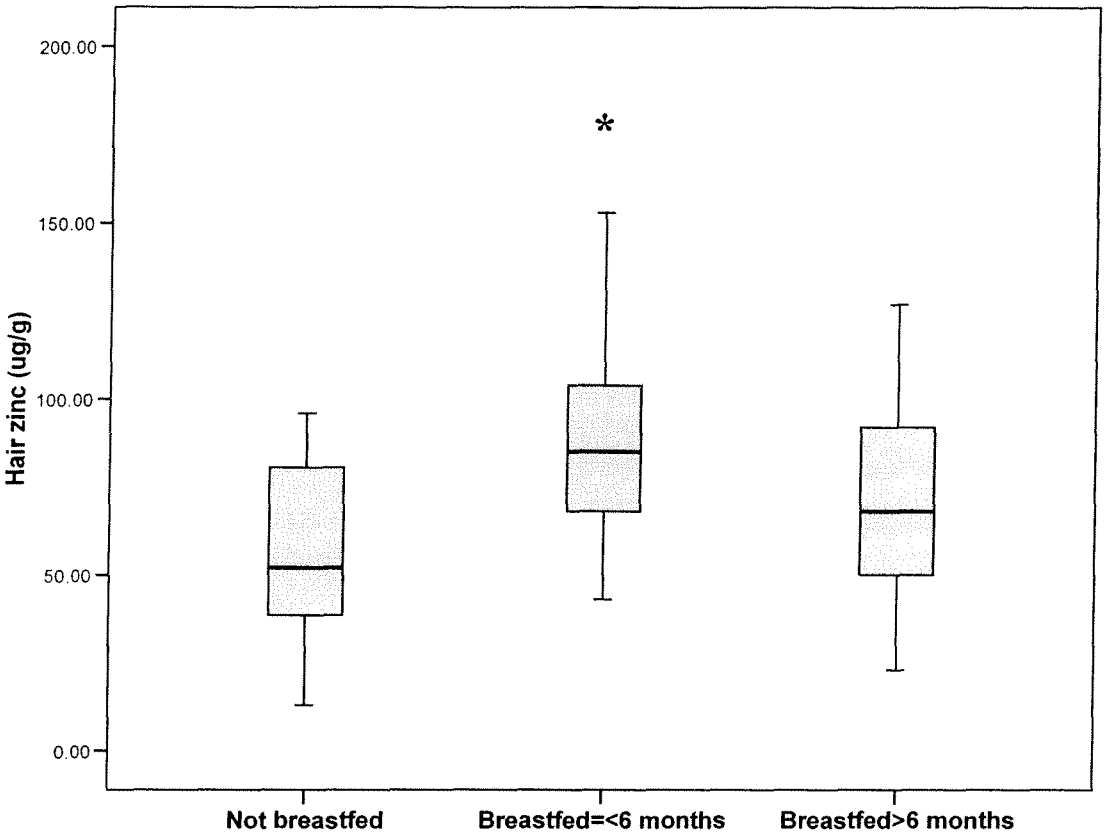
Variable	Unadjusted			Adjusted†	
	R^2	*p*	B* (95% CI)	R^2	P
Having been breastfed (n=66, 10)**	0.07	0.02	21(3.1, 39)	0.20	0.02
Prolonged†† breast feeding (n=41, 26)	0.11	0.01	-19(-33, -5.6)	0.27	0.01
Being described as "often sick" (n=15, 52)	0.55	0.00	-43(-36, -52)	0.58	0.00
Does not eat enough (n=23, 42)	0.02	0.29	6.8(-5.8, 19)	0.14	0.20
Being described as" unhealthy eater"(n=8, 57)	0.16	0.00	-30(-13, -47)	0.20	0.01
Being in a single parent household (n=19, 64)	0.03	0.13	11(-3.6, 27)	0.17	0.07
Height for age Z scores	0.00	0.83	0.53(-4.1, 5.1)	0.16	0.98
Weight for age Z scores	0.06	0.04	6.5(0.2, 13)	0.17	0.08
Weight for height Z scores	0.00	0.66	-1.3(-7.2, 4.6)	0.16	0.15

*Bs are the unstandardized regression coefficients.

†Adjusted regression for all variables (except for anthropometric variables) has been carried out using, age, sex, ethnicity, income and maternal education as covariates. For anthropometric variables the adjustments were carried out using ethnicity, family income and maternal education as covariates. The R^2 for socio-demographic variables alone was 0.08.

**The first and second numbers in brackets indicate number of children who had and did not have the variable in question, respectively. For example, the first number in the first row of the table, (66), is the number of the children who had been breast-fed, the second number, (10), is the number of children who had not been breast-fed.

†† The term "prolonged" is used to indicate "exclusive" breast-feeding beyond 6 months.



* Analysis of variance followed by Tukey's post-hoc analyses Indicated significant differences between this category and the other 2 categories of children ($P \leq 0.05$).

Figure II.II. Hair zinc level of 3 groups of children, categorized based on length of "exclusive" breast-feeding.

II.3.f. Contribution of all variables associating significantly with hair zinc to the overall variability in hair zinc of the inner city children

Inclusion of all dietary and non-dietary variables that had shown a significant association with hair zinc in a forward stepwise linear regression analysis revealed that being described as "often sick" and "having been breast-fed" were the only 2 significant predictors of hair zinc, with food intake no longer a significant component in this model (**Table II.VI**).

In addition to these 2 variables, other variables that (in univariate regression analyses) had already shown a significant association with hair zinc were entered in the above-mentioned stepwise regression model. These variables were "daily consumption of dairy", "daily consumption of milk", "prolonged breast-feeding", and being described as "eats unhealthy". Nevertheless, these variables did not seem to be significant predictors of hair zinc level, as they were eliminated from the final model produced by stepwise regression analyses. The overall model accounted for only 5% of the variation in hair zinc (R^2=0.05, P=0.00).

Table II.VI. Stepwise linear regression analyses of hair zinc and the variables significantly associating with it.

Variables	B*	SE	β	R^2 change	P
Constant	-22	15	----	----	0.15
Often sick	36	5.0	0.7	0.46	0.00
Breastfeeding	16	6.9	0.2	0.04	0.03

*Bs are unstandardized co-efficients.
For the whole model; R^2=0.50, P=0.00.

II.4. DISCUSSION

Results of this study revealed a very low mean of hair zinc for this group of low-income preschoolers. The occurrence of low hair zinc (<70 μg/g) was widespread (46%), and the mean of the hair zinc was significantly lower than the previously reported (Smit Vanderkooy & Gibson, 1987) mean for middle-income Canadian preschoolers (75±30 μg/g versus 116±34, respectively, p=0.00) (**Figure II.I**). We did not observe any sex or age-based differences in the mean hair zinc level or any differences in the occurrence of low hair zinc between the 2 sexes (**Table II.II**).

On the other hand, our population mean was comparable with the levels reported for 2 groups of low-income American children who were selected based on their lower growth percentiles (height-for-age<10^{th} percentile) (Hambidge et al, 1976, Walravens et al, 1983). This low population mean, along with the fact that 46% of our children had hair zinc <70 μg/g, made the overall zinc status of this population a concern. Our children were not recruited based on any inclusion criteria related specifically to suspected zinc deficiency, such as short stature, low zinc intake, or slow growth velocity, and they were a group of apparently healthy low-income preschoolers. The only common factor between our study children and the children of the 2 aforementioned studies, in whom the existence of zinc deficiency was confirmed following the growth response of the children to a period of zinc supplementation, was their socio-economic status. This not only indicates that low hair zinc might be common in groups of low-income children, but also suggests a potential zinc deficiency in our study children.

Despite the comparability of the hair zinc of our children with 2 groups of short low-income children (Hambidge et al, 1976, and Walravens, Krebs & Hambidge, 1983),

the mean ± SD of height-for-age Z score of our study population was normal (**Table II. I**) and comparable to that of low-income children of NHANES II (-0.19 and -0.15, respectively) (Yip, Scanlon & Trowbridge, 1993), as was the nutritional status of the study population (indicated by children's WAZ and WHZ scores).

Income is a strong and consistent determinant of health and indeed is first among the 12 identified in Health Canada's model of population health. Low-income children are known to be at higher risk for nutritional deficiencies. Perhaps this is, in part, due to some characteristic eating patterns of low-income children that predispose them to a given deficiency more than the children of a higher income group. One eating pattern characteristic of our children was a high consumption of dairy. Milk drinks were a large part of this high consumption. Indeed, drinking large amounts of milk was common among these children, reaching as high as 6 glasses per day for some children. Milk and milk products, although the second major source of energy for young North American children, are one of the food groups where children's intake often does not meet the recommended levels. Cycle 2.2 of the Canadian Community Health Survey, CCHS, the most recent survey of Canadians' eating habits, indicates that dairy products are generally under-consumed and more than one-third (33%) of the children in the 2-9 years age group did not have the recommended 2 servings of milk products a day, based on the data collected by the 24-hours food recall (Health Canada, 2004). For our study children this proportion was much lower (18% versus 33%), while a large portion of our children (53%) exceeded the recommended 2 servings/day by more than 1 serving a day (**Table II.IV**). The results of the recent nationwide survey also indicate that the 2-9 years age group (of mixed income) was the only age group in which the average number of

servings for dairy was met and was actually slightly higher than the present recommendations and minimum recommendation of CFGHE (2.31 vs. 2). Perhaps this age propensity for "dairy" combined with the relative affordability of dairy (Shanklin & Wie, 2001) is one of the factors contributing to the observed high dairy consumption among this group of low-income children.

In our study high dairy intake also revealed a significant negative association with the hair zinc of children (**Table II.III**). Dairy products are high in calcium and some studies have shown that excessive calcium intake can interfere with zinc absorption through the capacity of calcium to potentiate complex formation between zinc and ligands present in the diet, such as phytate (Oberleas, Muhrer & O'Dell, 1966). This is consistent with 2 studies of Canadian children. The first showed an inverse relationship between calcium intake and hair zinc (Smit Vanderkooy & Gibson, 1987), while the second showed that dietary [Ca x (phytate/Zn)] millimolar ratios were inversely associated with the zinc status (Gibson et al, 1991). Consistent with this inverse association, we also observed a significant difference between the hair zinc of children who had a dairy intake > 3 servings/day (exceeding the present recommendations by more than 1 serving and exceeding the upper limit of previous recommendations by CFGHE) and those who did not exceed 3 servings/day (68±27 and 82±30 μg/g, respectively, p=0.04). This finding confirms the previously reported negative association of excess calcium with zinc nutriture. It also extends this finding from the level of the mineral-mineral to the food-mineral level.

Another common food feature among our study children was prolonged "exclusive" breast-feeding (feeding child with breast milk only). Data on the effect of

income on breast-feeding are inconsistent (Dennis, 2002 and Dubois & Girard, 2003). Dubois & Girard's (2003) analysis of the Longitudinal Study of Child Development in Quebec (ELDEQ; 1998-2002, n=2,223), clearly indicates that the mother's age, followed by her education, are the 2 strongest determinants of "exclusive" breast-feeding at 4 months, and shows that these 2 factors positively affect the length of breast feeding and over-ride the effects of other factors governing family environment including family income. However, this study and most of the literature on social determinants of "exclusive" breast-feeding focus on feeding up to 6 months, which is the focus of the public health recommendations (WHO, 2008). We do not have an evidence-based explanation in our study for what factor(s) contributed to this characteristic of "exclusive" breast-feeding past 6 months.

Whatever the underlying reason for extending the duration of breast-feeding, the study did show a negative association with children's hair zinc (**Table II.V**), while breast-feeding *per se* was positively associated with hair zinc. Furthermore, the children who were 'exclusively' breast-fed for >6 months had significantly lower hair zinc than those who were fed for less than this period (70.2±28 versus 89.7±28 μg/g, respectively, p=0.01) (**Figure II.II**). The World Health Organization (WHO, 2008) recommends exclusive breast-feeding up to 6 months. This is based on some data suggesting potential risks with 'exclusive' breast-feeding beyond 6 months, including growth faltering and deficiency of some micronutrients. The bioavailability of zinc from human milk is significantly high (Lonnerdal, Keen & Hurley, 1981 and Sandstrom, Cederblad & Lonnerdale, 1983), however, the zinc content of milk undergoes a sharp decline over the first 6 postpartum months (sometimes dropping to as low as 25% of the initial supply)

(Krebs & Hambidge, 1986). Beyond 6 months, breast milk is not able to supply an infant with adequate zinc. At this point the introduction of other foods, particularly animal protein and cereals are needed to meet the high zinc requirement of a rapidly growing infant. Growth-limiting zinc deficiency has been shown in infants principally fed with human milk even after the age of 4 months (Walravens et al, 1992). Although hair zinc does reveal the zinc status over the previous few months, chronic mild zinc deficiency in childhood has hair zinc concentrations that are consistently low. It is possible that some of these children had low hair zinc from early infancy and, given their high physiological requirement during the time of accelerated growth, they never recovered from it.

Whether as an indicator of a chronic sub-optimal zinc status, inherited from the early years of life, or as a sub-optimal zinc status resulting from the dietary insufficiencies of later years, this low hair zinc may have some negative impact on the general health status of children. The adverse effect of zinc deficiency on various aspects of the immune system has been documented (Shankar & Prasad, 1998). This well-recognized adverse effect was reflected in our study in the strong and significant negative association between being described as "often sick" and the hair zinc (R^2 =0.58, P=0.00) (**Table II.V**). As an acute phase response element, plasma zinc is redistributed to other tissues in response to acute infection/inflammation (Prasad, 1998). Conceivably, reduced hair zinc can be the net result of an ongoing decline in the plasma zinc of children who are "often sick". Conversely, a lower zinc status, as indicated by lower hair zinc, can interfere with the function of the immune system, a highly zinc-dependent system, and be the cause of being "often sick". Other studies of preschoolers have indicated lower hair

zinc among children who are frequently sick with upper respiratory tract infections (Von Wouwe, Wolff & Van Gelderen, 1986 and Lombeck et al, 1988).

In this study there was no evidence of any association between hair zinc and growth status (**Table II.V**), an association shown in some studies (Hambidge 1972, Smit Vanderkooy & Gibson, 1987 and Gibson et al, 1989). Although the anthropometric data did not support the existence of any overt growth retardation, this does not eliminate the possibility of the existence of MZD among our study children. Children with marginally low hair zinc and normal growth parameters have been shown to exhibit a differential growth response (compared to growth in the placebo group) when supplemented with zinc (Ruz et al, 1997).

When all of the variables that revealed significant association with hair zinc were inserted in a stepwise linear regression analysis, being described as "often sick" and "having been breast-fed" remained significant components of the final model as the only two variables that could explain part of the variability in children's hair zinc **(Table II.VI)**. The collective model could account for only 5% of the variability in hair zinc level (R^2 =0.05, p=0.00).

Overall, our results indicate widespread low hair zinc among this group of low-income children. The biochemical evidence, combined with some predisposing dietary factors, (i.e. the excessive calcium intake), and some indirect evidence of impaired immunity (i.e. often sick with flu and common infections), suggests the likely existence of MZD among these children, even though their growth parameters did not provide evidence to support this. This assessment can only be confirmed or ruled out by carefully designed supplementation studies.

This study has some limitations that should be acknowledged. First, the food frequency questionnaire was not validated to collect data on zinc consumption. We thus refrained from exploring the zinc consumption of children based on the nutrient analysis of the FFQ. In addition, all of our data, including the dietary data, were self-reported. Unlike adults who are known to under-report their food intake, caregivers over-report the children's intake (Devaney et al, 2004). While part of the reason for this over reporting may be social desirability, part of it may also arise from over- estimating the serving size. Research has shown that most people are unaware of what constitutes an appropriate portion size (Young & Nestle, 1998). We had tried to minimize this shortcoming of the FFQ by using plastic food models and, to increase the accuracy of our dietary data, we collected it from a food frequency questionnaire completed through an interview administered by trained dietitians and research personnel rather than by parents.

Secondly, our study sample was small and we could not explore any age and/or sex based differences.

Thirdly, selection bias may have limited the generalization of our study's findings. Our children were a group of low-income children recruited in a conveniently from selected community centers of selected neighborhoods. Therefore, our findings may not be generalizable to all low-income preschoolers of the city.

Lastly, as in any cross-sectional study, our findings only indicate associations and the observed relationships cannot be taken as a cause and effect relationship or lack of it.

This study provides some important reference values on hair zinc level of low-income Canadian preschoolers. Our findings also add to the present literature by confirming an association of hair zinc with some previously reported variables and identifying its

association with some new variables. These findings need to be confirmed with larger studies of apparently healthy low-income preschoolers.

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CHAPTER III[2]

HAIR ZINC STATUS AND DETERMINANTS OF HAIR ZINC OF VANCOUVER PRESCHOOLERS AND THE USEFULNESS OF THESE DETERMINANTS AS A SCREENING TOOL FOR MARGINAL ZINC DEFICIENCY

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III. 1. INTRODUCTION

Marginal zinc deficiency (MZD) is the very early stage in the spectrum of zinc deficiency when the body's zinc supply is chronically and marginally short of meeting the physiologic needs (Prasad, 1988). During this stage most of the clinical signs and definite diagnostic symptoms of zinc deficiency such as dermal lesions, alopecia and severe growth retardation, are absent. The few symptoms present in MZD are either subtle like stunted growth, or unspecific like loss of appetite, which can be attributed to many other health problems.

Due to this lack of clear and specific signs and symptoms the deficiency can easily go unnoticed by caregivers and consequently remain undiagnosed by healthcare professionals. Though at present there is no precise information as to the number of people affected, judging from the predisposing dietary factors of MZD, it is believed that this deficiency is the most common form of zinc deficiency in developed countries and worldwide (Prentice, 1993).

Marginal zinc deficiency is a particular concern when it comes to children. Its adverse effect on growth and development, subtle as it is, has been well documented (Hambidge et al, 1972, Walravens, Krebs & Hambidge, 1983, Gibson et al, 1989-a and Ploysangam, Falciglia & Brehm, 1997). It is possible that these ill effects are not confined to childhood, since the effect of early childhood health experiences in later life, on well being, coping skills and competence, is very powerful (Currie & Madrian, 1999). A healthy child population not only speaks highly of the present health status of its society, but also indicates a reduced cost of future health care, increased productivity and an enhanced economy (Sardell, 1990). Because of the adverse effect of MZD on

children's health and well being, and the impact of children's well being on society, MZD is, indeed, an issue of significance for a healthy society.

Canadians are among the healthiest of the world's societies (WHO, 1999). Yet here, in a country with high standards of living, major health disparities still exist. These inequities create a gap within the population and affect the health status of the whole country. The larger the gap, the lower the health status of the overall population (Wilkinson, 1996 and Wilkinson & Marmot, 1998). In order to reduce health inequities and thus improve further the health status of all Canadians, Health Canada has identified and adopted the "population health approach" as its approach to policy and program development for public health (Public Health Agency, 2002).

The population health approach, as the phrase suggests, addresses the health status of the population, as opposed to that of individuals. Since our understanding of what makes and keeps people healthy is continuously evolving, this approach to evaluating health status considers the entire range of both individual and collective factors affecting a population. These elements, 1) focusing on the population and 2) addressing a variety of health determinants, are 2 out of the 8 key elements upon which the population health approach is based (Public Health Agency, 2002).

The knowledge of determinants (both social and environmental) of a given health issue can aid population health promotion in 2 important ways. It can be useful in taking measures that can inhibit the emergence and establishment of environmental, economic, social and behavioral conditions known to increase the risk of disease. It can also be useful in constructing the profile of the at-risk individuals/groups which can, subsequently, serve as a detection tool in the hands of health care professionals. While

the former is known as primary prevention and the latter as secondary prevention, they are both considered effective upstream interventions for public health promotion.

Upstream intervention, which is central to the population health approach (Federal/Provincial/Territorial Advisory Committee on Population Health, 1999), can aid in directing investments to those areas that have the greatest potential for the enhancement of health. It can also help in maximizing that potential vis a vis cost-saving concerns. The population health approach is grounded in the conviction that the earlier in the causal stream action is taken, the greater the likelihood for gain in population health. With this in mind, the most effective interventions may be those that aim not just at the onset of disease but also at the conditions that pertain prior to the onset.

An essential requirement for such intervention is a detection tool. It can often be a biological marker sufficiently sensitive and reliable to detect the disease at an early stage. Such a biomarker should also be one widely recognized and in common use by practitioners.

The collective results of supplementation studies confirming the existence of MZD in children of various developed countries suggest that, where protein energy malnutrition does not exist, hair zinc is a good indicator of this deficiency among children (Gibson, 1989). It is sensitive enough for detection even in the very early stage of zinc deficiency (Hambidge, 1980). In addition, owing to the structural characteristics of a hair follicle which isolates the element within itself, hair zinc is immune to fluctuations resulting from other physiological events in the body (Hopps, 1977). However, despite its sensitivity, specificity, and relatively prevalent use in research, health practitioners seldom use this biomarker.

Alternatively, in the absence of a single sensitive and specific biomarker that is also commonly recognized and widely used, the profile of an at-risk individual can be of great help. Such a profile, constructed from the determinants of that health issue/disease, can be used to construct a questionnaire which when validated can become a screening tool. However, an indicator of the disease is needed in order to identify its determinants and their interactions. Hair zinc can fulfill this role by acting as an indicator of zinc status in studying the determinants of MZD.

At present our knowledge of the determinants of MZD is very limited. In Canada the last study conducted on the zinc status of a group of healthy Canadian preschoolers dates back to the 1980s (Smit Vander Kooy & Gibson, 1987). This deficiency (MZD) was later confirmed through a zinc-supplementation study (Gibson et al, 1989-a). In more recent years our own study of a group of low-income preschoolers of Vancouver indicated widespread low hair zinc while identifying a few potential determinants of low hair zinc status (Chapter II of this thesis). This has generated in us concern as well as curiosity regarding the overall zinc status of Vancouver preschoolers. Combined, they posed the need for a new and large-scale study that would address these issues.

Therefore, with these knowledge gaps in mind and using hair zinc as the biomarker of zinc status, this study was undertaken to examine the zinc status of Vancouver preschoolers through a large and representative sample. In addition, we planned to explore and identify the determinants of sub-optimal zinc status in an attempt to construct a short and simple questionnaire that could serve as a screening tool for low hair zinc/MZD. We intended, then, to validate the developed questionnaire. The specific objectives of this study were:

1. To assess the hair zinc status and the extent of low hair zinc in Vancouver preschoolers through a large and representative sample from the city.

2. To explore the relationship between the 5 main demographic and socioeconomic factors (age, sex, ethnicity, family income and maternal education) and hair zinc levels.

3. To examine the relationship between some additional family characteristics (family size, and composition, food security and paternal education) and hair zinc levels.

4. To investigate the relationship between some eating behaviors such as the intake frequency of meat, dairy, cereals and grains, as well as variables such as "being concerned about child's eating", "adequacy of child's eating", "pickiness", and "unhealthy eating" with the children's hair zinc level.

5. To examine the relationship of parental perceptions of some health-related variables (such as overall health, frequency of a child being sick, breast-feeding pattern, and intake of supplements containing iron), and behavior-related variables (such as the scores of activity level and attentional focusing) with the hair zinc of children.

6. To examine the relationship between the indices of growth (HAZ and WHZ scores) and nutrition (WHZ scores) with the children's hair zinc.

7. To determine which of the variables significantly associated with hair zinc, can predict the hair zinc levels.

8. To verify which of the variables associating significantly with low hair zinc status can predict hair zinc status (i.e. low hair zinc).

9. To assess the collective usefulness of the variables identified under objective 8 (and the questionnaire constructed based on this model), in predicting the hair zinc status of

children by exploring the classification tables and the Receiver's Operating Characteristic (ROC) curve of the model.

It was our belief that the outcome of this study will make an important contribution to the MZD literature by expanding our understanding of the factors in children and their environment and social setting that are associated with (and may therefore influence) their hair zinc status. It will also provide information on the hair zinc/zinc status of the city's preschoolers while making some reference values available for future researchers working on MZD in apparently healthy Canadian preschoolers and preschoolers of industrialized societies in general.

III.2. METHODS AND MATERIALS

III.2.a.Study design

The study, which was approved by the Human Ethics Committees of the University of British Columbia (Appendix III-1), was designed as a citywide survey representing all 23 neighborhoods of the city of Vancouver. Daycare and preschool centers were planned as the sources for our recruitment. Through the cooperation of the West Coast Childcare Resource Center we obtained the list of all licensed daycares and preschool centers in all 23 neighborhoods of the city. From this database, depending on the number of subjects needed to represent each neighborhood (more information on our sampling procedure is provided later), we selected a few daycares and/or preschool centers as potential contact centers. In studying data from the 2001 census, it was apparent that there is a wide variation in the socio-economic status (SES) even within the individual neighborhoods. In order to have a fair representation from each neighborhood, they were broken down into smaller units referred to as dissemination areas (DA) (the

basic building block of Canada's census geography composed of one or more neighboring blocks having a population of 400-700 people). The Geographic Information System (GIS) of the Human Early Learning Partnership (HELP) produced maps of each neighborhood with their DAs color-coded indicating the SES status of that DA. The potential participating centers (daycare and preschool centers) were identified on the map of each neighborhood. Care was taken to have a fair representation of each color (each SES) when choosing the centers to participate. A sample neighborhood map is enclosed (Appendix III-2).

III.2.b. Contacting the potential centers to participate

Once the list of potential centers was completed, they were contacted by telephone. During the initial call, the research student, after introducing herself and her affiliation with the University of British Columbia (UBC) explained the reason for her call. Then, the survey, the people, and the institutions involved (UBC and HELP) were introduced. At this point the research student asked for the name of the person in charge of the center and whether the center would be willing to participate, so that a package could be put in the mail for them. Some centers (n=13) expressed an unwillingness to participate for varying reasons. Some, whose boards were comprised solely of parents, expressed a general lack of interest in participating in research. Others indicated that the survey would coincide, and therefore conflict, with other activities already scheduled, such as religious or ethnic holidays. With each refusal a new center, within the same neighborhood DA, was selected for contact. It was also during these phone calls that inquiry was made as to the enrolment number, and the data already provided by the West Coast Child Care Resource Center was then updated.

In total, 55 centers agreed to participate, while 13 refused (55/68=81% response rate). Following this initial contact, the survey information package was mailed to those centers that had expressed an interest in participating. It contained a supporting letter from the Executive Director of West Coast Child Care Resource Centre (Appendix III-3), a letter from the principal investigator of the project (Appendix III-4) and a schematic presentation outlining the sequence of the survey events together with their allotted time intervals (presented as **Figure III.I** in the upcoming sections).

III.2.c. Meeting with centers prior to the commencement of the survey

The survey was planned in 4 waves, each starting and ending in a staggered manner. Waves 1, 2, 3, and 4 covered 6, 5, 5, and 7 neighborhoods of the city, respectively. Second phone calls were made to the participating centers 2 weeks prior to the survey start requesting a meeting with as many childcare workers as possible.

During this visit:

- The survey and the survey team were introduced to the childcare professionals.

- The time line for the survey and the activities involved were discussed.

- The significance of their role as the middle person and their impact on the response rate was acknowledged and their cooperation requested.

- Their questions were answered and their concerns received. As a result, some center-specific changes in the survey process were made when necessary.

-Contact information of the survey team was provided and communication of the centers with the survey team throughout the process was encouraged.

- The "initial letter of contact" (Appendix III-5) was given out for distribution in a week's time.

III.2.d. Inclusion criteria

The inclusion criteria for our survey were: 1) residency in the city of Vancouver, 2) being healthy (not diagnosed with any major or chronic illness), and 3) being between 24-71 months old.

III.2.e. Survey Tool

The survey tool was a 41- item questionnaire covering 4 subjects: the child's background, family background, child's eating habits, and child's behavior. The following 3 sections outline the process of development and validation of this questionnaire and describe its components.

III.2.e.A. Development of the survey questionnaire

From a thorough search of the literature, all of the variables that have been shown to correlate with the hair zinc of children were identified. The shortest, clearest version of the question for each relevant variable was then synthesized from existing items. In order to elicit a specific response, (Czaja & Blair, 1996) and also to attain a higher response rate (Passmore et al, 2002), the respondents were given specific response categories to choose from. After constructing a question for each of the variables, all of the questions were then compiled to create the survey instrument.

III.2.e.B. Components of the survey questionnaire

The following sections will explain the different areas on which the survey questionnaire collected information (in the order that they appear in the questionnaire), and will describe the questions designed for each area.

III.2.e.B.1. Socio-Demographic Information

This information was collected through 2 groups of questions arranged under 2 headings: the child's background and family background. Under these headings we collected information on the child's sex, date and place of birth, ethnicity of parents, maternal and paternal education, family income, family size and composition (number of adults and number of children living in the house).

Since in our previous study in Vancouver inner city (chapter II of this thesis) we had noticed a significantly different hair zinc level and status in children < 4 and those ≥ 4 years, we used this age breakdown for the initial categorization of the subjects of this study as well.

The most recent census has reported a median income of $39,400 for the city of Vancouver (Statistics Canada, 2005). We categorized the income level into 4 categories (increments of 20,000) with 2 below and 2 above this median.

III.2.e.B.2. Information on child's breast-feeding history and perceived health status

We asked parents to provide some information on their breast-feeding history through 3 questions;

1) The first question asked them whether or not the child was breast-fed and, if so, to provide the duration of breast feeding by filling in the numbers in 2 blanks (from the time she/he was ____months to the time she/he was _____months old).

2) The second question asked about whether or not the child received infant formula and, if so, to provide the length of time that formula was fed by filling in the numbers in 2 blanks (from the time she/he was ____months to the time she/he was __months old).

3) The third question asked about the age of weaning by requesting the parents to fill in the weaning age in the blank provided (when she/he was ____months old).

Using the 3 above-mentioned questions we figured out the duration of "exclusive" breast-feeding.

We also requested the caregivers to answer 2 further questions; one asked parents to describe their child's health using 1 of the 4 adjectives: very good, good, fair and poor. The second asked them to record the frequency (times per month or year) of their children being sick with flu, fever, cold and other common infections. We used both these questions as indices of the children's overall health status and, ultimately as, proxies of their immune system function.

III.2.e.B.3. Information on supplement intake

In order to obtain information on the use of supplements, as to their brand name and frequency of use, a 3 part question was designed and placed under the first category of the survey questionnaire, namely the "child's background".

1) The first of the 3 parts asked whether or not the child took or takes any supplement(s).

2) The second part inquired as to the period of time over which the supplement had been consumed (e.g. from the time that child was 18 months up until she was 4 years old).

3) The third part asked for the name(s) of the supplement(s) given to the child.

In cases where multi-mineral supplements were reported, we constructed a list and visited local pharmacies and grocery stores to examine the supplement shelves so as to verify if and how much iron and zinc they contained.

III.2.e.B.4. Information on children's eating behavior

This information was collected under the heading of "Child's Eating Habits". In an effort to obtain a high response rate, we, in general, used questions that were straightforward and easy to answer without requiring a lot of time commitment. This basic principle applied to our dietary questions as well. We did not use a comprehensive assessment of diet. Under the heading of "child's eating habits", there were 5 major sections each of which contained multiple (between 1-10) questions to collect information on the following topics;

- The first section contained 7 questions that assessed the parents' perception of their child's eating behavior. It commenced by asking the parents if they are concerned about their children's eating in general, then provided 7 stated options from which to choose. These options were some of the common eating characteristics/problems of young children (such as being a picky eater, not eating enough etc.) identified in our meetings with community dietitians. The parents could select more than one behavior. Space was also provided in this first question for concerned parents to write about any additional eating behavior/problem in their children which was not provided as an option.

- The second section was a single question collecting information on the consumption frequency of fluids (everything but water) intake. Although no study of zinc has assessed any associations between poor zinc nutriture and fluid consumption, such an association has been documented for some other micronutrients (Guenther, 1986 and Williams, 2000). Estimation of fluid consumption was done to enable us to explore these associations.

- The third section comprised of 6 questions, collected information on the consumption frequency of meat, fish and poultry.
- The fourth section comprised of 10 questions, collected information on the consumption frequency of cereals and grains.
- The fifth section comprised of 3 questions, collected information on the consumption frequency of dairy products.

The 3rd, 4th, and 5th sections were designed to obtain information on the bioavailable sources of zinc i.e. meat, fish and poultry, and sources of inhibitors of zinc absorption i.e. dairy as a major source of calcium and cereals and grains as major sources of phytate. These 3 sections had asked questions on the usual consumption frequency of a variety of foods belonging to these food groups. The questions provided response options in the form of boxes (consumed daily/weekly/monthly) to check and blanks to fill in (once, twice, 3 times...). Portion size was not ascertained. As with the other parts of the survey questionnaire, the dietary section was subjected to multiple revisions and changes based on the outcome of our multiple focus groups.

III.2.e.B.5. Food Security Questions

This section comprised 3 pre-validated questions as to how often the family members experienced some aspect of hunger and/or concern about food, in order to measure food security in the survey families. They were adapted from the Cornell/Radimer food insecurity questionnaire (Olson et al, 1997). These pre-validated questions are highly sensitive, widely used, and likely to identify families affected by food insecurity and hunger. The National Population Health Survey, NPHS, (1998-1999) also used these questions. Similar to the scoring system used in NPHS, the respondents in

our survey were considered to be living in a food insecure household if they responded "Yes" (either sometimes or often) to at least 1 of the following 3 question/statements (Che & Chen, 2001);

In the past 12 months:

Q1: Were you and others worried that food would run out before you got money to buy more?

