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URINARY STATUS OF MAJOR MACRO-MINERALS OF LEBANESE SCHOOL AGED CHILDREN

by
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The optimal growth and development of children has been attributed to several combined factors. Adequate nutrition and mineral intake especially of calcium (Ca), magnesium (Mg), and phosphorus (P) play an important role in this aspect. A deficient diet as well as some metabolic diseases prohibit normal metabolism and affect the child health and nutritional status. Nutrient deficiencies are usually assessed through dietary assessment methods, such as validated food frequency questionnaires (FFQs), which can be biased and do not account for mineral bioavailability and other limiting factors. Ca, Mg, and P serum levels are tightly regulated and maintained in a narrow range. Hence, discrepancies only appear in extreme situations.

The determination of the status and reference values for Ca, Mg, and P urinary excretion in 6-10 year old Lebanese children is proposed in this study.

Using a multi-stage cluster sampling at district, school, and class levels, a sample size of 1403 children was selected. Personal information, anthropometric measurements, and non-fasting urine samples were collected. Before analysis, urine samples were acidified to increase solubility and reduce possible precipitation. Ca, Mg, P, and Creatinine (Crea) were analyzed.

The means Ca/Crea, Mg/Crea, and P/Crea were not significantly different between genders. When compared between age groups, only P/Crea was statistically significant. Ca, Mg, and P statuses varied with school types, highlighting potential impact of socio-economic level on nutritional status. The 5^{th} percentile barely changed with age for all mineral ratios. The 95^{th} percentile fluctuates proportionally with age in Ca/Crea and P/Crea emphasizing similar bone retention rates.

Geographical variability between countries proves the necessity of having Lebanon-specific pediatric references. Establishing these cutoffs for Lebanese children will allow health professionals to have objective screening methods for mineral status. This will help in the prevention of deficiencies and the detection of Ca, Mg, and P-related metabolic diseases.

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ABBREVIATIONS

-	Minus
%	Percent
&	and
/	Per
<	Less than
>	Greater than
±	Plus or minus
µl	Microliter
µS	Average number of students per school
ºC	Degree Celsius
a.m.	Before noon
AUB	American University of Beirut
BMI	Body Mass Index
Ca	Calcium
cm	Centimeter
Crea	Creatinine
CV	Coefficient of variance
d	Day
DEFF	Estimated Design Effect
et al.	and others
FAO	Food and Agriculture Organization
g	Gram
h	Hour
H_2O	Distilled Water
HCl	Hydrochloric acid
kg	Kilogram
m	Meter
M	Mol
MEHE	Ministry of education and higher Education
Mg	Magnesium
mg	Milligram
ml	Milliliter
mmol	Millimol
n	Sample Size
N/A	Not applicable
P	Phosphorus
p.m.	After noon
PPS	Probability Proportionate to Size
QC	Quality control
RDA	Recommended Dietary Allowance
RNI	Recommended Nutrient Intake
SD	Standard Deviation
SE	Standard Error
UNICEF	United Nations Children's Funds

USD	U.S. dollars
v	Volume
vs.	Versus
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Calcium (Ca), magnesium (Mg), and phosphorus (P), together, play a key role in the development of bones (Institute of Medicine, 1997), assure optimal skeletal growth in children (Heaney, Weaver, & Heaney, 2006), and prevent the development of some chronic diseases (refer to chapter II). Each mineral, Ca, Mg, and P is involved in different mechanisms in the body and consequently each has its own paramount physiological functions.

Metabolic abnormalities induce alterations in the regulation of these three minerals which have repercussions on different specific biomarkers' concentrations. Knowing that the kidney is the main organ that regulates the homeostasis of Ca, Mg, and P, urinary concentrations of these minerals fluctuate accordingly.

Elevated urinary Ca excretion is a sign of hyperparathyroidism, hyperthyroidism, osteoporosis or any bone damaging diseases such as myeloma, renal tubular acidosis, vitamin D intoxication, Paget's disease, and can be a risk factor of lithiasis. Hypocalciuria is mostly seen when having a constant low Ca intake. Additionally, several pathological causes could be behind this low Ca excretion: steatorrhea, hypoparathyroidism, pseudohypoparathyroidism, nephrosis, etc. (Foley & Boccuzzi, 2010). A reduced urinary Mg output is normally seen when having a chronic renal disease or a decreased renal function in case of dehydration, Addison's disease, or diabetic acidosis. Additionally, Mg deficient diets, malabsorption syndromes (specifically in alcoholism), and cases of hypoparathyroidism result in a low Mg

excretion. Conversely, elevated Mg levels in urine excluding the scenarios of some drug therapy (like diuretics), may be caused by chronic glomerulonephritis, aldosteronism, and Bartter's syndrome (Fischbach & Dunning, 2009; Mayo Foundation for Medical Education and Research, 2014). Increased urine P is associated with renal tubular damage (Fanconi syndrome), familial hypophosphatemia, hyperparathyroidism, vitamin D-resistant rickets, nonrenal acidosis (because of the buffering mechanism of P), paraplegia, and vitamin D toxicity. Low urinary P excretions can be indicative of hypoparathyroidism (or parathyroidectomy), pseudohypoparathyroidism, and low P diets (Chernecky & Berger, 2012).

The investigation of mineral metabolic disorders requires comparing Ca, Mg, and P excretions to normal reference values. In adults, the norms are set and agreed upon. Normal levels of Ca in urine when consuming a regular diet are between 100 and 300 mg/d, when on a low Ca diet 50-150 mg/d, and when on a Ca free diet 5-40 mg/d (Fischbach & Dunning, 2009; Foley & Boccuzzi, 2010). A ratio of 0.14 mg/mg for Ca/Crea in adults is expected and values exceeding 0.2 are considered high (Foley & Boccuzzi, 2010). Mg normal levels in 24-hour (h) urine collection range between 75 and 150 mg/d and in ratio to Crea range between 0.03 and 0.1 mg/mg (Mayo Foundation for Medical Education and Research, 2014). Normal P excretion levels in adults range between 400 and 1300 mg/d (Duh & Cook, 2005). The values strictly fall below 1000 mg/d when a low P diet is adopted (Chernecky & Berger, 2012). No clear reference values for P/Crea levels in urine are found.

Pediatricians and health concerned personnel face a problem in adopting reference values for children. Many gaps exist regarding Ca, Mg, and P reference values in urine

since they vary according to child's age and geographical residence (Foley & Boccuzzi, 2010; Safarinejad, 2003).

As reference values are not available for Lebanese children, this study aims to determine age-related reference values for urinary Ca/Crea, Mg/Crea, and P/Crea ratios as well as to assess Ca, Mg, and P statuses in healthy 6 to 10 year old Lebanese students. Establishing these cutoffs for children will allow health professionals to have objective screening methods for mineral status. This will help in the screening of deficiencies and the detection of Ca, Mg, and P-related metabolic diseases.

This thesis consists of 6 chapters. The following chapter, the literature review presents background information about each of the three minerals and urine assessment method. Chapter II, materials and methods, describes the study design and details related to the data collection and laboratory and statistical analyses. Chapters III and IV discusses our results, their interpretation, and discussion. Chapter IV also includes comparisons of our results with different other results and presents our study's limitations. Finally, the final chapter summarizes the findings and gives a brief conclusion.

CHAPTER II

LITERATURE REVIEW

This chapter begins by elaborating information about each of calcium (Ca), magnesium (Mg), and phosphorus (P) in terms of definition, sources, absorption, balance and homeostasis, functions, deficiencies, and recommendations. It highlights the gaps and shows the need of such a study in Lebanon. It also indicates studies that validate the use of spot urine samples as a method of assessment in children.

A. Calcium

1. Calcium in Human Body

Ca is the most abundant mineral element in the body: with about 30 g being present in the skeleton at birth (Abrams, 2001) and levels reaching 1200 g which accounts for about 2% of bodyweight in adulthood (Joint FAO & WHO, 2005). Ninety nine percent of Ca is found in the skeleton and teeth, mainly as hydroxyapatite, an inorganic crystalline structure made up of Ca and P [$Ca_{10}(PO_4)6(OH)_2$] (Institute of Medicine, 1997), which provides rigidity. Less than 1 % of total body Ca is present in soft tissues and body fluids (Theobald, 2005), and is responsible of all metabolic effects.

2. Food Sources and Bioavailability of Calcium

Due to different nutrients' interactions, Ca bioavailability of some food sources is higher than others. Several explanations have been attributed to the high

bioavailability of Ca in milk, naturally rich in Ca. Lactose (milk disaccharide), casein (milk protein), and some non digestible oligosaccharides such as inulin (sometimes added to milk and dairy products) seem to prevent the formation of insoluble salts thus, prevent Ca precipitation (Cashman, 2002). Ca content is somehow high in several vegetable sources like spinach; however, the existence of oxalic acid lowers Ca absorption from 30% (in milk) to around 5% (Charles, 1992). Some researchers believe that Ca content of food has greater impact than Ca bioavailability since the latter only affects short term Ca absorption (Institute of Medicine, 1997), and the body has the capacity to cope on a chronic basis and regulate Ca absorption. Table 1 presents food sources of Ca regardless of the rate of absorption.

Table 1. *Selective Food Sources of Calcium*

Food	Ca content (mg) per 100 g
Dried Basil	2240
Dried Thyme	1890
Non Fat Milk Powder	1257
Parmesan Cheese (Hard)	1184
Gruyere Cheese	1011
Roasted Sesame Seeds (Whole)	989
Swiss Cheese	961
Cheddar Cheese	675
Feta Cheese	493
Tahini	426
Sardine (Canned with bones)	382
Grape Leaves (Raw)	363
Soybeans (Raw)	277
Almonds	269

Adapted from "U.S. Department of Agriculture, Agricultural Research Service. 2014. USDA National Nutrient Database for Standard Reference, Release 26. Nutrient List Home Page, http://ndb.nal.usda.gov/ndb/nutrients/index"

3. Calcium Absorption

Ca is absorbed most efficiently at the level of the duodenum (Shils & Shike, 2006) by active transport (important at low Ca intake), against the concentration gradient (important at high intake), and by passive diffusion across the intestinal epithelium (Ross *et al.*, 2011). As previously said, Ca bioavailability depends on physiological and dietary variables (Theobald, 2005). Ca intake and vitamin D status are known to directly affect Ca absorption. The fractional Ca absorption is inversely related to Ca intake from food (Institute of Medicine, 1997). For instance, a decrease in Ca intake is concomitant with an increase in Ca absorption and vice versa. 1,25-dihydroxyvitamin D by its turn, with its intestinal vitamin D receptor (VDR) (Del Valle *et al.*, 2011), controls the active transport of Ca absorption. Calbidin, known as vitamin D dependent Ca binding protein is essential for the transport of Ca from the brush border membrane to the basolateral membrane. Thus, inadequate Vitamin D status leads to a decline in Ca absorption. Additionally, a competition occurs at the level of the intestine between Ca and oxalate, and Ca and phytate which is an organic storage form of phosphorus in cereals and seeds (Viveros *et al.*, 2000). This competition negatively affects intestinal Ca absorption (Shils & Shike, 2006). Moreover, other components like age, gastric acidity, growth, pregnancy, lactation, diseases, protein, salt, fat, caffeine, alcohol, and some minerals (like phosphorus, lead, magnesium, etc.) affect Ca absorption (Obeid, 2013b). Taking into consideration all these variables, true Ca absorption, estimated to vary between 30 and 50%, is difficult to measure especially due to the existence of intra- and inter-individual variability (McDowell, 2003). An approximate estimation of intestinal Ca absorption in adults is 30% (Awumey & Bukoski, 2006). Unexpectedly, studies on children and adolescents using stable isotopes

showed that Ca absorption in these age groups does not significantly exceed 30 % (Peacock, 1991).

4. Calcium Balance and Homeostasis[1]

The body aims to keep a tight normal extracellular fluid/plasma Ca concentration that assures a normal intracellular Ca level at the same time. The regulation of Ca, known as Ca homeostasis, requires three calciotropic hormones (parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxyvitamin D_3) and targets three major organs: intestine, bones, and kidneys (Mundy & Guise, 1999). The interplay between the organs and the extracellular fluid involves bones remodeling (osteoclastic bone resorption or osteoblastic bone formation), Ca absorption (increased or decreased intestinal absorption), and Ca regulation at the kidney level (increased renal excretion or tubular reabsorption). A low Ca level stimulates PTH secretion which triggers the regulation of plasma Ca concentration through different direct and indirect pathways. At the level of the kidney, PTH (synthesized by the parathyroid gland) stimulates Ca tubular (mainly proximal tubule at 70% (Del Valle, 2011)) reabsorption (decreasing Ca loss in urine) and kidney secretion of calcitriol (1,25-dihydroxyvitamin D_3). Calcitriol itself activates the VDR in the gut which increases intestinal Ca absorption (Peacock, 2010). Additionally, PTH triggers bone resorption through increasing phosphorus excretion. Once Ca levels are back to normal, a negative feedback on the parathyroid gland inhibits PTH release. Calcitonin (synthesized by the thyroid gland) opposes the effect of PTH on bones, preventing their resorption. Hence, calcitonin levels are proportional to food intake and inversely related to PTH levels. All of the above show

[1] Bohn (2003) defines homeostasis as "the ability to maintain a dynamic equilibrium of a compound in the body and its tissues".

that serum Ca level is tightly regulated and maintained in a narrow range. Only in severe cases of hyperparathyroidism, malnutrition, and other extreme circumstances, serum Ca concentration fluctuates above or below the normal range (Institute of Medicine, 1997).

5. Functions of Calcium

Ca is known to be essential for bones and teeth rigidity. However, the functions do not reside on bone mineralization only, since Ca ions play an important role in most metabolic processes (Joint FAO & WHO, 2005). Ca works as an intracellular messenger for protein synthesis, regulation of several enzymes, blood clotting, nerve conduction, muscle contraction, etc. Ca has been found to reduce the occurrence and severity of different diseases. Proper Ca levels seem to prevent the development of colon cancer (Appleton, Bristol, & Williamson, 1986; Sorenson, Slattery, & Ford, 1988), dyslipidemia (Denke, Fox, & Schulte, 1993; Jacqmain *et al.*, 2003), and hypertension (Hatton & McCarron, 1994; Zemel, 2001). The antiobesity effect of "dietary" Ca discussed by several authors (Davies *et al.*, 2000; Zemel *et al.*, 2005) is not conclusive to date and needs to be further assessed.

6. Role of Calcium in Children

The total Ca in the skeleton increases from 120 g at the age of two and reaches 400 g at the age of nine (Joint, FAO & WHO, 2005). This shows the importance of Ca in the development of children. Johnston *et al.* (1992) proved that, in prepubertal children, the higher the Ca intake the better the bone density. This high intake, if maintained, increases the peak of bone density and protects against osteoporosis. This

underlines the importance of Ca intake in prepubertal age, being a critical age for bone mineral deposition.

All the beneficial effects of Ca, its role in preventing diseases and maintaining good metabolic function indicate the need of an adequate Ca status at young age.

7. Calcium Deficiency

a. Prevalence

Few decades ago, the American population started observing a decline in milk and dairy products consumption (Albertson, Tobelmann, & Marquart, 1997). In fact has made over 90% of adolescent females consume less than the Recommended Dietary Allowance (RDA). Since 1977, the U.S witnessed a low Ca intake in the majority of its population (Morgan *et al.*, 1985). In Portugal, Moreira *et al.* (2005) reported having around one third of the population (7-9 year old children) having a Ca intake below the DRI. In parallel to these results, a study performed on Lebanese 10-16 year old students (Salamoun *et al.*, 2005) showed that only 12% of the population meets the adequate intake (AI) of Ca of this specific age group (1300 mg) and 16% meets the Vitamin D AI, the latter affecting Ca absorption. Countries of all continents, America, Europe, Asia-the Middle East, and Asia-the Far East report low Ca intakes in all age groups. In Japan, most preschool children do not meet the Ca AI due to low intakes of milk and dairy products (Shibata *et al.*, 2008). Consistent results are found in South African children as well (Eyberg, Pettifor, & Moodley, 1985). It is worth noting that most, if not all, studies assessing Ca intake estimated it using the dietary assessment methods like food frequency questionnaires, 24-h recalls, and food records. These methods rely on short term memory, estimations, categorizations, matching and other limiting

requirements. Table 2 clearly presents several studies assessing Ca intake in different young populations, their findings, and methods used.

Table 2. *Calcium Intake in Selected Countries*

Country/Year of collection	Reference Number	Sample Size	Method Used	Age (yr)	Calcium Intake (mg/d)		
					Both Sexes	Male	Female
Britain/ 1997-1998	131	1701	Weighed dietary record	4-18		784	652
Japan/ 1999-2000	118	90	Food record + 24-h recall	3-5	425		
Lebanon/ 1999-2000	112	385	FFQ	10-16	816		
Netherlands/ 1996	14	500	FFQ	4-20	1180		
Portugal/ 2002-2003	89	3044	24-h recall	7-9		1174	1126
USA/ 1976-1980	42	4342	24-h recall + FFQ	4-6		1063	953
				7-10		1083	949
USA/ 1977-1978	90	3790	Dietary record	6-11	929		
USA/ 1980-1982	4	146	Food diary	11-12			811
1983-1985		144					814
1986-1988		112					837
1990-1992		73					781
USA/ 1999-2000	147	962	24-h recall	6-11	889	915	860

b. Populations at Risk

During the first two years of life, puberty, and adolescence, it is of paramount importance to ensure a net positive Ca balance because Ca deposition in bones is most efficient at the time intervals. For that reason, these particular ages are at high risk of Ca inadequacy and deficiency. Other susceptible populations include such as pregnant and lactating women, post-menopausal women, elderly (Joint FAO & WHO, 2005), lactose intolerant people and individuals who follow very restricted diets such as vegan and macrobiotics (Jacobs & Dwyer, 1988).

c. Consequences

Meeting Ca requirements is important to maintain a proper skeletal integrity. A

chronic deficiency of Ca intake leads to impaired bone mineralization and increased risk of fractures. This can be seen in the development of rickets in children, and osteoporosis in adults (Nordin, 1997). Additionally, as seen before, Ca prevents several diseases and Ca deficiency seems to trigger these particular health problems like kidney stones, colon cancer, hypertension, etc. (Obeid, 2013b).

8. Recommended Calcium Intake

Ca recommendations differ with sex, age, and some physiological conditions. Table 3 shows the recommended intakes according to different needs.

Table 3. *Recommended Calcium Intakes for Individuals*

Group	**Recommended Intake**	**Upper Limit of Intake ***
	mg/d	
Infants		
0-6 months human milk fed	300	Not established
0-6 months cow milk fed	400	Not established
7-12 months	400	Not established
1-3 years	500	2500
4-6 years	600	2500
7-9 years	700	2500
Adolescents		
10-18 years	1300[a]	2500
Males		
19 -65 years	1000	2500
> 65 years	1300	2500
Females		
19 years-Menopause	1000	2500
Postmenopause	1300	2500
Pregnant (last semester)	1200	
Lactating	1000	

Retrieved from "Joint, F.A.O. & World Health Organization. (2005). Vitamin and mineral requirements in human nutrition, p.71. [a] Particularly during growth spurt.
* Retrieved from "Food and Nutrition Board, Institute of Medicine (1997). Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride".

B. Magnesium

1. Magnesium in Human Body

Mg is the fourth most abundant mineral and the second most abundant intracellular cation (Romani, 2013). It starts with 760 mg at birth and increases to 25 g in adulthood (Institute of Medicine, 1997). Around 60% of body Mg is found in the skeleton (part of the hydroxylapatite crystals), 40% in muscles and soft tissues, and only 1 % in the extracellular milieu (Romani, 2013).

2. Food Sources and Bioavailability of Magnesium

Mg is found in many food sources, and the ordinary diet usually provides adequate amounts (Mahan & Escott-Stump, 2004). High fiber foods are important sources of Mg, however, fibers lead to a low rate of Mg absorption, and it is crucial to give attention to nutrient interactions. Same effect on Mg absorption rate appears with high phytate foods since P has the ability to bind Mg thus reduces its absorption (Brink & Beynen, 1992). A competition between some Ca, Zn, and Mg at the level of the enterocytes also decreases Mg absorption (Spencer, Norris, & Williams, 1994). Disregarding all possible interactions, Table 4 shows the most popular food sources of Mg.