Q2: The food that you and others bought didn't last and there wasn't any money to get more.

Q3: You and others, because of not having money, could not eat balanced meals.

Three options were provided in choosing a response: "Often", "Sometimes", or "Never".

III.2.e.B.6. Behavioral Information

The Child Behavior Questionnaire, CBQ, is a behavior checklist developed by Dr. Mary Rothbart, University of Oregon (Rothbart, 1996). It is a highly differentiated assessment of temperament in early to middle childhood. In its original form, this tool measures 12 domains of behavior through 195 questions. The original CBQ was shortened in 2003 to measure the same domains of behavior through a reduced number of questions (n=85) (Putnam & Rothbart, 2003). Through a series of email communications with Dr. Rothbart and Samuel Putnam, a collaborating scientist who has had a key role in development of this tool, we took this version and extracted the questions pertaining to 2 behavior domains of interest in zinc deficiency: activity level and attentional focusing. The first score is the measurement of the level of gross motor activity including rate and extent of locomotion. The other score is defined as the measure of the tendency to maintain attentional focus upon task-related channels (Rothbart et al, 2001).

The questions addressing these 2 domains were further modified and shortened (by Dr. Rothbart's laboratory) in order to be more suitable for a survey. The final shortened questionnaire comprised of 13 questions measuring activity level (7 questions) and attentional focusing (6 questions).

As with the original tool, the questions measuring the same behavioral domains were separated from each other by a question measuring another behavior in order to prevent parents from getting into a mental groove that could potentially affect the validity of their responses.

III.2.e.C. Verifying the face validity of the questionnaire

We assessed the face validity of the survey questionnaire through 5 focus groups set up in Hastings Sunrise, University Lands, Kensington Cedar Cottage, Strathcona, and Downtown. These neighborhoods were chosen because collectively they included every level of literacy in the city with Hasting Sunrise and University Lands at the low and high ends of the literacy spectrum, respectively. Each group included 6 parents. After each group met, a modified version of the questionnaire was developed and tested in the next focus group. This was repeated until no new information emerged.

Efforts were made to include parents of different ethnicities in each group. Participants were recruited through day-care centers and personal contacts known to the research student. The snowballing method was also used. At the time of recruitment, the research student explained to the potential participants both the study and the organizations involved (UBC and HELP). Individuals willing to participate in a focus group were asked to provide contact information by filling out a form (Appendix III-6). An information sheet containing the day and time of the focus group, along with the

address of the focus group location, and the research student's phone number was provided to all recruited participants (Appendix. III-7). The recruited parents were called the day before to remind them of their appointment.

The focus groups began with the research student, as the group facilitator, re-explaining the purpose of the focus group and assuring the group of the confidentiality of the information and asking the participants to sign an informed consent form (Appendix III-8).

The parents were reimbursed for their transportation expenses (when applicable) and a gift certificate was presented to them at the end of each group. The outcome of the final focus group was sent to the supervisory committee for their final comments and then the final questionnaire (Appendix III-9) was given to 2 translators for translation into Punjabi and Cantonese. These questionnaires and accessory forms were then given to 2 other translators to translate them back to English. The research student reviewed the back-translated forms and met with the first set of translators to go over the problematic areas of the first translations. After a few rounds the final translated forms were produced (Appendix III-10 and 11 for the Punjabi and Cantonese survey questionnaires, respectively).

III.2.f. The survey sequence of events

The survey began by sending home the "initial letter of contact". One to 2 weeks after sending this letter to the parents, the centers distributed the "survey packages". These contained: "subject's information letter" (Appendix III-12) reiterating the information provided in the "initial letter of contact", a "consent form" (Appendix III-13), and the "survey questionnaire". A cardboard box labeled "UBC Survey" on all 4

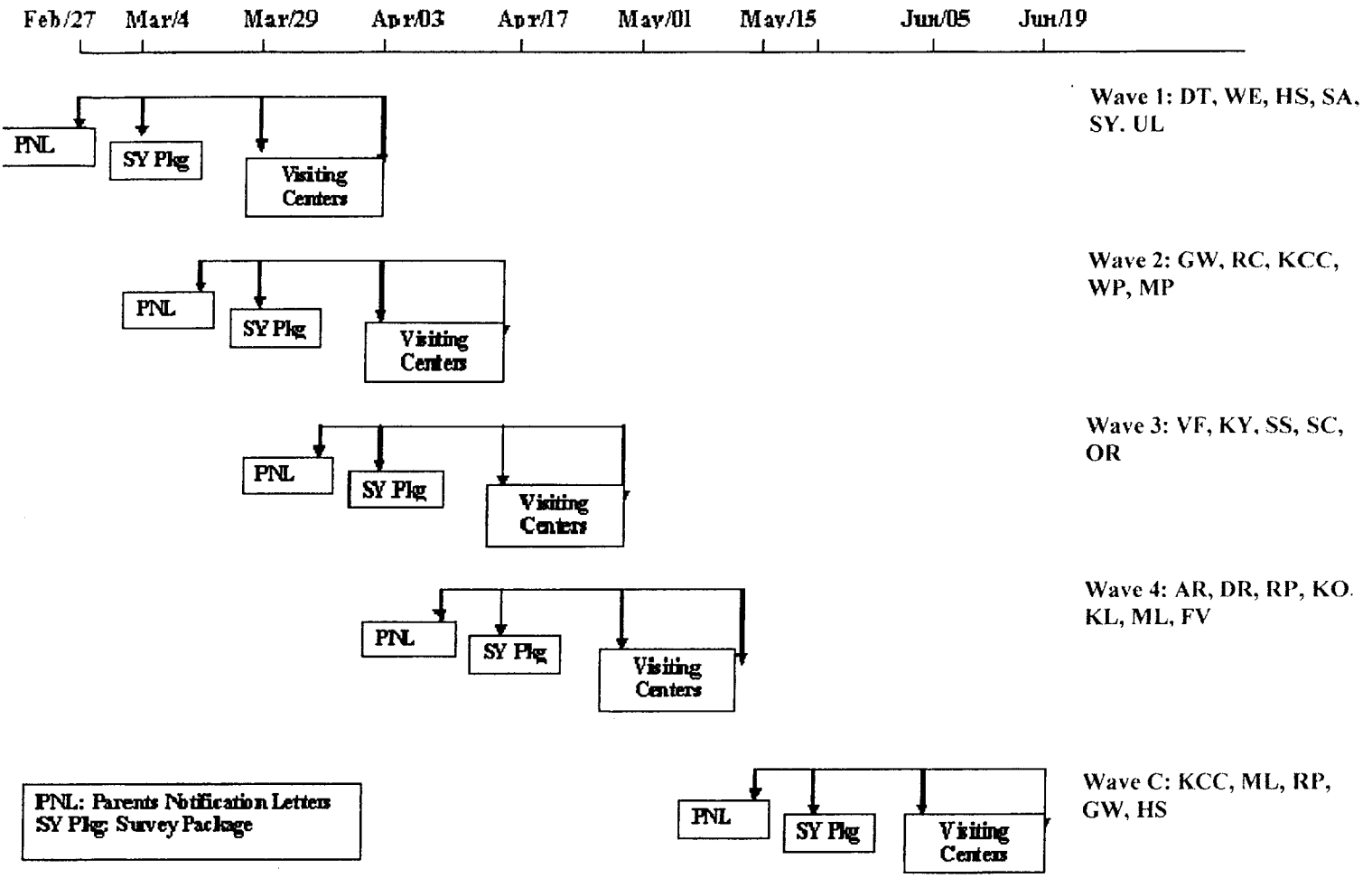
sides was placed at the "sign in/sign out" station at each center as a continuing reminder to the parents to drop off their completed survey questionnaires along with their consent forms. A log sheet was provided beside the box for parents to sign their study code (printed on each envelope) together with the date of their survey return (Appendix III-14).

One week after distribution of the survey packages, a "reminder letter" (Appendix III-15) was sent to the homes of the children encouraging and reminding the caregivers to return their completed surveys. The survey forms continued to be received during the second week. It was also during this week that the survey team and the daycare authorities tried to set up a time convenient for both parties to visit the centers in order to take anthropometric measures and collect the hair samples of the children for whom a consent form had been received. Earlier in the week of our visit, a sign was posted at various locations in the centers in order to announce our arrival and ensure the attendance of the participating children on that day (Appendix III-16).

Figure III.I is a schematic summary of this sequence of events. As shown, upon completion of the first 4 waves, a fifth wave, called wave-C, was designed to re-initiate the survey in a few neighborhoods where the total number of families responding to the survey did not meet the target numbers. Wave-C completed the survey in the 5 neighborhoods of Kensington Cedar Cottage, Hastings Sunrise, Grandview Woodlands, Marpole and Riley Park.

The recruitment method for some wave 1- 4 neighborhoods and most of the wave-C neighborhoods was different from our usual method. For them, where the rate of response was low and did not meet the target number of that neighborhood (KCC, SS,

GW, RP, ML, KO, HS, OR,), we contacted the coordinators of parent-child programs in community centers of those neighborhoods and requested their permission to drop in. We attended coffee-time and playtime programs where recruitment, survey questionnaire completion and sample collection were all accomplished together. We recruited 121 (17%) survey participants in this way. Through a series of one-way analyses of variance, ANOVA, we then compared the SES (age, sex, ethnicity, maternal education and family income) of these children and the children recruited by our usual recruitment method. No significant differences were observed.



DT= Downtown, We=West end, HS=Hasting Sunrise, SA=Strathcona, SY=Shaughnessy, UL=University Lands, GW=Grandview Woodlands, RC=Renfrew Collingwood, KCC=Kensington Cedar Cottage, WP=West Point Grey, MP=Mount Pleasant, VF=Victoria Fraserview, KY=Killarney, SS=Sunset, SC=South Cambie, OR=Oakridge, AR=Arbutus Ridge, DR=Dunbar, RP=Riley Park, KO=Kitsilano, KI=Kerrisdale, ML=Marpole, FV=Fariview,

Figure III.I. Schematic presentation of survey waves and their time-lines.

III.2.g. Sample Size Calculation

Initially we had planned to divide the returned surveys, randomly, into 2 equal batches to be used as follows:

1) One of the batches was to be used for exploring the socioeconomic, socio-demographic and food, health and behavior-related variables having significant association with low hair zinc. Then using the logistic regression analysis, the variables predictive of hair zinc were to be identified and used to construct a mini-questionnaire.

2) The second batch was to be used to validate this questionnaire.

However, upon completion of the survey, our preliminary analyses of the first batch of surveys indicated that the R^2 values of the variables significantly associating with hair zinc were too small to construct a useful questionnaire. It was our understanding that any questionnaire constructed based on these variables, would not have great predictive power and as such would not be of much use in practice. Therefore, the plan was modified and all of the returned surveys were used as one batch to increase the power of the study to explore as many variables as possible. The following is a description of the sample calculation for the initial plan.

Based on the total number of variables that we had included in our survey questionnaire, we predicted that the likely number of variables for inclusion in the mini-questionnaire would not exceed 10. Using this hypothetical maximum number, the following reasoning was used in estimating the sample size and the number of surveys required for distribution.

- As a general rule of thumb 10 affected subjects (i.e. subjects with low hair zinc) are considered an adequate size to test each variable through regression analysis (Van Belle, 2002). Therefore, for 10 variables to be tested, 100 affected children were going to be required.
- Considering 100 subjects for development of a questionnaire, and 100 for validation of the questionnaire, 200 affected subjects was required to complete both tasks.
- Results of our previous work in the city (Chapter II of this thesis) had indicated an occurrence rate of low hair zinc at 46%, while another Canadian study of this age group has reported 24% (Smit Vanderkooy & Gibson, 1987). Taking the midpoint as the expected rate of occurrence gave us 35%. [46+24]/2= 35%.
- Assuming, then, a 35% occurrence rate, in order to have 200 affected children we needed 572 children overall. [200x100]/35=572
- Assuming 50% response rate, to have 572 returned required that 1144 be sent out.

[572 x100]/50=1144

- Provision of 12% additional subjects to compensate for the incomplete, lost surveys and etc, gave;

[1144x12]/100=138

Therefore the required number were: 1144+138 = 1282

Rounding upward, we needed to distribute 1300 surveys. **Table III.I** is the summary of the number of surveys to be given out in each one of the 23 neighborhoods.

Overall, 2550 survey packages were sent out and 772 were returned (30% response). Out

of the returned surveys 719 had a competed survey questionnaire and hair sample result (attrition rate of 7%).

Table.III.I: A summary of the estimated number of survey packages needed to be given out in each neighborhood.

Neighborhood	Number of preschoolers in the neighborhood	Percentage of Vancouver preschoolers	Number of surveys to be given
Shaughnessy	270	1.11	14
South Cambie	310	1.27	17
Strathcona	345	1.42	18
Oakridge	375	1.54	20
Arbutus-Ridge	410	1.68	22
Kerridsale	420	1.72	22
West point Grey	510	2.09	27
University lands	515	2.11	27
West End	655	2.69	35
Marpole	810	3.32	43
Downtown	835	3.43	45
Fairview	955	3.92	51
Dunbar	995	4.08	53
Victoria-Fraser view	1105	4.54	59
Riley park	1200	4.93	64
Killarney	1215	4.99	65
Mount Pleasant	1260	5.17	67
Kitsilano	1355	5.56	72
Grandview-Woodlands	1525	6.26	81
Hastings-Sunrise	1875	7.70	100
Sunset	2285	9.38	122
Renfrew-Collingswood	2470	10.14	132
Kensington Cedar Cottage	2670	10.96	142
Total Vancouver	24365	100	1300

III.2.h. Pre-testing the survey

After all the survey components were in place, and prior to the survey commencement, the questionnaire, as well as the designed plans and procedures, were tested in 1 of the daycares of the Shaughnessy neighborhood. The survey began and ended as outlined. Based on the feedback, and our own observations, a few minor changes were made. Out of 50 surveys given out, only 10 were returned (20% response) giving us a first indication that our expected 50% response rate for the sample size calculation was optimistic. We then decided (depending on the size of the required sample from each neighborhood) to add from 1 to 4 centers to the centers list.

III.2.i. Reliability of the questionnaire

Having the questionnaire completed by 10% (n=72) of the survey respondents at 2 different points in time allowed assessment of the reliability of the questionnaire. The second questionnaire, relying on the log sheet and the return of the first questionnaire, was given out in such a way that the subjects had 3 weeks between filling out the 2 questionnaires. The following steps were taken in order to complete this part of the study. Seventy-two subjects out of the survey participants were randomly sampled and the reliability package was given to them through the centers. This package contained: another copy of the questionnaire, a covering letter (Appendix III-17) and a gift certificate. The return from the reliability study was very high. To get the desired number of 72 subjects we had to send out 80 reliability packages only (return rate 90%). The results of the reliability test for different components of the survey questionnaire will be presented in their respective result sections.

III.2.j. Incentive

We included small incentives for participants at various points of the survey. Gift certificates (in the value of $15) were provided for the participants of the focus groups and the reliability study. In addition, at the end of the survey, a raffle of 35 baskets was held (one for every 22 surveys returned thus giving a 5% chance of winning) with items relevant to preschoolers and caregivers. They were delivered to the winners' respective centers, along with a letter that requested them to call the survey line and confirm the receipt of the basket (Appendix III-18). A few photographs of the winners are enclosed (Appendix III-19).

III.2.k. "Thank you" letters

The gratitude of the research team was expressed to all of the participating parents through a thank you letter given to all participating children on the day of the sample collection (Appendix III-20), along with a sticker given to each child. Upon completion of the survey, a thank you card was mailed to all participating centers (Appendix III-21) as well as to West Coast Family Services (Appendix III-22).

III.2.l. Educational pamphlets

To express further appreciation to the survey participants, an educational pamphlet on zinc, its dietary sources and the amounts required for children of different age groups, was prepared and supplied to all participating centers to be made available to their families. The on-line Practice-based Evidence in Nutrition, PEN, developed by Dietitians of Canada (Dietitians of Canada, 2006) was consulted for preparation of this pamphlet. A draft of this pamphlet was edited by Karol Traviss, the dietetic education coordinator, and Dr. Susan Barr, a member of the supervisory committee, and was then

put through 3 focus groups held in Kiwassa Neighborhood House and Marpole Family Place where educational attainment is generally low. Through these focus groups the level of literacy and ease of comprehension of the pamphlet were tested and the necessary adjustments made prior to printing the final product (Appendix III-23).

III.2.m. Biochemical data

A pair of stainless steel scissors wiped with ethanol swabs (between subjects) to avoid cross contamination was used to cut the hair samples. Following the protocol proposed by the International Atomic Energy Agency, IAEA, (Deppisch et al, 1999), and adopted by the Center of Disease Control, CDC, and the US Environmental Protection Agency, EPA, they were cut from 3-4 locations at the back of the head and as close to the scalp as possible. Only the first 1-2 centimeters proximal to the scalp were taken, the remainder being discarded. The collected samples were each placed in a pre-labeled coin-envelope. They were all inspected and cross-matched with a list of the consented children of the center visited that day. Samples were delivered to JR Labs in Burnaby on a weekly basis along with a log sheet listing all samples with their study code (Appendix III-24).

The samples were processed (Puchyr et al, 1998) and analyzed for zinc content by inductively coupled plasma mass spectrometry, ICP-MS (EPA, 1994). They were analyzed as single samples with a duplicate after every 10 samples. The within assay CV for this method was 3.3% at a mean of 157.3 μg zinc/g hair, while the between assay CV was 6.0% at a mean of 157.7 μg zinc/g hair.

The cutoff of 70 μg/g, the one most commonly used cutoff which has been proposed by Hambidge (1972) and defined as 3 SD below the adult mean, was used to define low hair zinc in this study.

III.2.n. Anthropometrics

Trained research personnel measured the children's height and weight and recorded them on a log sheet prepared in advance (Appendix III-25). A measuring rod, aligned and secured on any wall in the room that was flush with an uncarpeted floor, was employed to measure the height as each child stood shoeless with back to the wall, feet together and looking straight ahead. Heights were measured to the nearest 0.1 cm. Body weight was measured to the nearest 0.1 kg using a lithium electronic scale (Taylor Precision Products, L.P, Model 7300) with the scale placed on a hard floor and children standing on it shoeless and wearing light indoor clothing only. All the readings were done in multiples until 2 consecutive numbers were identical or not different by more than 0.5 unit of measurement. When 2 consecutive readings were not identical, the average of the 2 readings was recorded.

The reference tables of the Center for Disease Control (CDC, 2000) were used to calculate the weight for age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) z scores of all children.

III.2.o. Statistical analyses

The data were analyzed using the Statistical Package for Social Science, SPSS, (version 13.0, 2005). Using this software, descriptive statistics (such as mean and standard deviation) were computed for all variables.

In order to assess the relationship between each of the variables of interest (5 main socioeconomic and socio-demographic variables, some family characteristics, frequency of the intake of some food groups, parental perceptions of some nutrition related, health-related and behavior-related variables and intake of iron supplements) and

hair zinc levels, univariate regression analyses were carried out with hair zinc as the outcome variable.

In order to examine how the variables associating significantly with hair zinc collectively predicted hair zinc, a forward stepwise linear regression analysis was carried out with hair zinc level as the outcome variable and all of the variables that were significantly associated with hair zinc level as predictors. The inclusion criterion for predictors in this analysis was having $p \leq 0.05$ in the adjusted regression analysis.

In order to identify the variables that may have usefulness in predicting the hair zinc status of children, and as a result the construction of a screening questionnaire, we set up a logistic regression analysis with hair zinc (binary data) as the independent variable and all of the variables that associated significantly with low hair zinc as predictive variables. The inclusion criterion for this analysis was having $p \leq 0.05$ in the *chi square* analysis of the occurrence of low hair zinc, when children were classified based on a given variable (for example being described as "unhealthy eater" was included in this analysis because the occurrence of low hair zinc in children who were described as "unhealthy eater" was significantly higher than that in children who were not described so). In cases of the variables pertaining to the consumption frequency of food groups as well as the scores of 2 behavior domains, (which were continuous data), variables were inserted into the logistic regression analysis only if the difference in the consumption frequency of a given food or the score of a given behavior of the low hair zinc and normal hair zinc children was significant ($p<0.05$). For both these regression analyses (the step-wise and logistic regression analyses), the collinearity of the variables inserted into the models were verified. Collinearity between two components of a regression

model could "blow up" the standard deviation and result in elimination of one of the two variables from the final model produced.

In order to examine the utility of the logistic regression model produced in predicting the hair zinc status of children, the classification table and the Receiver Operating Characteristic (ROC) curve for the fitted logistic regression model was constructed.

The differences between the hair zinc levels of different sexes and age groups were verified using ANOVA, followed by *Tukey's post hoc* analyses, when applicable. When the outcome variable and the variable of interest were both in categorical form, the differences were examined using *Chi square* analysis. The groups with different occurrence of low hair zinc were differentiated using continuity corrected *chi-square* (adjusted chi square) (e.g. difference in the occurrence of low hair zinc between different ethnic groups). The chance of having low hair zinc for children in different categories (such as for boys and girls) was computed using risk analysis statistics. For all analyses the level of significance was set at ≤ 0.05.

III.3. RESULTS

III.3.a. Survey population

A description of the study population has been given in **Table III.II**. As shown, boys and girls were equally represented, while the representation of younger (<4 years old) preschoolers was slightly less than the older (≥4 years old) ones.

As **Table III.II** indicates children with Caucasian (42%), Chinese (31%) and East Indian (8%) ancestry made up the 3 main ethnicities of the survey. More than half of the survey mothers (58%) held a college or university degree, while 7% of them had not completed high school. Income of the survey families varied within the range of <$20,000->$60,000/year with the largest portion of the families at >$60,000 and 11% at <$20,000/year.

Anthropometric data indicated normal growth and nutritional status for the survey population. About 50% of HAZ and 51% of WAZ scores of the study population were between -0.67 and 0.67, corresponding to the 25^{th} and 75^{th} percentiles. The rate of stunting (HAZ<-2) and malnutrition (WAZ<-2) (Walravens, Krebs & Hambidge, 1983 and Gibson et al, 1989), at 2% (n=13) and 2.3% (n=16), respectively, was not higher than the expected baseline.

Table III. II. Socio-demographic description of study population (N=719).

Socio-demographic variables	Number (%)*
Sex	
Boys	360 (50)
Girls	359 (50)
Age	
< 4 years	305 (42)
≥4 years	400 (56)
Missing	*14 (2)*
Ethnicity	
Caucasian	305 (42)
Chinese	223 (31)
East Indian	54 (8)
Other ethnicities†	134 (19)
Missing	*3 (0.0)*
Maternal education	
<High school	49 (7)
High school	72 (10)
Some college	170 (24)
College or university degree	420 (58)
Missing	*8 (1)*
Income	
<20,000/year	67 (9)
20-39,000/year	120 (17)
40-60,000/year	94 (13)
>60,000/year	310 (43)
Missing	*128 (18)*
Anthropometrics**	**Mean ±SD**
HAZ scores	0.09 ± 1.0
WAZ scores	0.24 ± 1.2
WHZ scores	0.25 ± 1.6

*Indicate percentages of the total survey population

†Other ethnicities: 32 Filipinos, 19 Japanese, 18 identified themselves as Asians, 11 Vietnamese, 10 Latin, 7 Korean, 6 Aboriginal, 5 Middle Eastern, and 26 miscellaneous.

**Only 662 children had anthropometric data.

HAZ: Height-for-age Z scores

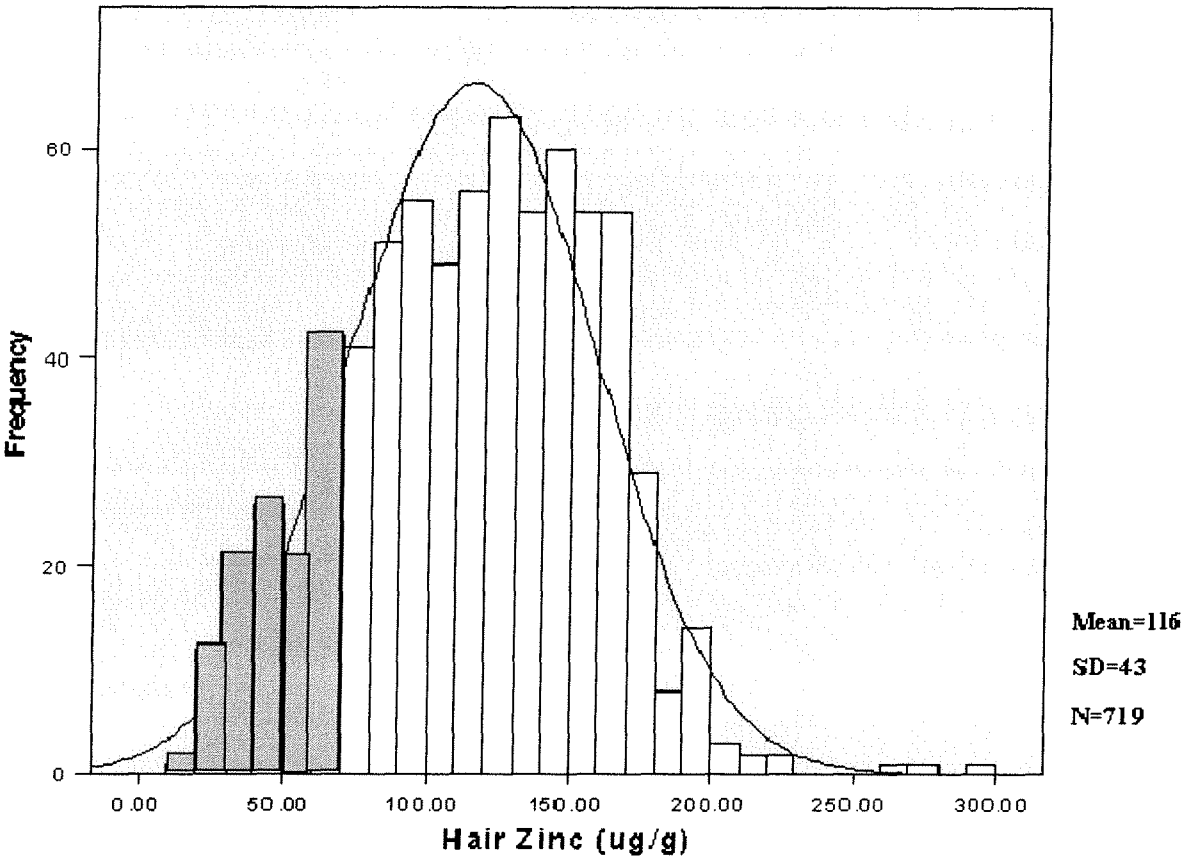
WAZ: Weight-for-age Z scores

WHZ: weight-for-height Z scores

III. 3.b. Hair zinc status of the survey population

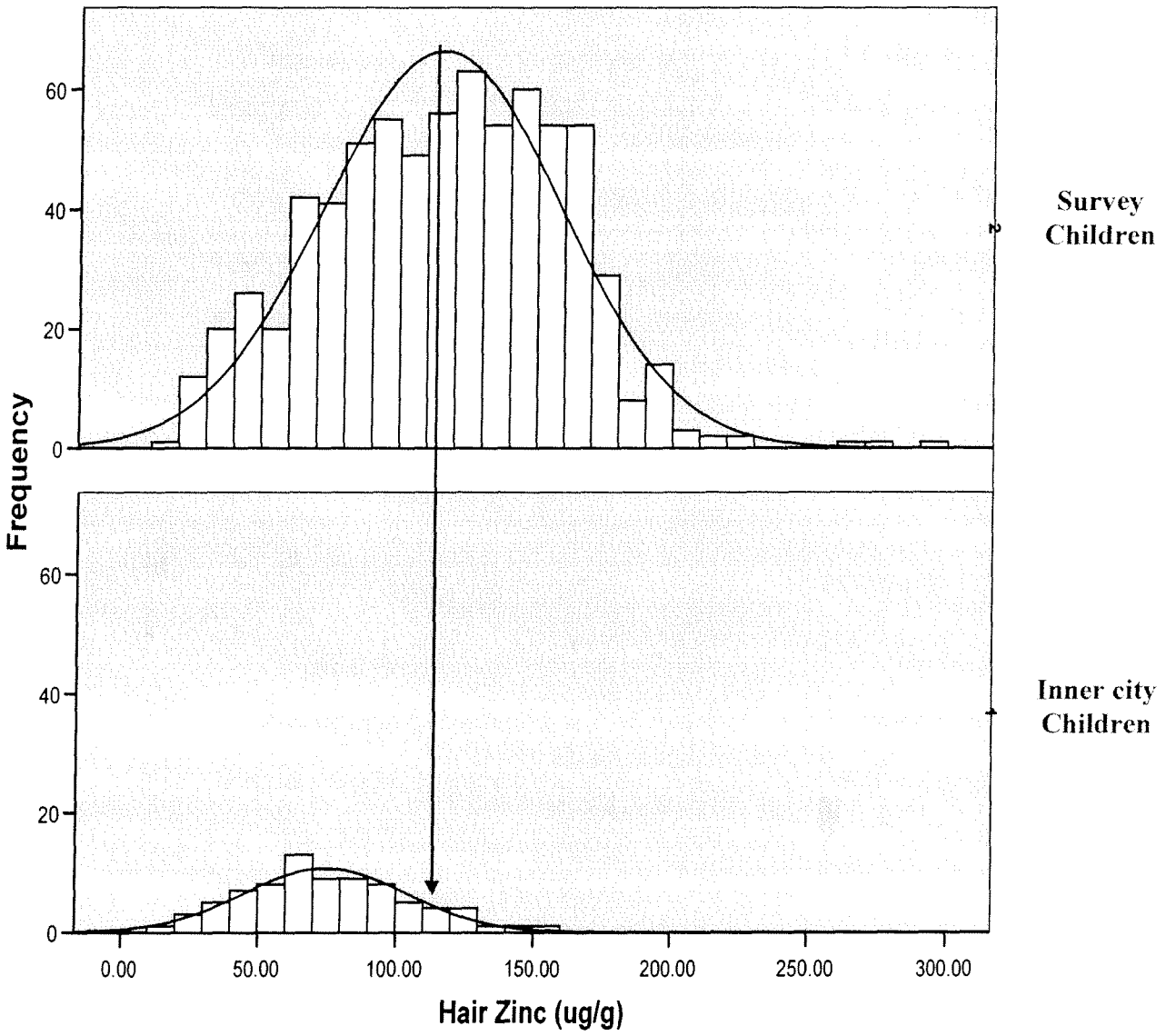
Hair zinc of the survey population was normally distributed with a mean (±SD) of 116±43 μg/g, while the hair zinc of the children varied within a wide range of 17-297 μg/g (**Figure III.II**).

Figure III.III is a schematic comparison of the hair zinc distribution of the survey with that of the inner city children (Chapter II of the thesis). The mean (±SD) of the survey population was significantly higher than that of the inner city children and corresponded to the 90^{th} percentile in the hair zinc distribution curve of the inner city children.



The shaded area depicts the fraction of population with hair zinc below cutoff value of 70 µg/g.

Figure III.II. Frequency distribution and descriptive statistics of the hair zinc level of the study population.



The arrow demonstrates the mean of survey corresponding to the 90th percentile of inner city children.

Figure III.III. Comparison of hair zinc distribution of the present study with that of low -income inner city preschoolers in Vancouver.

The overall prevalence of low hair zinc was 17% (121/719) (**Table III.III**). As displayed, there were inter-neighborhood differences in the mean (±SD) of hair zinc, and the occurrence of low hair zinc. However, due to the lack of statistical power, we refrained from any further analysis of data at the neighborhood level. There were also differences in the response rate of neighborhoods, in some cases as much as 10-fold. Some of the possible contributing factors to this fluctuation in response rate could be education level of the caregivers, centers' and parents' previous experience with research, and their previous knowledge of or exposure to HELP, one of the two key institutions involved in the survey.

Table III. III. Mean (±SD) hair zinc, and rate of low hair zinc in neighborhoods and their contribution to survey participants

Neighborhood	City hildren (%)*	Survey :hildren (%)	Response Rate (%)	Low hair zinc # (%)	Hair zinc$ (µg/g)
Survey (n=719)	100	100	30.3	121 (17)	116 ± 43
Grandview-Woodlands (n=36)	6.25	5.01	29	8 (22)	93 ± 32
Marpole (n=21)	3.30	2.92	80	7 (33)	98 ±44
Shaughnessy (n=10)	1.11	1.39	26	3(30)	99±44
Kensington Cedar Cottage (n=51)	10.96	7.09	32	13(26)	104±42
Victoria Fraser(n=49)	4.54	6.8	51	12 (25)	107 ±46
Downtown (n=18)	3.43	2.5	16	1 (6)	109 ± 36
Riley park (n=34)	4.93	4.73	68	10 (29)	111 ±62
Hastings Sunrise (n=46)	7.70	6.40	29	8 (17)	112 ± 32
Renfrew Collingwood (n=92)	10.14	12.8	30	14 (15)	112 ±40
Dunbar (n=30)	4.06	4.17	34	3 (10)	113 ± 36
Kerrisdale (n=11)	1.72	1.53	22	2 (18)	113 ±40
Kitsilano (n=41)	5.56	5.7	78	8 (20)	114 ±46
Fairview (n=23)	3.92	3.2	28	5 (22)	116 ± 41
West Point Grey (n=13)	2.09	2.09	19	2 (13)	117 ±42
West End (n=26)	2.69	3.62	38	4 (15)	117 ±46
Mount Pleasant (n=34)	5.17	4.72	27	6 (18)	121 ±37
University Lands (n=25)	2.11	3.48	35	2 (8)	122 ±36
South Cambie (n=10)	1.27	2.36	34	2 (20)	122 ±43
Oakridge (n=12)	1.54	1.67	8	1 (8)	123 ±40
Sunset (n=58)	9.38	8.1	22	5 (9)	128 ±44
Arbutus Ridge (n=8)	1.68	1.11	16	1 (12)	135 ± 47
Killarney (n=59)	4.99	8.21	39	3 (5)	138 ±37
Strathcona (n=10)	1.42	1.39	20	1 (10)	148 ±47

Extracted from census 2001 with kind assistance of the late Peter Schuab, Geographer, HELP.