3. Magnesium Absorption

Mg is absorbed in the small intestine, primarily at the level of the jejunum and ileum (Vormann, 2003). Literature has shown that intestinal Mg absorption is not fixed and varies from 30 % (Quamme, 2008; Seo & Park, 2008) to 35 % (Mahan & Escott-Stump, 2004) and 38 % (Fine *et al.*, 1991) up to 60 % when adopting a constant diet

(Schwartz, Spencer, & Welsh, 1984). Like Ca, both transcellular (active transport), mediated by transient receptor potential ion channel TRPM6 and TRPM7 transport proteins (Quamme, 2008) and paracellular (passive diffusion) mechanisms were indentified in the absorption of Mg. A high acute intake of Mg activates the paracellular pathway since the transcellular one is saturable (Bohn, 2003). The average Mg absorption of a typical diet is estimated to be around 50 % (Institute of Medicine, 1997; Joint FAO & WHO, 2005). Studies on children and adolescents concluded a mean Mg absorption rate between 42.8 (Abrams, 1997) and 52% (Andon *et al.*, 1996) which is quite similar to the rate in adults. Exceeding the recommended intakes of some minerals like Ca, P, etc. (Bohn, 2003), and having pharmacological doses of some others like Zn (Spencer, Norris, & Williams, 1994) may also decrease Mg absorption, as said previously. Gastric acidity, some diseases, and Mg intake itself affect as well Mg absorption (Bohn, 2003).

Table 4. *Selective Food Sources of Magnesium*

Food	Mg content (mg) per 100 g
Dried Basil	711
Roasted Pumpkin Seeds	550
Dried Watermelon Seeds	515
Dry Cocoa Powder	499
Roasted Sesame Seeds (Whole)	356
Instant Coffee Powder	327
Granular Caviar (Black and Red)	300
Cashew Nuts	292
Almonds	270
Molasses	242
Lima Beans (Raw)	224
Fava Beans (Raw)	192

Adapted from "U.S. Department of Agriculture, Agricultural Research Service. 2014. USDA National Nutrient Database for Standard Reference, Release 26. Nutrient List Home Page, http://ndb.nal.usda.gov/ndb/nutrients/index".

4. Magnesium Balance and Homeostasis

There is no identified homeostatic system for the regulation of serum Mg, but the latter concentration is noticeably constant (Mahan & Escott-Stump, 2004). Mg balance is accomplished through a harmony between intestinal absorption and renal excretion (Quamme, 2008). Although Alfrey and colleagues (Alfrey, Miller, & Butkus, 1974) found a correlation of 0.96 between Mg concentration and bone density, the latter is sometimes ignored when Mg homeostasis is discussed. Bones seem to partially contribute to the regulation of serum Mg concentration since 30 % of Mg in bones is exchangeable with serum (Seo & Park, 2008). A low Mg concentration triggers exchangeable Mg release from bones; whereas, a high Mg concentration induces Mg deposition on bone surface. Since part of Mg absorption occurs through uncontrollable passive diffusion, the intestine can't strictly regulate Mg absorption. Therefore, Mg balance is primarily controlled by the kidneys (Bohn, 2003). High intake of Mg results in increasing urinary Mg excretion and a low intake stimulates tubular reabsorption (Mahan & Escott-Stump, 2004) mainly (at 60%) in the cortical thick ascending limb of the loop of Henle (Quamme & Rouffignac, 2000).

Intracellular (IC) Mg concentration is constantly maintained except in extreme circumstances like hypoxia or severe malnutrition (Seo & Park, 2008). Hence, normal intracellular Mg levels might be accompanied by a low serum Mg (Vorman, 2003). The regulation of the intracellular milieu is done by chelation and buffering processes (De Rouffignac & Quamme, 1994).

5. Functions of Magnesium

Mg is metabolically very active; it works as a cofactor for over 300 enzyme systems. It is needed for PTH secretion, vitamin D hydroxylation, protein folding, and RNA and DNA synthesis (Joint FAO & WHO, 2005). Due to its high affinity to phosphate, Mg contributes to the phosphorylation of ATP; hence, Mg is indirectly involved in energy metabolism (Bohn, 2003). The pathological effect of Mg depletion has been documented. Clinical and epidemiological studies showed an inverse association between Mg intake and several pathologies such as cardiovascular diseases (Abraham *et al.*, 1987; Champagne, 2008), hypertension (Jee *et al.*, 2002), lung disease (Britton, 1994), insulin resistance (Cahill *et al.*, 2013), and metabolic syndrome (Champagne, 2008; He *et al.*, 2006). Therefore, it is not surprising that many clinical disorders are associated with magnesium deficiency (Witkowski, Hubert, & Mazur, 2011).

6. Role of Magnesium in Children

Few papers discuss the importance of Mg in children. The same effect of Mg on adults' health is magnified on children's health. Mg is essential for ATP, thus it is directly involved in energy metabolism and growth. It is needed for muscles and bones, for immunity and cardiac health. Mg, having a history of prevention against several non-communicable diseases is at high interest in the epidemic phenomenon of children obesity and its consequences.

7. Magnesium Deficiency

a. Prevalence

Mg intake in western diets seems to be enough to prevent deficiency but low to assure normal serum level (Vormann, 2003). Thus, pathological effects of Mg deficiency are generally infrequent but meeting the recommendations is rather hard. Many authors reported a low Mg intake in different countries. Galan *et al.* (1997) and Ford and Mokdad (2003) assessed Mg intake in French adults using 24-h dietary records and in American adults using 24-h recall; respectively. Both studies reported a Mg intake below the RDAs for the majority of their populations. Furthermore, according to Morgan *et al.* (1985), the average Mg intake of all the U.S. population (sample population excluding 0-5 year-old children), assessed through three-day dietary records, is below the RDAs. In Japan, according to Shibata *et al.* (2008) some children from 3 to 5 years old are at risk of Mg deficiency. Although Mg requirements are not that hard to reach, Mg intake still fails to meet them in different populations and age groups.

b. Populations at Risk

Mg deficiency is infrequent in children and even less common in adults (Joint FAO & WHO, 2005). Despites this fact, some susceptible populations need special attention when it comes to Mg. Due to its association with ATP, Mg is depleted when ATP is produced and used (Institute of Medicine, 1997). Although, there is no solid evidence of Mg deficiency in chronic endurance activity, athletes are considered at risk of Mg depletion. In addition, during growth, the needs of all nutrients especially Mg are markedly increased. This fact makes all children in growing period at risk of Mg

inadequacy. Knowing that Mg is required for nutrient metabolism (specifically phosphorylation), a careful care should be provided to people during their recovery from moderate to severe malnutrition when Mg is extensively used (Joint FAO & WHO, 2005).

c. Consequences

Since Mg is involved in most body reactions, the symptoms of Mg deficiency are generalized and non-specific. Mg deficiency is known to alter all the mechanisms that require Mg for their functions. Neuromuscular (muscular weaknesses, muscular spasm, tetany, hyperirritability, hyperexcitability), gastrointestinal (nausea and anorexia that lead to weight loss), skeletal (vitamin D resistance and bone alteration due to PTH secretion difficulties), and cardiovascular (cardiac arrhythmia, hypertension) alterations are signs of Mg deficiency (Joint FAO & WHO, 2005).

8. Recommended Magnesium Intake

Since Mg is tightly related to lean body mass and ATP, it is not surprising to find a difference in the recommendations of Mg intake between men and women and some physiological cases like growth, pregnancy and lactation. Table 5 shows the recommended Mg intake in all ages and physiological stages.

C. Phosphorus

1. Phosphorus in Human Body

The sixth most abundant mineral in the body, P, constitutes 1 to 1.4 % of lean body mass and 0.65 to 1.1 % of total body mass (Heaney, 2012; Institute of Medicine,

1997), which accounts for 630 g in adults. P is not proportionally distributed in the body; 85% of total body P is present in the skeleton, 14 % in soft tissues and muscles, and less than 1 % is found in blood (Obeid, 2013c).

Table 5. *Recommended Magnesium Intakes for Individuals*

Group	**Recommended Nutrient Intake (RNI)**	**Upper Limit of Intake***
	mg/d	
Infants		
0-6 months human milk fed	26	Not established
0-6 months cow milk fed	36	Not established
7-12 months	54	Not established
1-3 years	60	65
4-6 years	76	110
7-9 years	100	110
Males		
10-18 years	230	350
19-65 years	260	350
> 65 years	224	350
Females		
10-18 years	220	350
19-65 years	220	350
> 65 years	190	350
Pregnant	220	350
Lactating	270	350

Retrieved from "Joint, F.A.O. & World Health Organization. (2005). Vitamin and mineral requirements in human nutrition, p.224.
* The upper limit levels are set for supplemental Mg intake. Retrieved from "Food and Nutrition Board, Institute of Medicine (1997). Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride".

2. Sources and Bioavailability of Phosphorus

The majority of natural foods are the major sources of P (Mahan & Escott-Stump, 2004), however, since it is found in different food sources, additives in processed foods contribute to a significant yet uncontrollable quantity of daily P intake (Sullivan, Leon, & Sehgal, 2007; Uribarri & Calvo, 2003).Table 6 shows occurring P

content in foods and Appendix I illustrates the main GRAS[2] food additives that contain P. P bioavailability is fairly good in most foods except seeds and cereals. P in seeds and cereals is found in its phytate form and not in its organic ester form like in other food. P in its phytate form is not directly available because the human body lacks phytase, the enzyme responsible for phytate hydrolysis. Bacteria and yeast naturally produce phytase. This fact allows the colonic bacteria to release some absorbable P (Heaney, 2012).

Table 6. *Selected Food Sources of Phosphorus*

Food	P content (mg) per 100 g
Roasted Pumpkin Seeds	1174
Roasted Sunflower Seeds	1155
Non Fat Milk Powder	968
Tahini	790
Cocoa Powder	734
Goat Cheese (Hard)	729
Soybeans (Raw)	704
Parmesan Cheese (Hard)	694
Gruyere Cheese	605
Pork Bacon	533
Sardines (canned with bones)	490
Veal Liver	483
Lamb Brain	384

Adapted from "U.S. Department of Agriculture, Agricultural Research Service. 2014. USDA National Nutrient Database for Standard Reference, Release 26. Nutrient List Home Page, http://ndb.nal.usda.gov/ndb/nutrients/index".

3. Phosphorus Absorption

The proximal portion of the duodenum has the lowest pH compared to other parts of the intestine and thus has the highest rate of absorption. The lower the intestinal pH, the better the P solubility, and the higher the P absorption (Mahan & Escott-Stump,

[2] The Food and Drugs Administration defines GRAS as any substance intentionally added to food and **G**enerally **R**ecognized **A**s **S**afe

2004). Like the other minerals, P is absorbed in both active transport using the luminal sodium phosphate cotransporter type 2b, facilitated by 1,25-dihydroxyvitamin D (Institute of Medicine, 1997), and passive diffusion (Uribarri, 2007). The absorption of P depends on several factors like the amount of P in the diet, its bioavailability, gastric acidity, intake of other nutrients, etc. Ca in its upper limit dose is known to affect P absorption. A ratio Ca/P has been established to eliminate possible interactions reducing these nutrients absorptions. It has been postulated that there is no apparent adaptive mechanism to enhance P absorption when having low intake (Institute of Medicine, 1997). P absorption is linearly related to P intake by the range of 4-30 mg/kg/d (Allen & Wood, 1994). Nonetheless, Segawa and colleagues (2004) demonstrated the presence of an upregulation of sodium-phosphate cotransporter IIb when P intake is low. The net P absorption in a mixed diet is estimated to vary between 55 and 70 % in adults and 65 to 90 % in infants and children (Institute of Medicine, 1997).

4. Phosphorus Balance and Homeostasis

P regulation is done through an interplay between intestinal absorption, renal excretion, and bone remodeling. Some researchers highlighted the importance of intracellular P in the regulation of serum P, while others did not. Irving (1973) reported that intracellular contribution of P (where PTH is supposed to control P shifts between intra and extra-cellular fluids) is even greater than the contribution of bones. However, the main organ that manipulates and maintains stable serum phosphate levels is the kidney. Around 80 % of absorbed P is reabsorbed at the level of the proximal convoluted tubule of the kidneys (Weinman & Lederer, 2012). P homeostasis is presented in Figure 1 (Uribarri, 2007). Several endocrine factors play important roles in

the control of phosphatemia: Parathyroid Hormone (PTH), Vitamin D, and phosphatonins (particularly Fibroblast growth factor-23 (FGF-23), secreted frizzled related protein-4 (sFRP-4), Matrix extracellular phosphoglycoprotein (MEPE), and fibroblast growth factor 7 (FGD-7)) and some other hormones (Berndt & Kumar, 2009; Kuro-o, 2010; McDowell, 2003; Uribarri, 2007).

Several reversible control mechanisms are involved to keep extracellular P levels constant. The small intestine is the first organ of regulation. Three important physiological factors are known to mostly affect P absorption: dietary P intake, Vitamin D, and PTH (Penido & Alon, 2012). P absorption is increased up to a certain level when P intake is increased.When the latter is low, a postulated theory supposing an upregulation of P absorption is adopted (Segawa *et al.*, 2004). Although Vitamin D-independent increase of diffusional mechanism of P absorption extensively exists at high intake (Williams & DeLuca, 2007), 1,25-dihydroxyvitamin D plays a key role in the stimulation of intestinal P absorption by the regulation of the inorganic P Na receptor IIb. PTH affects gut absorption indirectly by stimulating $1,25(OH)_2D_3$ synthesis (Marks, Debnam, & Unwin, 2010).

Bone remodeling is an additional factor that regulates phosphatemia. FGF-23 primarily and PTH are the key hormones that determine the state of bone activity. FGF-23 appears to protect cells from hyperphosphatemia which negatively affects osteoblastic activity (Tiosano & Hochberg, 2009). Conversely, PTH stimulates osteoclasts to release P into the extracellular milieu.

The kidneys are the major regulators of irreversible P homeostasis through the control of P urinary excretion. This fact explains P restriction in patients with renal insufficiency. An interplay between endocrine factors results in either increasing

excretion or reabsorption. The main factors known to increase renal P reabsorption are 1,25-dihydroxyvitamin D, P depletion, and some hormones like insulin, thyroid hormone, growth hormone, and estrogen. Some factors in parallel stimulate tubular P excretion as PTH and phosphatonins (Uribarri, 2007).

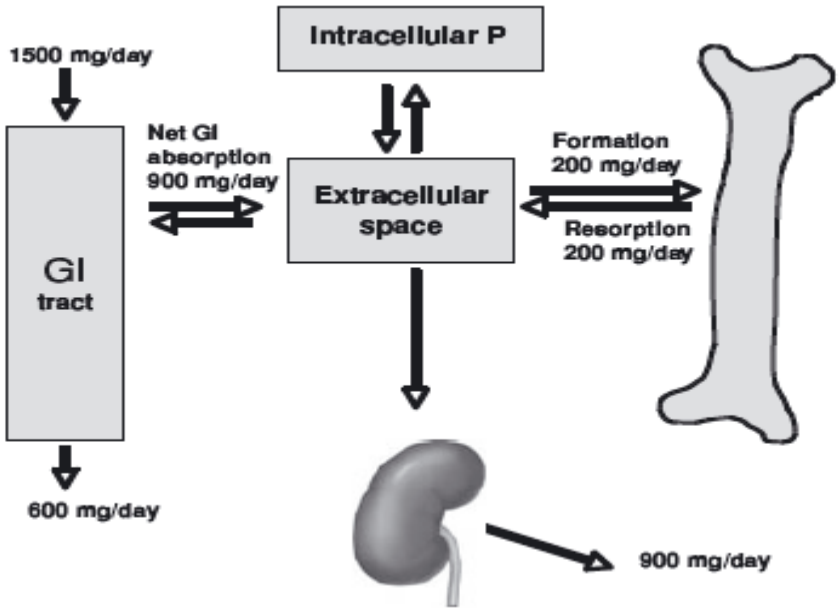


Figure 1. *Phosphorus Homeostasis*
Retrieved from Uribarri, J. (2007). Phosphorus homeostasis in normal health and in chronic kidney disease patients with special emphasis on dietary phosphorus intake. *Seminars in Dialysis, 20*(4), p. 296.

5. Functions of Phosphorus

P is important for bones structure and mineralization being part of bone apatite. Additionally, most, if not all, metabolic reactions require P. Particularly, P is required for carbohydrate (Ditzel & Lervang, 2009; Haap *et al.*, 2006; Haglin, 2001). A decreased phosphate serum level can be associated with increased insulin resistance and hyperglycemia (Haap *et al.*, 2006; Haglin, 2001). It is directly related to the functions of enzymes and hormones. Moreover, P is integrated in the structure of ATP,

phospholipids, phosphoproteins, and nucleic acids which make it essential for genetic transmission. Additionally, P works as a buffer in blood and other fluids in the body (McDowell, 2003). Hypophosphatemia might be one of the underlying conditions behind several diseases like hypertension, low serum HDL levels (Haglin, 2001), and the onset of obesity (Obeid, 2013a).

6. Role of Phosphorus in Children

P is paramount un adult health and it is even more crucial in growing individuals because it is involved in all body metabolisms.

P is particularly important in children's bone health since it is part of the apatite. Furthermore, P is known to be essential for the oxygen carrying capacity of hemoglobin through 2,3- diphosphoglycerate (Drezner, 2002), especially in young ages.

7. Phosphorus Deficiency

a. Prevalence

Dietary deficiency and diet induced hypophosphatemia are rare in healthy subjects due to the large P reservoir in the body (Kiple & Ornelas, 2000) (in muscles, bones, cells, etc.) and to the wide distribution of P in food (Mahan & Escott-Stump, 2004). Usually, low serum P levels are caused by metabolic illness or abnormalities and not by low intake (Heaney, 2012). P intake is assumed to be high by some authors (Calvo, 1994; Calvo & Uribarri, 2013) and not sufficient by others (Obeid, 2013a). High P intake doesn't imply high P availability and a high serum levels. Obeid (2013a) postulated having a low P availability caused by the westernization of diet. The excessive consumption of high carbohydrate foods like refined cereals, potatoes, and

sugars, stimulate insulin which triggers the uptake of P by the peripheral tissues. Additionally, when having a high carbs meal, P will be oriented towards compound phosphorylation. Thus, serum P levels are not necessarily indicative of intake because P can be used up by different metabolic mechanisms. Sugiyama *et al.* (2009), using duplicate diets, found P deficiency among 3-5 year old Japanese children. Similarly in the US, Fiorito *et al.* (2006) found a suboptimal intake of P in 9 and 11 year old girls living in central Pennsylvania. Inversely, 2 to 4-fold excess of P intake in Polish adults as well as in Greek children was reported through duplicate diets and dietary records, respectively (Roma-Giannikou, 1997; Skibniewska, 2001). Lombardi-Boccia *et al.* (2003) reported that in Italy, the mean P intake exceeded the RDAs. Regarding the conflicted data, the assessment of P should be done by evaluating P status and not just intake.

b. Populations at Risk

P deficiency is very unlikely to be caused by the diet. Vegans are considered vulnerable populations since they follow a very restricted diet in terms of dairy and meat products. Their main source of P is phytate which is not easily absorbed. Even though P intake and absorption in vegans is low and can be insufficient, hypophosphatemia and its underlying symptoms are very improbable (Kiple & Ornelas, 2000). Hypophosphatemia is life threatening in alcoholics, diabetics with ketoacidosis, and in patients in refeeding states where calorie-rich supplements are given without P supplementations (Heaney, 2012).

c. Consequences

Limitations in P supplies induce a “generalized impairments of body functions” because P is metabolically very active and it is involved in practically most of the body metabolisms, (MacDowell, 2003) Hypophasphatemia is associated with alterations of several organs functions including the nervous system, blood, GI tract, kidneys, bones, and muscles. Thus the following manifestations might appear when having P deficiency: Confusion, paralysis, hemolysis, impaired oxygen transport, impaired clot formation, decreased nerve conduction, dysphagia, myalgia, weakness, rhabdomyolysis, cardiac failure, respiratory failure, osteomalagia and rickets, renal tubular acidosis, etc. (Berndt & Kumar, 2009; Obeid, 2013).