III.3.c. Associations of the 5 main socio-demographic and socio-economic variables with hair zinc

Table III.IV summarizes the hair zinc level together with the occurrence of low hair zinc based on the 5 socio-demographic and socio-economic variables. As shown, there was a significant difference in the hair zinc of the children as well as the occurrence of low hair zinc based on age, ethnicity, and maternal education. Sex-based differences were also observed in the occurrence of low hair zinc. However, the mean hair zinc for the 2 sexes was comparable (118±39 and 113 ±47 μg/g for boys and girls, respectively, p=0.12).

The age-based differences observed in the hair zinc level and the occurrence of the low hair zinc will be elaborated upon later.

As **Table III.IV** indicates the rate of low hair zinc among different ethnicities varied within a range of 0% for East Indian children to 26% for Chinese children, while the Caucasian children had a rate in between (13%). The hair color was not associated with the zinc level (R^2 =0.00, p=0.22) and the hair zinc level of dark (black and brown) and light (blond) hair samples were statistically comparable (116±44 versus 112±40 μg/g for dark and light hair samples, respectively, p=0.22). There were no significant differences in the ratio of different age or/sex groups among these 3 ethnicities (Appendix III-27).

The hair zinc level as well as the occurrence of low hair zinc in children of the 2 lowest categories of maternal education was significantly different from that of the children in the 2 highest categories. The income level was not associated with the hair zinc level or the occurrence of low hair zinc among the survey children.

The results of the reliability test of the survey tool indicated substantial to perfect agreement (Saw & Ng, 2001) between the socio-economic and socio-demographic data collected at 2 points of time (R^2 varying within the range of 0.67-1.00, for income, maternal education, date of birth, ethnicity, and sex).

Table III.IV. A socio-demographic and socio-economic description of the study population and the hair zinc levels (X±SD) and the occurrence of low hair zinc based on these variables among them (n=719).

Variables	n	Hair Zinc (µg/g)			Low hair zinc*		
	# (%)	Mean ±SD	F	p	# (%)	χ^2	p
Sex							
Boys	360 (50)	118 ± 39			45 (12)^x		
			2.5	0.12		9.7	0.00
Girls	359 (50)	113 ± 47			76 (21)^y		
Missing	*0*						
Age							
< 4 years	305(43)	104 ± 45^a			77(25)^x		
			39	0.00		27	0.00
≥4 years	400(57)	124 ± 40^b			42(11)^y		
Missing	*14*						
Ethnicity							
Caucasian	305 (42)	116 ± 41^a			41(13)^x		
Chinese	223 (31)	104 ± 43^b			58 (26)^y		
			16	0.00		27	0.00
East Indian	54 (8)	154 ± 37^c			0 (0)^z		
Other ethnicities†	134 (19)	117 ± 41^a			21(16)^x		
Missing	*3*						
Maternal education							
<High school	53 (7)	96 ± 49^a			16(30)^x		
High school	72 (10)	104 ± 45^a			22(30)^x		
			7.0	0.00		21	0.00
Some college	170 (24)	120 ± 43^b			20(12)^y		
College or university degree	416 (59)	118 ± 41^b			61(15)^y		
Missing	*8*						
Income							
<20,000/year	67 (11)	113 ± 50			15 (22)		
20-40,000/year	120 (20)	113 ± 42			23 (19)		
			0.9	0.43		4.0	0.26
40-60,000/year	94 (16)	121 ± 46			14 (15)		
>60,000/year	310 (53)	118 ± 41			43 (14)		
Missing	*128*						

* Low hair zinc=Hair zinc<70µg/g.

†Other ethnicities: 32 Filipinos, 19 Japanese, 18 self-identified as Asians, 11 Vietnamese, 10 Latin, 7 Korean, 6 Aboriginal, 5 Middle Eastern and 26 miscellaneous.

a,b,c : Indicate statistically significant differences in the hair zinc level of different categories of a given variable, verified by one-way ANOVA followed by *Tukey's post hoc* analysis, if applicable.

x,y,z: Indicate statistically significant differences in the occurrence of low hair zinc in different categories of a given variable, verified by adjusted *Chi square* analysis, if applicable.

There were 705 children who had both demographic information (full birth date and sex) and biochemical data available. The hair zinc mean±SD was 115±43 μg/g. The scatter plot of children's hair zinc against their age indicated a trend of increasing hair zinc with increasing age (**Figure III.IV**). The sample collection time (March-June) was not associated with the hair zinc level (R^2 =0.01, p=0.07) (no trend was observed between the hair zinc level and the month of year that sample was collected).

To explore these differences further, the children's age was categorized in increments of 12 months. We observed a significant increase in hair zinc upon completion of the fourth year of life (from 107±42 μg/g for children in 36-<48 months category to 125±40 μg/g, for children in 48-<60 months category) and remaining high thereafter (**Table III.V**). This trend applied to both sexes, individually and collectively.

The sex-by-age interaction on hair zinc level was not significant as verified by ANOVA (R^2 =0.05, P=0.31).

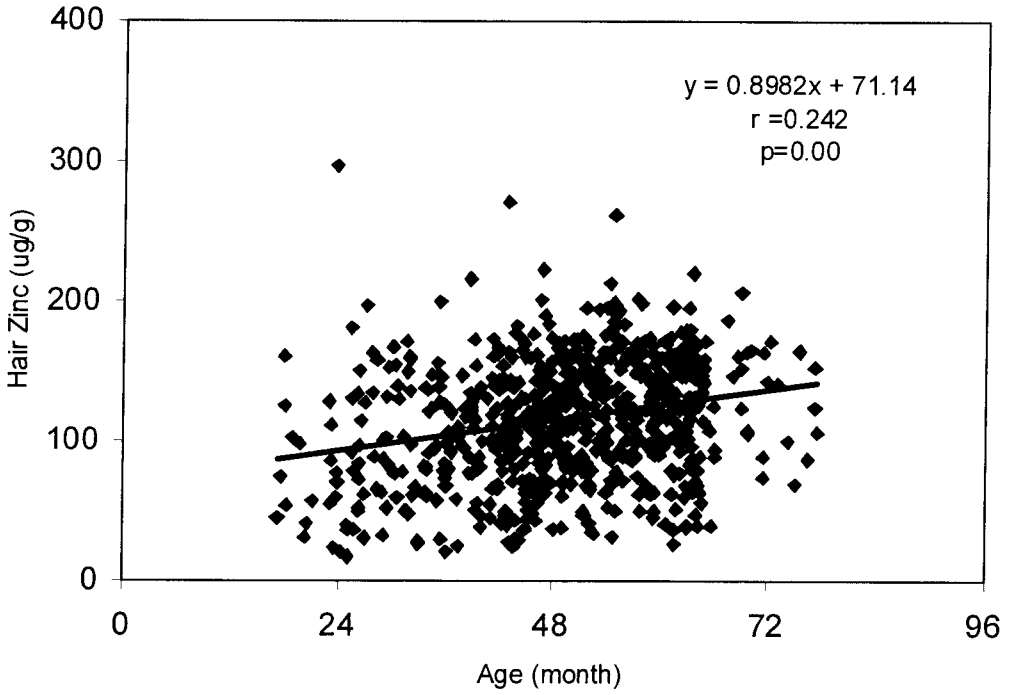


Figure III. IV. Scatter plot of hair zinc against age of the children (n=705).

Table III.V. Influence of age on hair zinc (μg/g) of boys and girls of the survey (n=705).

	All*		Boys		Girls	
Age (months)	n	Hair zinc (μg/g)	N	Hair zinc (μg/g)	n	Hair zinc (μg/g)
24-<36	103	98±49^{x}	43	105±46^{x}	60	93±52^{x}
36-<48	203	107±42^{x}	106	107±39^{x}	97	107±46^{xy}
48- <60	234	125±40^{y}	116	125±37^{y}	118	124±43^{z}
60-<72	165	123±40^{y}	88	127±34^{y}	77	119±45^{zy}
All	705	115±43	353	118±39	352	113±47

*In total 705 children had both date of birth and sex recorded and hair zinc data available. There were no sex-based differences in hair zinc level overall or by age group.

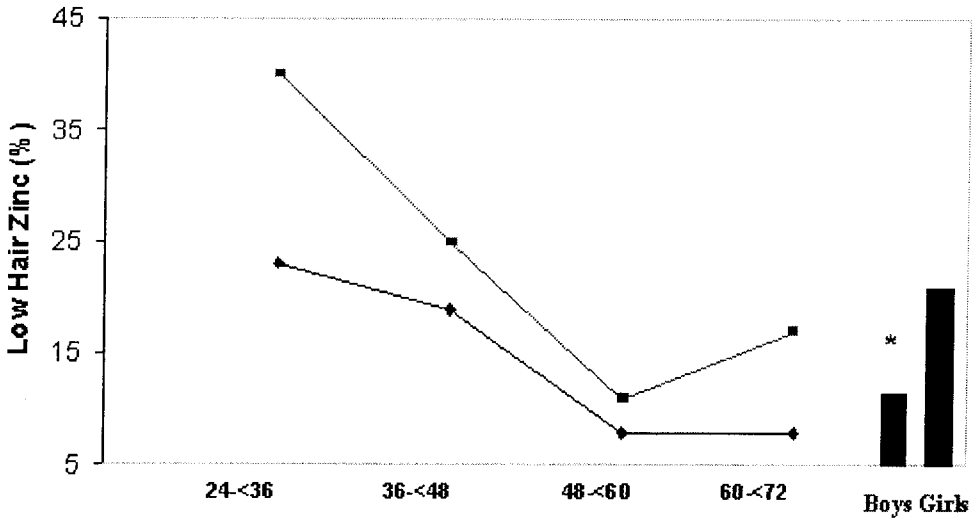
Superscripts x, y, z... are used to indicate statistically significant differences between the consecutive values in a given column.

Values in a column not having a common superscript are statistically different (P<0.05).

In agreement with the changes in the mean of hair zinc with age, for both sexes, a significant decrease was observed in the occurrence of low hair zinc with age. In both sexes, a significant decrease was observed in going from children in the age category of 36-<48 to the category of 48-<60 months of age, as verified by *Chi square* analyses (for boys: 19% versus 8%, χ^2 =5.2, df=1, p=0.02 and for girls: 25% versus 11%, χ^2 =7.0, df=1,p=0.01) (**Figure III.V**). The occurrence of low hair zinc appeared to plateau after completion of the 4th year of life in both sexes.

In addition, as represented by the 2 bars in **Figure III.V**, the overall rate of low hair zinc was significantly higher in girls than in boys (21% and 12%, respectively, χ^2 =8.6, df=1, p=0.00). The sex-by-age interaction on occurrence of low hair zinc was not significant as verified by ANOVA (R^2 =0.05, P=0.31).

The risk analysis of having low hair zinc for the 2 sexes indicated a significantly higher risk for girls versus boys (OR=1.9, 95%CI [1.2, 2.8]) and for children <4years versus those ≥4 years (OR=2.9, 95%CI [1.9, 4.3]).



* Indicates significantly different occurrence of low hair zinc for two sexes (χ^2=8.6, df=1, p=0.00)

Figure III.V. Comparison of the occurrence of low hair zinc in different age categories of girls (the upper line) and boys (the lower line).

The associations between hair zinc level and each of the 5 main demographic or socioeconomic variables (age, sex, ethnicity, maternal education, and family income) were assessed through univariate linear regression analyses. **Table III.VI** summarizes the results of these analyses. As shown, age, ethnicity and maternal education indicated significant associations with the children's hair zinc level. These associations remained significant even when all 5 socio-demographic and socio-economic variables were inserted in the regression model simultaneously. The overall model accounted for 12% of the variability in hair zinc (R^2=0.12, p=0.00).

Table III.VI. Relationship between hair zinc and the 5 main socio-demographic and socio-economic factors in the study population (n=719).

Variable	B* (95% CI)	R^2	*P*
Age (months)	0.9 (0.6, 1.2)	0.06	0.00
Sex	-5.1 (-11, 1.3)	0.00	0.12
Ethnicity		0.08	0.00
Caucasian	116 (112,121)		
Chinese	104 (99-110)		
East Indian	154 (143-165)		
Other ethnicities	117 (110-124)		
Maternal education		0.02	0.00
<High school	96 (85, 108)		
High school	104 (94, 114)		
Some college	120 (113, 126)		
Held a degree	118 (114, 123)		
Family income		0.01	0.42
<20,000/year	113 (102, 123)		
20-40,000/year	113 (105, 121)		
40-60,000/year	121 (112, 130)		
>60,000/year	118 (113, 123)		

*Bs are unstandardized coefficients.

When all 5 variables were inserted in the regression model, simultaneously; age, ethnicity and maternal education remained as significant variables of this model. For the overall model R^2 =0.12, P=0.00.

III.3.d. Association of some family characteristics with hair zinc of the children

Table III. VII presents data on some family characteristics and the hair zinc level and occurrence of low hair zinc based on these characteristics. About 14% of the survey families were food insecure (as assessed by a "yes" response to any of the 3 questions).

As the table indicates, most of the fathers (59%) were university graduates while 8% had not completed high school. About 72% of the survey families were 2 adult households, while 10% of the survey children lived in a lone-parent household and 18% in households with more than 2 adults. The majority of survey families had 2-4 members. About 24% of survey families had between 5-7 members, and a small fraction (2%) were larger families having $\geq$8 members. Significant differences in the hair zinc level were apparent based on "being in a single parent household", "number of adults at home" and "family size". Differences in the occurrence of low hair zinc were observed based on the number of adults at home and being in a single parent household, only.

The results of the reliability test of the survey tool for collecting data on family characteristics indicated a good agreement between the 2 sets of data collected by the questionnaire at 2 points of time (R^2 varying between the range of 0.64-0.83).

Table III.VII. Description of some family characteristics as well as the hair zinc level and occurrence of low hair zinc based on these characteristics (n=719).

Variables	n # (%)	Hair Zinc (µg/g) Mean ±SD	F	p	Low hair zinc* # (%)	χ^2	p
Food Insecurity							
Food secure	575(86)	114±44	0.1	0.80	94(16)	1.6	0.20
Food insecure	92(14)	115±42			20(22)		
Missing	*52*						
Father's education							
<High school	52(8)	104 ± 45			12(23)		
High school	78(11)	115 ± 47			17(22)		
Some college	157(22)	117 ± 43	1.4	0.24	22(14)	3.7	0.30
Holds degree(s)	418(59)	116 ± 43			69(16)		
Missing	*14*						
Lone parent household							
Yes	70(10)	103±45[a]			19(27)[x]		
No	649(90)	117±43[b]	6.0	0.02	102(16)[y]	5.9	0.02
Missing	*0*						
Number of adults at home							
1 adult	70(10)	103±43[a]			19(27)[x]		
2 adults	522(72)	114±43[a]			92(18)[y]		
3 –4 adults	108(15)	128±37[b]	5.5	0.00	5(5)[z]	18	0.00
5-6 adults	19(3)	124±64[ab]			5(26)[xy]		
Missing	*0*						
Family size							
2-4 person	528(74)	113±42[a]			92(17)		
5-7 person	172(24)	120±44[a]	6.5	0.00	27(17)	1.1	0.59
≥8 person	13(2)	152±56[b]			1(13)		
Missing	*6*						

*Low hair zinc=Hair zinc≤70µg/g.

a,b,c : Indicate statistically significant differences in the hair zinc level of different categories of a given variable, verified by one-way ANOVA followed by *Tukey's post hoc* analysis, if applicable.

x,y,z: Indicate statistically significant differences in the occurrence of low hair zinc in different categories of a given variable, verified by adjusted *Chi square* analysis, if applicable.

To explore the associations of family characteristics with the children's hair zinc, linear regression analyses were carried out between the hair zinc level and these variables. As **Table III.VIII** shows, "number of adults at home" "being in a lone parent household" and "family size" had significant associations with hair zinc. These variables accounted for a very small proportion of variance (accounting for 3%, 2% and 3% of the variance, respectively). When the adjustments for the 5 main socio-demographic and socio-economic factors were carried out, "being in a lone parent household" no longer indicated a significant association with hair zinc.

Educational attainments of the father and food security of the family were not associated with the children's hair zinc.

Table III.VIII.. Relationship of hair zinc with some family characteristics.

	Unadjusted			Adjusted*	
Variables	B†(95% CI)	R^2	p	R^{2**}	P
Father's education	Mean (95% CI)	0.0	0.24	0.12	0.12
<High school	104 (92, 115)				
High school	115 (106, 125)				
Some college	117 (110, 124)				
Held a degree	116 (112, 120)				
Food security	1.2 (-8.1, 11)	0.00	0.80	0.10	0.90
Being in a lone parent household	-13 (-24, -2.6)	0.01	0.01	0.12	0.07
Number of adults at home†† (per each adult)	7.3 (3.6, 11)	0.02	0.00	0.13	0.05
Family size†† (per each person in the family)	4.3 (1.9, 6.8)	0.02	0.00	0.13	0.02

* Adjusted regression analyses have been carried out using age, sex, ethnicity, maternal education and family income as covariates.

†Bs are unstandardized coefficients.

**R^2 represent the R^2 s of adjusting covariates (R^2 =0.12) combined with R^2 of the new variable in the adjusted regression analyses.

††Two variables of "number of adults at home" and "family size" were the only continuous variables. The 2 variables of food security and being in a lone-parent household were categorical data in a binary form (yes/No).

III.3.e. Association of eating behaviors with hair zinc of the children

We collected data on the consumption frequency of some main food groups. We also explored the differences in the intake frequency of these food groups based on hair zinc status (**Table IX.A**). Consumption frequency of dairy, milk, cereals and grains, and whole grains of low hair zinc children were significantly higher than that of normal hair zinc children.

We collected data on the caregivers' perception of the children's eating behavior. **Table III.IX.B.** displays the hair zinc level and occurrence of low hair zinc based on them. Our data indicated differences in hair zinc as well as the occurrence of low hair zinc based on being described as "eating unhealthy" and "not eating enough".

To explore this further, we examined the associations of hair zinc with these variables and with the intake frequency of some food groups through linear regression analyses (**Table III.X**). Our data indicated inverse associations between hair zinc and the intake frequency of "dairy" and "milk". In addition, being described as "eating unhealthy" and "not eating enough" had significant negative associations with hair zinc as well.

In general, there was a moderate to substantial agreement (Saw & Ng, 2001) between the 2 sets of data collected on the consumption of food groups with R^2 varying within the range of 0.67-0.77.

Table III. IXA. Comparison of the daily consumption frequency of some main food groups of low hair zinc and normal hair zinc children (n=719)

Food groups	Low hair zinc children* (n=121)	Normal hair zinc children (n=598)	P
Meat, fish, and poultry	1.5±1.1	1.4 ± 1.1	0.42
Dairy	3.7±1.5	3.3±1.6	0.01
Milk	2.3±1.2	1.9±1.1	0.00
Cereals and grains	3.6±1.6	3.1±1.4	0.01
Whole grains	1.7±1.6	1.5±1.2	0.02
Fluids	3.3±1.5	3.4±1.4	0.45

* Values indicate number of servings/day.

Table III.IX.B. Description of some food behaviors and the hair zinc level and occurrence of low hair zinc based on these behaviors (n=719).

	N	Hair Zinc (µg/g)			Low hair zinc*		
	# (%)	Mean ±SD	F	p	# (%)	χ^2	p
Concerned about child's eating;							
Yes	360(51)	114 ± 44	1.2	0.28	66(18)	1.2	0.28
No	352(49)	117 ± 42			55(15)		
Described as eating unhealthy							
Yes	79(11)	103 ±44[a]	7.9	0.01	19(24)[x]	3.3	0.01
No	640(89)	117 ± 43 [b]			102(16)[y]		
Described as picky eater							
Yes	223(32)	115 ± 43	0.1	0.73	38(13)	0.1	0.08
No	486(68)	116 ± 43			83(15)		
Described as not eating enough							
Yes	157(22)	109 ± 48 [a]	5.1	0.02	38(24)[x]	7.8	0.01
No	562(88)	117 ± 42 [b]			83(15)[y]		

*Low hair zinc=Hair zinc<70µg/g.

a,b,c : Indicate statistically significant differences in the hair zinc level of different categories of a given variable, verified by one-way ANOVA followed by *Tukey's* post hoc analysis, if applicable.

x,y,z: Indicate statistically significant differences in the occurrence of low hair zinc in different categories of a given variable, verified by adjusted *Chi square* analysis, if applicable.

Table III.X. Regression analyses of hair zinc (μg /g) with some eating behavior (n=705).

	Unadjusted			Adjusted*	
Food groups	B† (95% CI)	R^2	P	R^{2**}	p
Caregiver being concerned about child's eating	3.5(-2.8, 9.8)	0.00	0.28	0.12	0.70
Being described as eating unhealthy	14(4.3, 24)	0.01	0.01	0.13	0.04
Being described as picky eater	1.2(-5.6, 8.0)	0.00	0.73	0.12	0.69
Being described as not eating enough	8.8(1.1, 16)	0.01	0.02	0.13	0.04
Consumption frequency of meat, fish, and poultry	-1.6(-4.6, 1.3)	0.00	0.29	0.12	0.67
Consumption frequency of; Dairy	-3.6(-5.6, -1.6)	0.02	0.00	0.14	0.00
Milk	-7.2(-10, -4.5)	0.04	0.00	0.14	0.00
Consumption frequency of; Cereals& Grains	-2.9(-5.0, -0.8)	0.01	0.01	0.12	0.06
Whole grains & cereals	-2.0(-4.5, 0.4)	0.00	0.10	0.12	0.08

* Adjustments have been carried out using age, sex, ethnicity, maternal education and family income as covariates.

†Bs are unstandardized coefficients

**R^2 represent the R^2s of adjusting covariates (R^2=0.12) combined with R^2 of the new variable in the analysis.

III.3.f. Associations of breast-feeding, parental perception of children's health status and taking supplements containing iron with hair zinc of the children

Table III.XI.A displays descriptive data on the children's health and health behaviors (as perceived by the caregivers) and the hair zinc level and occurrence of low hair zinc based on these behaviors. As shown, children described as being frequently sick and having poor health had significantly lower hair zinc level and higher occurrence of low hair zinc than their counterparts.

No differences in the hair zinc level or the occurrence of low hair zinc was apparent based on the breast-feeding pattern.

Taking supplement(s) was common among our survey children. About 37% (240/671) of parents reported giving $\geq$1 supplement(s) to their children (Appendix III-31). About 29% (197/671) of the survey children were taking supplements containing iron. The children taking supplements containing iron not only had significantly lower hair zinc; they also revealed a higher occurrence of low hair zinc.

Since our data on scores of attentional focusing and activity and the anthropometric Z scores were all in the form of continuous data, we could not include these variables in **Table XI.A.** However, we compared the scores of the 2 behavior domains of CBQ and the anthropometric Z scores of the 2 groups of children with low and normal hair zinc level through one-way analyses of variance, ANOVA (**Table III.XI.B**). Our results indicated a significantly lower score of activity level for low hair zinc children.

Table III.XI.A Description of some health behaviors and the hair zinc level and occurrence of low hair zinc based on these behaviors.

	n	Hair Zinc (μg/g)			Low hair zinc*		
	# (%)	Mean ±SD	F	p	# (%)	χ^2	p
Described as frequently sick							
Yes	32(5)	95 ± 50 [a]	7.5	0.01	12(38)[x]	10	0.01
No	686(95)	117 ± 43 [b]			109(16)[y]		
Health status described poor							
Yes	57(8)	103 ± 53[a]	5.6	0.02	18(32)[x]	9.6	0.00
No	662(92)	117 ± 42[b]			103(16)[y]		
Breastfeeding							
Not fed	79	111±42			13(17)		
Fed≤6 months	409	117 ± 45	1.0	0.38	68(17)	0.1	0.95
Fed>6 months	230	114 ± 42			40(17)		
Taking supplements containing iron							
Yes	197(29)	112 ± 44[a]	4.1	0.04	39(20)[x]	3.9	0.03
No	474(71)	119 ± 42[b]			65(14)[y]		

*Low hair zinc=Hair zinc<70μg/g.

a,b,c : are used to indicate statistically significant differences in the hair zinc level of different subcategories of a variable, as verified by one way ANOVA followed by *Tukey's post hoc* analysis, if applicable.

x,y,z: are used to indicate statistically significant differences in the occurrence of low hair zinc in different subcategories of a variable, as verified by *Chi square* analysis.

Table III.XIB. Comparison of the scores of attentional focusing and activity level of low hair zinc and normal hair zinc children=719

Variable	Low hair zinc* children (n=121)	Normal hair zinc children (n=598)	P
CBQ behavioral domains (n=121, 598)			
Attentional focusing	28±6	29±6	0.26
Activity level	30±7	33±6	0.00
Anthropometric Z scpres (n=110, 554)			
HAZ	-0.03±0.9	0.08±0.9	0.30
WAZ	0.21±1.5	0.24±1.1	0.27
WHZ	0.20±2.0	0.26±1.3	0.65

CBQ: Child Behavior Questionnaire

* Low hair zinc= Hair zinc <70µg/g.

HAZ: Height-for-age Z scores

WAZ: Weight-for-age Z scores

WHZ: Weight-for-height Z scores

We explored associations between the children's hair zinc and all of the above-mentioned variables. We observed that being described as "having poor health" and "being frequently sick" as well as "taking supplements containing iron" had negative associations with the children's hair zinc. (**Table III.XII**). When the analysis was adjusted for socio-demographic variables, however, only the association of "taking supplements containing iron" with hair zinc remained significant.

Our data also indicated that the scores of "activity level", but not the "attentional focusing", associated with hair zinc in a positive manner.

The internal consistency of the short CBQ, verified by Dr. Rothbart's research laboratory was á = 0.71 and 0.81 for activity level and attentional focusing, respectively (Putnam, personal email communications), while the results of our own reliability test indicated substantial agreements between the data collected on the children's behavior at 2 points of time (R^2 =0.68 and 0.72, for" activity level" and "attentional focusing", respectively).

There were also substantial agreements between the 2 sets of data collected on the parents' perception of their child's health status with R^2 varying within the range 0.62-0.85.

When we explored the existence of any possible association between "taking supplements containing iron" and eating behaviors (Appendix III-28) of the children we noticed a significant association between "taking supplements containing iron" and 3 variables of "caregiver being concerned about child's eating", being described as "not eating enough" and being described as "picky eater" among which being described as "not eating enough" had already been shown associating significantly with hair zinc. Risk

analysis indicated that the children described as "not eating enough" were 40% more likely to be given supplements containing iron (χ^2 =2.9, OR=1.4, 95%CI=[1.1, 2.1).

Table III.XII. Regression analyses of hair zinc (μg /g) with breast-feeding, and some health-related and behavior-related variables.

	Unadjusted			Adjusted*	
Variables	B† (95% CI)	R^2	p	R^{2**}	p
Described as frequently sick	21(6.1, 37)	0.01	0.01	0.12	0.07
Described as having poor health	-14(-26, -2.4)	0.01	0.02	0.12	0.08
Breastfeeding:	Mean (95% CI)	0.00	0.38	0.12	0.06
Not fed	111(101, 121)				
Fed≤6 months	117(113, 121)				
Fed>6 months	114(108, 119)				
Scores of CBQ:					
Attentional focusing	88 (85, 92)	0.00	0.44	0.12	0.29
Activity level	3.5 (3.4, 3.6)	0.02	0.00	0.13	0.05
Taking supplements containing iron	119(115, 123)	0.01	0.04	0.13	0.01
Anthropometric Z scores:					
HAZ	-0.5(-3.9, 2.9)	0.00	0.79	0.09	0.92
WAZ	0.8(-1.7, 3.3)	0.00	0.54	0.09	0.19
WHZ	0.6(-1.7, 3.3)	0.00	0.63	0.09	0.27

* Adjustments have been carried out using age, sex, ethnicity, maternal education and family income as covariates.

†Bs are unstandardized co-efficients.

"R^2 represent the R^2 s of adjusting covariates (R^2 =0.12) combined with R^2 of the new variable in the analysis.

Data on anthropometric Z scores and scores of attentional focusing and activity level were in the form of continuous data.

CBQ denotes Child Behavior Questionnaire, while HAZ, WAZ, and WHZ denote Height-for-age Z scores, Weight-for-age Z scores, and Weight-for-height Z scores, respectively.

III.3.g. Contribution of all variables associating significantly with hair zinc, to the overall variability in hair zinc

We set up a stepwise linear regression analysis with all of the previously mentioned variables that had shown a significant association with hair zinc. The inclusion criteria for entering this analysis was having $P \leq 0.05$ in adjusted regression analyses of that variable (independent variable) with hair zinc (dependent variable).

The result of this analysis revealed that age, maternal education, family size, frequency of daily milk consumption, being described as "eating unhealthy", scores of activity level, and taking supplements containing iron were the significant predictors of hair zinc. The model produced by these variables could account for about 15% of the variability in hair zinc (**Table III.XIII**).

In addition to these variables, others entered in the stepwise regression analysis were ethnicity, "number of adults at home", being described as "not eating enough", and "frequency of dairy consumption". Nevertheless, these variables did not seem to be significant independent predictors of hair zinc level, as they were eliminated from the final model produced.

One likely reason for the elimination of "number of adults at home" from the final model could be its strong correlation with "family size" ($r = 0.51$, $p = 0.00$). Likewise the strong correlation of "frequency of dairy consumption" and the "frequency of milk consumption" ($r = 0.62$, $p = 0.00$) could be a possible reason for elimination of the "frequency of dairy consumption" from the final model.

Table III.XIII. Stepwise linear regression analyses of hair zinc and the variables significantly associating with it.

Predictors in the model	B*	SE	β	R^2 change	P
Constant	*-27*	*18*	---	-----	*0.13*
Age	0.8	0.1	0.2	0.05	0.00
Maternal education	6.8	1.8	0.2	0.03	0.00
Family size	4.7	1.2	0.1	0.02	0.00
Frequency of daily milk consumption	-4.6	1.4	-0.1	0.02	0.00
Being described as eating unhealthy	-16	5.3	0.1	0.01	0.01
Score of activity level	0.8	0.3	0.1	0.01	0.00
Taking supplements containing iron	-8.5	3.5	0.1	0.01	0.02

*B indicates unstandardized coefficient.

For the whole model; R^2=0.15, P=0.00.

R^2 change: Change in the R^2 of the model as the result of the insertion of that variable into the model

Other variables that were inserted in the analysis but did not become a component of the final model produced were ethnicity, "number of adults at home, being described as "not eating enough", "frequency of daily dairy consumption".

III.3.h. Exploring the usefulness of the model/questionnaire produced by these components in predicting hair zinc status

To produce a model that may have usefulness in predicting the hair zinc status of children, we set up a forward logistic regression analysis of hair zinc (binary outcome) with all of the variables significantly associating with low hair zinc status. The variables entering the analysis were: sex, age, ethnicity, maternal education, number of adults at home, being in a lone-parent household, described as "not eating enough", described as "eating unhealthy", consumption frequency of cereals, consumption frequency of whole grains, consumption frequency of dairy, consumption frequency of milk, scores of activity level, described as "having poor health", described as "being frequently sick" and taking supplements containing iron. **Table III.XIV** displays the results of this logistic regression analysis. In this model, sex, age, maternal education, number of adults at home, described as "not eating enough", consumption frequency of milk, scores of acitivity level, described as "being frequently sick", and taking supplements containing iron appeared as statistically significant predictors of low hair zinc.

To test the utility of this model in predicting the hair zinc status we constructed the classification table for classifying the study children based on their hair zinc status, when using (**Table III.XV.B**) and not using (**Tables III.XV.A**) this model. As shown, the model generates only a very small improvement in the overall percentage of children classified correctly (84.4% when not using the model versus 85.5% when using the model) and the model could only detect 14 of the 99 low hair zinc children. This finding i.e. the lack of the model's practical efficacy is further supported in the Receiver Operating Characteristic (ROC) curve for the fitted logistic regression model. We

conducted ROC analysis as a way of examining the detection power of the model. As **Figure III.IV** presents, the model produced did not have much practical utility as the area under the curve, AUC, was only 0.72. This area measures discrimination, that is, the ability of the model to correctly classify those with and without the disease. The value of 0.72 indicates that such model has only fair discrimination ability. For clinical purposes and screening tools, a model with AUC greater than 0.80 is desirable.

Table III.XIV. Logistic regression analysis of all factors associated significantly with low hair zinc (n=719).

Variables	B*	S.E	β	P
Constant	*0.1*	*1.0*	*-2.9*	*0.01*
Sex	1.7	0.2	0.5	0.04
Age	1.1	0.1	0.1	0.00
Maternal education	1.3	0.1	0.3	0.01
Number of adults at home	1.4	0.2	0.3	0.04
Described as "not eating enough"	0.6	0.3	-0.6	0.04
Consumption frequency of milk	0.8	0.1	-0.2	0.03
Score of activity level	1.1	0.0	0.0	0.02
Described as "being frequently sick"	0.3	0.5	-1.1	0.03
Taking supplements containing iron	0.6	0.3	-0.6	0.02

*B_S are standardized coefficients.

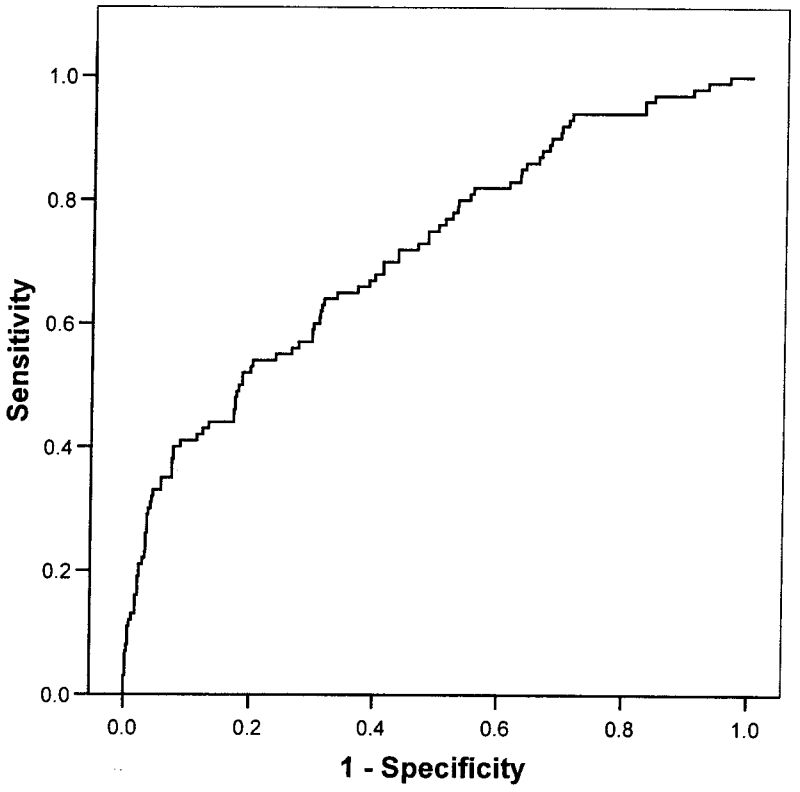
Other variables that were inserted in the analysis but did not become a component of the final model were: ethnicity, being in a lone-parent household, described as "eating unhealthy", consumption frequency of cereals, consumption frequency of whole grains, consumption frequency of dairy, and described as "having poor health."

Table III.XV.A. Classification of the survey children based on their hair zinc status without the use of the model produced by logistic regression analysis.

Predicted		Observed		
		Hair zinc status		Percentage Correct
		Low	Normal	
Hair zinc status	Low	0	0	
	Normal	100	541	
		0.00	100	84.4

Table III.XV.B. Classification of the survey children based on their hair zinc status with the use of the model produced by logistic regression analysis.

Predicted		Observed		
		Hair zinc status		Percentage Correct
		Low	Normal	
Hair zinc status	Low	16	9	
	Normal	84	532	
Overall Percentage		16	98.3	85.5



Predictive probability (Area under the curve)=0.72.

Figure III.VI. Receiver Operating Characteristic (ROC) curve of the final regression model to detect children with low hair zinc.

III.4. DISCUSSION

Our study explored the hair zinc status of Vancouver preschoolers by taking a 3% sample of this age group (n=719), with representation of the main ethnicities comparable to percentages reported for the city (Statistics Canada, 2001) (**Table III.II**). The study findings indicated that the average hair zinc in this population, together with the occurrence of low hair zinc (<70 μg/g) (**Figure III.II**), were comparable with the only available report for a group of apparently healthy Canadian preschoolers (Smit VanderKooy & Gibson, 1987) as well as with reports on children of other industrialized societies (Hambidge et al, 1976, and Lombeck et al, 1988).

Overall, our analyses indicated sex, age, maternal education, "the number of adults at home", being described as "not eating enough", consumption frequency of milk, scores of activity level, being described as "frequently sick", and taking supplements containing iron as the significant predictors of hair zinc status in the final logistic regression model produced (**Table III.XIV**). However, the predictive power of this model (and consequently any screening questionnaire constructed based on this model) was not strong enough to be of any practical use. As a result, the primary goal in this study i.e. constructing and validating a small and simple screening questionnaire could not be achieved. However, in working through the process that built up the final logistic regression model, we identified a number of factors in the children, their family environment and social setting that were significantly associated with hair zinc and hair zinc status and may potentially affect their zinc status.

We observed that the two sexes had a comparable hair zinc mean while having a significantly different occurrence of low hair zinc **(Table III.IV).** The occurrence of low

hair zinc among girls was so much higher that "being female" conferred a 90% higher risk on children. We could not explain this finding given that the majority of evidence in the zinc literature indicates male children as the more vulnerable sex to low hair zinc (Lambeck et al, 1988, Smit Vanderkooy & Gibson, 1987) and having a better response to zinc supplementation (Walravens & Hambidge, 1976, Hambidge et al., 1979, Heinersdorff & Tylor, 1979, Hambidge et al, 1979 and Walravens et al, 1983). The literature speculates on the higher physiological need in male as the underlying reason for these observed differences. However, in light of our finding and the findings of Zachiwieja and co-workers (1995) that have shown a higher hair zinc for boys, it is reasonable to believe that there may be other factors besides physiology at work here, factors that, unlike the physiology of male humans, differ for male children of different environments/studies. One such factor could be the sex-based differences in food intake that has been documented in some Canadian studies (Smit Vanderkooy & Gibson, 1987, Nakano, et al, 2005, and Kuhnlein et al, 2007) and in studies of other industrial societies (Ganjii, Hampl, & Betts, 2003, Glynn et al, 2005, and Touvier et al, 2006) as well. In our study, due to the lack of quantitative dietary data we could not verify the existence of such differences as an explanation of the observed differences in the hair zinc status of our survey boys and girls.

Our data also indicated an increase in hair zinc as the age of the children increased (**Figure III.IV**). When we categorized children into finer age categories (12 month increments) we noticed that the children's hair zinc increased from 107 to 125 μg/g at age 4 regardless of sex (**Table III.V**). Accordingly, again regardless of sex, the occurrence of low hair zinc declined with advancing age (**Figure III.V).** Zinc literature

indicates a change in hair zinc concentration with age (Klevay, 1970, Hambidge et al, 1972, Lombeck et al, 1988, Van Wouwe, 1995 and Meng, 1998). While there seems to be consensus on the decrease in hair zinc during infancy and then an increase during post-infancy, information on the nadir and zenith of this change is inconsistent. A result similar to ours has been reported in the cross-sectional study of Hambidge and co-workers (1972), which showed that the hair zinc for neonates was closely comparable with that of young adults, while for infants it was lower than that of young adults. These levels remained low until the children were 4 years of age, and then rose, whereas others (Klevay, 1970, Van Wouwe, 1995 and Sakai et al, 2000) have indicated a different age for this rise in hair zinc. These studies, however, either did not look at the preschool years individually, thus keeping all 0-5 years in one group (Klevay, 1970 and Van Wouwe, 1995), or had only a small sample size for each age segment (Sakai et al, 2000). Our study is the first sufficiently large study of this age group that has been able to sample an adequate number of children from each age cluster (2, 3, 4, 5, years old) and has explored their trends and differences. This large sample size, coupled with a superior method of hair zinc analysis ICP-MS, which has improved dramatically the measurement of trace elements in hair (Bass et al, 2001, and Miekeley et al, 1998), confers a high reliability on our results.

This age-based difference in hair zinc deserves further investigation. If this low hair zinc has no functional/clinical ramification, it may be a normal physiological level. If so, it may necessitate the validation of the commonly used hair zinc cutoff (<70 μg/g) in younger preschoolers and may challenge its usefulness as a biomarker of MZD among younger preschoolers. Hambidge and co-workers (1972), in their study of preschoolers,

documented associations between hair zinc and some clinical signs of zinc deficiency such as anorexia and poor growth among apparently healthy younger children (<4 years of age). However, this association was evident for children who had a hair zinc level below 30 μg/g, and was not apparent in children (below the age of 4) whose hair zinc was 30-70 μg/g. This observation clearly undermines the appropriateness of using the cutoff of 70 μg/g, for younger children. However, since that study (perhaps owing to the absence of any further research on this issue) researchers continue to include these 2 age groups in the same study using the same cutoff for both younger and older children (Hambidge et al, 1976, Walravens, Krebs & Hambidge, 1983, Lombeck et al 1988 and Van Wouwe, 1995).

In our study, we did not observe any differences in the indices of growth or nutrition between younger low hair zinc children (based on the cutoff of 30 μg/g) and their counterparts with normal hair zinc (Appendix III-29). Further, the lack of any associations between hair zinc status and the indices of growth and nutrition (the anthropometric Z scores) persisted, even when the hair zinc status of the younger children was assigned using the cutoff of 30 μg/g. Clearly some additional studies are warranted to further investigate this important issue.

Our survey also indicated differences in the hair zinc level as well as the occurrence of low hair zinc among children of different ethnicities (**Table III.IV**). The differences in the rate of low hair zinc varied largely, East Indian and Chinese children being at the lower and upper end of this range, respectively, this in light of the fact that there were no significant differences in the distribution of vulnerable sex and age group among the three main ethnic groups of the survey (Appendix III-27).

Caucasian children were positioned in the middle with a 13% occurrence of low hair zinc, significantly lower than the rate of 24% reported for the white middle income Canadian preschoolers of Smit Vanderkooy & Gibson (1987). Chinese survey children, on the other hand, had the lowest mean of hair zinc and the highest occurrence of low hair zinc, which resulted in more than a quarter of them showing hair zinc below the cutoff. Zinc deficiency has been a concern among Chinese in earlier years (Eggleton, 1940) and more recently too (Chen, 1985 and Sheng et al, 2006). Our dietary data (Appendix III-30) did not indicate any differences among ethnicities that might explain this high occurrence of low hair zinc among our Chinese children. In addition, our data measured the frequency of intake and not the total quantity of daily intake. For these reasons we were not able to link our biochemical data (the hair zinc measurements) to the dietary data. However, the general nature of the Chinese diet (a cereal and plant-based diet with small amounts of meat) may help to explain this finding. The 2 food groups "cereals and grains" and "fruits and vegetables" are often sources of zinc inhibitors such as phytate and fiber. Therefore, the status of zinc in the Chinese may be sub-optimal, due not only to a low supply of zinc, but also inhibition of whatever zinc is present in the diet. Many Chinese immigrants do not stop eating Chinese food, but rather layer Western foods and eating behavior over their stable Chinese pattern (Satia et al, 2000 and Roville-Sausse, 2005). In addition, a comparison of the mean hair zinc of our Chinese children with the previously reported values for Chinese children living in China (104±43 and 100±41, respectively) (Chen et al, 1985) may also be supporting evidence that the diet of our Chinese children may have been a traditional Chinese diet.

Unlike this finding, our data on the hair zinc status of our East Indian children were somewhat unexpected. We observed no low hair zinc child among our children with East Indian ethnicity. The published literature indicates the existence of widespread mild (Agte, Chiplonkar & Tarwadi, 2005) as well as severe (Chiplonkar et al, 2004, Bhandari et al, 2002 and Sazawal et al, 1996) zinc deficiency in East Indians living in India. There are no published data on the zinc nutriture of East Indian immigrants living in North America or other industrialized societies. However, sub-optimal iron status has been documented in East Indian immigrant populations residing in Canada (Bindra & Gibson, 1986), the United Kingdom (Robertson et al, 1982), and the USA (Ganapathy & Dhanda, 1980) and these findings have been attributed to the retention of traditional East Indian eating patterns among East Indian immigrants. These patterns include a predominance of lacto-ovo-vegetarianism and daily consumption of unleavened chapatti bread made from whole-wheat flour with or without added bran (Bindra & Gibson, 1986), which is very high in phytate. Such a diet with a lack of bioavailable dietary zinc and a provision of large amounts of zinc inhibitors (phytates), could easily lead to an unfavorable zinc status as well. In our study, only 28% (n=15) of our East Indian children were lacto-ovo-vegetarian. As for the other component of the traditional East Indian diet (the daily consumption of large amounts of high phytate bread) due to the limitations of our dietary tool we cannot comment further.

At this point we cannot be certain whether ethnicity *per se* is the explaining factor for these differences or other factors associated with ethnicity (such as ethnic food etc) are actually mediators. However, the fact that ethnicity did not remain as a significant component of our final stepwise or logistic regression models as the predictor of hair zinc

(**Table III. XIII and Table III.XIV**, respectively), suggests the likelihood of the latter rather than the former. Further studies with comprehensive dietary information are warranted in order to verify these findings and explore any possible dietary or other non-dietary explanation for them.

We observed a significant difference in the children's hair zinc based on maternal education (**Table III.IV**). There was no gradient effect (wherein the hair zinc status of children of each category of maternal education would have been better than the one above and worse than the one below it in the table) from maternal education and either the hair zinc level or the occurrence of low hair zinc. Both these variables, though, were significantly different in moving from children with maternal education $\leq$ high school (the first 2 categories of maternal education) to those with maternal education >high school (the last 2 categories of maternal education). Since dichotomizing data on a variable that has been measured by a continuous scale (i.e. the hair zinc) could potentially result in a loss of information and even increase a Type I error (Maxwell & Delaney, 1993), we decided to keep the information on hair zinc intact in its continuous form and explore associations between the hair zinc of children and the socio-economic and socio-demographic variables through regression analyses. The results of these analyses, while confirming the associations of age and ethnicity with hair zinc, indicated also a significant positive association between hair zinc and maternal education (**Table III.VI**). However, this variable (maternal education) could only explain 2% of the variance in hair zinc.

Other research has indicated the positive effect of maternal education on social development, emotional well-being and the physical health of children (Zill et al, 1995

and Zill et al, 1996). An avenue by which maternal education may affect a child's health is nutrition. Studies have shown more favorable eating patterns in children with higher maternal education (North & Emmet, 2000). Some studies have actually gone beyond this and shown evidence in support of the thesis that, for young children, maternal education may be the most important factor determining the adequacy of their nutrition (Watt, Dykes & Sheiham, 2001). One way through which the positive effect of maternal education on a child's nutrition can be played out is through an increase in family income, which, in turn, could result in higher buying power and increased accessibility of better food. However, this was not the likely path for the positive association of maternal education with the hair zinc level in our study, since income itself was not, as indicated, significantly associated with hair zinc (**Table III.VI**). Further, the positive association of maternal education with hair zinc remained significant, even after adjustments for the 5 socio-demographic variables, including family income were carried out. Therefore, the observed positive association of maternal education with hair zinc appeared to be independent of its potential enhancing effect on a family's income.

Nevertheless, increased parental education can have a positive effect on a child's nutrition in other ways. Research today does not support the contention that maternal education is just another surrogate for measuring social class or socio-economic status. In households with low maternal education it is not just the lack of financial resources that are at work but also a lack of knowledge coupled possibly with unhealthy parental food behaviors that pose a threat to a child's nutrition and health (Dubois, 2006). Educated parents are likely to have some knowledge of childhood health and nutrition. In addition, a better-educated mother would be more likely to eat healthy herself (Basiotis et al, 2002)

and influence the eating patterns of her child by modeling healthy behavior and setting a positive example.

The lack of any significant association between the hair zinc of children and their family income was an interesting finding of our study. Since our information on family income was the "recorded income" and not the "income per capita", all the above-mentioned analyses were repeated with "total family members" inserted as the covariate. We did not observe any difference in the results. Data from the US (Silva, 2001 and Skalicky et al, 2006) present supporting evidence for the existence of an inverse relationship between family income and some nutritional deficiencies. Some studies from Quebec have shown family socio-economic status to be related to some elements in the quality of a preschoolers' diet (Dubois & Girard, 2001). This lack of association between income and hair zinc level in our study, despite some pre-existing evidence on the association of income with nutritional deficiencies, was not an unexpected finding for a nutritional deficiency such as MZD. Research indicates that marginal zinc deficiency, unlike severe zinc deficiency, which usually co-occurs with caloric deficiency and hunger (Muller & Krawinkel, 2005), is usually but not always, the result of food consumption patterns rather than the quantity of food consumed (Gibson et al, 2001). This deficiency, although it may occur in underdeveloped and developing societies as well, is more prevalent in industrialized societies (Gibson et al, 1989, Chakar et al, 1993 and Nakamura, 1993), where food insecurity, although not non-existent, is not as large a concern. In other words, while extreme low income and food insecurity may predispose a child to MZD, food security will not necessarily protect him/her. This point was reflected

in our data, when we observed no significant associations between the hair zinc level of children and their families' food security (**Table III.VIII**).

The occurrence of food insecurity among our survey families was 14%, comparable to 10% reported by Statistics Canada (2001) for the Vancouver population. An additional explanation for the lack of association between food security and hair zinc may come from the fact that a food insecure family does not always equate to food insecure children (Che & Chen, 2001). Some Canadian studies indicate that younger children seem to be protected from poor quality diets in households with limited resources to acquire food (Glanville & McIntyre, 2006, Radimer et al, 1992 and McIntyre et al, 2003). Family food insecurity affects the status of food security of the children in some circumstances only. In support of the findings of these small scale studies, data from our recent National Population Health Survey, NPHS, 1998/1999 also indicate over half of the Canadian children in food insecure households were food secure, while (20%) of these children experienced food insecurity only to the level of worrying about food (marginal food security) and about 29% had actually experienced food insecurity to the point of compromising their diet (Che & Chen, 2001). In addition, in the classification we used to categorize our families' food security status (Che & Chen, 2001), answering "yes" to anyone of the three questions would result in being classified as food insecure. A family, which is in this category due to the giving of a positive answer to the first question, has experienced food insecurity only to the extent of worrying about food. This degree of food insecurity may not lead to any actual change in food intake.

We also observed a statistically significant difference between the mean (±SD) of the hair zinc of children from lone parent households and those who were not in a lone

parent household (**Table III.VII**), indicating that a child's nutrition may be affected by lone parenthood. Data analyses from the second National Health and Nutrition Examination Survey, NHANES II, and a comparison of the nutritional status of the preschoolers from intact and lone-parent families have provided some evidence linking lone parenthood with sub-optimal nutritional status (Bowering & Wynn, 1986). The usual association of lone parenthood with poverty (Dubois et al. 2000 and Family Pediatrics, 2003) did not appear to be the likely explanation for this observation.

Within lone parent households, there are other factors that may work adversely on a child's nutrition. Among them are the stress and anxiety level as speculated by Bowering & Wynn (1986) and perhaps the scarcity of time available for childcare. This shortage of time may have its deleterious effect on the quality of the care given to children including the nutritional care as in meal planning and allocating time for the proper preparation and cooking of nutritious meals. Congruent with this line of reasoning was a unique finding of a positive association between the children's hair zinc and "number of adults at home" (**Table III.VII**). Although observing this latter association strengthened our speculation of "time scarcity" as the possible underlying reason for the negative association between hair zinc and "being in a lone parent household", the negative association of "being in lone-parent household" with hair zinc was no longer significant when we adjusted the regression analysis. Additionally, "being in lone-parent household" was not a component of our final stepwise or logistic regression model.

The last observation of family characteristics was the positive association between children's hair zinc and family size (**Table III.VII**). This led us to suspect that this positive association may have stemmed from an increase in the "number of adults at

home". However, in the final stage of our statistical analyses, when we inserted "number of adults at home" and "family size" in the stepwise regression (**Table III.XIII**) analyses, only the "family size" remained as a significant component as the variable that could explain part of the variation in hair zinc level. On the other hand, "number of adults at home" was the significant component of the final model produced by logistic regression analysis (**Table III.XIV**), as one of the variables that could predict hair zinc status.

Through collecting data on caregivers' perceptions of their children's eating behaviors, we observed differences in the hair zinc level and the occurrence of low hair zinc based on being described as "eating unhealthy" (**Table III.IX.A**). This variable was also negatively associated with hair zinc of the children in linear regression analyses (**Table III.X**). In our survey, similar to our study of the low-income preschoolers of Vancouver, the survey parents had not described what is perceived as "unhealthy eating". Whatever this behavior (so described) may have been for individual parents of this group of children, our analysis of this behavior showed an association with one common health outcome - having lower mean hair zinc. In our final analysis, however, being described as "eating unhealthy" was a significant component of the stepwise regression analysis (**Table III.XIII**), but not a significant component of the model produced by logistic regression analysis (**Table III.XIV**).

Another parent-described eating behavior accompanied by significantly lower hair zinc level and higher occurrence of low hair zinc among children was "not eating enough". We asked parents whether or not they perceived their child as a "picky eater" or "not eating enough". We used these 2 eating behaviors as 2 proxies for loss of appetite, a clinical symptom often described in MZD (Mangian, Lig, & Shay, 1997, and Mantzoros

et al, 1998). Being described as "not eating enough" remained as a significant component of the final logistic regression model.

Among other eating behaviors we observed significant negative association between the children's hair zinc and the consumption frequency of milk and dairy (**Table III.X**). Very frequent milk consumption was a common dietary theme among our preschoolers. Our previous study of low-income preschoolers of Vancouver (chapter II of this thesis) had indicated frequent consumption to the point of over-consumption of dairy and milk. However, the dietary tool of our survey, unlike that of the inner city study was not detailed enough to differentiate between repeated milk consumption constituted as over consumption or just frequent consumption of small portions. Heavy milk consumption (Lino et al, 1999 and Kranz et al, 2006) and milk being a favorite food among preschoolers (Skinner et al, 1999) have been reported in the literature. Perhaps its ease of consumption and familiarity with its taste from infancy, when combined with parental approval, are key factors leading to the tendency of this age group towards frequent milk consumption.

On average young children consume only small amounts of food at meals. They, therefore, must have a nutrient-dense diet with variety in order to provide sufficient amounts of all nutrients for growth and development (Curran & Barnes, 2000). Frequent consumption of milk, similar to any other fluid, can displace other foods from a child's diet including the zinc-rich foods. Frequent consumption of milk and dairy, if resulting in over consumption, can also interfere with zinc absorption. Dairy foods contain large amounts of calcium, an element that can potentiate the complex formation between zinc and phytate and thus lead to zinc excretion. The adverse effect of over-consumption of

calcium on zinc status has clearly been shown in animal studies (Oberleas, 1966). Studies of Canadian children have also documented an inverse relationship between calcium intake and their hair zinc status (Smit Vanderkooy & Gibson, 1987 and Gibson et al, 1991). It is important to note that in both our final stepwise regression analysis and logistic regression analysis the consumption frequency of milk remained as a significant component, indicating its predictive power for hair zinc level and hair zinc status, respectively. However, it contributed only 2% to the overall explained variance.

From our information on parents' perceptions of their children's health and parents' report of their health behavior we learned that being described as "frequently sick" or "having poor health" was also inversely associated with hair zinc (**Table III.XII**). A strong inverse association was also observed between hair zinc and perceptions of parents as to their children's health as detailed in our previous work with low-income preschoolers of Vancouver (chapter II of this thesis). It is known that the immune system is highly zinc-dependent (Ibs & Rink, 2003) such that when the zinc supply to the body is inadequate the function of this system may well be compromised with a subsequent weakening of the child's resistance to common infections. Conversely, since zinc is an element of "acute phase response" and plasma zinc is redistributed to other tissues during infection/inflammation (Prasad, 1981), reduced hair zinc could be the net result of the ongoing decline in the plasma zinc of the children who were described as "often sick". Like any other cross-sectional study, all of our data were collected at one point of time and we cannot therefore be certain which one of these 2 events (the reduction in hair zinc and being frequently sick) occurred first. Regardless of the sequence of the events, the inverse association of hair zinc with frequency of common

infections has also been shown in studies of preschoolers in other developed countries (Von Wouwe et al, 1986 and Lombeck et al, 1988). In our study, being described as "frequently sick" remained as a predictor of hair zinc status by virtue of its appearance in our final logistic regression model (**Table III.XIV**), only.

In addition to the immune system, the zinc literature points also to the adverse effect of sub-optimal zinc status on other systems, among them the central nervous system and, as a result, the cognitive function. The results of data collected through CBQ on 2 behavioral domains of "attentional focusing" and "activity level" indicated that the scores of activity level were significantly lower for low hair zinc children compared to their normal hair zinc counterparts (**Table III.XI.B**). In addition, these scores were positively associated with the hair zinc level of the children (**Table III.XII**). Experimental animal studies (Golub et al, 1994 and 1996) and observational studies (Kirksey et al, 1994) and zinc supplementation trials (Ashworth et al, 1998, Bentley et al, 1997, Sazawal et al, 1996 and Sandstead et al, 1998) in humans have all documented the negative association of zinc deficiency with activity level and motor development. Our study provided an added evidence to this body of literature. This variable (the scores of activity level) remained as a significant component in both of our final regression models (**Tables III.XVIII and III.XIV**).

One of the noteworthy observations in our study was the widespread consumption of supplements, a large portion of which contained iron (Appendix III-31). Analyses of the hair zinc data based on consumption of supplements containing iron indicated a significantly lower hair zinc level and higher occurrence of low hair zinc among the children taking iron supplements (**Table III.XI.A**). Iron is known to have an antagonistic

interaction with zinc, and hence the potential to lower the biochemical zinc status (Solomons, 1986). The discovery of divalent metal transporter 1 (DMT1) (Gunshin et al, 1997) has provided an explanation for the competitive absorption of iron and zinc from the small intestine.

Although the adverse effect of iron supplementation on zinc absorption has been documented (Valberg, Flanagan, & Chamberlain, 1984, Sandstrom et al, 1985 and Troost et al, 2003), its consequence on the overall zinc status of the body has been inconsistent (Solomons et al, 1981, Solomons et al, 1983, Meadows et al, 1983, Solomons, 1986 and Troost et al, 2003). However, plasma Zn is tightly protected from the wide fluctuations experienced as a result of a broad change in zinc available to the body, and is thus maintained within a normal range (Hambidge & Krebs, 2001). For this reason any conclusions regarding the effect of iron supplements on zinc status are difficult to make with any certainty when plasma zinc is taken as the biomarker of zinc status. Such results could simply reflect the net result of the redistribution of zinc within the body. However, as noted, zinc in a hair follicle is not subject to redistribution in response to the ongoing metabolic events in the body (Hopps, 1977). Supporting evidence for this argument will be found in a study of New Zealand women where the plasma zinc concentrations were not significantly different for supplement takers while their mean hair zinc value was significantly lower (Gibson, 2001).

Although we observed as well a significant negative association between taking supplements containing iron and hair zinc level in our linear regression analyses (both adjusted and unadjusted) (**Table III.XII**), it is also noteworthy that the amount of iron in our supplements was very small, lying in the range of 4-18 mg/day. Most studies have

demonstrated iron and Zn interactions when the amount of supplemental iron exceeds 30 mg/d. The smallest iron concentration known to affect zinc status is 18 mg/d (Dawson et al, 1989). However, it is important to bear in mind that the subjects of this study were pregnant teens with a significantly high zinc requirement (Estimated Average Requirement of 10 mg/day for pregnancy, versus 4 mg/day for preschoolers) (Institute of Medicine, 2000). Furthermore, the supplements containing iron at the higher end of the range (Flintstones Children's chewable Multivitamin/Multmineral Supplement which provides 18mg of iron/tablet) also contained 15mg of zinc in each tablet. To sum up, considering the low concentration of iron in most supplements and the simultaneous provision of zinc in those with higher iron concentration, it was improbable that these supplements would have an adverse effect on zinc status. We speculated that the observed association might have originated from another variable.

In exploring our data further, we noticed that there was significant association between being described as "not eating enough" and taking supplements containing iron. In fact, the odds ratio of taking supplements containing iron among children who were described as "not eating enough" was 40% higher than the other children. It is possible that parents who had observed their children not eating enough administered these supplements as a way of safeguarding their health. However, taking supplements containing iron remained as the significant component of both our final regression models, while "eating unhealthy" was only a significant component of the logistic regression model produced (**Table III.XIV**).

At this point and within the limitations of our data, we cannot ascertain whether the observed negative association was the result of a mineral/mineral interaction or the

artifact of some other middle variable. However, the antagonistic effect of iron supplementation on zinc status is a concern that warrants further investigation. Although the Canadian Pediatrics Society (CPS) and the Dietitians of Canada recommend the intake of a wide variety of foods as a way of obtaining adequate vitamins and minerals rather than the intake of supplements (CPS, 2007), the intake of iron supplements for some children is crucial. Iron deficiency is a valid pediatric health concern even in developed countries (McCann & Ames, 2007), and despite global initiatives such as food fortification and enrichment, a large number of children in the US (CDC, 2002) as well as in Canada (Williams, 2000) still fail to meet their needs for this essential mineral. In view of the significance of zinc and iron for the human body and the consequences of their deficiencies on children's health and well being, further research on the possible interaction of these 2 minerals on each other is clearly warranted. This issue has a particular significance in a society such as ours where, based on our recent provincial survey, almost 1/3 of adults take supplements (Barr, 2004), and according to our survey over 1/3 of parents give supplements to their children (Appendix III-31).

The results of the stepwise regression analysis of hair zinc together with all of the variables in this study indicated age, maternal education, family size, consumption frequency of milk, being described as "eating unhealthy", scores of activity level, and taking supplements containing iron as the variables that could explain part of the variability in hair zinc level (**Table III.XIII).**

Similarly, subsequent to the identification of all of the dietary and non-dietary factors associated with low hair zinc, we constructed a logistic regression model with hair zinc (low versus normal) and all these variables. As displayed in **Table III.XIV** sex, age,

maternal education, number of adults at home, being described as "not eating enough", consumption frequency of milk, scores of activity level, described as "being frequently sick", and taking supplements containing iron were the significant components of the final model produced. However, as expected from the results of the regression analyses (the small R^2 value of these analyses) the collective model did not provide adequate predictive power. This was clearly reflected in the small improvement in the percentage of children classified correctly as normal/low hair zinc when this model was used (85.5% versus 84.4% with and without the use of model, respectively) (**Table III.XV.B** and **Table III.XV.A**). In other words, although this screening questionnaire identified most of the normal hair zinc children correctly (98.3% specificity), it managed to identify only 16 of the 100 low hair zinc children (16% sensitivity), thereby failing to fulfill the function of a first-stage screening tool to reduce the number of children needed to undergo subsequent exploratory and diagnostic tests. The positive predictive value of this model was 64% (16/25) while its negative predictive value was 86% (532/616) (**Table III.XV.A**). In total, only about 15% of the variability in hair zinc could be explained by the variables assessed in this study. The rest of the variability (85%) in hair zinc stems from factors not addressed in this study. Our study questions concerned risk factors that have been commonly acknowledged as risk factors of zinc deficiency in both marginal and severe forms. The predictive probability of a model based on such questions was calculated at 0.72 as indicated by the area under the curve of ROC (**Figure III.VI**). Obviously other factors that were not included in this study may be more predictive of low hair zinc and MZD.

The large variation in zinc absorption due to the presence or absence of other dietary and non-dietary enhancers and inhibitors such as the status of minerals like iron and lead may constitute some of these factors. In addition zinc is one of the most omnipresent and multifunctional minerals in the human body. Therefore many physiological and pathological events occurring in the body may affect its balance and status and outweigh the cumulative effect of some of the previously identified factors that were also explored in this study.

The zinc literature does not contain any previous studies attempting to construct a screening questionnaire and examine its utility. However, such attempts have been made for screening iron deficiency (Boutry & Needlman, 1996, Bogen et al, 2000, and Williams, 2000). These questionnaires are reported to have a high sensitivity (85%-95%) and a modest specificity (15%-49%). A simple dietary check list constructed by Williams (2000) based on the assessment of 152 children (8-26 months old) in the city of Vancouver in an attempt to serve as a first stage screening tool of iron deficiency anemia had 87% sensitivity and 49% specificity.

Although we could not reach the primary goal of this study in constructing and then validating a short questionnaire that could serve as a screening tool for marginal zinc deficiency, this study did contribute in several ways to the zinc literature. It updated our information on the hair zinc status and the prevalence of low hair zinc in an apparently healthy population of Canadian preschoolers 2 decades after the last Canadian study of this age group was published. The data also provided some important reference values for healthy children of North America and perhaps the children of industrialized societies in general. This study raised some key questions for zinc research such as the relationship

between hair zinc and age, and set the stage for future studies to further investigate this imperative issue. Finally, this study identified and drew to our attention factors in a child's social setting and family environment, which may predispose her/him to a sub-optimal hair zinc status.

However, as with other studies, ours has limitations, which should be kept in mind when generalizing its findings. One such limitation arises from our sampling method, which may have conferred "selection bias" to the findings. By using the preschool and daycare centers as a major source of recruitment, we may have excluded children of stay-home mothers who are not the consumers of these services. However, our recruitment of some children (n=121/719=17%) through drop-in sessions at community centers, where parents (stay-home parents) and children are engaged together in various activities, may have attenuated this bias.