8. Recommended Phosphorus Intake

P recommendations are known to be high in prepuberty and adolescence because of an increased speed of growth and needs of highly activated metabolic reactions. In adulthood, P needs seem to be constant regardless of the physiological states. Some authors (Obeid, 2013a) claimed that the actual recommendations of P are based on the lower limit of serum inorganic P in adults (Institute of medicine, 1997). If the middle range was taken, the RDAs would be greater and would better cover the needs. Till now, no modifications of the Estimated Average Requirements (EARs) were taken into account and the following table indicates the actual recommended intakes (Table 7).

Table 7. *Recommended Phosphorus Intakes for Individuals*

Group	EAR* of Phosphorus	Upper Limit of Intake
	mg/d	
Infants		
0-6 months	100	Not established
7-12 months	275	Not established
1-3 years	380	3000
4-8 years	405	3000
Adolescents		
9-18 years	1055	4000
Adults and Elderly		
19-70 years	580	4000
> 70 years	580	3000
Pregnant and Lactating	No increment	3500 and 4000

* Estimated Average Requirements
Retrieved from "Food and Nutrition Board, Institute of Medicine (1997). Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride".

D. Assessment of Status and Intake

The statuses of some macro-minerals like sodium (Na), potassium (K), and chloride (Cl) are primarily hormonally regulated at the level of the kidney(Renin-Agiotensine-Aldosterone System) (Laragh & Sealey, 2011), while the regulation of other minerals like Ca, Mg, and P relies on an interaction between hormonal and dietary patterns. In healthy conditions, with the absence of all metabolic diseases, Ca, Mg, and P intakes are determinants of statuses. This fact gave value to the assessment of intake of minerals through dietary assessment methods such as diet history, food frequency questionnaire (FFQ), 24-h recall, dietary record, duplicate diets, weighed food diary, etc. However, a great number of researchers questioned the efficacy and accuracy of using dietary assessment methods such as FFQ (Brown, 2006; Byers, 2001; Jardack, 2006; Kristal, Peters, & Potter, 2005), the most used method in the assessment of minerals intake. These methods rely on estimations, matching, categorizations, short and long term memory, and other limiting prerequisites which need lots of commitment

and are prone to subjective judgments. When it comes to children, these methods become harder and more biased. It is important to note that for a same meal, the composition differs between countries, families, and between different moments in time. The standardization of meal composition used to analyze the data of dietary assessment method is one of its greatest limitations. Moreover, mineral bioavailability, the rate of absorption, nutrients interactions (presence of inhibitors or stimulators), physiological status, and metabolic disturbances, are not accounted for in such types of assessments, since they only rely on types of food and portions. Therefore, an alternative, more objective and reliable method of mineral assessment, reflecting the young population's micronutrient status is needed.

Serum concentrations of Ca, Mg, and P are known to reflect severe conditions of malnutrition or diseases since they are almost stable in healthy conditions. It has been shown that Ca homeostasis is perfectly controlled and serum Ca concentration, being tightly regulated, is not a good indicator of Ca status (Peacock, 2010) and does not reflect Ca intake in healthy conditions. Although serum P is not as well tightly regulated as serum Ca (Irving, 1973), phosphatemia is still considered to be maintained in a narrow range (Kuro-o, 2012; Sauberlich, 1999). In normal conditions, dietary habits alone cannot cause alterations of serum phosphate levels (Heaney, 2012). In a national survey, De boer *et al.* noticed that an increment of 500 mg in dietary P was associated with a higher serum P concentration of 0.03 mg/dl (De Boer, Rue, & Kestenbaum, 2009). Thus, blood test is not the best indicator of phosphorus status or intake (Calvo & Carpenter, 2003). Assessing Mg status is open to doubt since no simple, accurate, and practical laboratory test reflects total body Mg or Mg status (Elin, 1994). Serum Mg is assumed to be a poor indicator because Mg level is homeostatically regulated primarily

by the kidney (gut and bones) which cannot help predict intracellular Mg depletion (Al Ghamdi, Cameron, & Sutton, 1994). Witkowski (2012) and colleagues affirmed that serum or plasma ionized Mg does not respond to variations in status or intake and thus cannot be taken as a standard method of assessment. Therefore, blood tests are not the proper methods to detect any discrepancy.

These three minerals share the same particularity: the kidney is the bandmaster of their homeostasis and it is the major organ that controls and balances their serum levels. Sauberlich (1999) showed that urinary Ca excretion is highly correlated with Ca intake. An increased Ca intake is concomitant with an increased Ca excretion and vice versa (Del Valle *et al.*, 2011; Ross *et al.*, 2011). This type of regulation is described in Mg (Bohn, 2003) and P as well, where urinary excretion is closely reflective to intake (Sauberlich, 1999). Consequently, urinary assessment appears to be a reliable indirect indicator of Ca, Mg, and P statuses reflecting intake in healthy individuals.

The assessment of minerals in urine has undergone development since the beginning of the century. The concept of 24-h urinary Ca assessment was proposed by Albright *et al* (1929).Due to the impracticality of this method in outpatients, spot urine was then used, whereby, the measurement of mineral in ratio to creatinine (Crea) was suggested (Nordin, 1959). Accordingly, the assessment of Ca, Mg, and P was done in pediatrics through the use of mineral to Crea ratio in sport urine specimens (Chen et al., 1994; Matos et al., 1997; Safarinejad, 2003). Several studies showed linear relationships between total mineral excretion and mineral/Crea ratios in children (Gokce, 1991; Reusz *et al.*, 1995; Safarinejad, 2003). Afterwards, this method was extensively performed in pediatrics due to its usefulness and practicability (Alconcher *et al.*, 1997; Chen *et al.*, 1994; Esbjorner & Jones, 1995; Matos *et al.*, 1997; Safarinejad, 2003;

Sorkhi & Aahmadi, 2005; Vachvanichsanong, Lebel, & Moore, 2000). In pediatrics, a positive correlation was found between first morning Ca/Crea ratio and 24-h Ca excretion (r=0.84) (Reusz *et al.*, 1995) and a stronger correlation was reported by Gokce *et al.* (1991) (r=0.946) when studying random spot urine specimens. Foley and Bossuzzi (2010) affirmed that the second urine void is most closely related to the total urinary Ca excretion. P/Crea ratios were as well positively correlated to 24-h P excretion (r=0.967) (Gorke *et al.*, 1991).

Based on all of the above and taking into consideration that dietary assessment methods have limited accuracy, serum mineral concentrations are tightly regulated, and 24-h urine collection is surrounded by all the impractical settings, we assessed Ca, Mg, and P in ratio to Crea in non fasting morning spot urine specimens in children.

CHAPTER III

MATERIAL AND METHODS

This chapter describes the study design. Details about the number of subjects, participant characteristics, sampling method, and field information are presented. Laboratory work and sample analyses are also outlined. In addition, this section includes a part dedicated for statistical analysis and definition of statistical variables.

A. Study Population

According the World Bank data, in 2012, the average enrollment rate of children in elementary schools was 93 %. Therefore, a representative sample of Lebanese 6-10 year old school children was picked.

A cross-sectional study was carried out between March 2013 and January 2014 using a proportionate cluster sampling method. The study aimed to obtain a representative sample of elementary school children in Lebanon. A list of elementary schools in Lebanon was obtained from the ministry of education and higher education (MEHE). The list contained different specifications of each school: the name, number of elementary children, location (district), and type (public, private and private free). An attempt was made to recruit children in proportion to their population size in each district.

Thirty-six schools were contacted in order to obtain the required number of children, but 26 only responded.The number of children recruited from each school was relative to the number of children in the school. These schools were chosen from the eight districts of Lebanon (North, Akkar, Bekaa, Baalbeck/Hermel, South, Nabatiyeh,

Mount Lebanon, and Beirut) according to the population load in each district, to reach a representative. One thousand four hundred and three (1403) healthy children (781 males and 622 females) of ages 6 to 10 were randomly selected.

Subject recruitment was done by directly approaching schools in coordination with the Ministry of Education and Higher Education (MEHE). Parents were asked to keep normal conditions of the children; neither to modify their diets nor their lifestyles including physical activity. Our study only included Lebanese 6-10 year old school children enrolled in elementary classes. Children having chronic or acute illness and those receiving any medical treatments were excluded from the study. Consent and assent forms[3] were respectively signed by the participants' parents and researchers prior to collection.

The study protocol was approved by the Institutional Review Board of the American University of Beirut.

B. Sampling Selection (Gorstein *et al*., 2007)

To keep a high degree of representativeness, the probability proportionate to size (PPS) method was adopted.

First, the sample size was determined using the following formula:

$$n=\frac{1.96\times1.96\times p\ (1-p)(DEFF)}{d\times d}$$

n: sample size.

p: estimate of the expected proportion; "if the expected proportion p of an indicator is not known, usually the value of 0.5 (or 50%) is used because it produces the largest sample size".

[3] Arabic and English forms are attached in the appendix section (APPENDIX I to IV)

d: desired level of absolute precision; the confidence interval is set at 95 %; thus the acceptable range of error is 5 %, making d=0.05.

DEFF: a design effect of 4 was used as sample size required for cluster surveys larger than the required for surveys using random sampling.

Following this formula, the sample size was found to be 1537.

Second, to determine the number of clusters (schools), we used: $nS=\frac{n}{\mu S \times pp}$.

nS: total number of schools to sample in a survey.

n: total sample size for the number of individuals, being 1537.

μS: average number of students per school.

pp: proportion of population in target group.

Thus, our calculations revealed a total number of schools of 36.

Multi-stage cluster sampling was then used at the Mohafazat (District) level, school level and class level. Number of schools required per district was calculated proportional to total number of schools per district.

The types of schools in each district were determined regarding the proportional distribution of schools in this particular district. Table 8 illustrates the number and the type of schools needed in each district.

In total we have 17 public, 13 private, and 6 private free schools.

Within districts, schools were selected using simple random selection from a school listing.

Then, to determine the number of students needed from each school, we compared the number of students (in Grades 1 to 5) in the selected schools to the total number of students in these classes in Lebanon (12000 students). In a school of 91 students between Grade 1 to Grade 5 we must recruit 11 students ([91×1500]/12000=11).

Finally, in each school, a random sampling was adopted. Each student aged between 6 and 10 years old had the same probability of being selected in the study.

Table 8. *Types and Numbers of Schools Needed in Each District*

District	Number of schools	Public	Private	Private Free
Akkar	5	3	1	1
Baalbeck/Hermel	3	1	1	1
Beirut	3	1	2	-
Bekaa	3	2	1	-
Mount Lebanon	5	1	3	1
Nabatiyeh	4	2	1	1
North	8	4	3	1
South	5	3	1	1

C. Procedures

1. Contacting Schools

Several procedures took place prior to data collection,. All public schools were notified about the project by the Director of Guidance and Counseling in the MEHE and all private and private free schools were directly contacted by the researches. An official letter and a consent form were given to the directors of the schools on a first visit. After having the acceptance of participation, consent forms were sent to all students in Grades 1 to 5 consistent with our age range (6-10 years old), and only students with signed consents participated in the study on the second visit.

2. Codes and Labels

Codes were divided into two, one related to the school and the other to the student. Codes for schools were given in the following way: LL-NN (L refers to a letter representing the district and N refers to a number representing the student). Table 9

shows the 8 different codes according to districts. Schools were then enumerated from 1 to 36. The codes of schools that did not participate were not given to other schools in order to prevent the occurrence of any mistake. Student IDs varied from 0001 to 1500. Accordingly, each participate had a double code. For example, the first participant in the first school in the south was labeled "S-01-0001".

Table 9. *Codes of Schools According to Districts*

Regions	Akkar	Beirut	Bekaa	Baalbeck/ Hermel	Mount-Lebanon	Nabatiye	North	South
Code	A	B	BK	H	ML	NB	N	S

3. Materials of Collection

All needed equipment and supplies were ordered 6 months prior to field trips. Table 10 lists the equipments used in this study.

Table 10. *Equipment and Supplies Used in This Study*

Items	**Equipment/Supplies**
General supplies	Personal Information Sheet
	Assent Forms
	Clip Board*
	Pens*
	2 Permanent Markers
	Stickers
	Field Manual
	Disposable Surgical Gloves*
	2 Hand Sanitizers
	Stapler and Staples
Specimens Collection	Disposable Urine Cups
	Sealable Plastic Bags*
	4 Ice packs
	2 Portable Coolers
Anthropometry	2 Digital Calibrated Scales
	1 Portable Stadiometer

Laboratory	Screw-capped tubes for urine storage* (2, 15, 50 mL) Disposable Pipette* (for transferring urine from cups to tubes) Tube Racks* Large Scotch Tape (for labeling) Biohazard Bags*

* the list of needed utensils was adapted from "Gorstein, J., Sullivan, K., Parvanta, I., & Begin, F. (2007). Sample size calculations *Indications and methods for cross sectional surveys of vitamin and mineral status of populattions* (pp. 28-37). The Micronutrient Initiative (Ottawa) and the Centers for Disease Control and Prevention (Atlanta)".

D. Data Collection

Four researchers collected students personal information (name, student ID number, age, and class), anthropometric measurements (weight and height), and urine samples. The research team was trained for ethical, professional, and standardized field work.

1. Anthropometric Measurements

Weight was measured using Tanita scale (± 0.1 Kg) in light indoor clothing. Height was measured using a Shorrboard portable stadiometer (± 0.1 cm) received from UNICEF. Children kept their footwear on, consequently, 1 cm was subtracted from the height when wearing tennis shoes; 2 cm were subtracted when wearing winter boots. BMI was calculated using the following formula weight/height^2 and expressed in kg/m^2.

2. Nutritional Status

According to WHO criteria, based on BMI for age z-scores growth charts (2007) for 5-19 year old boys and girls[4], percentiles and BMI classifications were calculated. Children were distributed in five BMI for age categories: "Severe thinness"

[4] Found in appendix (APPENDIX V and VI)

for a BMI below the 3rd percentile, "thinness" for a BMI for age between the 3rd and the 15th percentile, "normal" for a BMI between the 15th and the 85th percentile, "overweight" for a BMI between the 85th and the 97th percentile, and "obese" for a BMI above the 97th percentile.

3. Urine Collection and storage

The researchers provided all the necessary information about the study and urine collection to each participant aside. An assent form was signed by each student afterwards. Non fasting random urine samples were collected in chemicals free cups between 9 a.m. and 1 p.m. and were transported to the laboratory on ice. Urine specimens were stored in - 20 °C freezers until the date of analysis.

4. Biochemical Measurements

Before mineral analysis, urine samples were defrosted and acidified (with a diluted HCl solution) to prevent mineral precipitation which falsely lowers the results (Foley & Boccuzzi, 2010). A 75 µl of diluted hydrochloric acid (HCl) solution of a ratio of 1:5, prepared using [36 M of HCl:H_2O in 1:5(v/v)] ,was added to 5 ml of urine;The average pH of the specimen became between 3 and 4 which is a compatible pH for all minerals tests as described in the instruction sheets of technical use. This specific volume of acidic solution was determined after several trials over a significant number of samples taken from all districts.

After acidification, urine specimens were homogenized using vortex then centrifuged for 10 minutes at a speed of 3500RPM at 20 °C using EPPENDORF Centrifuge 5810R. Creatinine (Crea) analysis followed the same procedure without acidification.

Calcium (Ca), magnesium (Mg), phosphorus (P), and Crea were measured using Vitros 350 analyzer (Ortho Clinical Diagnostics, Johnson and Johnson, 50-100 Holmers Farm Way, High Wycombe, Buckinghamshire, HP 12 4DP, United Kingdom) in the NFSC department (AUB, Beirut, Lebanon). Values below the lower detection limit were defined as 0.04 mg/dl for Ca and 1 mg/dl for Mg. Values are reported in mg/dl. The conversion from mg to mmol was done by dividing the values by molecular weights of the analyzed variables (Ca, Mg, P, and Crea).

a. Calcium (144)

Test type: Colorimetric.

The Ca forms a complex with Arsenazo III dye, causing a shift in the absorption maximum. After incubation, the reflection density of the colored complex is measured spectrophotometrically. The amount of colored complex formed is proportional to the Ca concentration in the sample.

b. Magnesium (146)

Test type: Colorimetric.

Mg from the sample reacts with the formazan dye derivative in the reagent layer. The resulting Mg-dye complex causes a shift in the dye absorption maximum. The amount of dye complex formed is proportional to the Mg concentration present in the sample and is measured by reflection density.

c. Phosphorus (147)

Test type: Colorimetric.

The analysis is based on the reaction of inorganic phosphate with ammonium molybdate to form an ammonium phosphomolybdate complex at acidic pH, as described by Fiske and Subbarow (1925). p-Methylaminophenol sulfate, an organic reductant reported by Gomori (1942), reduces the complex to form a stable heteropolymolybdenum blue chromophore. P in the specimen forms a complex with ammonium molybdate. This complex is reduced by p-methylaminophenol sulfate to give a blue complex. The concentration of P in the sample is determined by measuring the heteropolymolybdenum blue complex by reflectance spectrophotometry.

d. Creatinine (145)

Test type: Two-point rate.

Crea diffuses to the reagent layer, where it is hydrolyzed to creatine in the rate-determining step. The creatine is converted to sarcosine and urea by creatine amidinohydrolase. The sarcosine, in the presence of sarcosine oxidase, is oxidized to glycine, formaldehyde, and hydrogen peroxide. The final reaction involves the peroxidase-catalyzed oxidation of a leuco dye to produce a colored product. Following addition of the sample, the slide is incubated. During the initial reaction phase, endogenous creatine in the sample is oxidized. The resulting change in reflection density is measured at 2 time points. The difference in reflection density is proportional to the concentration of Crea present in the sample.

5. Quality Control

Pooled urine specimens, collected from 3 healthy adults (25 year old women) to increase the volume, were mixed and aliquoted in small volumes to show consistent

and systematic results. These prepared urine samples were used as quality control (QC) to determine the coefficient of variance (cv) for between and within runs (Table 11). Every 30 tests were interrupted by QC measurements of all variables (Ca, Mg, P, and Crea), having a minimum average of 3 QC per run. The dispersions (cv) of between and within runs were at worse 5.5 % and 2.2 %, respectively, showing relevant and persistent results indicating a credible and reliable method of assessment.

Table 11. *Quality Control's Coefficient of Variance.*

Test	Between Runs CV (%)	Within Runs CV (%)
Ca	5.5	0.73
Mg	5.1	2.24
P	2.4	1.33
Cr	3.8	1.77

6. Mineral to Creatinine ratio

Mineral results are expressed in ratio to Crea as Ca/Crea, Mg/Crea, and P/Crea.

7. Predictive Values

Predictive values of total Crea excretion per 24 h were calculated (in mmol/d and converted into mg/d) based on the equation of Remer, Neubert, & Maser-Gluth (2002):

$$\log y = 0.0102x - 0.6854$$

(y: 24-h Crea expressed in mmol/d; x: height expressed in cm).

This was based on data from 3-18 year old children in which height was highly correlated with Crea excretion ($R^2 = 0.87$ and $P < 0.0001$). Thereafter, total Ca, Mg, and P excretions per 24-h were estimated.

E. Statistics

1. Data Entry

Data were collected on paper forms. Subject personal information was typed out on the Statistical Package for the Social Sciences (SPSS) 21. Biochemical results were entered primarily on Microsoft Office Excel 2007 and then copied on SPSS. A double data entry process was applied in order to minimize the occurrence of errors. Data cleaning was then done to identify and correct all possible mistakes.

2. Statistical Analysis

Statistical analysis was performed using Mini-Tab 16 for Windows. Statistical significance was set at p-value < 0.05. Results are presented as means ± standard deviation (SD). Frequencies and descriptive statistics were performed for the different variables. Data was stratified by gender and/or by 5 age groups (6-7, 7-8, 8-9, 9-10, and 10-11). Some statistical analysis was done dividing the results by districts or school types. Two-sample t-test was used to identify statistical significant differences between genders. One way ANOVA with 95 % of individual confidence interval evaluated differences among age groups. The subgroup analysis, differences among groups and categories, was performed using Fisher's test with 95 % of confidence interval. Pearson's Correlation was used to assess correlations between minerals, Crea, and other variables.

Six subjects were excluded due to insufficient urine volume to assess all biochemical variables. Four subjects (outliers) for Ca, two for Mg, and only 1 for P were excluded based on Anderson-Darling normality test.

F. Definition of Variables

Collected data were organized in both SPSS (version 21) and Excel sheets. Variables of interest including personal information, anthropometric, demographic, and biochemical data were drawn. The variables used in the statistical analysis and their description are presented in Appendix VIII.

- School Types

The Lebanese population benefits from the availability of three different types of schools. The percentages of public, private and private free schools are the following: 47 %, 37 % and 13 % (CERD, 2009-2010), indicating a disproportionate distribution all over Lebanon, The remaining 3 % are designated to the Palestinian refugees in Lebanon, thus they do not meet our inclusion criteria. Based on this distribution, the majority of our clusters were selected from public schools.

Private free schools are free of charge targeting people with varied socio-economical status who desire to enroll their children in religious schools (mostly). The variations of socio-economical status of families whose children are enrolled in private free schools depend upon the social level of the school that is determined by sponsor-ship and funds. Public schools in Lebanon, especially in the primary classes are designated for people who do not afford private school fees.

- Districts

Since 2003, according to the "Center de Resources sur le développement local" (Localiban, 2009), Lebanon has been divided into 8 districts (Akkar, Baalbeck/Hermel, Beirut, Bekaa, Mount Lebanon, Nabatiyeh, North, and South) instead of 6 (North

included Akkar and Bekaa included Baalbeck/Hermel). Our data was collected and entered as described above, considering the codes of the new 8 districts. However, even recent data describing the demographic structure of the Lebanese population takes into consideration the old district divisions. Thus, our results were reported following both distributions.

CHAPTER IV

RESULTS

This chapter presents the outcome of the study. The results are segregated in different sections. The sections describe baseline characteristics, sex, age, schools, and districts related differences as well as urinary mineral excretions in ratios and their reference values. A 24-h urinary excretion of Ca, Mg, and P, presented in the following page, is estimated for the whole population, as described in the previous chapter.

A. Baseline Characteristics

The study involved 1403 children, 781 boys and 622 girls, aged from 6 to 10 years old. They were classified in 5 age groups (6-7, 7-8, 8-9, 9-10 and 10-11). The mean weight and height of the whole sample was 28.3±7.9 kg and 127.0±9.7 cm; respectively. The mean BMI was 17.25±2.74 kg/m^2. Table 12 shows, according to WHO cutoffs, the nutritional status of our population.

Table 12. *Nutritional Status of Elementary School Lebanese Children (6-10 years old)*

Nutritional Status	**Sample (n)**	**Percentage (%)**
Sever Thinness	9	0.6
Thinness	76	5.4
Normal	911	65.0
Overweight	225	16.0
Obese	182	13.0

As illustrated in Figure 2, only 6% of our population was underweight. This fact reduces malnutrition related biases. The detailed subject information of the data split by age group and gender is found in Table 13. As indicated in Table 14, there was no sex related statistical significant difference for weight. However, a significant difference in height between girls and boys was found with a P value of 0.049, with the girls being 1 cm taller. This difference lost its significance when presented as BMI. When segregated by age, the differences of weight, height, and BMI were highly significant. Table 15 shows age group significant difference from 6 to 10 years old for weight and height. As for BMI, 2 age categories (8-9 and 9-10) were considered similar.

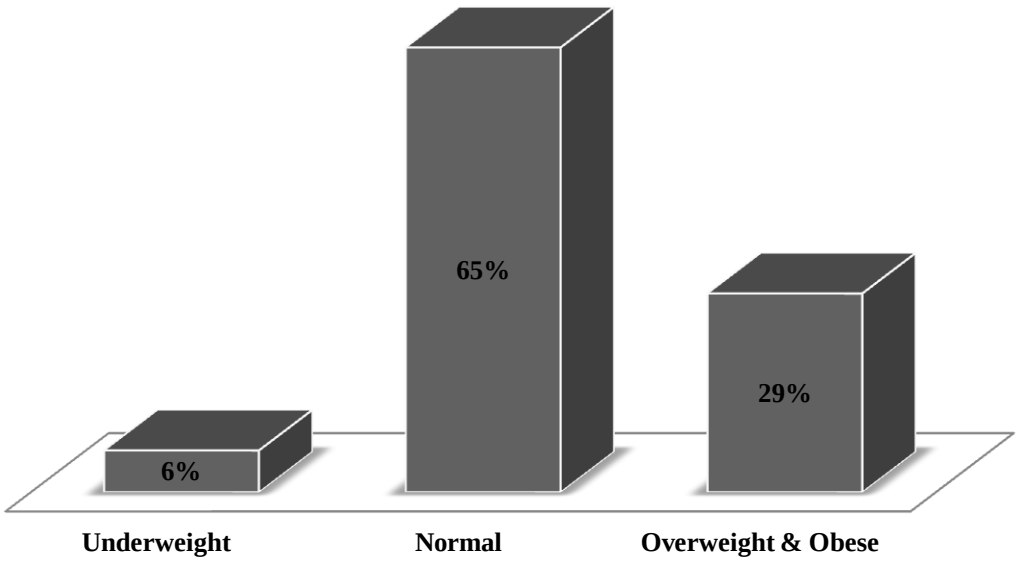


Figure 2. *Nutritional Status of Elementary School Lebanese Children (6-10 years old)*

Table 13. *Anthropometric Characteristics of Elementary School Lebanese Children (6-10 years old)*

Age (years)	Sample		Weight (kg)	Height (cm)	BMI (kg/m^2)
	Gender	n			
6-7	M	208	22.7±4.3	117.1±5.8	16.4±2.1
	F	122	22.2±4.1	116.4±5.4	16.3±2.0
	T	330	22.5±4.2	116.9±5.7	16.4±2.1
7-8	M	194	26.7±4.9	124.6±6.0	17.1±2.3
	F	154	26.6±5.4	124.2±5.8	17.1±2.3
	T	348	26.6±2.3	124.4±5.9	17.1±2.3
8-9	M	156	28.6±6.1	128.3±5.6	17.3±2.6
	F	147	28.0±6.2	128.1±6.3	16.9±2.5
	T	303	28.3±6.1	128.2±6.0	17.1±2.5
9-10	M	113	31.7±7.4	132.9±6.0	17.8±3.1
	F	101	30.8±6.5	132.4±6.6	17.4±2.6
	T	214	31.2±7.0	132.7±6.3	17.6±2.9
10-11	M	110	36.6±9.7	139.2±6.5	18.7±4.0
	F	98	38.1±9.7	141.3±8.3	18.9±3.4
	T	208	37.3±9.7	140.1±7.5	18.8±3.7
Total	M	781	28.1±7.7	126.6±9.4	17.3±2.8
	F	622	28.2±8.1	127.6±10.1	17.2±2.7
	T	1403	28.3±7.9	127.0±9.7	17.3±2.7

All the values in the table are represented as mean±SD.
M, F, and T refer respectively to Male, Female, and Total.

Table 14. *Sex Related Difference of Anthropometric Characteristics of Elementary School Lebanese Children (6-10 years old)*

Variable	**Boys (781)**	**Girls (622)**	**p-value**
Weight	28.1±7.7	28.6±8.1	0.291
Height	126.6±9.4	127.6±10.1	0.049
BMI	17.3±2.8	17.2±2.7	0.714

All values are reported as mean±SD.
The number presented between brackets is the sample size.
t-test is used comparing genders and significance is set at $p<0.05$.

Table 15. *Age Related Difference of Anthropometric Characteristics of Elementary School Lebanese Children (6-10 years old)*

Age (years)	**Anthropometric Characteristics**		
	Height (cm)	Weight (kg)	BMI (kg/m^2)
6-7	116.9 ± 5.7^{a}	22.5 ± 4.2^{a}	16.4 ± 2.1^{a}
7-8	124.4 ± 5.9^{b}	26.6 ± 5.1^{b}	17.1 ± 2.3^{b}
8-9	128.2 ± 6.0^{c}	28.3 ± 6.1^{c}	17.1 ± 2.5^{c}
9-10	132.7 ± 6.3^{d}	31.2 ± 7.0^{d}	17.6 ± 2.9^{c}
10-11	140.1 ± 7.5^{e}	37.3 ± 9.7^{e}	18.8 ± 3.7^{d}
p-value	<0.001	<0.001	<0.001

All values are reported as mean±SD.
One way ANOVA is used to detect baseline characteristics significant differences between age groups. Significance is set at $p<0.05$.
The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

Schools and districts distribution strongly depended on the response rate. The under-representativeness of Mount Lebanon for instance is directly related to the low response rate of private schools in this district. Tables 16 and 17 present our population repartitions in schools and districts and compare them to the percentages in Lebanon.

Table 16. *Selected Schools Repartitions*

School Types	Clusters (n)	Percentage (%)	Response Rate (%)	Percentages in Lebanon (%)
Public	16	61.5	94.1	47
Private	8	30.8	72.7	37
Private Free	2	7.7	33.3	13
Total	26	100	72.2	97*

*The remaining 3% is for UNRWA schools (excluded criteria).
Central Administration for Statistics, Ministry of Social Affairs,UNDP (2004-2005).

Table 17. *Selected Elementary School Lebanese Pupils (6-10 years old) from Each District*

District	Sample (n)	Percentage (%)
Akar	155	11.0
Beirut	230	16.4
Bekaa	168	12.0
Hermel	53	3.8
Mount Lebanon	160	11.4
North	247	17.6
Nabatiyeh	206	14.7
South	184	13.1

Selected Elementary School Lebanese Pupils (6-10 years old) from Each District (Old Districts Distributions)

District	Sample (n)	Percentage (%)	Lebanese Population load*
Beirut	230	16.4	10.4
Bekaa	221	15.8	12.5
Mount Lebanon	160	11.4	40.0
North	402	28.6	20.5
Nabatiyeh	206	14.7	5.9
South	184	13.1	10.7

*Central Administration for Statistics, Ministry of Social Affairs,UNDP (2004-2005).

B. Biochemical Measurements

1. Ratios

The mean Ca/Crea for the total population was found to be 0.084±0.101 mg/mg. Total Mg/Crea and P/Crea were also calculated to give 0.122±0.075 and 0.692±0.417; respectively (Table 18). Sex related differences of the ratios are presented in Table 18. No significant difference was detected. All ratio related values were presented for the whole population since no sex related difference was detected. Correlations between Ca/Crea, Mg/Crea, and P/Crea are given in Table 19. Significant positive correlation was found between all ratios ($p < 0.001$). Best correlation was found between Ca/Crea and Mg/Crea ($r = 0.489$).

Table 18. *Ca, Mg, and P Excretions in Ratio to Crea of Elementary School Lebanese Children (6-10 years old)*

Minerals Ratios (mg/mg)	**Total**	**Boys (777)**	**Girls (619)**	**p-value**
Ca/Crea	0.084±0.101	0.087±0.112	0.080±0.086	0.161
Mg/Crea	0.122±0.075	0.12±0.07	0.12±0.08	0.706
P/Crea	0.692±0.417	0.687±0.412	0.698±0.424	0.604

All values are reported as mean±SD.
The number presented between brackets defines the sample size.
t-test is used comparing genders. Significance is set at $p<0.05$ for the difference between genders.

Table 19. *Ca, Mg, and P Ratios Correlation Matrix*

	Ca/Crea	**Mg/Crea**	**P/Crea**
Ca/Crea		0.489***	0.253***
Mg/Crea	0.489***		0.463***
P/Crea	0.253***	0.463***	

Pearson correlation * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

2. Predicted 24-h Excretions

As previously mentioned, the 24-h urinary excretion of all biochemical markers was estimated. Table 20 demonstrates 24-h predicted values of Ca, Mg, and P for the total population. Based on the differences in height, total Crea excretion was found to be different between genders, girls having higher excretions. Total Ca, Mg, and P excretions were similar between them. All values describing total mineral excretions were presented for the whole population since no sex differences were proven. Correlations between total 24-h mineral and Crea excretion are shown in Table 21. Similar significant positive results were detected between all variables. The strongest correlation was found, similar to the ratios, between Ca and Mg. Positive correlation between Crea and all minerals were affirmed.

Table 20. *Predicted 24-h Ca, Mg, P, and Crea Excretions of Elementary School Lebanese Children (6-10 years old)*

Predicted 24-h Excretions	**Total**		**Boys (777)**	**Girls (619)**	**p-value**
	mg/d	**mg/kg/d**	**mg/d**		
Ca	38.95±46.72	1.416±1.716	40.4±50.9	37.1±40.8	0.185
Mg	57.15±37.03	2.073±1.296	56.1±36.5	58.5±37.7	0.228
P	321.9±206.6	11.767±7.305	317±203	328±211	0.331
Crea	473.837±113.328	16.988±2.145	468±107	481±120	0.03

All values are reported as mean±SD.
The number presented between brackets is the sample size.
t-test is used comparing genders and significance is set at $p<0.05$ for the difference between genders.

Table 21. *Predictive 24-h Minerals and Creatinine Excretions Correlations*

	Calcium	**Magnesium**	**Phosphorus**	**Creatinine**
Calcium		0.515***	0.3***	0.123***
Magnesium	0.515***		0.512***	0.278***
Phosphorus	0.300***	0.512***		0.240***
Creatinine	0.123***	0.278***	0.240***	

Pearson correlation * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3. Age related differences

a. Calcium

Ca decreased in ratio to Crea with age. This trend wasn't statistically significant. Inversely, total 24-h Ca excretions increased significantly as age increased. No sharp increment was detected between two consecutive age categories. The difference accentuated when the groups were not successive. Table 22 emphasizes age related difference of urinary Ca excretion.

Table 22. *Age Related Differences of Ca Excretions of Elementary School Lebanese Children (6-10 years old)*

Age (yrs)	**Samples (n)**	**Ca/Crea (mg/mg)**	**Predictions of daily Ca excretion**	
			mg/d	mg/kg/d
6-7	325	0.091±0.122	33.04±43.74[a]	1.502±2.034
7-8	347	0.083±0.103	35.8±44.18[ab]	1.373±1.705
8-9	302	0.087±0.091	41.98±44.27[ab]	1.496±1.567
9-10	212	0.078±0.090	41.58±50.09[bc]	1.352±1.553
10-11	207	0.075±0.087	46.43±53.67[c]	1.299±1.554
p-value		0.373	0.007	0.564

All values are reported as mean±SD.
One way ANOVA is used to detect Ca excretions differences between age groups. Significance is set at $p<0.05$.
The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

Figure 3 clearly illustrates the population distribution of Ca/Crea and total Ca excretion expressed per day and per kilogram of body weight per day.

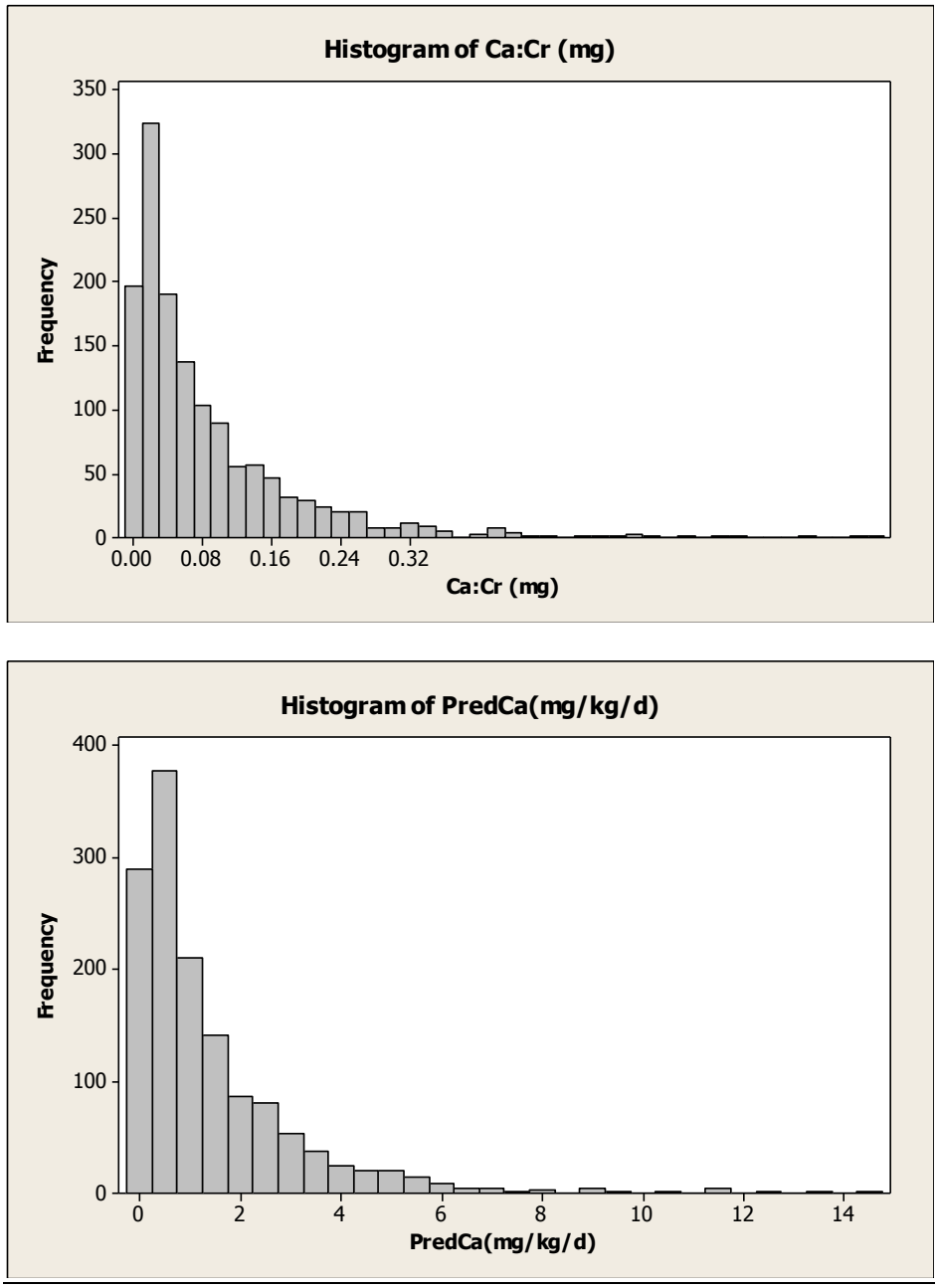


Figure 3. *Population Distribution of Ca/Crea and Predicted 24-h Ca Excretions of Elementary School Lebanese Children (6-10 years old)*

b. Magnesium

Urinary Mg/Crea (mg/kg/d) showed a continuous decrease with age. The decline in ratios was not significant between age groups as seen in Table 23. The total 24-h Mg excretions were assumed to be inversely related to age. Statistics highlighted the age of 10 years old to be totally different than the other ages. Figure 4 illustrates the population distributions of Mg/Crea and 24-h total Mg excretion.