Another factor that may have introduced some bias to our study is the selection bias due to non-response. The overall response rate of our survey was 30% with 70% of the parents receiving our survey package choosing not to respond. Non-response in survey research can pose a threat to the generalization of results, if respondents and non-respondents differ systematically (Barriball & While, 1999). We did not have any way of comparing the characteristics of non-respondents with respondents. However, in general, it has been shown that non-respondents have a lower socioeconomic (Groves & Couper, 1992, Mishra et al, 1993 and Goyder, Warriner, & Miller, 2002), worse health (Jackson, Chambless & Yang, 1996 and Shahar, Folsom & Jackson, 1996) and a better self-reported health (Mishra et al, 1993) status than respondents. One possible implication of

this error on the findings of our study would be under-estimation of the prevalence of low hair zinc due to the participation of the healthier fraction of the population.

One last methodological shortcoming of our study was our survey questionnaire not offering any visual aid for the serving sizes of different food groups to the participants. Such an aid would have enabled us to have some idea about the overall daily intake of food groups and compare the intake of our participants to the recommendations made by Eating Well with Canada's Food Guide for Healthy Eating, CFGHE (Health Canada, 2007) and then draw some valid and meaningful conclusions.

Finally, it is important to keep in mind that this was a cross-sectional survey with a limitation inherent in all cross-sectional studies. All of the relationships observed were those of association. As a result, we did not and cannot establish the existence of any causal relationship, or lack of it.

III.5.BIBLIOGRAPHY

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CHAPTER IV

CONCLUSION

IV.1. GENERAL CONCLUSIONS

This study, being the first conducted in the last 20 years on the zinc status of Canadian preschoolers, provides important hair zinc reference values for healthy Canadian preschoolers. Due to its relatively large sample size and with the participants being healthy free-living children, these values may also serve as a reference for healthy preschoolers of industrialized societies. Our study makes, as well, a significant contribution to our understanding of hair zinc and factors associating with it.

First and foremost, we observed the occurrence of low hair zinc in 46% of our low-income inner city children (**Chapter II**), and in 17% of our survey participants (a representative sample of the whole city) (**Chapter III**). The much higher population mean of the survey children compared to that of the low-income inner city children when combined with the much lower incidence of low hair zinc for the survey children (17% versus 46% in the inner city sample) conveyed a relatively better picture of zinc status for the preschoolers of the city. In addition, this comparison reiterated the well-recognized fact that children of low-income families are at higher risk of sub-optimal nutritional status. Radical differences (similar to ours) in the occurrence of low hair zinc between low-income (Hambidge et al, 1972) and middle income (Hambidge et al 1976) children of the same society have also been shown in American preschoolers (3-5 years) (48% versus 19%, respectively). The comparability of these numbers with ours (46% versus 17%, respectively) as well as the similarity of the differences observed between the 2 income classes of these 2 sets of data, are quite remarkable. The higher occurrence of low hair zinc among low-income inner city preschoolers compared to the survey (the overall city) was not unexpected. Nevertheless, the extent of the occurrence of low hair

zinc (46%) was alarming. Almost 1 out of every 2 apparently healthy low-income preschoolers from inner city of Vancouver, a major metropolitan center in one of the healthiest and most affluent countries of the world (WHO, 1991), had low hair zinc and, potentially, MZD. It is evidence like this that puts the assertion of marginal zinc deficiency as the most common hidden health problem in children (Hakim et al, 2006) into context.

Although the occurrence of low hair zinc was significantly higher among the low-income and potentially food insecure inner city children (46% versus 17%, χ^2=34 df=1, p=0.00), there was no association between income and hair zinc within either of the samples. Taken together, our results indicated that the recognized "gradient" effect of income on health status (Hertzman, 1998) might not govern hair zinc status (MZD). All socio-economic classes are at risk for the occurrence of low hair zinc and the differences only become measurable in extreme conditions. Comparing the results of our survey combined with that of our inner city study (Chapter II) and the study of Broughton et al (2006) which explored food security among Vancouver inner city families (the same cohort used in chapter II of this thesis) provides some support for this statement. Broughton and co-workers estimated 50% food insecurity for that group of inner city families of Vancouver while we observed the occurrence of low hair zinc for the same cohort at 46%. Yet in our survey we observed the rate of food insecurity and low hair zinc at 14% and 17%, respectively. Looking at these 2 studies may provide a support to the statement that the impact of a decline in income level on hair zinc becomes measurable only when the decline is severe and the food security of the child is jeopardized.

Looking at the results of two studies also indicated that while low income children of first study had very low mean hair zinc (73μg/g), the low income children of survey had much higher mean hair zinc as a group (113 μg/g). There are few possible explanations for this observation. First, this could be the result of some differences in the living environment of children at the neighborhood level. The available research suggests that some favorable factors at neighborhood level could attenuate some of the negative effects of low SES on children (BC child atlas, 2005). Some examples of these factors, relevant to the issue of discussion, could be having community centers offering nutrition and health classes to caregivers, having food banks or other initiatives promoting early child development. Alternatively, the observed differences in the mean hair zinc of inner city children and low income children of survey could be the result of a measurement error, as the samples of two studies were analyzed in two different laboratories.

While considering the ill effects of poverty on health, it is reasonable to assume that the observed differences are real. But it is also possible that some differences in the design and methodology of the 2 studies may have produced the observed drastic differences. The methodological differences between these studies include the fact that the hair zinc analyses were carried out in different laboratories. In addition, the amount of hair samples collected in inner city study were just enough to carry out single analyses, whereas the samples of our survey were analyzed as single samples with a duplicate after every 10 samples. However, the small coefficient of variation of the survey samples (3.3% for within assay and 6.0% for between assay) may indicate that the results of single analyses may still be reliable.

In both of our studies we observed significant differences in hair zinc as well as the occurrence of low hair zinc between children younger than 4 years and those who were 4 and over. This finding raises a fundamental question: Could this lower hair zinc in younger preschoolers be a normal physiological level independent of diet or any other environmental factor? This is an important question to address because, if it is, this will undermine the usefulness of having the same cutoff for younger children. While this critical question remains to be answered, perhaps comparing the results of studies with different age groups may not be advisable.

Furthermore, we observed differences in the occurrence of low hair zinc based on sex. Unlike the conclusion in most zinc literature (Hambidge et al, 1976, Gibson & DeWolfe, 1979, Smit Vanderkooy & Gibson, 1987 and Sky-Peck, 1990) indicating male children as the sex at higher risk for low hair zinc, our data showed the female preschoolers as the more vulnerable sex (a higher occurrence of low hair zinc for girls but a comparable mean hair zinc for the 2 sexes). Previous studies speculated a greater physiological need in males based on evidence from animal studies (Liptrap et al, 1970 and Swenerton & Hurley, 1968) as the reason for the observed differences. However, our finding, combined with the findings of other studies similar to ours, which have not found a significant difference in hair zinc between the 2 sexes (Sakai, Warishi & Nikiyama, 2000) or have actually observed higher hair zinc for male children (Zachiwieja et al, 1995) points to the inadequacy of physiology by itself as the explanatory factor for this observation.

Our finding that Chinese children have a higher susceptibility for low hair zinc was not unexpected in view of the nature of a traditional Chinese diet, which was

supposedly consumed by these children. However, we also made the unanticipated observation that not a single one of our fifty-four East Indian children was low in hair zinc. Like sex differences in our study, differences in the hair zinc level and the occurrence of low hair zinc among different ethnic groups could not be explained based on the limited dietary information gathered from the children. Whether these differences were due to the eating patterns of the ethnic groups and our dietary questionnaire was not sensitive enough to capture them, or due to some inherent factors in each ethnicity, we cannot answer.

Our study also indicated an inverse association between taking supplements containing iron and hair zinc. When we explored the relationship between taking supplements containing iron and other eating behaviors, we observed positive associations between "taking supplements containing iron" and "caregiver being concerned about child's nutrition", being described as "picky eater" and being described as "not eating enough". Taken together, we speculated that the negative association of hair zinc with taking supplements containing iron might have stemmed from the fact that most of the iron takers were children whose nutrition was a concern of the caregivers, and children who had been described as a "picky eater" or "not eating enough". Due to perceiving child with these eating characteristics, therefore, the caregivers had begun giving them these supplements (in order to provide the nutrients that they thought the children were not getting through their diet). However, in our final logistic regression model, both variables of "taking supplements containing iron" and being described as "not eating enough" remained as significant (albeit very weak) predictors of hair zinc status.

While the literature has documented the ill effect of iron supplements on zinc absorption as well as zinc status, we refrained from drawing such a conclusion based on our data due to the very small concentration of iron taken by our children (4-18mg/day), as well as the fact that iron was often con-consumed with zinc. On the other hand this study is the first population-based study to document such an association. Therefore, and in view of the significance of the iron and zinc balance in human health, it is reasonable to raise this issue as another question generated by this study that requires to be addressed properly.

Finally, we showed associations of hair zinc with some health and behavior related variables. In both groups of children (the low income children of inner city as well as the survey children), there were negative associations between being described as "frequently sick" and the children's hair zinc. In our survey children we also noticed a positive association between their hair zinc and the activity level score. Evidence from human studies, though scarce, does refer to decreased activity level as a consequence of zinc deficiency. The literature does not offer a plausible explanation for this finding.

In this study there was also no evidence of any association between hair zinc and growth status, an association shown in many, but not all, studies of MZD. Although the anthropometric data did not support the existence of any overt growth retardation, this does not eliminate the possibility of the existence of MZD among our study for the following reasons.

First, children with marginally low hair zinc and normal growth parameters have been shown to exhibit a differential growth response (compared to growth in the placebo group) when supplemented with zinc (Ruz et al, 1997). Since zinc has no

pharmacological growth promoting effect and it results in growth only when the growth had been hindered due to the shortage of zinc in the human body, this growth response indicates a pre-existing zinc deficiency. Therefore, in our study, while the existence of measurable anthropometric evidence could support the diagnosis of MZD, the absence of such data does not eliminate this diagnosis.

Second, there was a suggestion of a compromised immune system among some of these children. The low hair zinc children were more often described as having poor health and being frequently sick. The literature has also documented a more frequent occurrence of cold and common infections among children with MZD.

Third, we observed a significantly lower activity level score among our low hair zinc children. This reduced activity level has also been recognized in children with suboptimal zinc nutriture.

This evidence taken together, we believe that at least a fraction of these low hair zinc children were potentially marginally zinc deficient. At this point and within the constraints of our data, this is the extent of the conclusion we can draw. The confirmation or elimination of this possibility (the existence of MZD among these children) can only be substantiated through a zinc supplementation study.

IV.2. STRENGTHS AND LIMITATIONS OF THE STUDY

Our citywide survey (chapter III) was the first large study of zinc status in Canada. There are a few characteristics in our sampling (and methodology in general) that could have improved the quality of our data and increased the credibility of our findings.

We invested a great deal of care and deliberation in our sampling strategy. The significance of this survey is not just in its large sample (n=719), but also in the representativeness of this sample. Through the use of information from the 2001 census (Statistics Canada, 2001), and employing a stratified sampling method (details in chapter III) we ensured that every neighborhood in the city of Vancouver was adequately represented. In addition by means of useful maps produced by the combined effort of the UBC data library and the Geographic Information System, GIS, of the Human Early Learning Partnership (HELP) (Appendix III-2) we ensured that the intra-neighborhood income differences were carefully considered and properly reflected in the subjects recruited from each neighborhood. We believe the survey had a very fair representation of children of all neighborhoods and all income classes within them.

The other area in the methodology of our citywide survey in which a great deal of care was invested was in the hair sample collection itself. It has been shown that the collection of a hair sample is a critical factor in the credibility of the hair zinc results. In order to ensure strict adherence to one protocol, and in an attempt to minimize the inter-examiner error (a bias arising as a result of subtle differences in the methods of different examiners) all of our samples were collected by the research student only. Although this extended the sample collection over a longer time period (March-June), it clearly assured the reliability of the sample collection.

Finally, the large sample size of the survey and the fact that all the participants were apparently healthy free-living children may permit the generalization of our findings to many North American preschool populations with similar ethnic make up.

As with any study, ours had its limitations. First, we recruited most of our survey children (83%) through the preschool and daycare centers of the city, and only 15% (Personal communication with Ms. Diane Liscumb, West Coast Family Services) of the families with preschoolers use these services. Using this database we excluded the children who are not enrolled in these services. It is reasonable to assume, therefore, that the children of stay-home mothers were excluded by our sampling method. A large portion of such parents could have been unemployed due to a lack of training, skill and/or education. We might, therefore, have skewed the maternal education of the study participants towards the higher end of the spectrum and thus introduced a selection bias into the study. Perhaps the high maternal education observed among a large proportion of our survey participants is indicative of this bias (58% versus 38% and 42% reported for adult females 20-34 and 35-44 years old, respectively) (Statistics Canada, 2001).

Another factor to bear in mind when considering the extent to which our findings can be generalized is the moderate response rate of the survey. Our overall response rate was 30%, with the other 70% of the families receiving our survey package not replying. Non-response in a survey can pose a threat to the generalization of results if respondents and non-respondents differ systemically (Barriball & While, 1999). We did not have any way of verifying the existence of any systemic differences between the respondents and non-respondents. The research evidence provides support for significant differences in the socio-economic (Groves & Couper, 1992 and Mishra et al 1993) as well as the health (Shahar, Folsom, & Jackson, 1996 and Mishra et al, 1993) status between respondents and non-respondents.

The third possible limitation of our study was that most of our data was parent reported. Therefore, the validity of the data depended on the truthfulness and memory of the responding caregiver/parent. The first factor, truthfulness, particularly, becomes an issue when it comes to reporting the food intake of the children. Despite the fact that the estimates of adult food and energy intake is often under-reported (Krall & Dwyer, 1987) parents may over report their children's intake (Devaney et al, 2004) for the simple reason of social acceptability. In addition, it may be difficult for them to keep in mind that what is served and what was consumed are not often the same. Lastly, parents may vary in their idea of individual serving sizes (Young et al, 1998), and what is 1 serving size for one parent may not be the same for another.

As for reliance of our data on a parent's memory, research in this area indicates that the caregiver's recall of events regarding their children can indeed be considered a reliable source and has demonstrated a very good correlation with some gold standards such as a pediatrician's record of events (Pless & Pless, 1995). The high R^2 values of the associations of the 2 sets of data collected during our reliability study are also testimony to this statement.

One limitation specific to the study of the inner city was the small quantity of the hair samples which did not allow any analysis to be run in duplicate.

Finally, as it is with any cross sectional study, our data indicates associations at the very best. Therefore, we cannot make any conclusion regarding the existence of any casual relationship between the variables studied or for that matter, or lack of it.

IV.3. FUTURE DIRECTIONS

This work, while providing some useful information about the zinc status of Vancouver's preschoolers and hair zinc in general has generated some very important questions that need to be answered. One such is the hair zinc status of low-income preschoolers of the city (**Chapter II**) where we observed widespread low hair zinc among the inner-city children. Further studies are to be conducted to verify this in larger samples of low-income preschoolers. Carefully designed studies are also to be implemented to better understand the functional/clinical ramifications of low hair zinc for these children. For instance, longitudinal studies with serial anthropometric measurements and measure of growth velocity would enable one to understand the consequences of such low hair zinc on a child's physical growth, if any. Alternatively, supplementation studies would be able to determine if provision of zinc triggers any growth response in children, which would be confirmation for a pre-existing zinc deficiency.

Our study indicated children younger than 4 years old as those at higher risk of low hair zinc. The hair zinc of these children was significantly lower than that of the older children. It is critical to understand whether this observation is simply the normal physiological status of this stage of life or a deviation from the normal range due to different environmental factors such as nutrition. An answer to this question is extremely important, since, as discussed, if this observation is simply the result of the normal physiology of this stage of life, there is a need for setting up a different cut off and validating it in a large sample of children of this age group.

Lastly, differences observed in the hair zinc level of the children of different ethnicities (**Chapter III**) warrant further research. Studies with a more representative and larger sample of Chinese as well as East Indian children are to be conducted to confirm these findings. Such studies should gather detailed and elaborate dietary data and information on family background in order to better understand the children's diet as well as their social setting. Such studies would enable us to determine if ethnicity *per se* or other ethnicity-related factors (for example ethnic food) is the underlying explanation for the observed differences in hair zinc. It is possible that there are lessons to learn from the ethnicities with unexpected rate of low hair zinc.

All in all, this work has increased our understanding of the factors associated with the hair zinc of children and in doing so has brought into focus several important questions that may provide direction for future investigations which will further our knowledge of hair zinc and factors affecting it.

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APPENDICES

Appendix II-1: Certificate of ethic approval for the inner city study

17 January, 2004

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

Notice of Ethical Review: Memo

PRINCIPAL INVESTIGATOR	DEPARTMENT	[redacted]
Innis, S.M.	Paediatrics	C01-0539
INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT: BCCH Research Centre		
CO-INVESTIGATORS:		
SPONSORING AGENCIES: BC Ministry of Children and Family Development		
TITLE: Co-Morbidity of Iron Deficiency & Micronutrient Deficiency & Risk of Cognitive Delays in Preschool Children 18 Months- 5 Years of Age.		

The UBC CREB has reviewed your proposed study, and has issued a *Certificate of Approval.*

Please make sure the following items have been corrected/amended within 21 days. Ensure that any revisions to the consent form are made on the correct institutional letterhead and are either underlined or in **BOLD** text. A letter that explains how the requested changes have been addressed should accompany your response.

If needed for Clinical Trials, please ensure that the Consent Form Version Date is updated to reflect any required revisions and that the new version date is clearly indicated in your response. An updated Certificate of Approval will be issued accordingly.

All other responses will be acknowledged by the CREB Chair. **You may submit the revisions by email to Catherine Sutherland at c.sutherland@ors.ubc.ca**

Consent:
1. Include page numbering.
2. Include an invitation to participate and an explanation as to why they are being invited.
3. Page 3: Include the standard UBC CREB confidentiality statement as per UBC CREB Guidance Notes 39.7.1. Refer to http://www.ors.ubc.ca/ethics/forms/GNinitialapp.htm#Guide39.7
4. Page 2: Change "Director of Research Services" to "Research Subject Information Line". Phone number remains the same.
5. Include space for the printed names of all who sign the consent.

If you have any questions regarding these requirements, please call:
Dr. Peter Loewen, Chair, 604-822-7985
Dr. Alain Gagnon, Associate Chair, 604-875-3174
Dr. James McCormack, Associate Chair, 604-822-1710
Ms. Susan Chunick, Manager Ethical Reviews (ORS), 604-875-4149
For further information, refer to the UBC CREB's Guidance Notes and Policies at:
www.ors.ubc.ca/ethics/human_subjects.htm.

Please send all correspondence to: **Clinical Research Ethics Office, Room 210, Research Pavilion, 828 West 10th Avenue. Vancouver. BC Canada V5Z 1L8**

Appendix II-2.Recruitment questionnaire for the study of low-income preschoolers

Children's Eating and Nutrition Clinic

Clinic Sign-Up Form

Child's gender: Boy ❒

Girl ❒

Child's Date of birth / /

Year/mo/day

1. Are you concerned about your child's eating?

❒ Yes ❒ No

Why are you concerned? Please check all your concerns.

❒ Picky eater ❒ Doesn't eat often enough ❒ Won't try new foods

❒ Eats too much ❒ Always eats the same foods ❒ Eats unhealthy

❒ Eats too often ❒ Not enough variety in diet ❒ Doesn't eat enough

❒ Other _____

2. Does your child take a vitamin/mineral supplement?

❒ Yes ❒ No

Please list name of supplement(s) and how often taken.

_____

How long has he/she been taking it?

_____

❒ Yes ❒ No

To what food(s)?

4. Is your child often sick (flu, fever, diarrhea ...)

❒ Yes ❒ No

5. How would you describe your child's health:

- ❒ **Very healthy**
- ❒ **Average health**
- ❒ **Not so healthy**

6. Does your family ever eat meat, chicken or fish?

- ❒ **yes**
- ❒ **no**

7. Ethnic Background

Mother's Ethnicity_____ **Father's** Ethnicity_____

The time needed for this clinic will be about 60 minutes.

If you would like to participate in this study please write your name, address and phone number below.

❒ Yes/Maybe - I would like to take part in the research.

Name

_____

_____

Phone Number

_____

Address

_____

_____

Street number and name

_____

_____

City Province Postal Code

What time of day would you like your clinic appointment?

Wednesday, March 3

❒ Morning ❒ Afternoon

Specify time

_____

Thursday, March 4

❒ Morning ❒ Afternoon

Specify time

_____

We will call you to confirm your appointment time.

Appendix II-3: Add placed on the bulletin boards of the community centers inviting eligible families to attend the nutrition clinics

Children's Eating and Nutrition

Our study allows parents of children aged 2 to 5 years to bring children to a clinic to find out about their nutritional health. We are interested in finding out what young children eat today and how the foods they eat affect their health and growth.

Who should think about coming to the clinic?

❒ If your child is 2-5 years old and you live in Vancouver, please come.

What are the benefits of participating?

❒ Learn about the health of your child.

❒ Vitamin-mineral supplements for children, if a deficiency is found.

❒ Grocery store gift certificate for parents.

What happens if my child and I are involved?

❒ Your child will be weighed and measured.

❒ A blood sample will be drawn by a pediatric phlebotomist (a blood drawing specialist from BC Children's Hospital)

❒ You will be asked questions about your child.

Your information will be strictly confidential. No information that identifies you or your child will be given to anyone without your permission.

Where is the clinic?

Strathcona Community Centre, 601 Keefer Street

Britannia community centre 1661 Napier Street

When?

Wednesday, March 3 and Thursday, March 4, 2004 at Stratchona

AND Monday March the 29th and Tuesday March the 30th at Britannia

- **Clinic is open from 9am – 5 pm.**
- ***Your visit will take 30-60 minutes.***

To find out more or to register: we will be at Grandview, 2075 Woodland Drive on Monday February 23rd 4pm-6pm

REFRESHMENTS WILL BE SERVED

Appendix II-4: Informed Consent form for the study of low-income preschoolers

CO-MORBIDITY OF IRON DEFICIENCY AND MICRONUTRIENT DEFICIENCY

Informed Consent

Principal Investigator Dr. Sheila Innis
Department of Pediatrics, Faculty of Medicine
University of British Columbia

Emergency Phone Number (604) 875-2434

Site B.C.'s Children's Hospital

Summary of Project

Iron deficiency is often considered to be the most common nutritional deficiency in Canada and among children worldwide. Iron deficiency is an important health problem because it can lower children's activity and interaction with their environment. This can have important effects on learning. Many of the foods that are good sources of iron are also good sources of zinc. Zinc is important for growth, immune function and also has important effects on learning. We have recently found low zinc levels in about 4 in 10 preschool children who attended our nutrition research clinics. Zinc deficiency and iron deficiency are both easily treated. The purpose of this study is twofold. In the first part we will examine children's diets in a series of short questions and examine the relation of children's diets to the risk of iron and zinc deficiency. We would like to better understand how many children have zinc deficiency, and if zinc deficiency is due to inadequate zinc intake or if absorption is limited by large amounts of cereal fibers in children's diets.

I understand that by participating in this study I will:

1) Allow my child's weight, height, arm circumference and triceps skin fold thickness to be measured.
2) Provide information on my child's usual food intake, and complete a questionnaire on some of my child's behaviour.
3) Allow a registered technologist to obtain a small blood sample from my child (about ½ teaspoon), and allow a small sample of my child's hair to be collected by myself or the technician present at the clinic.

The benefit that I will receive from this study is an assessment of my child's iron and zinc status. The participation of my child in this study will also contribute valuable information on how dietary intakes of preschool children are linked to iron and zinc

nutrition. My participation in this study will require about 60 minutes of my time. My name and my child's name will be treated confidentially by the use of code numbers and will not be used in any report of this study. I may refuse to participate or withdraw from this study at any time with no consequence to my child or me.

Risks
There are no known risks to participating in this study. A certified technologist or nurse will draw the small amount of blood. Minor discomfort and some temporary discoloration may occur at the site of the blood draw.

If I have any questions about this study I may contact Ziba Vaghri at 604-875-2492 or Dr. Sheila Innis at 604-875-2431 at any time.

Consent
The objectives and procedures of this study have been explained to me to my satisfaction. I understand that I may withdraw from this study at any time. If I have any concerns about my treatment or rights in this study I may telephone the Director of Research Services at 604-822-8598. I acknowledge that I have received a copy of the consent form for my own records.

Compensation for Injury
Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

I _____ voluntarily give consent for my child
(Parent, please print)

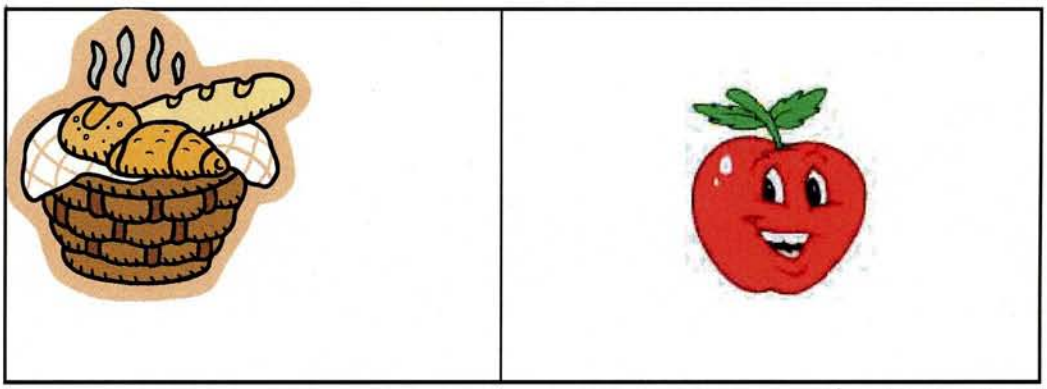
_____ to participate in the study entitled
(Please print) Co-Morbidity of Iron Deficiency and Micronutrient Deficiency, and Risk of **cognitive Delays in Preschool Children 18 months - 5 years of Age**
The parent(s) and the investigator are satisfied that the information contained in this consent form has been explained and that all questions have been answered.

Signed	**Date**
Witness	**Date**
Investigator's Signature	**Date**

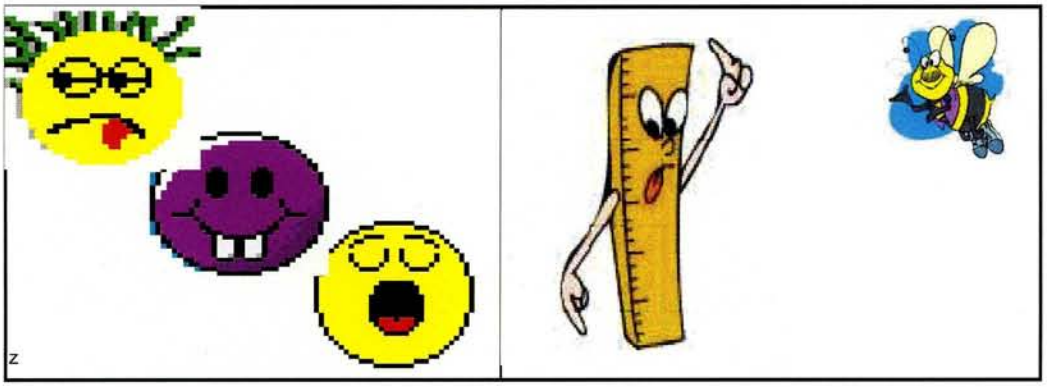
PASSPORT

Welcome to the Adventures to Good Health!

Travel and visit all four of our healthy friends and collect your prizes.



The Brave Bee



This is to certify that _____
has completed the Adventures to Good Health!

Congratulations!

Appendix II-6: An excerpt from the socio-demographic questionnaire of the study of "Food insecurity in inner city children", which collected data on maternal education and familyincome of the study participants

_Q3 Please look at the card and estimate in which of the following groups your total household income from all sources falls? Tell me the Code letter of the range (a card showing a table containing income levels for different family sizes is shown to the client-**will be attached to the final thesis**).

Household Income	Code
1. Response categories: <50% of LICO,	K
2, 50-100% of LICO	P
3. 100-150% of LICO,	B
4, >150% of LICO	N

ED_Q1 Which of the following best describes the highest level of education you have received:
1. less than high school completion
2. high school graduate
3. some college or university (including trade school)
4. college or university graduate

What is your postal code: _____

How many people live in your household as of today? _____

How many children live in the your household? _____

Appendix II-7: Measurement sheet used in the Nutrition Clinics

MEASUREMENT SHEET

1. Your child is a: Boy ☐ Girl ☐

2. What is your child's birth date?

Year/ Month/ day / /

3. What was your child's birth weight?

_____kg or _____lbs

4. Was anyone of the followings true about your pregnancy?

He/she was born full term ☐

He/she was born prematurely ☐ _____weeks of age

You gave birth to more than one child ☐ How many? _____ Babies

You were diagnosed with gestational diabetes ☐

You took vitamin and nutrient supplements ☐

You smoked yes ☐ no ☐ rarely ☐

5. Anthropometric measurements:

Wt: _____kg Ht: _____cm

Mom's height (self-reported): Dad's height (self-reported):

7. Does your child have any health condition or sickness that you are aware of? ☐ Yes ☐ No

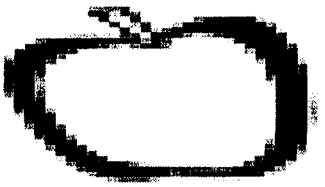
If yes, please specify:

8. Is your child taking any medication at present? ☐ Yes ☐ No

Appendix.II-8: Food Frequency questionnaire used in the study of low income preschoolers

FOOD FREQUENCY QUESTIONNAIRE

NUTRITION



RESEARCH

PROGRAM

Subject Code: zv042 _ _ _

Location of Visit:_____

Interviewer:_____

	Item Name		Brand/Homemade	Amount	Frequency
			DAIRY PRODUCTS		
			Please tell me how much cow's milk your child drinks and what type of milk		
1)	**Milk (drinking), cow's** Chocolate milk	circle	Homo, 2%, 1%, skim	☐ cup/ml	☐ Day
	Hot chocolate			☐ cup/ml	☐ Day
2)	Goat's milk			☐ cup/ml	☐ Day
3)	Soy milks/rice milks			☐ cup/ml	☐ Day
	Please tell me how much cheese your child ea in meals or sandwiches, do not include chees pasta or on pizza				
4)	Cheese, hard cheeses - cheddar, mozzarella, Swiss. Brie			☐ oz/g ☐ cup ☐ slice	☐ Day ☐ Week
5)	Cream cheese			☐ oz/g ☐ tsp ☐ Tsp	☐ Day ☐ Week ☐ Every 2 weeks
6)	Cottage cheese (1%, 2%, creamed or whole)			☐ oz/g ☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
7)	Processed cheese slices		☐ Brand	☐ Slices	☐ Day ☐ Week ☐ Every 2 weeks
8)	Cheese Spreads (eg. Cheez Whiz, Country Crock			☐ oz/g	☐ Day
10)	Feta cheese			☐ oz/g ☐ Piece	☐ Day ☐ Week ☐ Every 2 weeks
1)	Yogurt (see sample)			☐ oz/g ☐ cup/ml	☐ Day ☐ Week ☐ Every 2 weeks
13)	Minigo (Yoplait fresh cheese product)			☐ oz/g ☐ cup/ml	☐ Day ☐ Week ☐ Every 2 weeks
14)	Ice cream/ Frozen yogurt			☐ cup/ml	☐ Day ☐ Week

	Item Name	Brand/Homemade	Amount	Frequency
				☐ Every 2 weeks
15)	Milkshakes/ Yogurt shakes _____	_____	____ ☐ Cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
16)	Sour cream, whipping cream _____	☐ Brand _____ ☐ Homemade	____ ☐ Cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
17)	Whipped Topping _____	_____	____ ☐ Oz/g	_____ ☐ Day
	-cool Whip, Nutri Whip _____	_____	____ ☐ tsp	_____ ☐ Week
	-Whipping cream _____	_____	____ ☐ Tbsp	_____ ☐ Every 2 weeks
	Other Dairy Products- eggnog, Caresses, fresh cheese, cream substitutes _____	_____	____ ☐ oz/g ☐ Cup/ml ☐ Piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks

EGGS: Please tell me how many eggs your child eats, boiled, scrambled, fried, or in omelettes. Do not include eggs in baked dishes

1)	Eggs _____	_____	____ ☐ number	_____ ☐ Day
2)	Egg yolk only _____	_____	____ ☐ number	_____ ☐ Day ☐ Week ☐ Every 2 weeks
3)	Egg whites only _____	_____	____ ☐ number	_____ ☐ Day ☐ Week ☐ Every 2 weeks

TABLE/COOKING FAT

1)	Margarine for spreading on breads, crackers or vegetables _____	Brand: Soft ☐ Hard☐ _____	____ ☐ oz/g ☐ tsp ☐ Tbsp	_____ ☐ Day ☐ Week
3)	Butter for spreading on breads, crackers or vegetables _____	☐ Brand _____	____ ☐ Oz/g ☐ tsp ☐ Tbsp	_____ ☐ Day ☐ Week ☐ Every 2 weeks

Please tell me about oils you use in cooking.