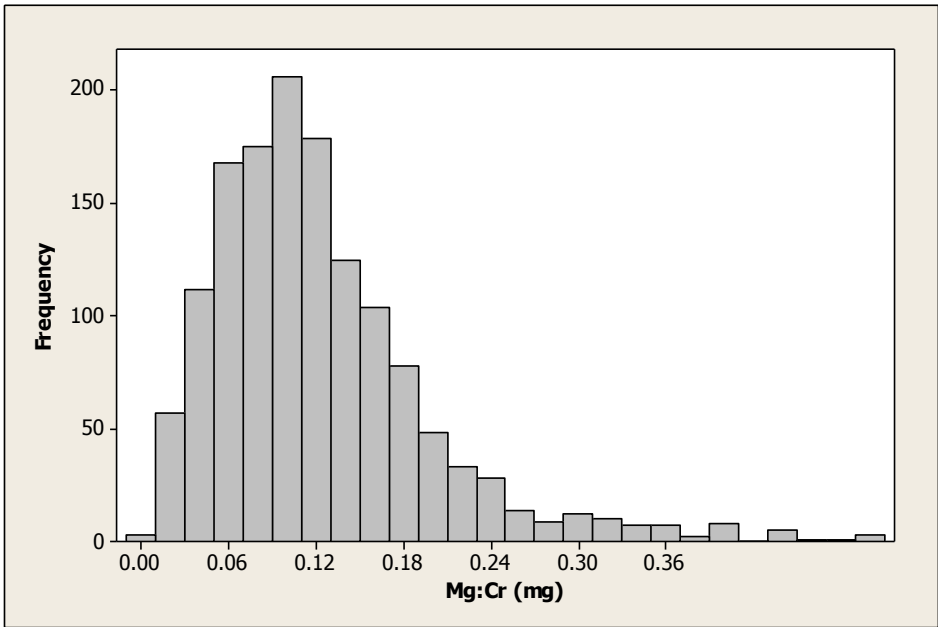
Table 23. *Age Related Difference of Mg Excretions of Elementary School Lebanese Children (6-10 years old)*

Age (yrs)	**Samples (n)**	**Mg/Crea (mg/mg)**	**Predictions of daily Mg excretion**	
			mg/d	mg/kg/d
6-7	325	0.126±0.074	45.92±27.00[a]	2.09±1.2
7-8	347	0.125±0.079	54.20±33.16[ab]	2.09±1.3
8-9	301	0.122±0.077	58.99±40.02[bc]	2.11±1.4
9-10	214	0.120±0.070	64.46±40.72[c]	2.08±1.2
10-11	208	0.111±0.069	69.44±42.50[d]	1.97±1.4
p-value		0.172	<0.001	0.803

All values are reported as mean±SD.
One way ANOVA is used to detect Mg excretions differences between age groups. Significance is set at $p<0.05$.
The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

c. Phosphorus

The mean urinary P/Crea were significantly higher in younger ages. As represented in Table 24, at 9 and 10 years old, the variances started being more pronounced. P excretion was significantly different between the three older age groups whether expressed as ratios or as total excretion, which was opposite to the results found for Ca and Mg. Table 24 segregated P excretion by age and presented the results with their differences. The distribution of P excretion for the whole sample is presented in Figure 5.



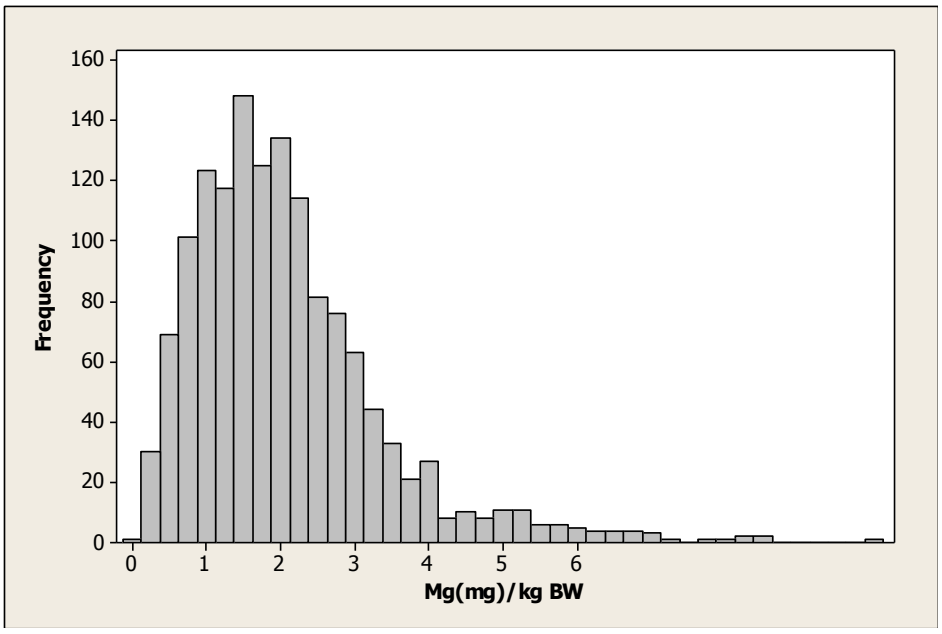
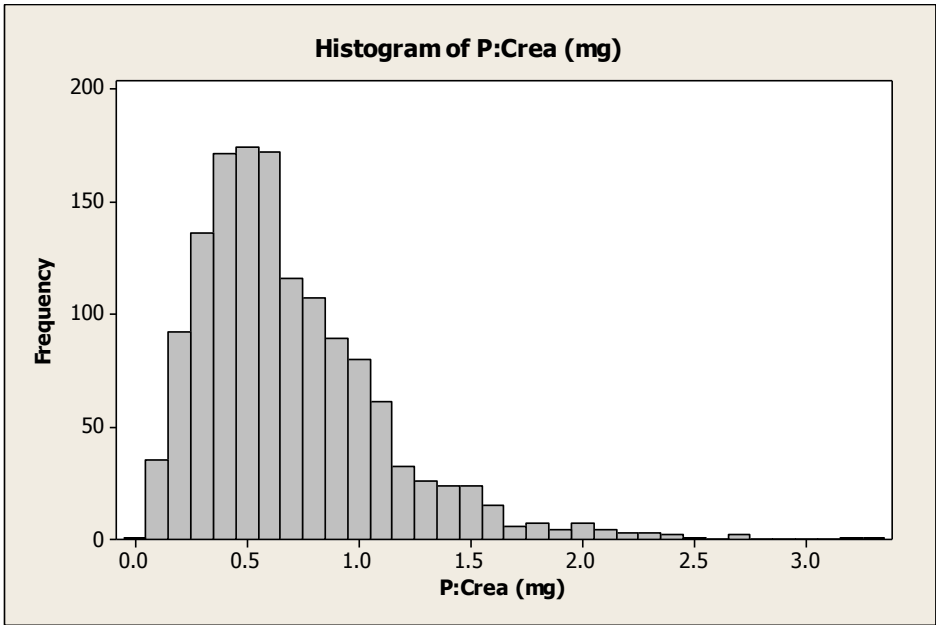


Figure 4. *Population Distribution of Mg/Crea and Predicted 24-h Mg Excretions of Elementary School Lebanese Children (6-10 years old)*

Table 24. *Age Related Difference of P Excretions of Elementary School Lebanese Children (6-10 years old)*

Age (yrs)	**Samples (n)**	**P/Crea (mg/mg)**	**Predictions of daily P excretion**	
			mg/d	mg/kg/d
6-7	326	0.759±0.410[a]	274.9±151.0[a]	12.562±7.069[a]
7-8	347	0.708±0.400[a]	307.8±172.8[a]	11.812±6.745[ab]
8-9	302	0.730±0.454[ab]	351.5±231.6[a]	12.610±8.171[a]
9-10	213	0.642±0.435[b]	344.4±249.6[b]	11.159±7.608[bc]
10-11	208	0.556±0.345[c]	353.1±231.3[c]	9.843±6.531[c]
p-value		<0.001	<0.001	<0.001

All values are reported as mean±SD.
One way ANOVA is used to detect P excretions differences between age groups. Significance is set at $p<0.05$.
The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.



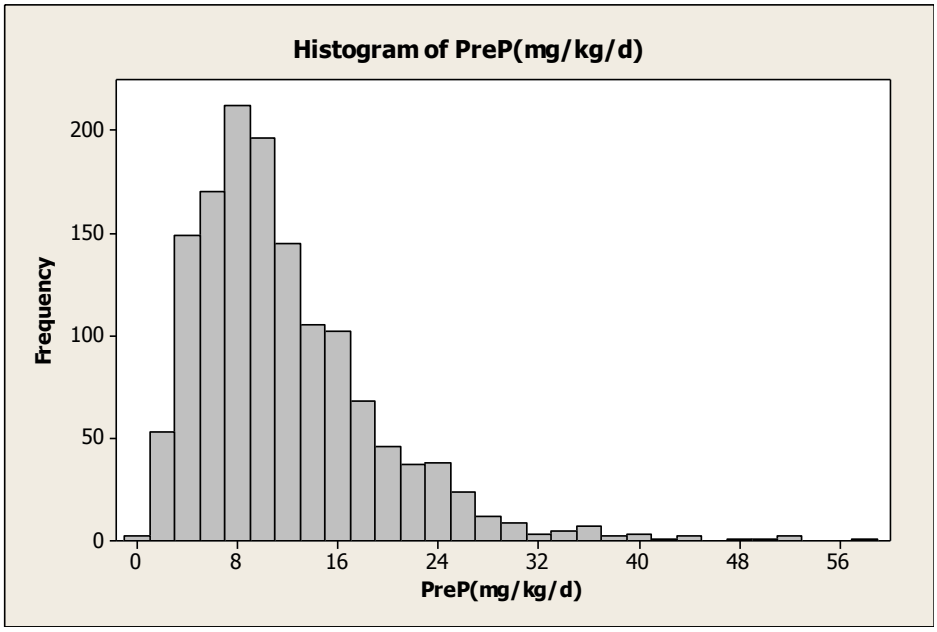


Figure 5. *Population Distribution of P/Crea and Predicted 24-h P Excretions of Elementary School Lebanese Children (6-10 years old)*

4. Schools

When comparing mineral excretion in ratio to Crea in different school types (Table 25), we found that only a significant difference was detected between public and private schools, the latter have higher P/Crea ratios, thus higher P excretion. However, when comparing predicted 24-h mineral excretion, significance appeared in Ca, Mg, and P (Table 26). In Ca and Mg, public and private free shared similar excretion different from private schools. P excretion varied according to school type.

Table 25. *Ca, Mg, and P Excretions in Ratio to Crea in Different School Types in Lebanon*

School Type	Ca/Crea	Mg/Crea	P/Crea
Public (666)	0.077± 0.098	0.119±0.076	0.634±0.389[a]
Private (509)	0.091±0.094	0.127±0.078	0.744±0.467[b]
Private Free (220)	0.085±0.123	0.118±0.062	0.747±0.355[b]
p-value	0.057	0.114	<0.001

All values are reported as mean±SD (mg/mg).
One way ANOVA is used to detect districts differences between age groups. Significance is set at $p<0.05$. The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

Table 26. *Predictions of 24-h Ca, Mg, and P Excretions in Different School Types in Lebanon*

School Type	Predicted 24-h Ca excretion	Predicted 24-h Mg excretion	Predicted 24-h P excretion
Public (666)	35.46±44.57[a]	55.19±36.42[a]	287.1±171.9[a]
Private (509)	44.51±48.33[b]	61.88±40.99[b]	364.5±255.1[b]
Private Free (220)	35.85±46.69[a]	51.65±25.68[a]	327.8±151.9[c]
p-value	0.02	0.001	<0.001

All values are reported as mean±SD (mg/d).
One way ANOVA is used to detect districts differences between age groups. Significance is set at $p<0.05$. The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

5. Districts

Student mineral excretion was distinct when expressed in ratios to Crea as well as when 24-h excretion was estimated. Tables 27 and 28 stated in detail Ca, Mg, and P excretion in different districts showing remarkable dissimilarities.

Table 27. *Ca, Mg, and P Excretions in Ratio to Crea in the Lebanese Districts*

District	Ca/Crea	Mg/Crea	P/Crea
Akar (154)	0.072±0.093[a]	0.128±0.071[a]	0.752±0.483[ab]
Beirut (228)	0.091±0.106[ab]	0.107±0.062[bc]	0.541±0.254[c]
Bekaa (167)	0.108±0.112[b]	0.146±0.090[d]	0.961±0.575[d]
Hermel (53)	0.098±0.107[ab]	0.164±0.083[d]	0.659±0.415[bc]
Mount Lebanon (160)	0.085±0.087[a]	0.146±0.088[d]	0.786±0.405[a]
North (246)	0.070±0.082[a]	0.095±0.066[c]	0.600±0.369[c]
Nabatiye (204)	0.087±0.127[a]	0.121±0.064[a]	0.733±0.358[ab]
South (184)	0.073±0.087[a]	0.118±0.068[ab]	0.593±0.333[c]
p-value	0.004	<0.001	<0.001

All values are reported as mean±SD (mg/mg).
The number presented between brackets is the sample size.
One way ANOVA is used to detect districts differences between age groups. Significance is set at $p<0.05$.
The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

6. Correlations between Variables

Correlations of Ca, Mg, and P in ratio to Crea with Ca/P, weigh, height and BMI were studied. Significant associations were noted and described in Table 29. A significant negative correlation was found between Mg/Crea and P/Crea and weight. This means that, as weight increased, Mg and P in ratio to Crea decreased and vice versa. Moreover, height was inversely associated with Ca/Crea, Mg/Crea, and P/Crea. As for BMI, a negative correlation was shown only for P ratio between all mineral ratios. Significant strong positive associations linked BMI with weight and height.

Table 28. *Predictions of 24-h Ca, Mg, and P Excretions in the Lebanese Districts*

District	Predicted 24-h Ca excretion	Predicted 24-h Mg excretion	Predicted 24-h P excretion
Akar (154)	29.38±37.61[a]	52.31±29.76[a]	298.4±192.7[ab]
Beirut (228)	39.49±44.4b[c]	46.75±25.13[ab]	238.1±118.3[c]
Bekaa (167)	57.32±61.98[d]	77.06±50.92[c]	510.6±328.6[d]
Hermel (53)	47.33±59.77[bd]	74.99±45.42[c]	297.3±206.7[ab]
Mount Lebanon (160)	41.62±44.84[b]	70.40±43.38[cd]	377.6±194.5[e]
North (246)	31.99±37.5[ac]	43.79±30.17[b]	271.1±160.0[bc]
Nabatiye (204)	35.56±48.61[abc]	51.86±25.57[a]	315.3±149.7[a]
South (184)	38.04±42.14[abc]	63.14±36.73[d]	309.6±165.0[a]
p-value	<0.001	<0.001	<0.001

All values are reported as mean±SD (mg/d).
The number presented between brackets is the sample size.
One way ANOVA is used to detect districts differences between age groups. Significance is set at $p<0.05$.
The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

Table 29. *Correlation Matrix*

	Ca/Crea	Mg/Crea	P/Crea	Ca/P	Weight	Height	BMI
Weight	-0.035	-0.078**	-0.124***	0.014	1	0.811***	0.859***
Height	-0.061*	-0.083**	-0.137***	0.020	0.811***	1	0.416***
BMI	-0.007	-0.051	-0.084**	0.008	0.859***	0.416***	1

Pearson correlation * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
All ratios are presented in mg/mg, weight in kg, height in cm, and BMI in kg/m^2.

Table 30. *Reference Values of Ca, Mg, and P Excretions in Ratio to Crea for Elementary School Lebanese Children (6-10 years)*

Age	**Urinary Ca/Crea**			**Urinary Mg/Crea**			**Urinary P/Crea**		
(years)	5^{th}	50^{th}	95^{th}	5^{th}	50^{th}	95^{th}	5^{th}	50^{th}	95^{th}
6-7	0.000	0.050	0.310	0.030	0.120	0.260	0.241	0.665	1.543
7-8	0.000	0.050	0.256	0.030	0.110	0.280	0.210	0.620	1.470
8-9	0.000	0.050	0.269	0.030	0.110	0.278	0.220	0.620	1.516
9-10	0.000	0.040	0.304	0.030	0.110	0.253	0.170	0.550	1.507
10-11	0.000	0.040	0.230	0.030	0.100	0.246	0.170	0.480	1.218
Total	0.000	0.050	0.270	0.030	0.110	0.260	0.190	0.600	1.480

All the values are reported in mg/mg.

7. Reference Values

The 5th, 50th and 95th percentiles were calculated for Ca, Mg, and P in ratio to Crea. Table 30 represents these reference values as an average for pediatric population, as well as values specific for different age categories.

a. Calcium

Ca 5th percentile was 0 for the total population and for all age groups. The 50th percentile for the whole population as well as for some age groups (6-7, 7-8 and 8-9) was 0.05. At the ages of 9 and 10 years, the median decreased from 0.05 to 0.04. The upper limit of Ca/Crea showed an unsystematic trend of increase and decrease. It started at 0.310 at the age of 6, decreased to 0.256 at the age of 7, and then gradually increased reaching 0.304 at the age of 9. The sharpest decline was found between ages 9 and 10. All of the previously mentioned values are found in Table 30 and illustrated in Figure 6.

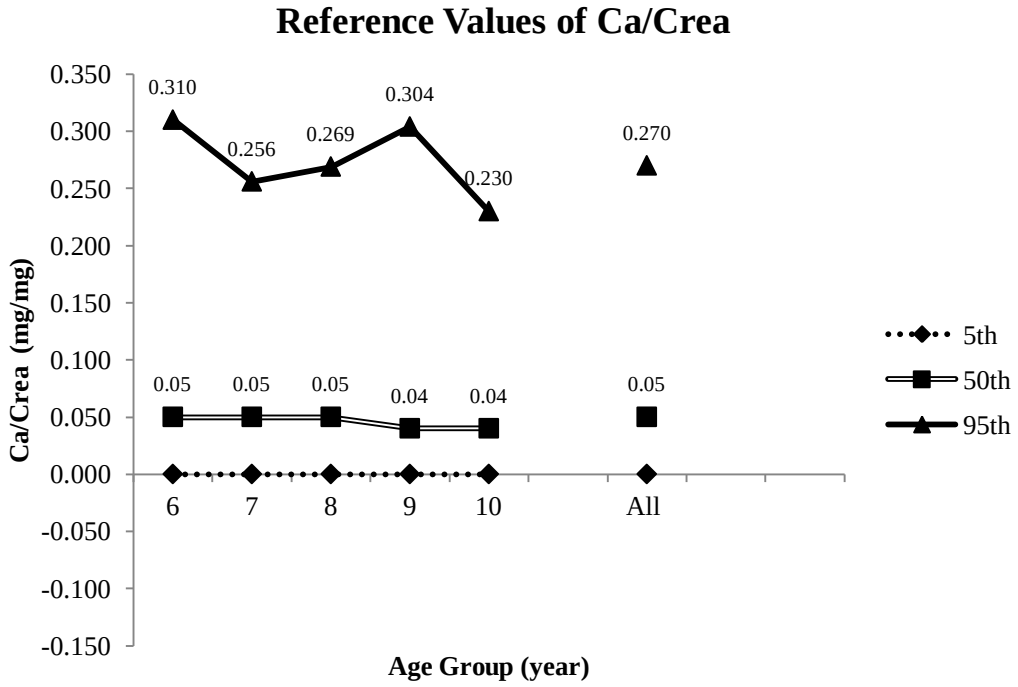


Figure 6. *Reference Values of Ca Excretions in Ratio to Crea for Elementary School Lebanese Children (6-10 years)*

b. Magnesium

According to Table 30 and in Figure 7, Mg/Crea in its lower cutoff was maintained constant in all ages. For the total population the 5th percentile was 0.03. The middle value of the sample was 0.11. It smoothly declined from 0.12 at the age of 6 to reach 0.10 at the age of 10. The 50th percentile remained constant at 0.11. The 95th percentile of 7 year olds was slightly higher than 6 year olds. The ratio decreased from 0.280 at age 7 to 0.246 at age 10. In 6 to 10 year old children, the 95th percentile of Mg/Crea was 0.260.

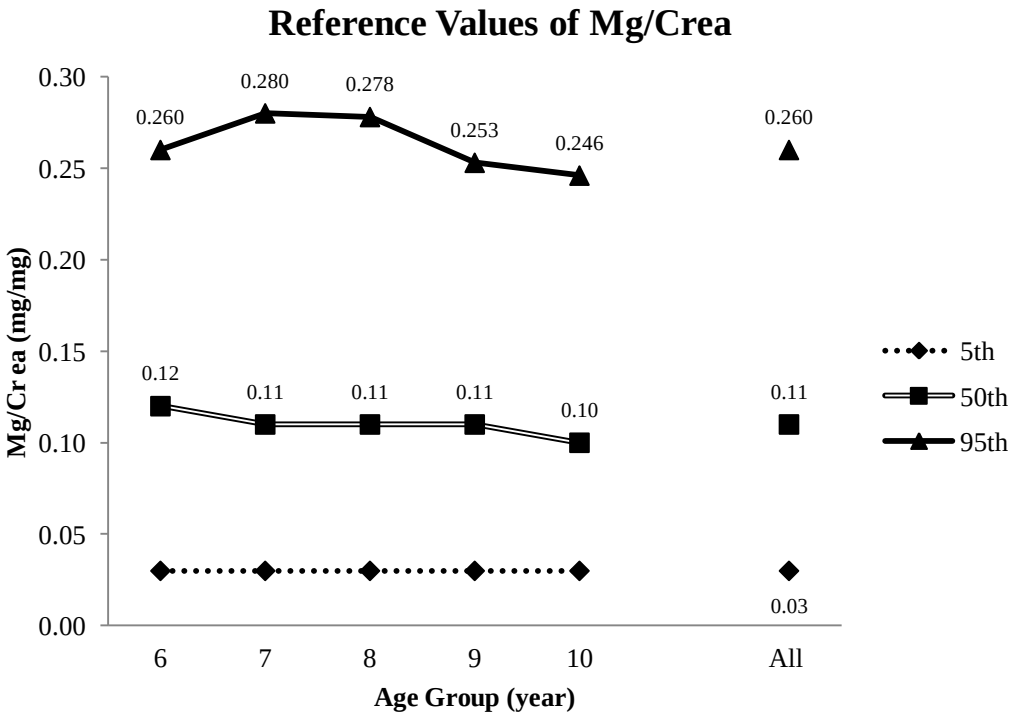


Figure 7. *Reference Values of Mg Excretions in Ratio to Crea for Elementary School Lebanese Children (6-10 years)*

c. *Phosphorus*

Compared to the flow of the upper cutoff of Ca, the 95th percentile of P/Crea decreased in the first two age groups then slightly increased to 1.507 at age 9. Consecutively, the ratio remarkably decreased to 1.218 at the age of 10. Overall, the 95th percentile was 1.480. The 50th percentile decreased with age from 0.67 to 0.48 and had an average of 0.60. The same trend was followed by the 5th percentile of P/Crea. It smoothly decreased to 0.17 and had a mean of 0.60. All the details about P/Crea reference values are found in Table 30 and Figure 8.

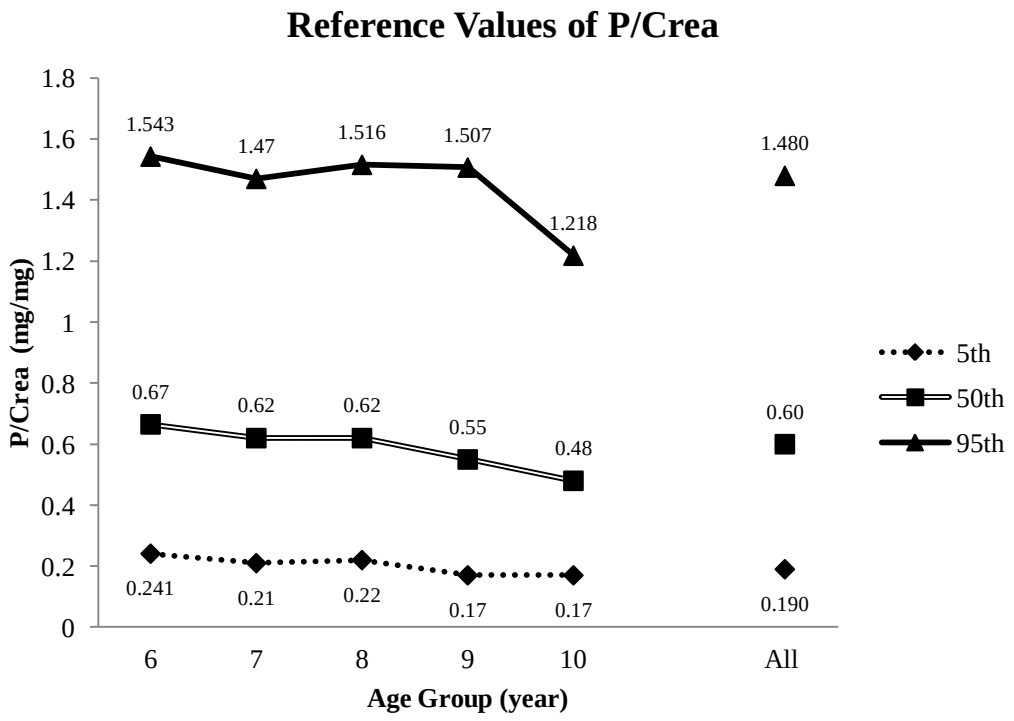


Figure 8. *Reference Values of P Excretions in Ratio to Crea for Elementary School Lebanese Children (6-10 years)*

8. Calcium to Phosphorus

Ca to P ratio was studied in our population and was reported in 2 different units mg/mg and mmol/mmol. A p-value of 0.366 indicated a lack of sex related difference (Table 31). In contrast to minerals to Crea ratios, Ca to P ratio showed no significant difference when studied by age groups (Table 32).

Table 31. *Sex Related Difference of Ca to P Ratio of Elementary School Lebanese Children (6-10 years)*

Ca/P	Total (1392)	Boys (776)	Girls (616)	p-value
mg/mg	0.146±0.190	0.150±0.197	0.141±0.181	0.366
mmol/mmol	0.113±0.147	0.116±0.153	0.109±0.140	

All values are reported as mean±SD.
The number presented between brackets defines the sample size.
t-test is used comparing genders and significance is set at p-value <0.05 for the difference between genders.

Table 32. *Age Related Difference of Ca to P Ratio of Elementary School Lebanese Children (6-10 years)*

Age (yrs)	Samples (n)	Ca/P	
		mg/mg	mmol/mmol
6-7	325	0.141±0.185	0.109±0.143
7-8	347	0.133±0.175	0.103±0.135
8-9	302	0.147±0.188	0.114±0.146
9-10	211	0.159±0.209	0.123±0.162
10-11	207	0.161±0.207	0.125±0.160
p-value		0.382	

All values are reported as mean±SD.
One way ANOVA is used to detect P excretions differences between age groups.

Figure 9 illustrates our population's distribution of Ca/P in mg/mg as well as in mmol/mmol. The regression of Ca/P with weight and height was not significant. The following are the regression equations (ratio was expressed in mg, the weight in kg, and the height in cm):

Ca/P = 0.136 + 0.000341 weight; r= 0.014; p= 0.599

Ca/P = 0.0963 + 0.000390 height; r= 0.020; p= 0.457

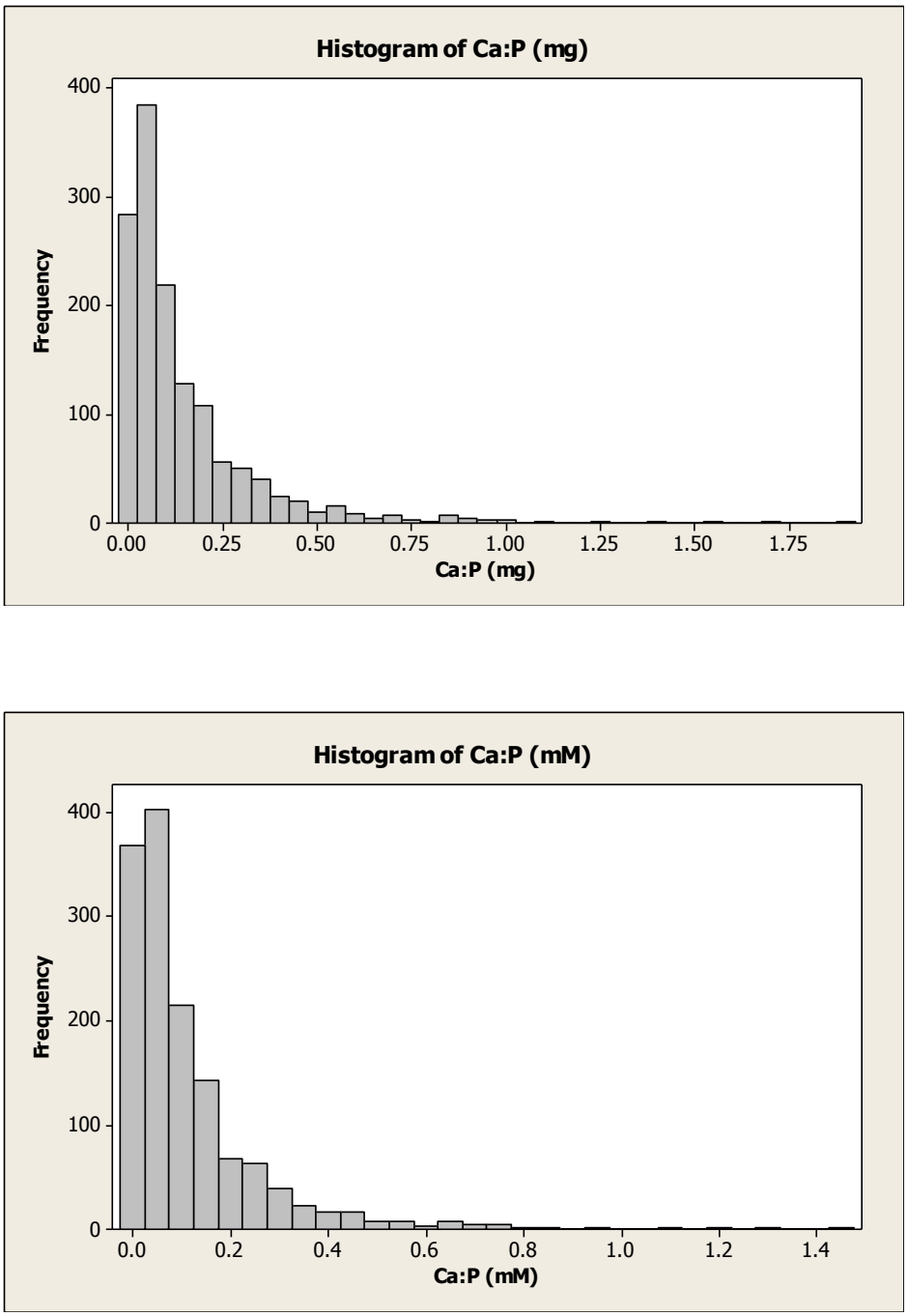


Figure 9. *Population Distribution of Ca/P Excretions of Elementary School Lebanese Children (6-10 years old)*

CHAPTER V

DISCUSSION

The nutritional status of the Lebanese children has not been fully studied. Knowledge in pediatric nutrition seems to be restricted to anthropometric measurements and the focus on body fluid (plasma and urine) biomarkers is still limited. The anthropometric status of the Lebanese children has been studied throughout the years. Apparently, with the trend of food westernization and the changes in lifestyles (working mothers, facilitated transportation, video games, etc.), the percentages of overweight and obese children are progressively increasing. A cross sectional study by Sibai *et al.* (2003) found, that the percentage of overweight and obese Lebanese 3-19 year old children was 23.4%, noting that children younger than 10 had lower rates than adolescents.

Nasreddine and colleagues found a noticeable increment (26.0 % vs. 30.9 %) in overweight in 6-9 year old children when comparing between the prevalence in 1997 and 2009,. Our study showed exactly same percentage of overweight and obese males (31.0 %) and lower percentage of overweight and obese females (26.5 %). As shown in Graph 1, the percentage of overweight and obese children in Lebanese elementary schools was 29 %. Our results were in line with other previous results. This fact showed that the baseline characteristics of our population were valid.

Our estimated daily Crea excretions of the age group of 6 to 10 years old varied from 367±50 to 637±112 with a mean of 16. 99±2.145 mg/kg/d. Our results were similar to others authors' findings (Table 33). Ghazali & Barratt (1974) stated that, in

mid-childhood, the average Crea excretion is 18 mg/kg/d, which is close to our mean. This comparison shows another indicator of a valid sample population.

Table 33. *Total Creatinine Excretions in Different Studies*

Country (Study)	**Sample Size**	**Age**	**Mean±SD**
			mg/d
Ohio, USA (31)	74	9	639±120
Boston, USA (32)	35	1-12	500±200
England (68)	100	8-10	688/593
Germany (84)	160	6	396 (3.50 mmol/d)
	147	7	452 (4.00 mmol/d)
	141	8	517 (4.57 mmol/d)
	144	9	517 (4.57 mmol/d)
	118	10	661 (5.84 mmol/d)
Lebanon (our study)	**All**	**All**	**474±113**
	330	6-7	367±50
	348	7-8	438±61
	303	8-9	479±69
	214	9-10	532±80
	208	10-11	637±112

The mean Ca/Crea in our population (0.087±0.101 mg/mg) was fairly close to the American Caucasians and Turkish children with an average of 0.08 and 0.092±0.123 mg/mg; respectively (So *et al.*, 2001; Sonmez *et al.*, 2007). Children from Germany (Remer *et al*., 2002), Korea (Choi *et al.,* 2013), and Southern Thailand (Vachvanichsanong *et al*., 2000) had higher means. African-Americans (0.04 mg/mg) (So *et al.*, 2001) and Iranian children (0.039±0.044) (Safarinejad, 2003) had ratios around half our values. In line with others' findings, we found no significant difference between both genders (p= 0.161) (Choi *et al*., 2013; Safarinejad, 2003; Sonmez *et al.*, 2007; Sorkhi & Aahmadi, 2005; Vachvanichsanong *et al.*, 2000). Significance inverse correlation of Ca/Crea with age is contradicted. While our study showed a trend of decrease with age, several other studies described significant higher values for younger

ages, (Esbjörner & Jones, 1995; Matos *et al.*, 1997; Safarinejad, 2003).

The estimation of total Ca excretions was 23.9 % lower than the Taiwanese values (1.86 ± 1.16 mg/kg/d) (Chen *et al.*, 1994) and 40.4 % lower than Argentineans', Southern Italians', and Londoners' values (2.377±1.492, 2.3±1.7 and 2.36±0.66 mg/kg/d; respectively reported by Alconcher *et al.* (1997), De Santo *et al.* (1992), and Ghazali & Barrat (1974)). Sex difference, when adjusted by weight, wasn't significant between boys and girls. This result was supported by Slev *et al.* (2010) but not by Alconcher *et al.* (1997). Children hypercalciuria was defined as urinary Ca excretion > 4 mg/kg/d (Alconcher *et al.*, 1997; Choi *et al.*, 2013; Ghazali & Barratt, 1974; Sonmez *et al.*, 2007). When applied on our population, this criterion highlitghed102 hypercalciuric children (7.3 %), noting that the classification was made using predicted 24-h urinary.

The 5^{th} percentile, defined as the lower limit, was not a priority of interest in research since it reflects low intake rather than the existence of metabolic problem. The 5^{th} percentile, when reported, seemed to barely vary with age (Matos *et al.*, 1996). Our results showed fixed values of 0.000 for Ca/Crea in all ages (taking into consideration the detection limit of the method used). In Spain (Saes-Torres *et al.*, 2014) and Switzerland (Matos *et al.*, 1996), the 5^{th} percentile of Ca/Crea was almost constant with a value of 0.01. Lower 5^{th} percentile for Ca/Crea (0.006) was described in Turkey (Sonmez *et al.*, 2007).

The upper limit (95^{th} percentiles) of our sample population for Ca/Crea was 0.270. Figure 10 illustrates country related differences of Ca/Crea 95^{th} percentile.

Most of the studies reported very close reference values for Ca/Crea that varied from 0.25 to 0.29. A meta-analysis by Metz (2006) to determine Ca/Crea reference values in pediatrics combined 14 studies and 4386 urine samples from children younger than 19

years old; the results were compiled to give an upper limit for Ca ratio of 0.70 mmol/mmol or 0.248 mg/mg for 5-10 year old children which is quite close to the reference value we found.

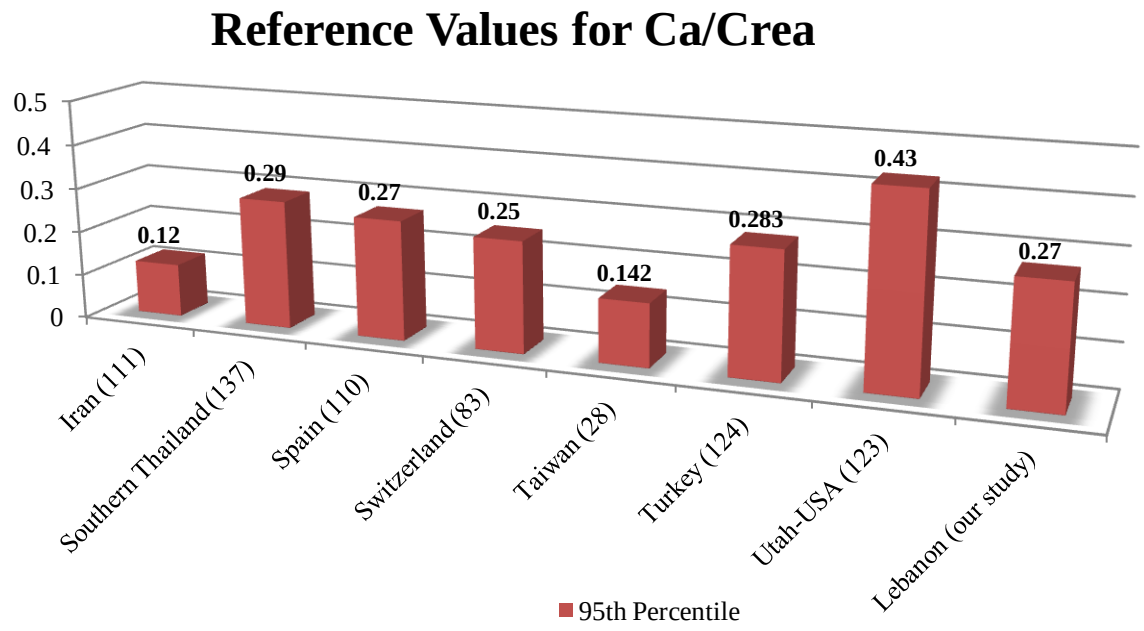


Figure 10. *Ca/Crea 95th Percentile of Different Countries*

When comparing our results to previous ones performed in other countries, we found that the mean Mg/Crea ratio of the Lebanese 6-10 year old elementary school children (0.122±0.07 mg/mg) was higher than in Taiwanese (7-10 years old) children (Chen *et al.*, 1994) with a mean of 0.027±0.015 mg/mg and Iranian (7-10 years old) children with a mean of 0.035±0.015 mg/mg (Safarinejad, 2003), but lower than the results presented by Ghazali &Barratt (0.21±0.1 mg/mg for 1-15 year old children living in London) and Mircetic and colleagues (0.75±0.05 mmol/mmol (0.161±0.011 mg/mg) for 3-14 year old students living in Rutherford NJ, USA). In contrast to our

results, other studies reported higher Mg/Crea in girls than boys (Chen *et al.*, 1994 and Safarinejad, 2003). Mg/Crea decreased with age yet no significant difference was detected between age groups. This fact was supported by Chen *et al.* (1994) and Safarinejad (2003) who found a remarkable decrease in the ratio in children older than 10.

The estimations of the 24-h Mg excretions (2.07±1.3 mg/kg) were similar to the results reached in Italy (Cimitile) 2.06±0.78 mg/kg (De Santo *et al.*, 1992) and a bit lower than the results found in London (Ghazali &Barratt, 1974) 2.82±0.79 mg/kg. Lower total Mg excretions were reported in France (Touitou *et al.,* 2010) and Taiwan (Chen *et al.,* 1994) with a mean of 0.205±0.003 (mean±SE) and 0.464±0.243 mg/kg/d; respectively. Predicted 24-h Mg excretions differed with age but lost its significance when expressed by weight. This fact was contradicted by De Santo *et al.* who found that total Mg excretions were lower in children older than 9 years old.

We reported stable values of 0.030 mg/mg for Mg/Crea in all age groups. Spanish and Lebanese children shared the same lower limit values (Saes-Torres *et al.,* 2014). The 5^{th} percentiles of Swiss children were 0.06 and 0.05mg/mg for 5 to 7 and 7 to 10 years old; respectively (Matos *et al,* 1996). No major differences were found when comparing our lower reference values for Mg/Crea with other previous results.

In line with our results, Saes-Torres *et al.* (2014) reported close 95^{th} percentiles for Mg/Crea in Spain (0.28). Figure 11 illustrates the geographical differences for pediatric Mg/Crea.