5)	Oils (example In cooking pancakes and frying foods _____	☐ Brand _____ ☐ Homemade	____ ☐ tsp ☐ Tbsp	_____ ☐ Day ☐ Week

	Item Name	Brand/Homemade	Amount	Frequency
				☐ Every 2 weeks

Please tell me about oils you use in salads:

6)	Salads, home made	Oil used:	☐ tsp ☐ Tbsp ☐ Cup/ml	☐ Day ☐ Week ☐ Every 2 weeks
7)	Salad dressing, purchased	Oil/Vinegar ☐	☐ tsp ☐ Tbsp	☐ Day
		Creamy ☐		☐ Week ☐ Every 2 weeks
	Mayonnaise, Miracle Whip	Low fat ☐	☐ tsp	☐ Day
		Regular ☐	☐ Tbsp	☐ Week ☐ Every 2 weeks
8)	Peanut butter **Specify**		☐ tsp	☐ Day
	- tahini, nut butters		☐ Tbsp	☐ Week ☐ Every 2 weeks
10)	Other Table/Cooking Fats – cereal cream, sour cream		☐ tsp ☐ Tbsp ☐ Cup/ml	☐ Day ☐ Week ☐ Every 2 weeks

BREADS/CEREALs: Please tell us about the type of bread your child eats

1)	Bread **Specify**		☐ Piece	☐ Day
	(Including pita/bagels) corn, rye, sourdough, chapatti, roti	☐ White ☐Whole wheat		☐ Week
2)		☐Multigrain		
		☐ Rye		
		☐ Sourdough		
		☐ Corn		
	Buns/Rolls (Including hamburger/hot dog buns) include **only** buns for hamburgers and hotdogs made at home		☐ Piece	☐ Day ☐ Week
3)	Tortillas	**Flour** ☐ White ☐ Whole wheat	☐ 6" ☐ 8" ☐ 12"	☐ Day ☐ Week ☐ every 2 Weeks
	DO Not include tortillas in purchased fast foods			
	Tacos	☐ Corn		
		☐ Soft Wheat (?)		

	Item Name		Brand/Homemade	Amount	Frequency
	Pita		☐ White		
			☐ Whole wheat		
	Bagels		☐ White		
			☐ Whole wheat		
			☐ Multigrain		☐ Day
	Breadsticks/Croutons			☐ Piece	☐ Week
			☐ Homemade		
4)	Cereals, cold breakfast e.g. corn flakes, rice krispies, corn pops			☐ Cup	☐ Day ☐ Week
5)	Cereals, cooked		☐ Oats (porridge) ☐ Cream of wheat ☐ ?	☐ Cup	☐ Day ☐ Week
6)	Wheat germ (Used in/on foods)		☐ Brand ☐ Homemade	☐ tsp ☐ Tsp	☐ Day ☐ Week ☐ Every 2 weeks
7)	Bran, on foods			☐ tsp	☐ Day
				☐ Tbsp	☐ Week
					☐ Every 2 weeks
	Milk added to cereals	**Specify** For each cup of cereal----------→	☐1 cup milk ☐ 1/2 cup milk ☐ 1/4 cup milk ☐ No milk		
	Sugar added to cereal	**Specify** For each cup of cereal----------→	--------tsp sugar ------Tbsp sugar ☐ No sugar		
	Baked Goods				
	Breakfast goods; Pancakes/Waffles. **Specify**		Surup_____Tbsp tsp ☐ No syrup	☐ 2" ☐ 4" ☐ 5" ☐ 6"	☐ Day ☐ Week ☐ Every 2 weeks
	Muffins, bran			☐Small ☐ regular ☐Large (Deli size)	☐ Day ☐ Week ☐ Every 2 weeks
	Fruit muffins, blueberry, carrots,			☐ Small	☐ Day

	Item Name		Brand/Homemade	Amount	Frequency
				☐ regular ☐Large (Deli size)	☐ Week ☐ Every 2 weeks
	English Muffin			☐ Piece	☐ Day ☐ Week ☐ Every 2 weeks
10)	Scones		☐ Brand ☐ Homemade	☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
11)	Tea biscuits		☐ Brand ☐ Homemade	☐ Piece	☐ Day ☐ Week ☐ Every 2 weeks
	Pop tarts/ Toaster Pastries		☐ Brand	☐ Piece	☐ Day ☐ Week ☐ Every 2 weeks
12)	Donuts, Fritters			☐ Piece	☐ Day ☐ Week ☐ Every 2 weeks
13)	Danish, Pastries			☐ Piece	☐ Day ☐ Week ☐ Every 2 weeks
14)	Croissants			☐ Oz/g ☐ Cup/ml ☐ Piece	☐ Day ☐ Week ☐ Every 2 weeks
15)	Cakes, white		☐ With icing ☐ Without icing	☐ 2" square ☐ 1/8 cake	☐ Day ☐ Week ☐ Every 2 weeks
16)	Cakes, chocolate		☐ With icing ☐ Without icing		☐ Day ☐ Week ☐ Every 2 weeks
	Fruit tarts			☐ Slice (1/8")	☐ Day ☐ Week ☐ Every 2 weeks
	Custard tarts			☐ piece	☐ Day ☐ Week

Item Name		Brand/Homemade	Amount	Frequency
17) Cookies	Specify ------ -→	☐ Plain	☐ piece	☐ Every 2 weeks ☐ Day
		☐ Oat meal ☐ Chocolate ☐ Fruit-filled ☐ Cream-filled		☐ Week ☐ Every 2 weeks
18) Cinnamon buns			☐ 1/4 Piece ☐ ½ piece	☐ Day ☐ Week ☐ Every 2 weeks
19) Wagon wheels			☐ 1/4 piece ☐ 1/2 piece	☐ Day ☐ Week ☐ Every 2 weeks
20) Instant noodle, pkg dry		☐ Brand	☐ 1/4 pkg ☐ 1/2 pkg	☐ Day ☐ Week ☐ Every 2 weeks
21) Cooked noodle				
Please tell me about the cooked noodles that your child eats; please include all the noodles that you use to make dishes **Except for macaroni and cheese**. Include spaghetti and lasagna and cannelloni **(tell me about the sauces later)**		☐ Brand ☐ Homemade	☐ cup/ml ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
22) Spaghetti, boiled			☐ Cup	☐ Day ☐ Week ☐ Every 2 weeks
23) Other noodles; fettuccini, rigatoni			☐ Cup	☐ Day ☐ Week ☐ Every 2 weeks
24) Rice noodles (Boiled)			☐ Cup	☐ Day ☐ Week ☐ Month
25) Macaroni and cheese, Home-made and packaged (e.g. KD)			☐ Cup ☐ 1/4 pkg ☐ 1/2pkg ☐ 3/4pkg ☐ 1 pkg	☐ Day ☐ Week ☐ Every 2 weeks
26) Canned noodles, with tomato sauce e.g. alphagetti, with tomato and meat e.g. ravioli			☐ Cup	☐ Day ☐ Week ☐ Every 2 weeks

Item Name	Brand/Homemade	Amount	Frequency
Lasagna/cannelloni, **with vegetables** ☐ Day			
_____	_____	___ ☐ Cup	___ ☐ Week ☐ Every 2 weeks
Lasagna/cannelloni **With meat**		☐ Cup	☐ Day
_____	_____	___	___ ☐ Week ☐ Every 2 weeks
Cooked rice	☐ White	☐ cup	☐ Day
_____	_____	___	___
_____	☐ Brown ☐ Wild	___	___ ☐ Week ☐ Every 2 weeks
Other grain products, couscous		☐ Cup	☐ Day
_____	_____	___	___ ☐ Week ☐ Every 2 weeks
_____	_____	___ ☐ oz/g ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
6) Lamb (including roast, chops, etc) _____	_____	___ ☐ oz/g ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
7) Chicken, turkey or other poultry _____	_____	___ ☐ oz/g ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
8) Chicken nuggets/ strips _____	_____ ☐ Brand ☐ Homemade	___ ☐ oz/g ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
9) Wild game (moose, deer, etc-fresh, f frozen, dried) _____	_____ ☐ Brand ☐ Homemade	___ ☐ oz/g ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
10) Canned fish and Shellfish, example. Tuna, salmon, sushi... _____	_____ ☐ Brand	___ ☐ oz/g ☐ cup ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
11) Fresh and frozen fish and shell fish _____	_____	___ ☐ oz/g ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks

Item Name	Brand/Homemade	Amount	Frequency
12) Other seafood - scallop, clams - Lobsters, mussels, oysters		___ ☐ oz/g ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
13) Deli meats; b bologna, salami, pepperoni, shell fish		___ ☐ oz/g ☐ slices	___ ☐ Day ☐ Week ☐ Every 2 weeks
14) Wieners, hot dogs, sausages		___ ☐ oz/g ☐number	___ ☐ Day ☐ Week ☐ Every 2 weeks

MEATALTERNATI VES

1) Firm or medium firm tofu or soybean curd	☐ Brand	___ ☐ oz/g ☐ cup ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
2) Soft or desert tofu	☐ Brand ☐ Homemade	___ ☐ oz/g ☐ cup ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks
3) Soy wiener/ vegetarian wiener	☐ Brand ☐ Homemade	___ ☐ oz/g ☐ cup ☐number	___ ☐ Day ☐ Week ☐ Every 2 weeks
4) Other meat replacements	☐ Brand	___ ☐ oz/g ☐ cup ☐ piece	___ ☐ Day ☐ Week ☐ Every 2 weeks

COMBINATION DISHES

CASSEROLS WITH MEAT, FISH AND POULTRY

1) Mixed dishes prepared, with beef (e.g. shepherd's pie, pot pie, chili, stew)	☐ Brand ☐ Homemade	___ ☐ oz/g ☐ cup ☐ Tbsp	___ ☐ Day ☐ Week ☐ Every 2 weeks
2) Mixed dishes made with fish (e.g., tuna casserole)	☐ Brand ☐ Homemade	___ ☐ oz/g ☐ cup	___ ☐ Day ☐ Week ☐ Every 2 weeks
3) Mixed dishes made with pork	☐ Brand ☐ Homemade	___ ☐ oz/g ☐ cup	___ ☐ Day ☐ Week ☐ Every 2 weeks
4) Mixed dishes made with lamb	☐ Brand ☐ Homemade	___ ☐ oz/g ☐ cup	___ ☐ Day ☐ Week ☐ Every 2 weeks
5) Pizza with meat		___ ☐ slice	___ ☐ Day

	Item Name	Brand/Homemade	Amount	Frequency
		☐ Brand ☐ Homemade		☐ Week ☐ Every 2 weeks
6)	Enchiladas and Taco with meat	☐ Brand ☐ Homemade	☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
7)	Filled buns, baked or steamed, meat filled		☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
8)	Luncheon Meats/ Spreads in sandwiches/subs	☐ Brand ☐ Homemade	☐ oz/g ☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks

COMBINATION DISHES WITH CHEESE

1)	Enchiladas, cheese filled	☐ Brand ☐ Homemade	☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
2)	Perogies (potato and cheese filled, or onion filled)	☐ Brand ☐ Homemade	☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
3)	Pizza and pizza pockets with cheese and no meat	☐ Brand ☐ Homemade	☐ piece ☐ slice	☐ Day ☐ Week ☐ Every 2 weeks
4)	Quiche without meat (with cheese)	☐ Brand ☐ Homemade	☐ oz/g ☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks

COMBINATION DISHES WITH VEGETABLES

1)	cooked lentils, beans, or peas (e.g. lentil stew or soup)	☐ Brand ☐ Homemade	☐ oz/g ☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
2)	Vegetarian pasta dishes	☐ Brand ☐ Homemade	☐ oz/g ☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
3)	Other mixed dishes	☐ Brand ☐ Homemade	☐ oz/g ☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks

	Item Name		Brand/Homemade	Amount	Frequency
	SOUPS				
1)	*Broth Type e.g.* Vegetable	_____	_____ ☐ Brand ☐ Homemade	____ ☐ cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
2)	Broth type; chicken, Beef and fish	_____	_____ ☐ Brand ☐ Homemade	____ ☐ cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
3)	Cream-type soups	_____	_____ ☐ Brand ☐ Homemade	____ ☐ cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
4)	Noodle soups	_____	_____ ☐ Brand ☐ Homemade	____ ☐ cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
5)	Other types of soup	_____	_____ ☐ Brand ☐ Homemade	____ ☐ oz/g ☐ cup/ml ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks
	VEGETABLE; CANNED, FRESH OR FROZEN				
1)	Broccoli	_____	_____	____ ☐ oz/g ☐ cup ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks
2)	Carrots	_____	_____	____ ☐ oz/g ☐ cup ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks
3)	Corn; cream or niblets	_____	_____	____ ☐ oz/g ☐ cup ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks
4)	Green peas	_____	_____	____ ☐ oz/g ☐ cup	_____ ☐ Day ☐ Week ☐ Every 2 weeks
5)	Spinach, cooked	_____	_____	____ ☐ oz/g ☐ cup	_____ ☐ Day ☐ Week ☐ Every 2 weeks
6)	Green beans, string beans, yellow beans	_____	_____	____ ☐ oz/g ☐ cup ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks
7)	Potatoes; mashed, baked, salad or boiled	_____	_____	____ ☐ oz/g ☐ cup	_____ ☐ Day ☐ Week

	Item Name	Brand/Homemade	Amount	Frequency
			☐ piece	☐ Every 2 weeks
8)	French fries, home fries, Pan fries		____ ☐ oz/g ☐ cup ☐ piece	____ ☐ Day ☐ Week ☐ Every 2 weeks
9)	Squash, all types		____ ☐ oz/g ☐ cup ☐ piece	____ ☐ Day ☐ Week ☐ Every 2 weeks
10)	Cabbage		____ ☐ oz/g ☐ cup	____ ☐ Day ☐ Week ☐ Every 2 weeks
11)	Brussel; sprouts		____ ☐ oz/g ☐ cup ☐ piece	____ ☐ Day ☐ Week ☐ Every 2 weeks
12)	Celery		____ ☐ oz/g ☐ cup ☐ sticks	____ ☐ Day ☐ Week ☐ Every 2 weeks
13)	Chick peas		____ ☐ oz/g ☐ cup	____ ☐ Day ☐ Week ☐ Every 2 weeks
14)	Lentils/split peas		____ ☐ oz/g ☐ cup	____ ☐ Day ☐ Week ☐ Every 2 weeks
15)	Kidney beans		____ ☐ oz/g ☐ cup	____ ☐ Day ☐ Week ☐ Every 2 weeks
16)	tomato		____ ☐ cup ☐ piece	____ ☐ Day ☐ Week ☐ Every 2 weeks
17)	Lettuce		____ ☐ cup ☐ leaves	____ ☐ Day ☐ Week ☐ Every 2 weeks
18)	Cucumber		____ ☐ cup ☐ slices	____ ☐ Day ☐ Week ☐ Every 2 weeks
19)	Peppers		____ ☐ cup ☐ piece	____ ☐ Day ☐ Week ☐ Every 2 weeks
20)	Other vegetables		____ ☐ cup ☐ piece	____ ☐ Day ☐ Week

	Item Name	Brand/Homemade	Amount	Frequency
				☐ Every 2 weeks
	FRUIT (CANNED, FRESH OR FROZEN)			
1)	Apples and apple sauces		☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
2)	Bananas		☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
3)	Oranges		☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
4)	Pears, peaches, nectarines and plums		☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
5)	Grapes		☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
6)	Raisins, prunes and other dried fruits	☐ Brand	☐ oz/g ☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
7)	Melon (eg. Cantaloupe, honeydew, watermelon)		☐ oz/g ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
8)	Lychee		☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
9)	Strawberries		☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
10)	Other berries (blueberries, raspberries)		☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
11)	Fruit cocktail or fresh fruit salad	☐ Brand ☐ Homemade	☐ cup	☐ Day ☐ Week ☐ Every 2 weeks
12)	Other fruits		☐ oz/g	☐ Day

	Item Name	Brand/Homemade	Amount	Frequency
			____ ☐ cup ☐ piece	____ ☐ Week ☐ Every 2 weeks

BEVERAGES

1)	***Pure orange Juice and*** grapefruit juice		____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
2)	Apple juice		____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
3)	Five alive		____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
4)	Other fruit juices (eg. Grape, pear, pineapple, papaya, cranberry)		____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
5)	Prune juice	☐ Brand ☐ Homemade	____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
6)	Tomato and mixed Vegetable juices (eg. V8 juice)	☐ Brand ☐ Homemade	____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
7)	Carrot juice	☐ Brand ☐ Homemade	____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
8)	Sweetened fruit drinks including crystals and boxed varieties (eg. Tang, Kool-Aid, Ribena)	☐ Brand ☐ Homemade	____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
9)	Pop (regular)	☐ Brand ☐ Homemade	____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
10)	Pop (diet)	☐ Brand ☐ Homemade	____ ☐ cup/ml	____ ☐ Day ☐ Week ☐ Every 2 weeks
11)	Carbonated fruit drinks (eg. Koala Springs)	☐ Brand ☐ Homemade	____ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Every 2 weeks
12)	Tea		____ ☐ cup/ml	____ ☐ Day ☐ Week

	Item Name	Brand/Homemade	Amount	Frequency
				☐ Every 2 weeks
13)	Coffee _____	_____	____ ☐ cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
14)	Other beverages _____	_____ ☐ Brand ☐ Homemade	____ ☐ cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks

DESSERTS

1)	***Custard*** _____	_____ ☐ Brand ☐ Homemade	____ ☐ cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
2)	Pudding _____	_____ ☐ Brand ☐ Homemade	____ ☐ cup/ml	_____ ☐ Day ☐ Week ☐ Every 2 weeks
3)	Jello _____	_____ ☐ Brand ☐ Homemade	____ ☐ cup/ml ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks
4)	Popsicle or Mr. Freezie _____	_____ ☐ Brand ☐ Homemade	____ ☐ oz/g ☐ cup/ml ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks

SNACKS

1)	***Plain or cheese crackers*** _____ (Ritz, cheese type and soda crackers)	_____ ☐ Brand ☐ Homemade	____ ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks
2)	Wheat crackers _____ (Stone wheat, thins, Triscuits, wholegrain, soda crackers)	_____ ☐ Brand ☐ Homemade	____ ☐ piece	_____ ☐ Day ☐ Week ☐ Every 2 weeks
3)	Potato chips, cheeses or tortilla chips _____	_____ ☐ Brand ☐ Homemade	____ ☐ piece ☐ pkg	_____ ☐ Day ☐ Week ☐ Every 2 weeks
4)	Popcorn _____	_____ ☐ Brand ☐ Homemade	____ ☐ oz/g ☐ cup	_____ ☐ Day ☐ Week ☐ Every 2 weeks
5)	Party snacks _____ - Nuts & Bolts, pretzels - Crunch N Munch	_____ ☐ Brand ☐ Homemade	☐ cup ____ ☐ piece	☐ Day _____ ☐ Week ☐ Every 2 weeks

	Item Name	Brand/Homemade	Amount	Frequency
6)	Walnuts	☐ Brand ☐ Homemade	☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
7)	Almonds	☐ Brand ☐ Homemade	☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
8)	Other nuts	☐ Brand ☐ Homemade	☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
9)	Seeds (eg, sunflower seeds)	☐ Brand ☐ Homemade	☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
10)	Other seeds	☐ Brand ☐ Homemade	☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
11)	Other snacks	☐ Brand ☐ Homemade	☐ oz/g ☐ cup ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
	JAMS, JELLIES,CANDIES			
1)	Candy	☐ Brand	☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
2)	Jam and jellies on bread	☐ Brand ☐ Homemade	☐ Tsp ☐ tsp	☐ Day ☐ Week ☐ Every 2 weeks
3)	Chocolate bar	☐ Brand ☐ Homemade	☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
4)	Granola bar	☐ Brand	☐ oz/g ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
5)	Fruit roll up, fruit leather	☐ Brand	☐ oz/g ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
6)	Suckers lolly pops etc	☐ Brand	☐ oz/g ☐ piece	☐ Day ☐ Week ☐ Every 2 weeks
	CONDIMENTS			

	Item Name		Brand/Homemade	Amount	Frequency
1)	*Tomato ketchup*		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp	☐ Day ☐ Week ☐ Every 2 weeks
2)	Chatni		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp	☐ Day ☐ Week ☐ Every 2 weeks
3)	Other sauces		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp	☐ Day ☐ Week ☐ Every 2 weeks
4)	***Other condiments***		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp	☐ Day ☐ Week ☐ Every 2 weeks
	PURCHASED INFANT, JUNIOR AND TODDLER FOODS				
1)	Cereal (eg. Rice, barley , oats or mixed)		☐ Brand	☐ Tsp ☐ tsp ☐ jar	☐ Day ☐ Week ☐ Every 2 weeks
2)	Cereals mixed with yogurt or/and fruits		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp ☐ jar	☐ Day ☐ Week ☐ Every 2 weeks
3)	Meat or poultry (eg, beef, pork, lamb, veal, ham, chicken or turkey)		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp ☐ jar	☐ Day ☐ Week ☐ Every 2 weeks
4)	***Liver***		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp ☐ jar	☐ Day ☐ Week ☐ Every 2 weeks
5)	Meat or poultry with rice or noodle dinner		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp ☐ jar	☐ Day ☐ Week ☐ Every 2 weeks
6)	Vegetable and meat		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp ☐ jar	☐ Day ☐ Week ☐ Every 2 weeks
7)	***Vegetables***		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp ☐ jar	☐ Day ☐ Week ☐ Every 2 weeks
8)	Fruits		☐ Brand ☐ Homemade	☐ Tsp ☐ tsp ☐ jar	☐ Day ☐ Week ☐ Every 2 weeks

	Item Name		Brand/Homemade	Amount	Frequency
9)	Prunes	_____	_____ ☐ Brand ☐ Homemade	____ ☐ Tsp ☐ tsp ☐ jar	_____ ☐ Day ☐ Week ☐ Every 2 weeks
10)	*Fruit desserts* (eg. Tutti Frutti)	_____	_____ ☐ Brand ☐ Homemade	____ ☐ Tsp ☐ tsp ☐ jar	_____ ☐ Day ☐ Week ☐ Every 2 weeks
11)	*Fruit yogurt desserts*	_____	_____ ☐ Brand ☐ Homemade	____ ☐ Tsp ☐ tsp ☐ jar	_____ ☐ Day ☐ Week ☐ Every 2 weeks
12)	Custard or pudding	_____	_____ ☐ Brand ☐ Homemade	____ ☐ Tsp ☐ tsp ☐ jar	_____ ☐ Day ☐ Week ☐ Every 2 weeks
13)	Other purchased baby foods	_____	_____ ☐ Brand ☐ Homemade	____ ☐ Tsp ☐ tsp ☐ jar	_____ ☐ Day ☐ Week ☐ Every 2 weeks

1) Did you breast-feed your child?

☐ Yes ☐ No

If yes, please specify (exclusively breast-fed);
;

1-2 months ☐ 4-6 months ☐ >6-10 months ☐ 12-18 months ☐

2) Are you currently breast-feeding?

☐ Yes Number of times per day _____ ☐ No

3) Are you currently giving your child a commercial infant formula?

☐ Yes (please go to question 4)

☐ No (please go to question 5)

4) What brand types of formula do you usually give your child?	Color of label	How many times per day or per week	How much does your child usually drink per day?
(1) _____	_____	_____times per ☐ day ☐ week	_____

(2) _____ _____ _____times per ☐ day
_____ ☐ week

(3) _____ _____ _____times per ☐ day
_____ ☐ week

5) Do you usually give your child a vitamin/mineral supplement?

☐ Yes (please go to Q5)
☐ No

6) What brands/Types of
Supplements?

(2) _____ ☐ day
☐ week
☐ month

(1) _____ ☐ day
☐ week
☐ month

(2) _____ ☐ day
☐ week
☐ month

7) Does your child takes a bottle?

At night	during day time naps	during the day
☐ Yes	☐ Yes	☐ Yes
☐ NO	☐ NO	☐ NO

8) What is usually in your child's bottle?

	At night	during day time
Milk; Homogenized	_____	_____
2%	_____	_____
1%	_____	_____
Skim	_____	_____
Juice	_____	_____
Water	_____	_____
Other	_____	_____

Appendix II-9. Comparison of the daily intake (number of serving/ day) of main food groups and their sub categories for inner city boys and girls (n=82)

Food group	Servings/day (Boys, n=43)	Servings/day (Girls, n=35)
Meat and alternatives	2.73±b1.4	3.12 ± 1.9
Cereals and Grains	7.19 ± 3.5	6.37 ± 3.7
Whole wheat C&G	1.33 ± 1.9	1.31 ± 2.1
Non-whole wheat C&G	5.85 ± 2.9	5.06 ± 2.6
Dairy	3.62 ±2.0	3.92 ± 2.4
Fruits and Vegetables	7.40 ± 5.6	6.65 ± 5.0

Appendix III-1: Certificate of ethic approval for "Population patterns of hair zinc"

The University of British Columbia
Office of Research Services

Clinical Research Ethics Board
Room 210, Research Pavilion, 828 West 10th Avenue,
Vancouver, BC V5Z 1L8

PRINCIPAL INVESTIGATOR	DEPARTMENT	NUMBER
Hertzman, C.	Health Care/Epidemiology	**C05-0463**

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT: UBC Campus

CO-INVESTIGATORS:

Barr, Susan, Family & Nutr Sci; Chapman, Gwenneth, Land & Food Systems; Vaghri, Ziba, Land & Food Systems; Wong, Hubert, Health Care/Epidemiology

SPONSORING AGENCIES: Unfunded Research

TITLE: Population Pattern of Hair Zinc; Dietary and Sociodemographic Determinants

Notice of Ethical Review: Memo

The UBC CREB has reviewed your proposed study, and has issued a *Certificate of Approval.*

Please make sure the following items have been corrected/amended within 21 days. Ensure that any revisions to the consent form are made on the correct institutional letterhead and are either underlined or in **BOLD** text. A letter that explains how the requested changes have been addressed should accompany your response.

If needed for Clinical Trials, please ensure that the Consent Form Version Date is updated to reflect any required revisions and that the new version date is clearly indicated in your response. An updated Certificate of Approval will be issued accordingly.

All other responses will be acknowledged by the CREB Chair. **You may submit the revisions by email to Ms. Farin Ramji at farin.ramji@ors.ubc.ca.** Thank you for your attention.

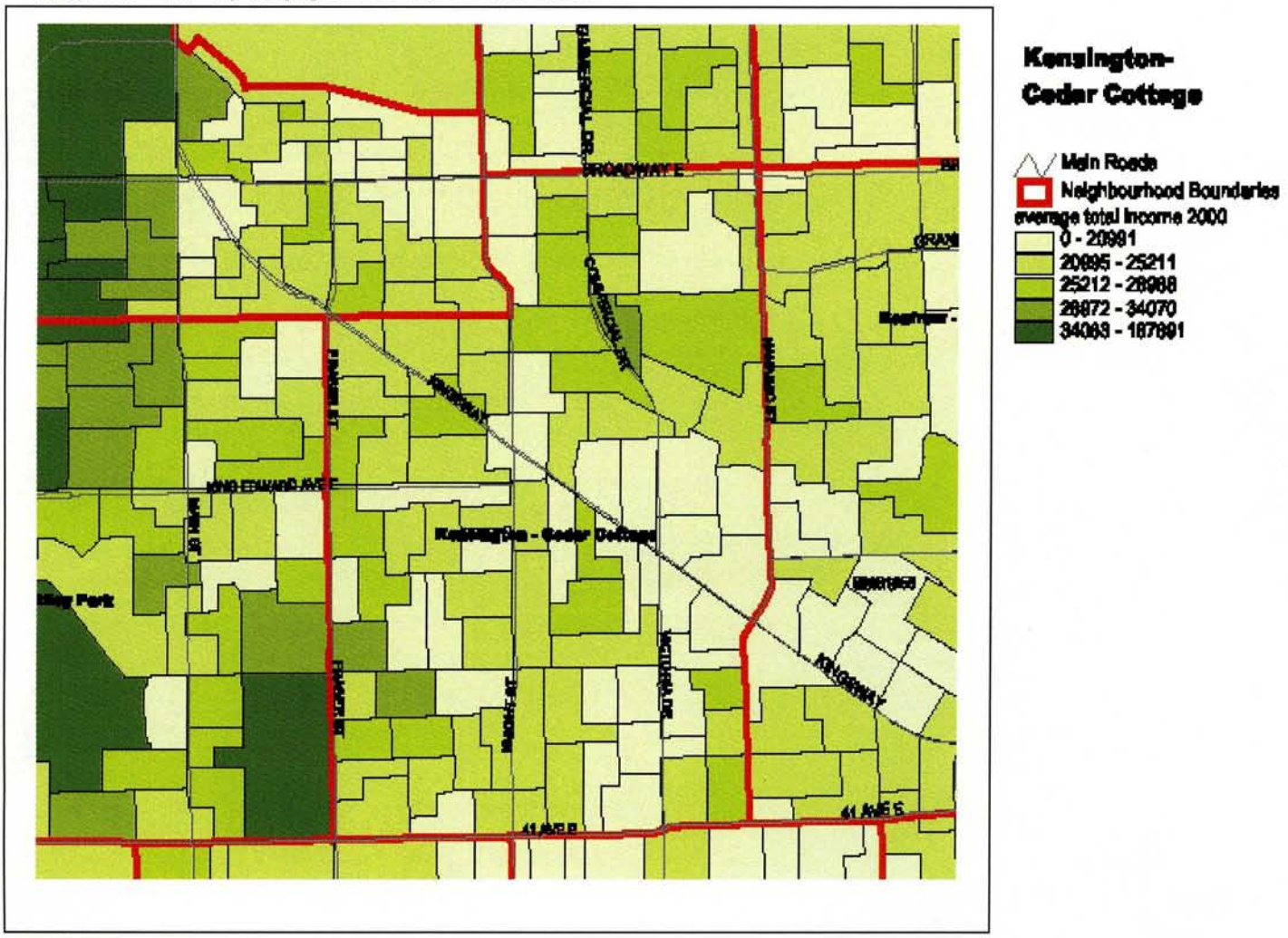
Please provide the CREB a copy of the information pamphlet for approval once it has been prepared.

If you have any questions regarding these requirements, please call:
Dr. Gail Bellward, Chair, 604-875-4111 ext. 62276
Dr. James McCormack, Associate Chair, 604-822-1710
Ms. Erin Skrapek, Manager, Clinical Ethical Reviews (ORS), 604-875-4149
For further information, refer to the UBC CREB's Guidance Notes and Policies at:
www.ors.ubc.ca/ethics/human_subjects.htm.

Please send all correspondence to: **Clinical Research Ethics Office, Room 210, Research Pavilion,**
828 West 10th Avenue, Vancouver, BC Canada V5Z 1L

Appendix III-2: Neighborhood Maps produced by the Geographic Information System, GIS, unit of the Human Early Learning Partnership, HELP, to facilitate planning the recruitment strategy

Average total income (2000) by census dissemination area.



map use forbidden for publication or dissemination

Appendix III-3: Letter of Support from the Executive Director of the West Coast Child Care Resource Center

3rd Floor, 210 W. Broadway
Vancouver, BC V5Y 3W2
Ph: 604.709.5661 Fax: 604.709.5662
Toll-free: 1.877.262.0022
Website: www.wstcoast.org

January 25, 2006

Dear Child Care Colleague:

Westcoast Child Care Resource Centre is pleased to lend its support to an important research study regarding the dietary status of preschool-aged children in Vancouver. This research study is being conducted by Ziba Vaghri, a UBC Doctoral student under the direction of Dr. Clyde Hertzman, the Director of Human Early Learning Partnership (HELP).

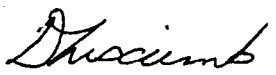
This study involves a city-wide survey of preschoolers to look into some of the dietary, health and socio-demographic factors related to zinc deficiency in young children. Zinc deficiency is quite common and can adversely affect school performance and a wide range of subsequent life events such as educational achievement, employment, income, and so on.. Previous work by UBC researchers has indicated that there are some factors that may place a child at a higher risk for this deficiency.

The survey involves a questionnaire that will go to parents. Following completion of the questionnaire and with parental consent, researchers will come into your child care centre to take height and weight measurements of children as well as a small hair sample for the purpose of hair zinc analysis. This analysis can be an index of child's zinc status.

You will be contacted in the near future for further discussion of this project, and how you can help parents become involved. Please feel free to direct any questions you might have regarding the study to the UBC researchers when they call or visit.

Westcoast Child Care Resource Centre asks you to consider participation in this research project. Such research is key to our understanding of the determinants of young children's health.

Sincerely'



Dianne Liscumb
Executive Director
Westcoast Child Care Resource Centre

Appendix III-4: Letter from the principal investigator of the project

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemi
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Dear child educators;

We are a group of researchers from the University of British Columbia planning to conduct a survey to investigate the "Population patterns of hair zinc in preschoolers" in the city of Vancouver. Your agency is key to helping us connect with parents of children 2-6 years old who may choose to participate in this study. Qualified professionals will treat participants and the information they provide with great care and confidentiality.

Zinc is an essential micronutrient available from many sources including meat, dairy, cereal and grain products. Inadequate zinc intake can result in zinc deficiency. It has been shown that even minor zinc deficiency, termed marginal zinc deficiency (MZD), can interfere with children's normal growth and development. MZD can be easily prevented if adequate amounts of zinc-rich foods are included in a child's diet. When properly diagnosed, the correction of MZD is, also, relatively simple. Since the hair zinc content in children is considered an index of the body's zinc content, it can be used in detecting MZD. Children with MZD often have lower hair zinc content. The main purpose of this study is to investigate the hair zinc status of a representative group of Vancouver preschoolers. The second purpose of this survey is to examine and understand the dietary and environmental factors that could predispose children to low hair zinc. The information collected through this survey will increase our awareness on the at-risk groups of children in our communities.