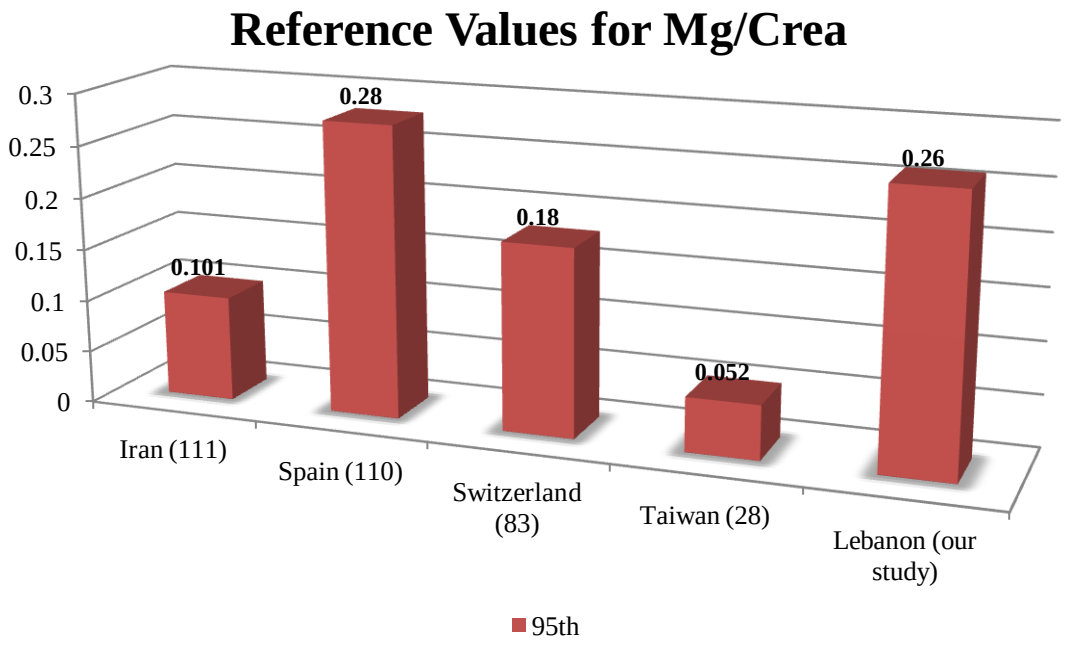


Figure 11. *Mg/Crea 95th Percentile of Different Countries*

P excretion has not been previously studied as much as Ca and Mg. Our mean P/Crea (0.692±0.417) was noticeably higher than the ratios reported in Iran (Safarinejad, 2003) and Taiwan (Chen *et al*, 1994) in 7-10 year old children and close to the ratio described in Florida by Malone *et al.* (0.72±0.08). Our results did not show sex related differences for P/Crea and predicted 24-h P excretions. This was supported by other authors (Chen *et al.*, 1994; Safarinejad, 2003; Matos *et al.*, 1996).

Our total P excretions (11.767 ±7.305 mg/kg/d) were lower than the results found by Chen *et al*. (15.87±4.03) and De Santo *et al.* (22.7±11.3) in Taiwan and Southern Italy. P excretion significantly deceased with age in ratio to Crea (Chen *et al.*, 1994; Safarinejad, 2003; Selv *et al.*, 2010; Matos *et al.*, 1996) and as total urinary excretions even when adjusted by weight. Malone *et al.* found no significant decrease of total P

excretion (mg/kg/d) as age increased, which contradicts our findings that are in line with Chen *et al.* and Matos *et al*.

The 5th percentile for P/Crea presented minor fluctuations (decrease) with age. A similar trend of decrease was shown in Switzerland (Matos *et al.*, 1996) among 5 to10 year olds (0.33 for 5-7 years and 0.32 for 7-10 years). Around double our results (0.44) were shown for P/Crea in Spain (Saes-Torres *et al.*, 2014).

The upper limits for urinary P/Crea were far from being clear since our results are quite close to the Spanish ones but so different from all others countries (Figure 12).

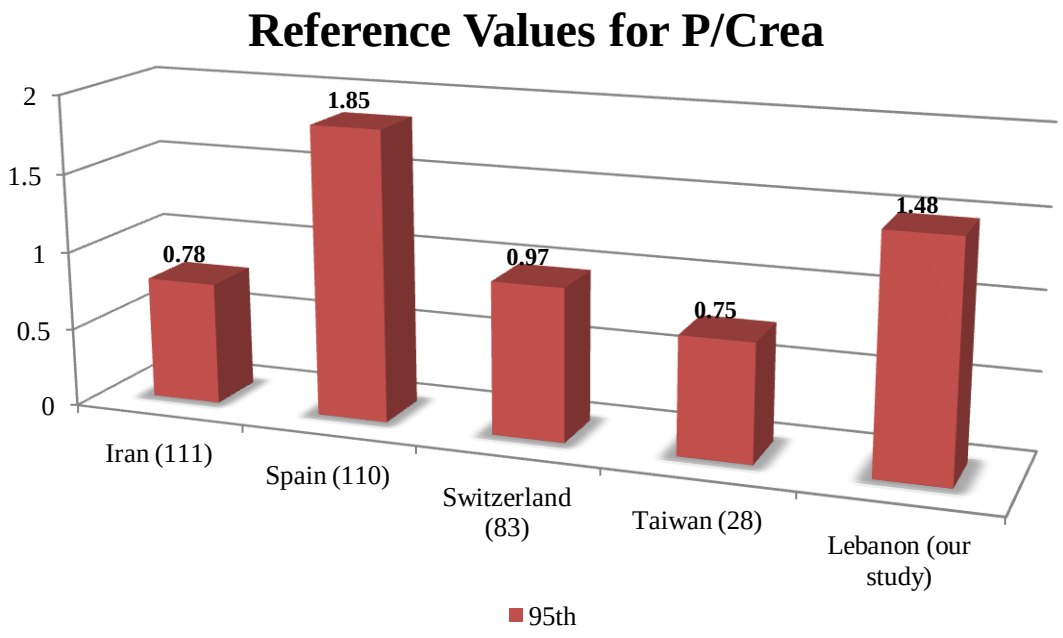


Figure 12. *P/Crea 95th Percentile of Different Countries*

The wide variation in the mean values implicates wide ranges for reference values and emphasizes on the importance of having country related cutoffs.

The differences in urinary Ca, Mg, and P excretions depend on race, geography, and age. However, several factors including mineral bioavailability, water content, sun exposure and vitamin D intake, fasting or non-fasting urine analysis, etc. are also responsible for the variation of means and accordingly their reference values.

Chen *et al.* (in Taiwan) were the only ones who analyzed fasting urine samples, consequently they reported lowest values for all minerals.

Food intake as well as mineral absorption and bioavailability take part in the explanation of urinary mineral excretion. According to the FAO stat (2011), the USA had particularly higher milk and dairy product consumption (373 kcal/capita/d) when compared to Lebanon (146 kcal/capita/d) or other countries (Spain and Iran). This fact justifies the high -even highest- urinary excretion reported by Mircetic *et al.* and Malone *et al.* in New Jersey and Florida.

One major component affecting Ca, Mg, and P absorption is the existence of phytate or phytic acid in the diet. No studies assessed the intake of phytate or phytic acid in the Lebanese diets, however, findings allow the estimations of their availability in the diet. The major cereals consumed in Lebanon are rice (from stews) and wheat (from bread, having a high bread consuming community). It is well known that the phytate content of bread depends on the preparation techniques. During fermentation, bacterial and yeast phytase hydrolyze phytic acid, reducing phytate bread content (Reddy & Sathe, 2001). Buddrick *et al.* (2013) showed a reduction of around 60 % of phytate in wheat bread when left to rise at 30 °C for 5 h (the optimal time for which we observe the best reduction in phytate as described by Lopez *et al.*, 2001). Furthermore, it is true that traditionally, whole grain cereals were part of our patterns, however, nowadays grains were mostly refined, and breads are prepared from white flour.

Our pita bread, similar to the one consumed in Kuwait, contains between 0.12 and 0.16 % of phytate when prepared with white flour (Reddy & Sathe, 2001). When compared with other types of breads, our pita bread contains lower phytate than Iranian breads for instance which hold up to 2.41 % of phyate. This fact explains the lower means and reference values of all mineral excretions in Iranians. It is worth noting that, according to the FAO stat (2011), wheat and wheat products contribute to more than third the energy intake of Iranians (1189 kcal/capita/d vs. 3058 kcal/capita/d).

It is important to highlight the geographical location of Lebanon, Turkey, and Spain on the Mediterranean Sea, all having a Mediterranean climate (similar seasonal fruits and vegetables, types of dietary patterns, etc.). It is not surprising to find that these countries share the same dietary characteristics thus, had very close means and reference values. The differences between our values and others reached in different countries can be explained not only by geographical location but also by difference in methodology such as age categorizations, sample sizes, analysis techniques, etc.

As the children get older (especially older than 9 years old), mineral/Crea ratios have decreased. This trend seemed to continue in teenagers as reported by Chen *et al.* (1994), Matos *et al.* (1997), and Safarinejad (2003). This fact could be related to a higher rate of mineral retention and bone deposition in puberty and adolescence since substantial amount of Mg and other bone minerals like Ca and P are known to be sharply retained by bones after the age of 12 (Joint FAO & WHO, 2005; Saes-Torres *et al*, 2014).

Ca/Crea and Mg/Crea were not different between school types. However, when the predictive values were compared, the whole image becomes clearer. Higher values

for all mineral ratios were found in children enrolled in private schools, which can be explained by a better economical status. This finding can be interpreted in two different ways. In fact, it is likely that a stronger coverage of nutritional education is provided in private schools. The keys points that any nutrition instructor would have stressed on in this age group, when explaining the food pyramid or Myplate for kids, is to assure having a daily cup of milk and to increase sources of Ca (like cheese and labneh), to have 4 to 5 servings of fruits and vegetables, etc. Additionally, a higher socio-economical status is usually concomitant with a better educational level of the parents, thus a better parental knowledge in health and nutrition. On the other hand, higher mineral excretions can be simply linked to higher food availability and consumption. A study by Nasreddine *et al.* (2014) showed that the socio-economical status, in Lebanese children, is associated with increased weight and adiposity due to increased food consumption. The previous theory can be easily adopted when we refer to the prevalence of overweight and obesity in private schools which was 1.86 folds compared to the public schools (37.18 % vs. 20.03%). Similar percentage was found in private free schools, reflecting a better socio-economical status than in public schools.

Milk and dairy products are the major sources of Ca and P together. Thus, when Ca/Crea parallels P/Crea, this implies that the population is consuming the same source; dairy products. This explanation can be concluded when we compare student's mineral excretion in schools and districts.

Public school students had lower values of Ca and P than private school students. The difference between private and private free schools regarding total P excretions can be related to P content in high P foods other than milk products, such as meats and processed foods which are usually more affordable to people with higher economical

status. Mg/Crea ratio didn't differ between schools, however, when it came to the total value, Mg excretions were higher in private schools compared to private free and public. This fact can be explained, on the one hand, by the stress on the importance of fruits and vegetables given in the nutrition sessions in private schools, and, on the other hand, the economical availability to have this variety on a daily basis.

When it comes to the difference in districts, the evaluation and interpretation of the results becomes harder, since Lebanon, due to its small area, has the characteristics of a city and not those of a country. Thus the dietary patterns were not meant to be categorically different. Nevertheless, some geographical characteristics can clarify different points. It wasn't surprising to find that Bekaa had the highest scores in both Ca and P, since it is the area where most -if not all- of the national dairy products are produced, which implies that the citizens consume the highest amount of milk products. Mg excretions were seen as expected to be higher in districts that include mountains and valleys (Bekaa, Hermel, Mount Lebanon) rather than sea (Beirut, North) since people living in such regions normally consume more seasonal fruits and vegetables.

As seen in WHO report, bone mineral content increases progressively with age. The increment in mineral concentration seems to be constant from the age of 4 till 12 in both males and females (Figure 13). Similar results were found by Theintz *et al.* (1992) when they assessed bone mineral density and bone mineral content in girls; boys having higher mean age. This fact implies that the accretion in this age range is fixed for Ca as well as for the other bone minerals, noting that the main minerals found in bones are respectively Ca, P, and Mg. Several factors, combined together, along with urinary mineral excretion, provide an idea (estimation) about the population's minerals intake.

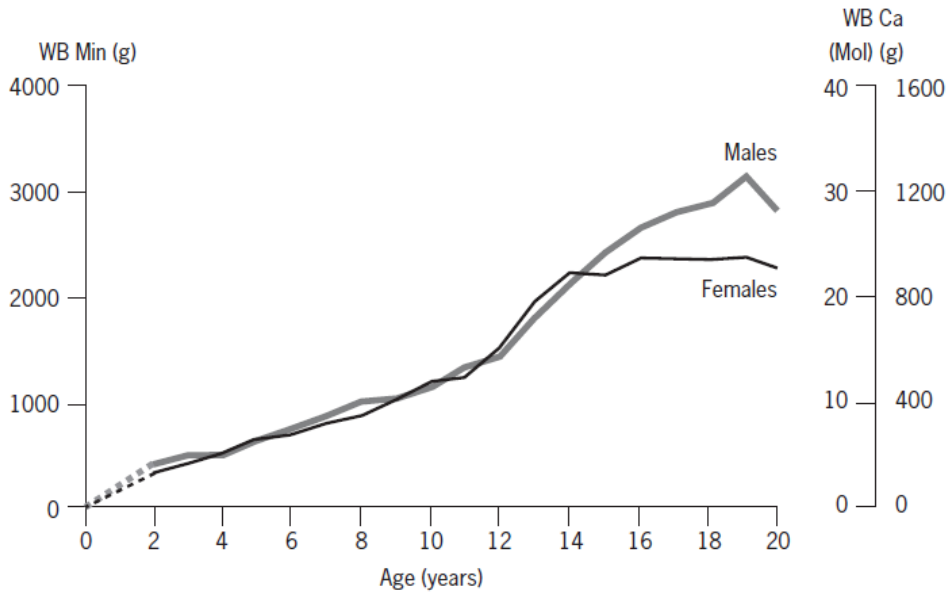


Figure 13. *Whole-Body Mineral and Whole-Body Ca as a Function of Age as Determined by Total-Body Dual-Energy X-Ray Absorptiometry*
Retreived from "Joint, F.A.O., & World Health Organization. (2005). Vitamin and mineral requirements in human nutrition, p. 60.

The postulated explanation is illustrated in Figure 14 below. The amount of urinary Ca excreted reflects part of the total Ca absorbed. To have the whole picture, Ca bone deposition and some insensible losses (mainly dermal losses) should be taken into account. Hence, the average daily net Ca absorbed is the sum of 1) the average daily Ca bone deposition in children which is around 120 mg/d 2) the 24-h urinary Ca excretion (which varies a lot between individuals), and 3) the insensible losses supposed to be around 40 mg/d (Joint FAO & WHO, 2005). In our population, the average daily net Ca absorption was found to be 198.95 mg estimating an average intake of 663 mg/d when the rate of absorption is around 30 %. The estimated Ca intake of our population was close to the RNI for 6-9 year olds (600 mg/d for 6 years and 700 mg/d for 7-9 years) but strictly below the recommended nutrient intake (RNI) for the age of 10 (1300 mg/d).

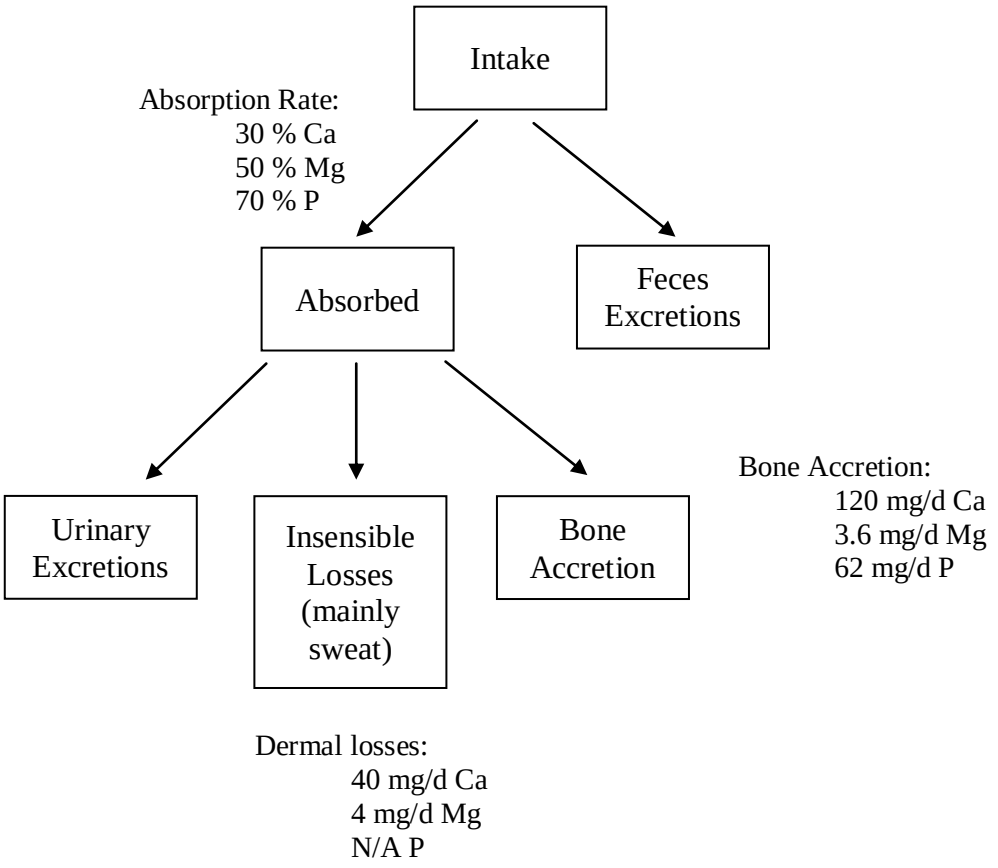


Figure 14. *Postulated Explanation of the Relation between Intake and Urinary Excretion*

When compared to the EAR (RDA-2SD), our estimated Ca intakes were way below EAR in all ages (800 mg/d for 6 to 8 years and 1040 mg/d for 9 and 10 years). According to FAO/WHO (2005), the RNI of Mg for 4-9 year old children is 4.0 mg/kg body weight, assuming a rate of 50 % for intestinal absorption. Our data showed a mean predicted 24-h Mg urinary excretion of 2.07±1.3 mg/kg of body weight. We can conclude that the mean estimated Mg intake (4.14 mg/d) met the RNI even without accounting for Mg accretion (3.6 mg/d) and dermal losses (the latter are usually about a tenth of the value for calcium (Seelig, 1964) supposed to be about 4 mg/d). Thus, we can conclude that our population seemed to have an adequate Mg intake.

Same explanation for Ca and Mg is applied on P noting that P content in sweat has not been studied in normal circumstances in children. The bone P deposition seems to be

equal to 62 mg/d (Institute of Medicine, 1997) in children between 4 and 8. The estimated P intake, with an absorption rate of 70 %, would then be around 548 mg/d, exceeding the EAR for 4-8 year olds (405 mg/d) but remaining below those for 9 and 10 year olds (1055 mg/d).

The intake of Ca and P has been established using a ratio Ca/P to predict risk of bone fracture and prevent osteoporosis. The same ratio in urine has not been standardized since several uncontrollable modifiers such as bone accretion and retention due to low intake take place. In fact, when having proper Ca and P intakes and when accounting for minerals absorption rates, bone accretion, and insensible losses, a ratio of 0.146 mg/mg or 0.113 mmol/mmol is far to be predicted. A postulated explanation of this low ratio can be vitamin D mediated. Despite the sunny weather that characterizes the MENA region, hypovitaminosis D remains a common health problem in this area (El Rassi, Baliki, & El-Hajj Fuleihan, 2012). This epidemic does not spare the pediatric population. As described in Salamoun *et al.* (2005), only 16 % of 10-16 year old Lebanese children met the AI of vitamin D (200 IU/d). A low vitamin D inhibits Ca absorption, triggers parathyroid hormone (PTH) secretion (DeLuca, 2004) which raises fibroblast growth factor 23 (FGF-23) (Isakova *et al.*, 2011) and stimulates P excretions (Penido & Alon, 2012). Therefore, a low vitamin D status can be behind a reduced Ca and increased P excretion, reducing Ca/P ratio.

Although additional effort should be done, our project added important value to the pediatric field. Nevertheless, we acknowledge facing several limitations. One of which is the detection limit of the method used.

Second, the selective response rate had a great negative impact. Even though the total acceptance of participation of schools was fairly good (72.2 %), the proportions between private and public schools misbalanced the equilibrium. With the coordination of the MEHE, almost all public schools, whereas only 10 out of 19 contacted private schools participated. Future analysis giving greater weight to private schools and some districts (like Mount Lebanon) would be considered.