In few weeks, you will be provided with an "announcement letter" addressed to the parents of these children. This letter, which is to be sent home with every child, will notify parents about the upcoming survey package. Subsequently, two weeks after this

letter, survey packages will be supplied to your centers to be handed to the parents of children when they drop off or pick up their children.

Few weeks after distributing the survey packages, we will need to come to your center (at a time that is convenient for your center). During this visit we will collect the hair samples and measure the heights and weights of children whose parents have consented to this. **We foresee that the survey will take 4-6 weeks.**

The information collected through this survey will better our understanding of zinc deficiency, one of the common and silent deficiencies of this age group. The success of this survey is largely dependent on the number of people participating in it.

With this letter I am asking for your support in this important research. You can extend your support to this research by encouraging the parents to participate.

Yours sincerely,

Clyde Hertzman MD, M.Sc, FRCPC
Director of Human Early Learning Partnership
University of British Columbia

Appendix III-5: Initial letter of contact for survey participants

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemi
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Dear parents;

In two weeks you will be receiving a survey package through your childcare center, which will invite you to participate in a survey to be undertaken by a group of researchers from the University of British Columbia. **We are inviting you to participate in this survey.** You have been selected because you have a child who is two years or older but not yet in school.

This survey, entitled "Population Pattern of Low Hair Zinc in Preschoolers", is a **part of the doctoral thesis of a UBC student, Ziba Vaghri.** It will be conducted through the University of British Columbia under the supervision **of myself, Dr. Clyde Hertzman, a UBC professor** and director of the Human Early Learning Partnership, HELP. The survey's purpose is to increase our understanding of zinc deficiency in preschool children both because it is a very common deficiency in childhood and also, one that to date remains largely unrecognized and is not easily detected.

Your participation will involve answering a short questionnaire. It will take 10-15 minutes. You will also need to read and sign a consent form to give us permission to measure your child's weight and height and to take a sample of his/her hair. We ask that

the completed questionnaire and consent form be returned to your center at your early convenience.

Taking a hair sample (taken at the center) is a simple procedure that will be explained to your child and will cause him/her no pain.

By participating in this survey you will be doing your part to expand the present state of knowledge of zinc deficiency among preschool children. Your child's hair will be analyzed for its zinc content and the result of this analysis along with the information provided in the questionnaire will be used to better understand some possible patterns associated with this deficiency. This information will be treated with **confidentiality.**

Sincerely,

Clyde Hertzman MD, M.Sc, FRCPC

Director of Human Early Learning Partnership

University of British Columbia

Appendix III-6: Recruitment form for the focus group participants

THE UNIVERSITY OF BRITISH COLUMBIA

Food, Nutrition and health
Faculty of Land and Food Systems
2205 East Mall
Vancouver, BC, Canada V6T 1Z4
Phone: 604-822-2502
Fax: 604-822-5143

Dear parent/guardian

We are planning a citywide survey of preschooler's health and nutrition. Before we initiate the survey we need to test our survey tool/questionnaire.

Would you be willing to participate in a small group discussion that will help us to pre-test our survey tool?

In these groups you, along with 3-4 other mothers, will be asked to complete a questionnaire and take part in a subsequent group discussion. After completion of this session you will receive a $15 grocery certificate. The researchers in this study will facilitate the discussion groups.

Would you like to participate in these focus groups?

If you answered YES, please provide your name and phone number

Name_____ Phone Number_____

Ethnicity_____ age of the child_____

And email this form to;

zibav@interchange.ubc.ca

For your convenience he groups will be set up close to your daycare center.

Do you need child minding? Yes ☐ No ☐

Appendix. III-7: Information sheet for recruited focus group participants

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemiology
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Dear focus group participant,

You are scheduled to attend a focus group on

January the_____ from _____ Am/Pm to _____ Am/Pm.

The focus groups will be held at:

If for any reason you are unable to attend please call **Ziba @ 604-812-1419** to reschedule.

We appreciate your support and look forward to seeing you again.

Sincerely,

Ziba Vaghri
Ph.D. Candidate
University of British Columbia

Appendix III-8: Informed Consent form for focus group participants

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemiology
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Informed consent:

I have accepted to participate in a focus group for the study of "population patterns of hair zinc". Ziba Vaghri, the student conducting the study, has explained to me, both, the purpose of this focus group and my role in it. I have read and understood the subject information and consent form.

- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this focus group is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I have read this form and I freely consent to participate in this focus group.
- I have been told that I will receive a dated and signed copy of this form.

Printed name of subject's legally acceptable representative — Signature
Date

Printed name of principal investigator/ designated representative — Signature
Date

Appendix III-9: Survey questionnaire

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemiology, Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772

Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Date 2006/ /

I. CHILD'S BACKGROUND

Gender: ☐ Boy ☐ Girl

Date of Birth: / /

Day/ month / year

Place of birth (country): _____

If not born in Canada, how long the child has been in Canada? _____ and _____

years **months**

1) Was your child fed with breast-milk? ☐ No ☐Yes

If yes, from the time she/he was_____months to the time she/he was_____months old

(Please write a number) (Please write a number)

2) Was your child fed with infant formula? ☐ No ☐Yes

If yes, from the time she/he was_____months to the time she/he was____months old

(Please write a number) (Please write a number)

3) When did you introduce food to your child?

When she/he was_____months old.

(Please write a number)

4) Does your child take or has taken a vitamin/mineral supplement? ☐No ☐Yes
If yes, from the time she/he was_____months to the time she/he was_____months old

(Please write a number)

(Please write a number)

5) Please write the name of supplement(s) your child takes or took in the past (the brand name)_____

6) How would you describe your child's health?

☐Very good ☐ Good ☐Fair ☐ Poor

7) How often does your child get sick with sick flu, fever, cold, diarrhea, cough etc?

☐ **Never** ☐

II. CHILD'S EATING HABITS

1) Are you concerned about your child's eating? ☐ **Yes** ☐ **No**

If "yes", why are you concerned? Please check all your concerns.

☐ Picky eater ☐Eats unhealthy foods	☐ Does not eat enough ☐ Eats too much	☐ Always eats the same foods ☐ Other **(specify)**_____

2) In total, how many glasses of liquid NOT COUNTING WATER, does your child drink in a day? (Milk, juices, soft drinks, **BREAST MILK**, and other drinks)

☐None ☐ One ☐ two ☐ three ☐ four ☐ five ☐ more than five

3) Does your child eat the following food?

Meat (beef, lamb, pork, veal, moose meat and…) ☐Yes ☐ No

Fish and shellfish (Includes crab, shrimps, lobsters, clams, scallops…) ☐ Yes ☐ No

Poultry (Includes chicken, duck, goose, turkey, etc) ☐ Yes ☐ No

How often?

Meat____ times ☐per day **OR** ☐ per week **OR** ☐per month

Fish and shellfish_____times ☐per day **OR** ☐ per week **OR** ☐per month

Poultry_____times ☐per day **OR** ☐ per week **OR** ☐per month

4) How many times does your child eat the following?

White bread _____times per ☐day ☐week ☐month

Brown bread_____times per ☐day ☐week ☐month

White rice_____times per ☐day ☐week ☐month

Brown and wild rice____times per ☐day ☐week ☐month

White pasta (noodles, rice noodles…)_____times per ☐day ☐week ☐month

Brown or whole-wheat pasta____times per ☐day ☐week ☐month

Breakfast cereal (non-whole grain)_____times per ☐day ☐week ☐month

Breakfast cereal (whole grain)_____times per ☐day ☐week ☐month

Other grain products (like couscous, tortillas…)____times per ☐day ☐week ☐month

Other whole grain products)_____times per ☐day ☐week ☐month

5) How many times does your child eat/drink the following dairy products?

Milk (**NOT COUNTING SOY MILK**)_____ times ☐per day **OR** ☐ per week

Cheese _____times ☐per day **OR** ☐ per week

Yogurt and yogurt drinks (fruit or plain)_____times ☐per day **OR** ☐ per week

6. My child eats lunch; ☐at home ☐with care giver

7.If she/he does not eat at home, do you pack her/him a lunch? ☐Yes

☐ No, the caregiver provides the lunch

III. FAMILY BACKGROUND

1)n What is the ethnic Background (example Chinese, First Nations, African American and....) of child's biological parents?

Mother_____ **Father**_____

2) What is the height of child's biological parents?

Mother _____ ☐ feet/in **Father** _____ ☐ feet/in

☐Centimeters

☐Centimeters

3) What is the total number of your family members living together? _____**Persons**

Please indicate the relationship of each adult to this child (example; one grandmother, one stepfather…).

4) What is the highest level of education completed?

Child's mother: **Child's father:**

☐ Grade ___ ☐ Grade ___

☐ High school diploma	☐ High school diploma
☐ College	☐ College
☐ University	☐ University

3) What is the total family income? (BEFORE TAX DEDUCTION)

☐ Less than $10,000/year	☐ $ 50,000-60,000/year
☐ $ 10, 000-20,000/year	☐ $ 60,000-70,000/year
☐ $ 20,000-30,000/year	☐ More than 70,000/year
☐ $ 30,000-40,000/year	☐ Do not wish to respond
☐ $ 40,000-50,000/year	☐ On income assistance

4) In the past 12 months:

4.1.Were you and others worried that food would run out before you got money to buy more?

☐Often true ☐ sometimes true ☐ never true ☐ don't know

4.2.The food that you and others bought just didn't last, and there wasn't any money to get more?

☐Often true ☐ sometimes true ☐ never true ☐ don't know

4.3.You and others, because of not having money, could not eat balanced meals?

☐Often true ☐ sometimes true ☐ never true ☐ don't know

5) Please record your postal code: V___-____

IV. CHILD BEHAVIOR QUESTIONNAIRE

Instructions: Please read carefully before starting:
We would like you to tell us what **your child's** reaction is likely to be in those situations. There are of course no "correct" ways of reacting; children differ widely in their reactions, and it is these differences we are trying to learn about. **Please be sure to circle a number or NA (not applied) for every item.**

My child:

1. Seems always in a big hurry to get from one place to another.

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

2. When practicing an activity, has a hard time keeping her/his mind on it

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

3. Tends to run rather than walk from room to room.

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

4. Will move from one task to another without completing any of them

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

5. When outside, often sits quietly.

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

6. When drawing or coloring in a book, shows strong concentration

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

7. Moves about actively (runs, climbs, jumps) when playing in the house

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

8. When building or putting something together, becomes very involved in what she/he is doing, and works for long periods.

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

9. Prefers quiet activities to active games.

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

10. Is easily distracted when listening to a story.

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

11.Is full of energy, even in the evening

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

12. Sometimes becomes absorbed in a picture book and looks at it for a long time.

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

13. Likes to sit quietly and watch people do things.

1	2	3	4	5	6	7	NA
Extremely untrue	Quite untrue	Slightly untrue	Neither true nor false	Slightly true	Quite true	Extremely true	

Many thanks for participating in our survey!

Appendix III-10: Survey questionnaire translated into Punjabi

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ਤਰੀਕ ੨੦੦੬/ /

੧.ਬੱਚੇ ਦਾ ਪਿਛੋਕੜ

ਲਿੰਗ: ☐ ਮੁੰਡਾ ☐ ਕੁੜੀ
ਜਨਮ ਦੀ ਤਰੀਖ: / /
ਦਿਨ ਮਹੀਨਾ ਸਾਲ
ਜਨਮ ਸਥਾਨ (ਦੇਸ਼): _____
ਜੇ ਕੈਨੇਡਾ ਵਿਚ ਪੈਦਾ ਨਹੀਂ ਹੋਇਆ, ਇਥੇ ਆਏ ਨੂੰ ਕਿਨੀ ਸਮਾਂ ਹੋਇਆ ਹੈ? ___ ਸਾਲ ਜਾਂ ___ ਮਹੀਨੇ

੧) ਬੱਚੇ ਦੇ ਵਾਲਾਂ ਦਾ ਰੰਗ ਕਿਹਤਾ ਹੈ? _____

੨) ਬੱਚੇ ਦੇ ਵਾਲ ਘੁੰਗਰਾਲੇ ਹਨ ਹਨ ਜਾਂ ਸਿਧੇ ਹਨ? _____

੩) ਕੀ ਬਚੱਾ ਪੂਰੇ ਟਾਈਮ ਤੇ ਹੋਇਆ ਸੀ? ☐ ਹਾਂ ☐ ਨਹੀਂ
ਜੇਕਰ ਨਹੀਂ ਤਾਂ ਕੀ ਓਹ ਸਮੇਂ ਤੋ ਪਹਿਲਾਂ ਹੋਇਆ ਸੀ ਅਤੇ ਉਹ ਗਰਭ ਦੇ ਕਿਹੜੇ ਮਹੀਨੇ ਵਿਚ ਪੈਦਾ ਹੋਇਆ ਸੀ? ___ਮਹੀਨ

੪) ਕੀ ਤੁਸੀਂ ਬੱਚੇ ਨੂੰ ਅਪਣਾ ਦੁਧ ਦਿਤਾ ਸੀ? ☐ ਨਹੀਂ ☐ ਹਾਂ
ਜੇਕਰ ਹਾਂ, ਤਾਂ (ਲਿਖੋ ਕਿਨਵੇਂ) _____ ਮਹੀਨੇ ਤੋਂ ___ ਮਹੀਨੇ ਤਕ

੫) ਕੀ ਤੁਸੀ ਅਪਨੇ ਬੱਚੇ ਨੂੰ ਫਾਰਮੂਲਾ ਪਿਆਯਾ ਸੀ? ☐ ਨਹੀਂ ☐ ਹਾਂ
ਜੇਕਰ ਹਾਂ, ਬੱਚੇ ਨੂੰ _____ ਮਹੀਨੇ ਤੋਂ _____ ਮਹੀਨੇ ਤਕ

੬) ਕਦੋਂ ਤੁਸੀ ਬੱਚੇ ਨੂੰ ਪਹਿਲੀ ਵਾਰ ਖਾਣਾ ਦਿਤਾ?
ਜਦੋਂ ਉਹ _____ ਮਹੀਨੇ ਦਾ ਸੀ

੭) ਕੀ ਤੁਹਾਡਾ ਬੱਚਾ ਵਿਟਾਮਨ ਜਾਂ ਖਨਿਜ ਸਪਲੀਮੇਂਟ ਲੈਂਦਾ ਹੈ ਜਾਂ ਪਹਿਲਾਂ ਕਦੇ ਲਏ ਹਨ?

☐ ਨਹੀਂ ☐ ਹਾਂ ਜੇਕਰ ਹਾਂ ਤਾਂ _____ ਮਹੀਨਿਆ ਤੋਂ _____ ਮਹੀਨਿਆ ਤਕ
(ਨੰਬਰ ਲਿਖੋ)

੮) ਕਿਰਪਾ ਕਰਕੇ ਵਿਟਾਮਨ/ਖਨਿਜ ਦਾ ਨਾਂ ਲਿਖੋ ਜੇ ਬੱਚਾ ਹੁਣ ਲੈਂਦਾ ਹੈ ਜਾਂ ਪਹਿਲਾਂ ਲੈਂਦਾ ਸੀ (ਕੰਪਨੀ ਦਾਂ ਨਾਂ) _____

੯) ਤੁਹਾਡੇ ਬੱਚੇ ਦੀ ਸੇਹਤ ਕਿਸ ਤਰਾਂ ਦੀ ਹੈ?

☐ ਬਹੁਤ ਹੀ ਚੰਗੀ ☐ ਬਹੁਤ ਚੰਗੀ ☐ ਚੰਗੀ ☐ ਮਾੜੀ

੧੦) ਤੁਹਾਡਾ ਬੱਚਾ ਫਲੂ, ਬੁਖਾਰ, ਠੰਡ, ਖੰਘ ਵਗੈਰਾਂ ਨਾਲ ਕਿੰਨੀ ਵਾਰ ਬੀਮਾਰ ਹੁੰਦਾ ਹੈ?

☐ ਬਹੁਤੀ ਵਾਰ ਨਹੀਂ (ਸਾਲ ਵਿਚ ਇਕ ਜਾਂ ਦੋ ਵਾਰੀ)

☐ ਕਈ ਵਾਰ _____ ਵਾਰੀ ਸਾਲ ਵਿਚ
(ਨੰਬਰ ਲਿਖੋ)

☐ ਬਹੁਤ ਵਾਰ _____ ਵਾਰੀ ਸਾਲ ਵਿਚ
(ਨੰਬਰ ਲਿਖੋ)

੨)ਬੱਚੇ ਦੇ ਖਾਣ-ਪੀਣ ਦੀਆਂ ਆਦਤਾਂ

੧) ਕੀ ਤੁਸੀ ਬੱਚੇ ਦੀ ਖੁਰਾਕ ਬਾਰੇ ਚਿੰਤਿਤ ਹੋ? ☐ ਹਾਂ ☐ ਨਹੀਂ
ਜੇਕਰ ਹਾਂ ਤਾਂ ਅਪਣੀ ਚਿੰਤਾ ਦੇ ਕਾਰਣ ਦਸੋ

☐ ਸਾਰਾ ਕੁਛ ਨਹੀਂ ਖਾਂਦਾ	☐ ਥੋੜਾ ਖਾਂਦਾ ਹੈ	☐ ਹਮੇਸ਼ਾ ਇਕ ਤਰਾਂ ਦਾ ਖਾਣਾ ਖਾਂਦਾ ਹੈ
☐ ਚੰਗੇ ਖਾਣੇ ਨਹੀਂ ਖਾਂਦਾ	☐ ਜ਼ਿਆਦਾ ਖਾਂਦਾ ਹੈ	☐ਹੋਰ ਕਾਰਣ ਹਨ_____

੨) ਸਾਰੇ ਦਿਣ ਵਿਚ ਕਿਨੇ ਗਿਲਾਸ ਤਰਲ ਪਦਾਰਥਾਂ(ਲਿਕਵਿਡ) ਦੇ ਪੀਂਦਾ ਹੈ(ਪਾਣੀ ਤੋਂ ਬਿਨਾਂ) ਦੂਧ, ਜੂਸ, ਸੋਡਾ ਆਦਿਕ ਪੀਣਾ ?

☐ ੦ ☐ ੧ ☐ ੨ ☐ ੩ ☐ ੪ ☐ ੫ ☐ ੫+

੩) ਕੀ ਤੁਹਾਡਾ ਬੱਚਾ ਹੇਠਾਂ ਲਿਖੇ ਖਾਣੇ ਖਾਂਦਾ ਹੈ?

ਮੀਟ (ਬੀਫ (ਗਾਂ), ਲੈਂਬ, ਪੋਰਕ, ਵੀਲ, ਮੂਸ ਆਦਿਕ) ☐ ਹਾਂ ☐ ਨਹੀਂ
ਮਛੀ ਅਤੇ ਸ਼ੈਲਫਿਸ਼ (ਟ੍ਰਾਊਟ, ਸ਼ਰਿੰਪ, ਲੌਬਸਟਰ, ਕਲੈਮ, ਸਕੈਲੋਪ) ☐ ਹਾਂ ☐ ਨਹੀਂ
ਪੋਲਟਰੀ (ਚਿਕਨ, ਡੱਕ, ਟ੍ਰਕੀ,)
ਕਿੰਨੇ ਚਰੀ?
ਮੀਟ _____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਮਛੀ ਅਤੇ ਸ਼ੈਲਫਿਸ਼ _____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਪੋਲਟਰੀ _____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ

੪) ਤੁਹਾਡਾ ਬੱਚਾ ਹੇਠਾਂ ਲਿਖੇ ਖਾਣੇ ਕਿਨੇ ਵਾਰੀ ਖਾਂਦਾ ਹੈ?

ਚਿਟੀ ਬ੍ਰੈਡ ____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਭੂਰੀ ਬ੍ਰੈਡ ____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਚਿਟੇ ਚੋਲ ____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਭੂਰੇ ਅਤੇ ਜੰਗਲੀ ਚੋਲ ____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਚਿਟਾ ਪਾਸਤਾ ____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਭੂਰਾ ਜਾਂ ਸਾਬਤ ਕਣਕ ਦਾ ਪਾਸਟਾ____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਨਾਸ਼ਤੇ ਦਾ ਸੀਰਿਅਲ (ਸਾਬਤ ਅਨਾਜ ਵਾਲਾ ਨਹੀਂ) ____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਨਾਸ਼ਤੇ ਦਾ ਸੀਰਿਅਲ (ਸਾਬਤ ਅਨਾਜ ਵਾਲਾ) ____ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਹੋਰ ਅਨਾਜ ਦੀਆਂ ਚੀਜਾਂ (ਕੂਸਕੂਸ, ਟੋਰਟੀਲਾਜ...) ____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਹੋਰ ਸਾਬਤ ਅਨਾਜ ਦੀਆਂ ਚੀਜਾਂ ____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿ

੫) ਤੁਹਾਡਾ ਬੱਚਾ ਹੇਠਾਂ ਲਿਖੀਆਂ ਦੁਧ ਦੀਆਂ ਚੀਜਾਂ ਕਿੰਨੀ ਵਾਰ ਖਾਂਦਾ ਹੈ?

ਦੁਧ(ਸੋਯਾ ਦੁਧ ਨਾਂ ਲਿਣੋ) _____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ
ਚੀਜ਼ ਜਾਂ ਪਨੀਰ _____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ
ਦਹੀਂ ਅਤੇ ਦਹੀਂ ਡਰਿੰਕਸ(ਫਲ ਵਾਲੇ ਜਾਂ ਸਾਦੇ) _____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ

੬) ਹਫਤੇ ਵਿਚ ____ਵਾਰ ਮੇਰਾ ਬੱਚਾ ਲੰਚ ਘਰ ਖਾਂਦਾ ਹੈ ਅਤੇ ____ਵਾਰ ਦੇਖਭਾਲ ਕਰਣ ਵਾਲਿਆਂ ਕੋਲ ਖਾਂਦ ਹੈ. (ਨੰਬਰ ਲਿਖੋ) (ਨੰਬਰ ਲਿਖੋ)

੭) ਜਦੋਂ ਬੱਚਾ ਘਰ ਲੰਚ ਨਹੀਂ ਖਾਂਦਾ ਕੀ ਤੁਸੀਂ ਉਸਦਾ ਲੰਚ ਨਾਲ ਭੇਜਦੇ ਹੋ?
☐ ਹਾਂ ☐ ਨਹੀਂ ਦੇਖਭਾਲ ਕਰਣ ਵਾਲੇ ਲੰਚ ਦੇਂਦੇ ਹਨ.

3) ਪਰਿਵਾਰ ਦਾ ਪਿਛੋਕੜ

੧) ਬੱਚੇ ਦੇ ਜਨਮ ਦੇਣ ਵਾਲੇ ਮਾਤਾ ਪਿਤਾ ਦਾ ਜਾਤੀ ਪਿਛੋਕੜ ਕੀ ਹੈ (ਜਿਸ ਤਰਾਂ ਕਿ ਈਸਟ ਇੰਡਿਅਨ, ਅਮੇਰਿਕਨ, ਚਾਈਨੀਜ਼...)
ਮਾਤਾ_____ ਪਿਤਾ_____

੨) ਬੱਚੇ ਨੂੰ ਜਨਮ ਦੇਣ ਵਾਲੇ ਮਾਤਾ ਪਿਤਾ ਦੀ ਲੰਬਾਈ ਕਿੰਨੀ ਹੈ?
ਮਾਤਾ_____☐ ਫੀਟ/ਇਨਚ ਪਿਤਾ_____☐ ਫੀਟ/ਇਨਚ
(ਨੰਬਰ ਲਿਖੋ) ☐ ਸੈਂਟੀਮੀਟਰ (ਨੰਬਰ ਲਿਖੋ) ☐ ਸੈਂਟੀਮੀਟਰ

੪) ਤੁਹਾਡਾ ਬੱਚਾ ਹੇਠਾਂ ਲਿਖੇ ਖਾਣੇ ਕਿਨੇ ਵਾਰੀ ਖਾਂਦਾ ਹੈ?

ਚਿਟੀ ਬ੍ਰੈਡ ___ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਭੂਰੀ ਬ੍ਰੈਡ ___ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਚਿਟੇ ਚੋਲ ___ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਭੂਰੇ ਅਤੇ ਜੰਗਲੀ ਚੋਲ ___ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਚਿਟਾ ਪਾਸਤਾ ___ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਭੂਰਾ ਜਾਂ ਸਾਬਤ ਕਣਕ ਦਾ ਪਾਸਟਾ___ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਨਾਸ਼ਤੇ ਦਾ ਸੀਰਿਅਲ (ਸਾਬਤ ਅਨਾਜ ਵਾਲਾ ਨਹੀਂ) ___ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਨਾਸ਼ਤੇ ਦਾ ਸੀਰਿਅਲ (ਸਾਬਤ ਅਨਾਜ ਵਾਲਾ) ___ ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਹੋਰ ਅਨਾਜ ਦੀਆਂ ਚੀਜਾਂ (ਕੂਸਕੂਸ, ਟੋਰਟੀਲਾਜ...) ___ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ
ਹੋਰ ਸਾਬਤ ਅਨਾਜ ਦੀਆਂ ਚੀਜਾਂ ___ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ ☐ ਇਕ ਮਹੀਨੇ ਵਿਚ

੫) ਤੁਹਾਡਾ ਬੱਚਾ ਹੇਠਾਂ ਲਿਖੀਆਂ ਦੂਧ ਦੀਆਂ ਚੀਜਾਂ ਕਿੰਨੀ ਵਾਰ ਖਾਂਦਾ ਹੈ?

ਦੂਧ(ਸੋਯਾ ਦੂਧ ਨਾਂ ਗਿਣੋ) _____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ
ਚੀਜ਼ ਜਾਂ ਪਨੀਰ _____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ
ਦਹੀਂ ਅਤੇ ਦਹੀਂ ਡਰਿੰਕਸ(ਫਲ ਵਾਲੇ ਜਾਂ ਸਾਦੇ) _____ਵਾਰ ☐ ਇਕ ਦਿਨ ਵਿਚ ☐ ਇਕ ਹਫਤੇ ਵਿਚ

੬) ਹਫਤੇ ਵਿਚ ___ਵਾਰ ਮੇਰਾ ਬੱਚਾ ਲੰਚ ਘਰ ਖਾਂਦਾ ਹੈ ਅਤੇ ___ਵਾਰ ਦੇਖਭਾਲ ਕਰਣ ਵਾਲਿਆਂ ਕੋਲ ਖਾਂਦ ਹੈ. (ਨੰਬਰ ਲਿਖੋ) (ਨੰਬਰ ਲਿਖੋ)

੭) ਜਦੋਂ ਬੱਚਾ ਘਰ ਲੰਚ ਨਹੀਂ ਖਾਂਦਾ ਕੀ ਤੁਸੀਂ ਉਸਦਾ ਲੰਚ ਨਾਲ ਭੇਜਦੇ ਹੋ?
☐ ਹਾਂ ☐ ਨਹੀਂ ਦੇਖਭਾਲ ਕਰਣ ਵਾਲੇ ਲੰਚ ਦੇਂਦੇ ਹਨ.

3) ਪਰਿਵਾਰ ਦਾ ਪਿਛੋਕੜ

੧) ਬੱਚੇ ਦੇ ਜਨਮ ਦੇਣ ਵਾਲੇ ਮਾਤਾ ਪਿਤਾ ਦਾ ਜਾਤੀ ਪਿਛੋਕੜ ਕੀ ਹੈ (ਜਿਸ ਤਰਾਂ ਕਿ ਈਸਟ ਇੰਡਿਅਨ, ਅਮੇਰਿਕਨ, ਚਾਣੀਨੀਜ਼...)
ਮਾਤਾ_____ ਪਿਤਾ_____

੨) ਬੱਚੇ ਨੂੰ ਜਨਮ ਦੇਣ ਵਾਲੇ ਮਾਤਾ ਪਿਤਾ ਦੀ ਲੰਬਾਈ ਕਿੰਨੀ ਹੈ?
ਮਾਤਾ_____☐ ਫੀਟ/ਇਨਚ ਪਿਤਾ_____☐ ਫੀਟ/ਇਨਚ
(ਨੰਬਰ ਲਿਖੋ) ☐ ਸੈਂਟੀਮੀਟਰ (ਨੰਬਰ ਲਿਖੋ) ☐ ਸੈਂਟੀਮੀਟਰ

੪)ਬੱਚੇ ਦੇ ਸੁਭਾਅ ਬਾਰੇ ਸਵਾਲ

ਹਿਦਾਇਤ: ਕਿਰਪਾ ਕਰਕੇ ਜਵਾਬ ਦੇਣ ਤੋਂ ਪਹਿਲਾਂ ਚੰਗੀ ਤਰਾਂ ਪੜ੍ਹ ਲਵੋ

ਅਸੀਂ ਚਾਹੁੰਦੇ ਹਾਂ ਕਿ ਤੁਸੀਂ ਸਾਨੂ ਦਸੋ ਕਿ ਹੇਠਾਂ ਲਿਖੇ ਹਾਲਾਤਾਂ ਨਾਲ ਤੁਹਾਡਾ ਬਚਾ ਕਿਸ ਤਰਾਂ ਪ੍ਰਭਾਵਿਤ ਹੁੰਦਾ ਹੈ. ਇਥੇ ਇਹ ਜਾਨਣਾ ਜਰੂਰੀ ਹੈ ਕਿ ਪ੍ਰਭਾਵਿਤ ਹੋਣ ਦਾ ਕੋਈ ਇਕ ਤਰੀਕਾ ਨਹੀਂ ਹੈ ਹਰ ਬੱਚੇ ਤੇ ਅਲਗ-ਅਲਗ ਤਰਾਂ ਅਸਰ ਹੁੰਦਾ ਹੈ. ਅਸੀਂ ਇਸ ਹੀ ਗਲ ਨੂੰ ਸਮਝਣ ਦੀ ਕੋਸ਼ਿਸ਼ ਕਰ ਰਹੇ ਹਾਂ,

ਕਿਰਪਾ ਕਰਕੇ ਹੇਠਾਂ ਲਿਖਿਆਂ ਗਲਾਂ ਦਾ ਜਵਾਬ ਕਿਸੇ ਇਕ ਨੰਬਰ ਜਾਂ ਲਾਗੂ ਨਹੀਂ ਹੁੰਦਾ ਦੇ ਦੁਆਲੇ ਘੇਰਾO ਬਣਾਓ.

ਮੇਰਾ ਬੱਚਾ:

੧) ਕਮੇਸ਼ਾ ਹੀ ਇਕ ਜਗਾ ਤੋਂ ਦੂਜੀ ਜਗਾ ਤੇ ਜਾਣ ਦੀ ਜਲਦੀ ਵਿਚ ਹੁੰਦਾ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੨) ਜਦੋਂ ਕਿਸੀ ਚੁਡੇਂਚੇ ਦੀ ਕਿਰਿਯਾ ਕਰਦਾ ਹੈ ਤਾਂ ਉਸਨੂੰ ਧਿਆਨ ਨਾਲ ਕਰਨਾਂ ਉਸਦੇ ਲਈ ਕਠਿਨ ਹੁੰਦਾ ਹੈ

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੩) ਇਕ ਕਮਰੇ ਤੋਂ ਦੂਜੇ ਕਮਰੇ ਵਿਚ ਰੁਕਕੇ ਜਾਣ ਦੇ ਬਜਾਏ ਭੱਜ ਕੇ ਜਾਂਦਾ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੪) ਇਕ ਕੱਮ ਸ਼ੁਰੂ ਕਰਦਾ ਹੈ ਅਤੇ ਉਸਨੂੰ ਪੂਰਾ ਕਰੇ ਬਗੈਰ ਹੋਰ ਸ਼ੁਰੂ ਕਰ ਲੈਂਦਾ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੫) ਜਦੋਂ ਬਾਹਰ ਹੁੰਦਾ ਹੈ ਤਾਂ ਚੁਪ ਕਰ ਕੇ ਬੈਠਾ ਰਹਿੰਦਾ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੬) ਜਦੋਂ ਚੁਕ ਵਿਚ ਕਲੱਰ ਜਾਂ ਫ੍ਰਾ ਕਰਦਾ ਹੈ ਤਾਂ ਕਾਫੀ ਧਿਆਨ ਦਿੰਦਾ ਲਗਦਾਂ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ

੭) ਜਦੋਂ ਘਰ ਦੇ ਅੰਦਰ ਖੇਡਦਾ ਹੈ ਚੁਸਤੀ ਨਾਲ ਫਿਰਦਾ ਹੈ(ਭੱਜਦਾ, ਉਪਰ ਚੜ੍ਹਦਾ ਅਤੇ ਟੱਪਦਾ)

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੮) ਜਦੋਂ ਕੁਝ ਬਨਾਉਂਦਾ ਜਾਂ ਇਕਠਾ ਕਰਦਾ ਹੈ ਤਾਂ ਕਾਫੀ ਰੁਝ ਜਾਂਦਾ ਹੈ ਅਤੇ ਬਹੁਤ ਦੇਰ ਤਕ ਲਗਾ ਰਹਿੰਦਾ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੯) ਭੱਜੇ ਫਿਰਣ ਵਾਲੀਆਂ ਖੇਡਾਂ ਨਾਲੋਂ ਸ਼ਾਂਤ ਰਹਿਣ ਵਾਲੀਆਂ ਖੇਡਾਂ ਜਿਆਦਾ ਪਸੰਦ ਕਰਦਾ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੧੦) ਜਦੋਂ ਕਹਾਣੀ ਸੁਣਦਾ ਹੋਇਆ ਧਿਆਨ ਇਧਰ-ਉਧਰ ਬਹੁਤ ਜਲਦੀ ਕਰ ਲੈਂਦਾ ਹੈ..

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੧੧) ਕਾਫੀ ਵਿਆਗੀਲ ਹੈ. ਸ਼ਾਮ ਤਕ ਵੀ ਨਹੀਂ ਥਕਦਾ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੧੨) ਕਈ ਵਾਰੀ ਫੋਟੋਆਂ ਵਾਲੀਆਂ ਕਿਤਾਬਾਂ ਨੂੰ ਲੰਬੇ ਸਮੇਂ ਤਕ ਬੜੇ ਧਿਆਨ ਨਾਲ ਵੇਖਦਾ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

੧੩) ਚੁਪ ਕਰ ਕੇ ਬੈਠਨਾਂ ਪਸੰਦ ਕਰਦਾ ਹੈ ਅਤੇ ਲੋਕਾਂ ਨੂੰ ਕੰਮ ਕਰਦੇ ਵੇਖਦਾ ਰਹਿੰਦਾ ਹੈ.

੧. ਬਿਲਕੁਲ ਗਲਤ ੨. ਕਾਫੀਗਲਤ ੩. ਥੋੜਾ ਗਲਤ ੪. ਨਾਂ ਸਹੀ ਨਾਂ ਗਲਤ
੫. ਥੋੜਾ ਸਹੀ ੬. ਕਾਫੀ ਸਹੀ ੭. ਬਿਲਕੁਲ ਸਹੀ ਇਹ ਗੱਲ ਨਹੀਂ ਢੁਕਦੀ

ਸਾਡੇ ਸਰਵੇਖਣ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਬਹੁਤ-ਬਹੁਤ ਧੱਨਵਾਦ !

☺

Appendix III-11: Survey questionnaire translated into Cantonese

Department of Health Care and Epide
Faculty of Medicine
Mather Building, 5804 Fairview Aver
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Date 2006/ /

I. 幼儿背景

性别: □男 □□ 女

出生日期:____/____/____(日/月/年)

出身地 (国家): _____

居住在加拿大的时间(非加拿大出身者):_____年，或_____月。

1. 孩子头发是什么颜色？_____

2. 他/她的头发是卷曲的还是直的？_____

3. 你的孩子是足月生产的吗？□是 □不是

如果不是，他/她就是个早产儿；他/她是在胚胎几个月时出生的？_____月
（请写数字）

4. 您的孩子是母乳哺乳吗? □ □不是

□□是, 从他/她 -------个月大到 -----个月大

(请写数字) (请写数字)

5 您喂孩子婴儿配方食物吗? □ □不是

□□是, 从他/她 -------个月大到 -----个月大

(请写数字) (请写数字)

6 何时开始喂孩子奶制品以外的食物?

从他/她 --------个月大

□是, 从他/她 --------个月大到 -----个月大

(请写数字) (请写数字)

8 请写出您的孩子所吃的或曾经吃过的维生素/矿物补充剂的名字(商标名).

_____

9 您觉得您孩子的健康状况如何?

□非常健康 □ 健康 □一般 □不太健康

10 您的孩子多长时间生病一次, 如感冒, 发烧, 腹泻, 咳嗽等?

□很少 (每年 1~2 次)

□每年多次。请详细说明, ____次/年 (请写数字)

□经常发生。请详细说明, ____次/月 (请写数字)

II. 幼儿的饮食习惯

1 您关注您孩子的饮食吗? □□ 是 □□ 不是

如果是, 为什么? 请检查哪方面是您所关注的:

□ 挑食	□ 吃得太少	□ 总吃同样的食物
□吃不健康的食物	□ 吃得太多	□ 其它 (详细说明)_____

2 您的孩子每天总共喝多少杯流质食物 不包括水? (奶类包括母乳, 果汁, 不含酒精饮料(soft drinks), 或其它饮料)

□ 没有 □ 一 □ 二 □ 三 □ 四 □ 五 □ 多于五

3 您的孩子吃下列食物吗?

肉类 (牛肉, 羊肉, 猪肉, 小牛肉, 鹿肉 和...) □ 是 □ 不是

鱼类和 贝壳类 (包含螃蟹, 虾, 龙虾, 蛤, 扇贝...) □ 是 □ 不是

禽类 (包含鸡, 鸭, 鹅, 火鸡等,) □ 是 □ 不是

多频繁?

禽类_____次　　□□每天　　□每星期　　□每月

4 您的孩子吃多少次下列食物?

白面包 _____次　　□每日　　□□每星期　　□□每月

黑面包_____ 次　　□每日　　□□每星期　　□□每月

白米_____次　　□每日　　□□每星期　　□□每月

糙米和菰米____次　　□每日　　□□每星期　　□□每月

白面制品 (面条, 米粉...)_____次　□每日　　□□每星期　　□□每月

黑面或全麦面制品____次　　□每日　　□□每星期　　□□每月

早餐谷物 (非完全谷类)_____次　□每日　　□□每星期　　□□每月

早餐谷物 (完全谷类)_____次　　□每日　　□□每星期　　□□每月

其它非全谷类制品 (如 燕粗麦粉, 玉米粉圆饼...)____次 □每日　　□每星期 □每月

其它全谷类制品 ____ 次 □每日　　□□每星期　　□□每月

5 您的孩子每天吃/喝下列乳制品多少次?

奶类 (不包括豆奶) _____ 次　　□ 每日　　□每星期

奶酪 _____次品　　□每日　　□□每 星期

酸奶酪和酸奶饮料 (水果或原味)_____次　□□每日　　□□ 每星期

6. 您的孩子吃午餐: _____次/星期在家　　□_____次/星期在寄托者处

7.如他/她不在家里吃午餐, 您给他/她带午饭吗? □□是　□□不, 寄托者提供午餐

III 家庭背景

1. 孩子生身父母的种族背景(如中国人, 土著人, 非洲人, 美洲人 和 ....)

母亲_____父亲_____

2. 孩子生身父母的身高? 母亲_____英尺, 父亲_____英尺

母亲_____厘米, 父亲_____厘米

3 共同生活在一起的家庭成员中共有_____孩子和_____成人?(请写数字)

母亲:	父亲:
□高中未毕业	□高中未毕业 □
□高中毕业 □	□高中毕业
□大专	□大专
□大学	□大学

5 您的家庭收入是多少(税前)?

□少于 $10,000/年 □ □ $ 50,000-60,000/年

□$ 10, 000-20,000/年 □ $ 60,000-70,000/年

□$ 20,000-30,000/年 □多于 70,000/年

□$ 30,000-40,000/年 □不希望回答

□$ 40,000-50,000/年 □ □没有收入补助

6 在过去十二个月中:

6.1 您是否曾担心在家中食物吃完后没钱购买食物?

□经常是 □ 有时侯是 □ 从不 □ 不知道

6.2 您所买的食物吃不了多长时间, 没钱买新的食物?

□经常是 □ 有时侯是 □ 从不 □ 不知道

6.3 因为没有钱, 您经常无法吃到均衡的膳食吗?

□经常是 □ 有时侯是 从不 □ 不知道

7 请提供您的邮编号码(postal code): V___-____

IV. 幼儿行为调查

指引 : 开始之前请仔细阅读:

请告诉我们您的孩子的行为表现属于下列哪一种情况.

没有所谓正确的行为表现, 每个孩子都是不同的.

我们也正在了解他们的行为表现.

请在每一个项目的数字或 NA (不适用、[illegible]

我的孩子:

1．好象总是很匆忙地从一个地方跑到另一个地方.

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

2. 进行一项活动时, 他/她很难集中注意力

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

3. 比走路更喜欢在房间中跑来跑去.

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

4. 没有完成一件事情就去做另一件事

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

5. 在户外时, 总是静静地坐着.

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

6. 当在本子上画画或填色时, 表现出很强的集中力

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

7. 在户内玩耍时更有活力 (跑, 爬, 跳)

1	2	3	4	5	6	7	NA
绝对	相当	有点	既不对	有点	相当	绝对	无从知

8. 玩积木或把东西堆放在一起时, 表现地非常投入, 且能玩很长时间.

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

9. 与活跃的游戏相比, 更喜欢安静的游戏.

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

10. 在听故事时很容易转移注意力吗?

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

11. 精力十足,即便在晚间也是.

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

12. 有时侯会长时间全神贯注地看一本图书.

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

13. 喜欢静静地坐着看别人做事情.

1	2	3	4	5	6	7	NA
绝对不真实	相当不真实	有点不真实	既不对也没错	有点真实	相当真实	绝对真实	无从知道

非常感谢您参加我们的调查活动!☺

Appendix III-12: Subject's Information letter

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemiology
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

SUBJECT INFORMATION AND CONSENT FORM FOR PARENTS/GUARDIANS

Study title: The population pattern of low hair zinc in preschoolers

Principal investigator: Dr. Clyde Hertzman, MD, M.Sc, FRCPC
Health Care and Epidemiology
University of British Columbia
Phone: (604) 822-3002 Fax: (604) 822-0640

Dear Parent/Guardian:

You are being invited to take part in this research study because you have a preschooler 24 - 71 months old and are residing in the city of Vancouver. Your participation is entirely voluntary, so it is up to you whether or not you wish to take part in this study. Before you decide, it is important for you to know that your written consent is required, and to understand what the research involves. This consent form, which you are asked to sign if you wish to participate, will tell you about the study, why the research is being done and what is involved in participation. If you do decide to take part in this study, you are still free to withdraw at any time without giving any reason for your decision, and this will not affect you or your child in any way. Please take time to read the following information carefully and to discuss it with your family or friends before you decide.

This project, comprising a part of the doctoral thesis of research student, Ziba Vaghri, is conducted through the University of British Columbia and in affiliation with the Human Early Learning Partnership and I, Dr. Clyde Hertzman a UBC professor, am the chief investigator of it.

Zinc is an essential micronutrient available from many sources including meat, dairy, cereal and grain products. Inadequate zinc intake can result in zinc deficiency. It has been shown that even minor zinc deficiency, termed marginal zinc deficiency (MZD), can interfere with children's normal growth and development. MZD can be easily prevented if adequate amounts of zinc-rich foods are included in a child's diet. When properly diagnosed, the correction of MZD is, also, relatively simple. Since the

hair zinc content in children is considered an index of the body's zinc content, it can be used in detecting MZD. Children with MZD often have lower hair zinc content. The main purpose of this study is to investigate the hair zinc status of a representative group of Vancouver preschoolers. The second purpose of this survey is to examine and understand the dietary and environmental factors that could predispose children to low hair zinc. The information collected through this survey will increase our awareness on the at-risk groups of children in our communities.

Please note that only one child from each household is eligible to participate. If you choose to participate, you will be invited to:

1. Read the consent form and sign it.

2. Answer the attached questionnaire, which will take 10-15 minutes. (You do not have to answer any questions on the questionnaire if you feel uncomfortable in answering).

3. Return the signed consent form together with the completed questionnaire to your childcare center.

By signing you agree to participate in the study and thereby give us permission to measure your child's height and weight, and take a small hair sample.

Upon receipt of your completed survey questionnaire and signed consent form, our research team will set up a day with daycare authorities to be present in the center and do the measurements and collect the hair samples of the children whose parents/guardians have consented. You will be notified about this date and if you wish to be present for the sample collection, you are welcome to do so, however; this is not a requirement. The procedure will be explained to your child and a tablespoonful of hair sample will be collected from the back of his/her head (3-4 different locations) while he/she is sitting on a chair. There is no pain or side effect for your child in this procedure.

Your name will be entered in a raffle draw (with a 1/20 chance to win a gift basket with clothing and accessories useful to your child and yourself). There may or may not be direct benefits to your child from taking part in this study. But the information learned from this study may be used in the future to benefit children affected by marginal zinc deficiency.

All of the information you provide, together with the result of your

child's hair analysis, will be confidential and will not be available to other research personnel. Your child will be given an identification number (the number at the top of this letter) and will be identified by this number only. All information will be kept in a locked filing cabinet in the principal investigator's office at the University of British Columbia. No information that discloses your identity will be released or published without your specific consent and no records, which identify you by name, or initials will be allowed to leave the Investigators' offices.

Two weeks following the return of your completed questionnaire to the childcare center, 10% of the subjects will be selected randomly (like the flip of a coin) and contacted again to repeat the survey questionnaire. This repeated task would help us to evaluate our survey questionnaire and further examine its ability to collect the information it is designed to collect. Even if you decide to participate in the main survey, you can decide not to participate in this second part of the survey. However, a $15 grocery voucher will be sent to you with the second questionnaire.

The research team will not share any part of the collected data or result of the hair analysis with the study parents/guardians individually. However, the results in the form of any future publications will be available to all through their daycare centers.

If you have any concern about your rights as a research subject while participating in this study, contact the 'Research Subject Information Line in the University of British Columbia Office of Research Services' at 604-822-8598. If you have any questions about this study before or during participation, you can contact Ziba Vaghri at (604) 812-1419 or Dr. Clyde Hertzman at (604) 822-3002. Sincerely,

Clyde Hertzman MD, M.Sc, FRCPC

Director of Human Early Learning Partnership

University of British Columbia

Appendix III-13: Informed Consent form for the survey participants

Informed consent:

- "I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I have read this form and I freely consent to participate in this study.
- I have read and understood that by signing this consent I will also give my permission to the research team to measure my child's weight and height and collect a spoonful of hair sample from him/her
- I have been told that I will receive a dated and signed copy of this form.
- I have been told that I will not receive the result of hair analysis of my child, since this information is only valuable to the research.

Printed name of subject's legally acceptable representative — Signature — Date

Printed name of witness — Signature — Date

Printed name of principal investigator/ designated representative — Signature — Date

Appendix III-14: Log Sheet of returned surveys

Please sign the log sheet when you drop your survey into the box.

THANK YOU FOR PARTICIPATING IN OUR SURVEY!

Study code	Date dropped	Comments

Appendix III-15: Reminder Letters

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemiology
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Dear parent/guardian;

While ago you received a survey package through your childcare center. The purpose of the survey, entitled "Population Patterns of Hair Zinc in Preschoolers", is to collect some information that will help us better understand zinc deficiency which is a common and silent deficiency in childhood.

To date we have not received your survey back. We are inviting you to take 10-15 to complete this survey and return it back to your center along with the signed consent form.

In appreciation of the time and effort you invest in this study, your name will be entered in a raffle draw to win a prize.

Sincerely,

Clyde Hertzman MD, M.Sc, FRCPC
Director of Human Early Learning Partnership
University of British Columbia

Appendix III-16: Notice posted in the centers announcing the day and time of the survey team visit for the hair sample collection and the height & weight measurements

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemiology
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

ATTENTION PARENTS

ON _____ WE WILL BE AT

YOUR CHILDCARE CENTER TO MEASURE YOUR CHILDREN AND

COLLECT THEIR HAIR SAMPLES.

PLEASE MAKE SURE YOUR CHILD IS IN THE CENTER IF HE/SHE IS A PARTICIPANT IN "CITYWIDE SURVEY OF PRESCHOOLERS".

Appendix III-17: Covering letter for the Reliability Package

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemi
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Dear parent/guardian;

A few weeks ago you participated in our survey entitled "Population Pattern of Low Hair Zinc in Preschoolers". In the covering letter you were informed that some of the participants would be asked to fill out the same questionnaire again. This task is repeated for a specific purpose, one that enables us to verify that the questionnaire is a valid tool for the research involved.

With this letter we would like to inform you that you have been selected **randomly (like the flip of the coin)** to fill the questionnaire again. Please take another 10-15 minutes to answer the questions and return it to your childcare center at your early convenience. Please note that this time no hair sample will be collected from your child.

As a token of our appreciation for your cooperation in this survey we have enclosed a grocery certificate in the value of $15. Once again, we appreciate the time and effort you invest in this important matter and we look forward to receiving your second questionnaire.

Sincerely,

Clyde Hertzman MD, M.Sc, FRCPC
Director of Human Early Learning Partnership
University of British Columbia

Appendix III-18: Letter to the gift basket winners

UNIVERSITY OF BRITISH COLUMBIA

"Citywide Survey of Preschoolers' Hair Zinc"

Dear parent,

Congratulations!

Upon completion of our citywide survey, we have drawn names of participants for gift baskets, and your name has been taken randomly as one of the winners of this draw.

Along with this letter you will receive a gift basket containing a few useful items for you and your little one.

In this basket, you will also find a gift certificate with a value of $20 from Safeway.

It will be greatly appreciated if you call our survey line at 604-827-4525 and confirm the receipt of your basket.

Enjoy your gifts and once again we thank you for participating in our study!

With warm regards,

Clyde Hertzman MD, M.Sc, FRCPC
Director of Human Early Learning Partnership
University of British Columbia

Ziba Vaghri, BN, M.Sc PhD candidate
University of British Columbia
Food, Nutrition and Health

Appendix III-19: Pictures from one of the basket draw winners

Appendix III-20: Thank you letter to the survey participants

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Health Care and Epidemiology
Faculty of Medicine
Mather Building, 5804 Fairview Avenue
Vancouver, B.C. V6T 1Z3

Tel: 604-822-2772
Fax: 604-822-4994
Website: www.healthcare.ubc.ca

Dear survey participant,

A few weeks ago you extended your kind support to us and participated in our survey entitled "Population Patterns of Hair Zinc in Preschoolers". Your contribution to the study, along with that of the others, has provided valuable information for our research on hair zinc status of preschoolers in Vancouver. We expect this information to further our understanding of zinc deficiency among our children.

With this letter we would like to acknowledge the significant contribution your participation has had on the success of our survey.

Thank you!

With warm regards and best wishes,

Clyde Hertzman MD, M.Sc, FRCPC
Director of Human Early Learning Partnership
University of British Columbia

Ziba Vaghri BN, M.Sc, PhD candidate
Faculty of Land and Food Systems
University of British Columbia

To Gail and the wonderful staff

of the Pacific Spirit Day Care:

Your cooperation and kind support of our survey helped greatly in bringing it to a successful completion.

Thank you for participating in our survey!

Thank you for making a difference!

On behalf of the "UBC city-wide preschooler survey team" and with warm regards,

Ziba Vaghri

July/2006

Appendix III-22: Thank you letter to West Coast Family Services

HELP Human Early Learning Partnership

THE UNIVERSITY OF BRITISH COLUMBIA
HELP is an interdisciplinary research institute within the Faculty of Graduate Studies

www.earlylearning.ubc.ca

Clyde Hertzman
Director
604.822.3002 Tel
604.822.0640 Fax
clyde.hertzman@ubc.ca

January 3, 2007
Dear Diane,

In 2006 we launched and completed a citywide survey of preschoolers' hair zinc. Your agency was key in helping us connect with parents of children 2-6 years old. Your office extended kind support to this survey by endorsing it via a letter written to the childcare professionals in the preschool centers within the Registry as well as by introducing our survey to the readers of your monthly newsletter. The survey was completed successfully with the participation of over 60 centers and more than 800 preschoolers.

We have developed an educational pamphlet that provides important information on zinc, its dietary sources, and ways to ensure its adequacy in children's diets. This pamphlet has been well received by the parents and caregivers of our focus groups set up to verify the ease of comprehension at level of literacy of the pamphlet.

This pamphlet will be supplied to the participating centers (only) within the next few weeks. But as a token of our appreciation for your support of the survey, we wish, by this letter, to inform you that an electronic version of the pamphlet can be made available to you, should you wish to supply the other centers under the Registry's jurisdiction.

Once again, thank you for your partnership in this important project!

Yours sincerely,

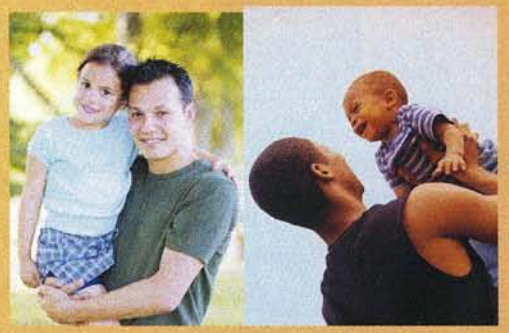
Clyde Hertzman MD, M.Sc, FRCPC
Director of Human Early Learning Partnership
University of British Columbia

Ziba Vaghri, BN, M.Sc, PhD Candidate
University of British Columbia
Food, Nutrition, and Health

Appendix III-23: The Educational Pamphlet

4. ZINC DEFICIENCY

- Zinc deficiency is usually due to low intake of foods high in Zinc.
- Children are most prone to this deficiency during periods of rapid growth.
- Zinc deficiency can be mild to severe.
- Zinc deficiency has been reported in children of developed countries such as Canada.



5. SYMPTOMS OF ZINC DEFICIENCY

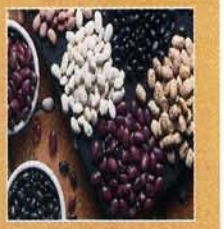
- People with Zinc deficiency may or may not have symptoms.
- As the deficiency sets in and body stores of Zinc are used up, some signs may be observed.
- Possible signs are poor appetite, weight loss, delayed healing of wounds, an impaired sense of taste and decreased growth rate.

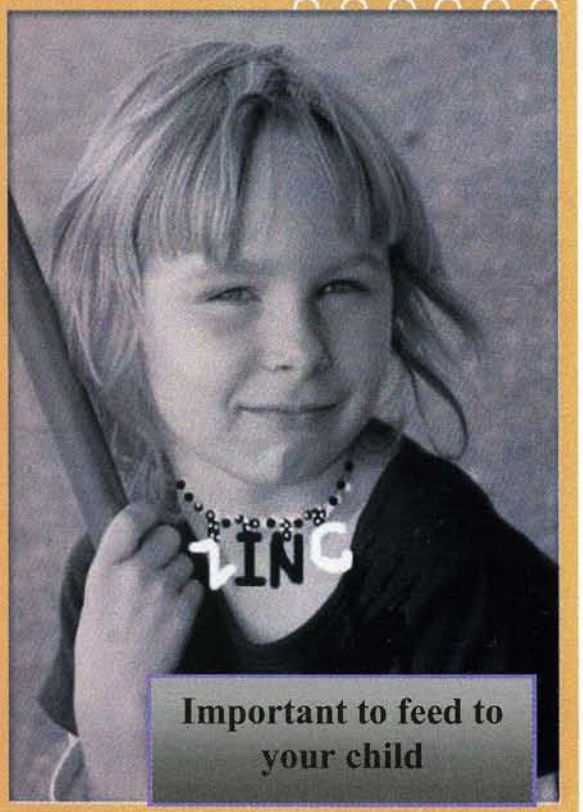
This educational material has been prepared by: Ziba Vaghri, a PhD candidate at the University of British Columbia, and Shadi Mojtabavi, a volunteer working on the team of "A Citywide Survey of Preschoolers' Hair zinc" at UBC.

The funding of this project was provided by the Human Early Learning Partnership; HELP.

For further information you can contact;
zibav@interchange.ubc.ca

DOES YOUR CHILD GET ENOUGH ZINC?





1. ZINC FACTS

- Zinc is an important mineral (like iron) that is found in many foods and is essential for good health.
- It is needed throughout life, and the need is high during the period of growth.
- Your child needs zinc for:
  1. For physical growth.
  2. For brain development.
  3. For wound healing.
  4. For maintaining a normal sense of taste and smell.
  5. As an immune booster for shortening the duration of colds, flues and other common infections.
  6. For fighting skin problems such as acne.

The body's need for Zinc can be met by eating a variety of foods rich in Zinc.

2. WHAT ARE GOOD SOURCES OF ZINC?

- Zinc can be found in both animal and plant food sources (higher amounts in animal products.)
- The primary Zinc source is animal products such as meat, shellfish and dairy products.
- Beef is particularly rich in Zinc.

- Plant-based sources include beans and lentils, nuts, seeds and wholegrain cereals.
- Vegetarians need to make sure to include plenty of Zinc-rich foods in their diet.

Some good sources of Zinc include:

Food	Serving	Zinc (mg)
Crab (cooked)	3 ounces* (85 grams)	4.6
Beef (cooked)	3 ounces	5.8
Pork	3 ounces	2.2
Chicken (dark meat cooked)	3 ounces	2.4
Turkey (dark meat, cooked)	3 ounces	3.5
Yogurt, fruit	1 cup	1.8
Cheese, cheddar	1 ounce	0.9
Milk	1 cup	1.0
Cashews	1 ounce	1.6
Almonds	1 ounce	1.0
Navy beans (cooked)	1/2 cup	3.8
Lentil (cooked)	1/2 cup	1.3
Kidney beans (cooked)	1/2 cup	1.0

*3 ounces serving of meat is about the size of a deck of cards

3. HOW MUCH ZINC DOES YOUR CHILD NEED?

At 0-6 months old he/she needs 2 mg per day
(Provided in breast milk)

7-12 months old he/she needs 3 mg per day

1-3 years old he/she needs 3 mg per day

4-8 years old he/she needs 5 mg per day

9-12 years old he/she needs 8 mg per day

Appendix III-24: Log Sheet for the Hair Samples delivered to the laboratory

University of British Columbia

Citywide survey of Preschoolers

Monday June the 19th
Batch #9
Total number of samples: __56_____

Subject code	Subject code	Subject code
SS03001	RP-BK-004	
SSO3002	RP-BK-006	
SS03003	RP-BK-20	
SS03004	RP-BK-034	
SS03005	RP-BK-036	
SS03006	RP-BK-O41	
SSO7OO4	RP-BK-O52	
SS07011	RP-BK-O58	
SSO7O12	KLO1O17	
SS07016	SS-IR-001	
GW04002	SS-IR-003	
GWO4003	SS-IR-004	
GW04005	SS-IR-005	
GWO4006	SS-IR-006	
GWO4008	SS-IR-007	
GWO4OO9	SS-IR-008	
GWO4O10	SS-IR-009	
GWO6OO1	SS-IR-011	
GW06002	SS-IR-012	
GW06003	SS-IR-013	
GWO6OO4	SS-IR-014	
GWO6005		
GW06006		
RP-G-001		
RP-G-OO2		
RP-G-003		
RP-G-004		
RP-G-006		
RP-G-OO7		
RP-G-OO8		
RP-G-OO9		
RP-G-010		
SS07011		
SSO7O12		
SS07016		

Appendix III-25: Log Sheet for height and weight

UNIVERSITY OF BRITISH COLUMBIA

Date:

Center: South Hill daycare (Branda)

Subject code	Name	Weight (lb)	Height (cm)	Remarks

Appendix III-26: Moments captured; Nutrition clinics and survey

Spring of 2004, Nutrition Clinics, research student entertaining a study participant (up) and measuring her height (down)

Spring of 2006, survey anthropometrist in one of the participating preschool centers

Appendix III.27. Hair zinc level of 5 main ethnicities of the study along with the gender and age breakdown of these ethnic groups

Variables	Caucasian (n=302)	Chinese (n=219)	E. Indian (n=54)
Hair Zinc (μg/g)	116 ±41[a]	104 ±43[b]	154 ± 37[c]
Gender distribution #(%)			
Boys	163 (54)	105(48)	23(43)
Girls	140(46)	117(52)	31(57)
Age distribution #(%)			
< 48 months	132 (44)	103 (47)	19 (35)
≥ 48 months	170(56)	116(53)	35(65)

Appendix III.28. Regression analyses of "taking supplements containing iron" with eating behaviors

Variables	Unadjusted			Adjusted*	
	R^2	p	B* (95% CI)	R^2	p
Concerned about child's eating	0.02	0.00	0.13(0.06, 0.20)	0.03	0.00
Described as eating unhealthy	0.01	0.01	0.07(0.02, 0.12)	0.02	0.13
Described as picky eater	0.02	0.00	0.13(0.06, 0.20)	0.05	0.00
Described as not eating enough	0.00	0.01	0.06(-0.01, 0.13)	0.03	0.01

* Adjustments have been carried out using age, gender, ethnicity, maternal education and family income as covariates.

$Bs are unstandardized co-efficients.

&R^2 represent the R^2 s of adjusting covariates (R^2 =0.12) combined with R^2 of the new variable in the analysis.

Appendix III-29. Comparison of the indices of growth (HAZ and WHZ) and nutrition (WAZ) of low hair zinc and normal hair zinc younger (<4 years old) children

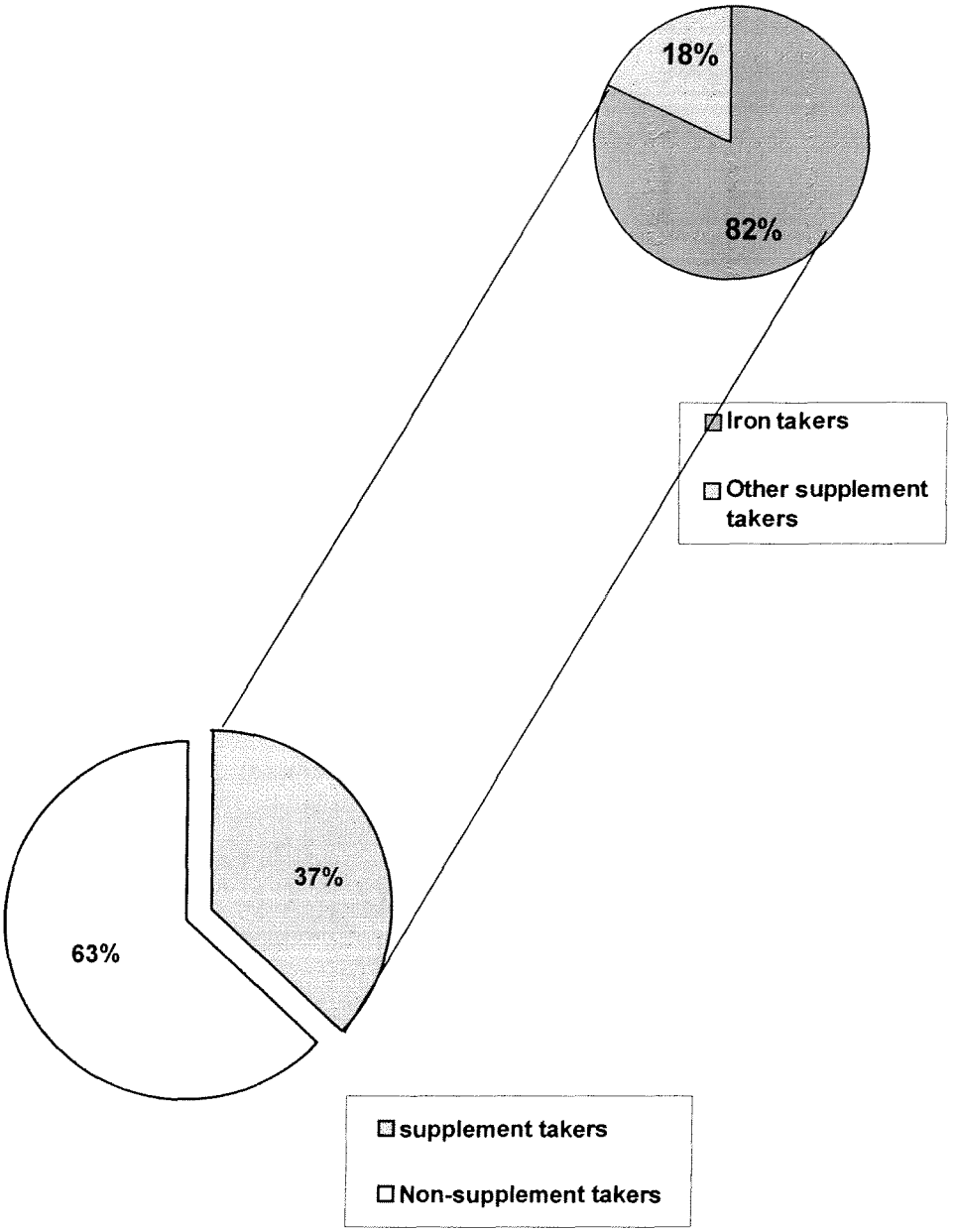
Z scores	Low hair zinc children (n=76)	Normal hair zinc children (n=229)	P
HAZ	0.03±0.6	0.16±1.0	0.67
WAZ	0.60±2.0	0.45±1.4	0.94
WHZ	0.61±2.5	0.41±1.7	0.71

Low hair zinc: Hair zinc <70µg/g
HAZ: Heigh-for-age Z scores
WAZ: Weight-for-age Z scores
WHZ: Weight-for-height Z scores

Appendix III-30. Mean ± SD of the daily intake frequency of some main food groups of survey children and the three main ethnic groups.

	All (n=719)	Caucasian (n=305)	Chinese (n=223)	East Indian (n=54)
Meat & alternatives	1.5 ± 1.1	1.9±0.7	1.9±1.2	1.0±1.2
Cereals and Grains	3.5 ± 1.6	3.1±1.2	3.2±1.6	3.3±1.7
Whole wheat C&G	1.9 ± 1.4	1.9±1.2[a*]	1.0±1.2[b]	1.6±1.5[a]
Dairy	38± 1.6	3.6±1.5	3.1±1.5	3.4±2.0
Milk	2.2 ± 1.2	2.0±1.1[a]	1.9±1.1[b]	1.8±1.3[ab]

Among the three ethnic groups values not sharing a common superscript (a,b,..) are significantly different



Appendix III-31. Distribution of supplement takers among the survey participants (bottom pie) and distribution of iron supplement takers among the supplement takers of the survey (top pie) (n=671).