Finally, we cannot exclude having a vitamin D related gap. Vitamin D is known to be tightly incorporated in the 3 minerals, specifically Ca and P metabolisms, affecting their retentions and excretions. Deficiency and inadequacy of vitamin D which is widespread in our region, might have lowered Ca/Crea values and raised P/Crea ones.

CHAPTER VI

SUMMARY AND CONCLUSION

This study provides a feasible and effective method of screening for Ca, Mg, and P metabolic abnormalities in pediatric population. It supplies age related normal reference values in healthy Lebanese 6 to 10 year old elementary school children for random urinary Ca/Crea, Mg/Crea, and P/Crea. The status of children in Lebanon was better than in various other countries. Lebanon had the closest mineral excretion means and reference values with respect to Turkey and Spain that share similar geographical and dietary characteristics. Differences between school types highlighted the variation of patterns with socio-economic status. Apparently, Mg intake seemed to be adequate compared to the recommendation. Regarding Ca and P, inadequacy of intake appeared in 9 and 10 year old children. A low Ca/P highlighted a possible vitamin D deficiency.

Our findings would be more convincing if future studies, taking into account 24-h urinary collection, validate our results. Future recommendations suggest excluding stunting and overweight children in setting reference values for the healthy pediatric population. After having set our country-related cutoffs, research screening for metabolic abnormalities and mineral deficiencies in our pediatric elementary school age children would be useful.

APPENDIX I

PHOSPHORUS GRAS FOOD ADDITIVES SUBSTSANCE

Phosphorus containing GRAS substances
Acetylated Distarch Phosphate
Ammonium phosphate dibasic
Ammonium phosphate monobasic
Calcium glycerophosphate (packaging)
Calcium hexametaphosphate
Calcium hypophosphate
Calcium phosphate dibasic
Calcium phosphate monobasic
Calcium phosphate tribasic
Calcium phytate
Calcium pyrophosphate
Dibasic magnesium phosphate
Ferric phosphate
Ferric pyrophosphate
Ferric sodium pyrophosphate
Hydropropyl distarch phosphate
Manganese glycerophosphate
Manganese glycerophsophate (packaging)
Manganous hypophosphite
Monostarch phosphate
Phosphoric acid
Potassium glycerophosphate
Potassium hypophosphite
Potassium phosphate dibasic
Potassium phosphate monobasic
Potassium phosphate tribasic
Potassium polymetaphosphate
Potassium pyrophosphate
Potassium tripolyphosphate
Riboflavin-5'-phosphate
Sodium acid pyrophosphate
Sodium aluminum phosphate, acidic
Sodium aluminum phosphate, basic
Sodium ferricitropyrophosphate
Sodium hexametaphosphate
Sodium hypophosphite
Sodium metaphosphate
Sodium phosphate dibasic
Sodium phosphate monobasic
Sodium phosphate tribasic
Sodium phosphoaluminate (packaging)
Sodium pyrophosphate

Sodium tetrametaphosphate
Sodium tetraphosphate
Sodium trimetaphosphate
Sodium tripolyphosphate
Tribasic magnesium phosphate

Adapted from the Food and Drug Administration, http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/SCOGS/ucm084104.htm, last updated 21/06/2013, retrieved on 7/7/2014.

APPENDIX II

ARABIC CONSENT FORM

عنوان البحث: تقييم حالة اليود في البول عند الأطفال في لبنان
إسم الباحث: د. عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.
الباحثين المساعدين: د. هلا غطاس/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.
منسقي البحث: دارين شاتيلا
العنوان: الجامعة الأميركية في بيروت، شارع الحمرا، بيروت – لبنان 01-350000
مكان إجراء البحث: المدارس الخاصة و الرسمية من كل لبنان.

هذا بيان موافقة للسماح لإبنك أو إبنتك بما أنك الوصي الشرعي المشاركة ببحث علمي سيجرى من قبل الجامعة الأميريكية في بيروت. الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) السماح لإبنك أو إبنتك المشاركة أم لا. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء مذكور في هذه الإستمارة أو عن هذه الدراسة ككل من طبيبك. مشاركة إبنك/إبنتك غير إلزامية. أذا تمت الموافقة على المشاركة، سوف يطلب من حضرتك كوصي شرعي على توقيع هذا البيان و سوف تحصل على نسخة لك من هذا البيان.

أ) هدف هذا البحث: اليود هو معدن أساسي و له دور مهم اذ يساعد في النمو الذهني عند الأطفال. ان نقص اليود هو واقع صحي يهدد الأطفال في العالم و لا توجد في لبنان معلومات عن نسبة نقص هذا المعدن و غيرها من المعادن الاساسية. مع أن لبنان اتبع برنامجا لتدعيم الملح باليود سنة ١٩٩٥ من قبل وزارة الصحة. ان هدف هذه الدراسة هو قياس نسبة اليود عند الاطفال الذين تتراوح أعمارهم بين ٥-١٠ سنوات و ذلك لضمان نجاح برنامج التدعيم و بالتالي التأكد من أن كمية اليود المستعملة في التدعيم هي كافية .إن هدف البحث هو تحديد المناطق التي تعاني من نقص في اليود في لبنان.إن هدف البحث أطروحة وستنشر في صحيفة طبية ومن الممكن تقديمها في المؤتمرات الأكاديمية.

ب) وصف الإجراءات والمشروع: ستتم عملية اختيار المشاركين قي الدراسة عن طريق الاتصال المباشر بمدير المدرسة التي تم اختيارها. سوف يعطى الأطفال الذين تم اختيارهم من كل مدرسة

بيان طلب موافقة للمشاركة في هذا البحث و استمارة لاعطاؤها للأهل أ, الوصي الشرعي. بعد الموافقة من قبل الأهل او الوصي الشرعي سوف يطلب منهم التوقيع على البيان و ملئ الاستمارة و ارجاعها مع الطفل الى المدرسة. و من ثم سوف يتم اختيار هؤلاء الأطفال لجمع عينات من البول المطلوب لهذا البحث. سوف يتم جمع عينات البول لقياس نسبة اليود، البوتاسيوم، الصوديوم، والفلورايد و الفسفور و الكالسيوم و المغنيزيوم و الكرياتنين في البول. و من خلال الاستمارات التي تم ملؤها من قبل الأهل أ, الوصي الشرعي سوف يتم قياس نسبة استعمال الملح المعالج باليود. هذه الاستمارات تتضمن ايضا معلومات عن الوضع الاجتماعي و الاقتصادي لعائلة الطفل.

سوف يطلب من الاشخاص الذين تم اختيارهم للمشاركة في هذا البحث متابعة تناولهم للطعام وممارسة نشاط بدني بشكل طبيعي خلال مدة الدراسة وتفادى النشاطات الكثيفة قبل ٢٤ ساعة من بدء الدراسة. سوف يطلب من الأشخاص الذين يعانون من أمراض مزمنة معينة عدم المشاركة في هذا البحث. كما سيتم استبعاد الأطفال الذين لا تتراوح أعمارهم بين ال٥ و ال١٠ سنوات.

سيتوجه الباحثون الى المدارس حيث سوف يتم قياس الوزن و الطول لكل طفل، و الحصول على الاستمارة التي تم ملؤها من قبل الاهل أو الوصي الشرعي، و الحصول على عينات من البول في عبوات مخصصة. هذه دراسة عشوائية و عدد الاطفال المطلوب مشاركتهم في هذا البحث هو ١٥٠٠ تلميذ تتراوح أعمارهم ما بين ال٥ و ال١٠ سنوات من أجل اكمال هذه الدراسة.

ج) المدة:**إن الوقت المقدر لانهاء البحث هو تقريبا سنة. مطلوب زيارة موقع الدراسة مرتين (مرة للحصول على موافقة المشاركة و مرة لاجراء البحث .مدة كلّ زيارة ٣٠ دقيقة تقريباً.**

يمكن لطفلك الانسحاب من البحث في أي وقت. إن أردت من طفلك التوقف عن المشاركة، ما من عقوبة تفرض عليك أو على طفلك ولن تخسر أي من الفوائد التي تملكها وقرارك لن يؤثر على أي علاقة مستقبلية لك أو لطفلك مع الجامعة الأمريكية في بيروت.

د) المخاطر والمضايقات والفوائد: **مشاركة طفلك في هذه الدراسةلا تتضمن أية مخاطر جسدية أو نفسية أكثر من مخاطر الحياة اليومية. لن تتقاضى أنت أو طفلك أي أجر لهذه الدراسة، و لكن اهمية هذه الدراسة تكمن عندما يتحدد نسبة نقص اليود عند الاطفال، سوف يحد من مشكلة**

طمور النموعند الاطفال. كما أن النتائج التي سوف يتم الحصول عليها ستمكننا من معرفة و ايجاد طرق لتغيير برامج تدعيم الملح باليود التي تستعمل منذ عام ١٩٩٥. سيتم الاخبار عن نتائج هذا البحث في نهاية الدراسة.

هـ) السرية: **سوف تبذل الجهود لتأمين سرية المعلومات التي تتعلق بإبنك، إسمك والمعرفات الأخرى لن تكون معلقة مع أجوبتك لضمان السرية. جميع المعلومات والمدونات ستحفظ في غرفة مغلقة أو حاسوب لديه رمز سري. الوصول إلى المعلومات مسموحٌ فقط للباحث الأساسي والباحثين الذين يعملون مباشرةً على الدراسة. جميع المعلومات ستدمر بشكل مسؤول من بعد الوقت المطلوب .سيحافظ على سريتك في جميع المعلومات المكتوبة والمنشورة عن نتائج هذا البحث. لن يستعمل إسمك أو أي معلومة متعلقة بهويتك في تقاريرنا أو مقالاتنا المنشورة.**
من الممكن أن توجد ظروف حيث يجب نشر معلوماتك السرية. مثلاً يمكن للمعلومات الشخصية المتعلقة باشتراكك أن تعطى لمجلس المراجعة المؤسسية في الجامعة الأمريكية في بيروت إن طُلبت و للجان الأخلاق المهنية المستقلة، ومفتشين من اللإدارات الحكومية المنظمة، مكتب حماية البحث الإنساني للولايات المتحدة أو أي وكالة تنظيمية فدرالية أو دولية أخرى، أو راعي البحث، إن وجد أو أي وكالة تسند البحث. بعد انتهاء البحث سوف يتم حفظ المعلومات من قبل الباحث المسؤول في مكان امن و سري لمدة ٣ سنوات بعدها تتلف بمسؤولية.

و) التعويض / الحافزة: **ليس هناك أية تكاليف مطلوبة منك أن تدفعها و لن تتقاضى أي أجر لهذه الدراسة، ولن تتقاضى أجر التنقل أو كلفة موقف السيارة الخ.**

ز) الدفع للإصابات ذات صلة بالبحث: **ما من تغطية لحصول الحوادث الغير متوقعة، في حال لم تكن هناك تغطية من قبل تأمين صحي أ, ضمان اجتماعي. إن تعرضت إلى إصابة جراء البحث، أو لأي سؤال عن الإصابات المتعلقة بالبحث، يرجى الاتصال بالدكتور عمر عبيد (01) 350000 مقسم 4440، email: oo01@aub.edu.lb**

ح) أسئلة ومعلومات الاتصال

١) لأي أسئلة أو أي مخاوف حول البحث، يمكنك الاتصال بالدكتور عمر عبيد، قسم التغذية وعلم الطعام الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان 350000 (01) مقسم 4440، email: oo01@aub.edu.lb.

٢) لأي أسئلة أو أي مخاوف حول حقك كمشارك في هذا البحث يمكنك الاتصال بالمكتب التالي في الجامعة الأمريكية في بيروت:مجلس المراجعة المؤسسية

أو 5445 مقسم 350000 (01) الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان email: irb@aub.edu.lb، 5440

ح) حقوق المشاركين:

مشاركة إبنك أ, إبنتك في هذا البحث طوعية. يمكن لابنك أو ابنتك مغادرة البحث في أي وقت من دون أي عقوبة. إن قرارك بعدم المشاركة لن يؤثر بأي شكل ممكن على علاقتك بالجامعة الأمريكية في بيروت.

إذا اخترت السماح لطفلك الاشتراك في هذه الدراسة، يمكنك وقف اشتراكه فى اى وقت بدون عقوبة او فقدان الاستحقاقات. بتوقيع هذا البيان, لن تتخلى عن اى حقوق قانونية أو شخصية اذا قمت أنت أو طفلك بالمشاركة فى هذه الدراسة.

مجلس المراجعة المؤسسية في الجامعة الأمريكية في بيروت المسؤولة عن الابحاث التي تتعلق بالبشر قد قامت باستعراض هذا المشروع البحثى,و وجدت أنه مقبول حسب القضاء اللبناني و اللوائح الفيدرالية في الولايات المتحدة الأمريكية . إن الجامعة الامريكية لها سياسات تهدف الى حماية حقوق ورفاهية المشاركين فى البحث.

هل لديك أي أسئلة حول المعلومات الواردة أعلاه؟ هل ترغب في المشاركة في هذه الدراسة؟

<u>الاتصال في المستقبل:</u>

هل ترغب في الاتصال بك للمشاركة في أبحاث أخرى في المستقبل؟ نعم _____ لا _____

ملاحظة: للباحث الحق الكامل بايقاف أي مشارك عن متابعة مشاركته في هذا البحث.

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. وبناء عليه فأنني، حرا مختارا، أجيز الموافقة لابني أو ابنتي تحت وصيتي إجراء هذا البحث و أوافق أن يشارك فيه .

لن أتخلى عن أية حقوق قانونية عند امضائي لهذا البيان كما أنني سأستلم نسخة من هذا البيان.

إسم المشترك _____ التاريخ_____توقيع المشترك_____

الإسم المطبوع للشخص المأذون للموافقة من أجل الشخص:_____

العلاقة بالشخص: _____

إمضاء الشخص المأذون للموافقة:_____ التاريخ:_____

توثيق الموافقة:

الإسم المطبوع للشخص الذي يطلب الموافقة:_____

إمضاء الشخص الذي يطلب الموافقة:_____

التاريخ و الوقت:_____

الباحثون:

لقد شرحت كل التفاصيل التي تتعلق بهذا البحث لأهل الطفل المشارك أو للوصي الشرعي قبل الحصول على امضاء الأخير. لا يوجد فراغات في هذه الوثيقةز و قد تم اعطاء نسخة لأهل الطفل المشارك أو للوصي الشرعي.

لإسم المطبوع للشخص المأذون للموافقة من أجل الشخص:_____

إمضاء الشخص المأذون للموافقة:_____

التاريخ والوقت:_____

APPENDIX III

ENGLISH CONSENT FORM

Permission for Child to Participate in Research
AUB

Study Title: The assessment of urinary iodine status of children in Lebanon
Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut
Co-Investigator: Dr. Hala Ghattas/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut
Researchers: Dareen Shatila/
Address : American University Beirut, Cairo Street, Hamra, Beirut – Lebanon/01 – 350000
Where the study will be conducted: Schools all over Lebanon (private and governmental schools)

This is a permission form for your child/child for whom you are legal guardian to participate in a research study. It contains important information about this study and what to expect if you decide to permit your child/child for whom you are legal guardian to participate.
Your child's participation is voluntary.
Please consider the information carefully before you decide to allow your child to participate. If you decide to permit participation, you will be asked to sign this form and will receive a copy of the form.

A. Purpose of the Research Study: Iodine is an essential trace mineral that plays an important physiological role in the body. In Lebanon, endemic goiter was previously reported to be a serious public health problem with the greatest incidence occurring in the high mountain valleys. Currently, Lebanon is still identified as a country with mild to moderate iodine deficiency. Salt iodination was partially initiated in 1992 and was implemented in the year 1995 in a uniform manner by the Ministry of Health. No comprehensive study was conducted to determine urinary iodine status of schoolchildren since the introduction of salt iodination (1992-1995). Therefore it is essential to conduct a study in order to determine the iodine status in children. This would be of importance to determine the success of the salt iodization program and to determine whether the level of iodination is sufficient. Recent data regarding iodine status exists for Lebanon is lacking. Thus, the objectives of the following study are to: assess the prevalence of IDD and ascertain the extent and severity of the problem and identify high-risk areas.

B. Project/Procedures Description: Subjects' recruitment will be done by direct approaching to the school director. The selected children from each school will be given consent forms and questionnaires to share it with their parents for approval and filling

the questionnaire once approved. Children of signed consent forms will be recruited afterwards to collect the urine samples needed for this research.
Urine samples will be collected for the measurement of urinary iodine, sodium, potassium fluoride, phosphorus, magnesium, calcium and creatinine concentrations. The assessment of iodine status will include concurrent assessment of household use of iodized salt through surveys that will be filled by parents. These surveys will also include information regarding the socioeconomic status of the participants.
In this study subjects will be asked to maintain their regular dietary and physical activity habits during the entire study course, as well as any unusual strenuous exercise 24 hours prior to the study. Exclusion criteria include: any significant medical diseases and subjects out of the age range (5-10years).
Researchers will go to schools where: anthropometric measurements (height, weight) will be taken, a socio-demographic questionnaire will be collected after being filled from the children's parents or legal guardian and a sample of urine will be collected in special tubes.
This study is a randomized study and a total of 1500 schoolchildren (age between 5 to 10 years) would be required; from 35 schools recruited randomly from all over Lebanon; for its completion.

C. Duration: The estimated time to complete this study is approximately one year. The researchers will have to visit the allocated schools spread all over Lebanon. Only two visits will be needed one for asking for the parents' or legal guardian's permission for participation in this study and one to undergo the study. The duration of each visit will be approximately 30 minutes.

Your child may leave the study at any time. If you decide to stop your child's participation in the study, there will be no penalty to you, or your child and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship, or that of your child, with AUB.

D. **Risks, Discomforts and Benefits:** Your child participation in this study does not involve any physical risk or emotional risk to you beyond the risks of daily life.

You or your child will receive no direct benefits from participating in this research; however, when the prevalence of IDD is detected, growth retardation can be prevented. Moreover, the results obtained are interesting in increasing our knowledge and in the modification of the fortification method used since 1995. This significant new finding will be conveyed to subjects.

E. Confidentiality: Efforts will be made to keep your child's study-related information confidential. All data from this study will be maintained in a secure locked drawer in a locked office or on a password protected computer. Data will only be reported in the aggregate. No names of individual children will be disclosed in any reports or presentations of this research. However, there may be circumstances where this information must be released. For example, personal information regarding your child's participation in this study may be disclosed if required by law. Also, your child's research data may be reviewed by the following groups (as applicable to the research):

• U.S. Office for Human Research Protections or other federal, state, or international regulatory agencies, required;
• The AUB Institutional Review Board or Office of Human Research Protections;
• The sponsor, if any, or agency supporting the study.

After the conclusion of the study, the Principal Investigator will retain all original study data in a secure location for at least three years to meet institutional archiving requirements. After this period, data will be responsibly destroyed.

F. Compensation/Incentive: No costs have to be paid by you. There will neither be anticipated expenses for participating and costs for transportation, parking etc will not be reimbursed.

G. Payment for Research-related Injury: In case of any adverse event as a result of the study, there will be no compensation to cover such expenses, in case it is not covered by a third party or governmental insurance.

If you are injured as result of participating in this study or for questions about a study-related injury, you may contact Dr. Omar Obeid at 01/355555-ext 4440 or send him an email at oo01@aub.edu.lb.

H. Contact Information and Questions:

1) If you have any questions or concerns about the research you may contact:
Dr. Omar Obeid, 01/355555-ext 4440; oo01@aub.edu.lb.
2) If you have any questions, concerns or complaints about your rights as a participant in this research, you can contact the following office at AUB:
Social & Behavioral Sciences Institutional Review Board: irb@aub.edu.lb, 00961 1 350000-ext 5440 or 5445

I. Participant Rights: You may refuse to allow your child to participate in this study without penalty or loss of benefits to which you are otherwise entitled. If you are a student or employee at AUB, your decision about whether or not you allow your child to participate in this research will not affect your grades or employment status. If you choose to allow your child to participate in the study, you may discontinue his/her participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you or your child may have as a participant in this study. The Institutional Review Board responsible for human subjects' research at AUB has reviewed this research project and found it to be acceptable, according to applicable Lebanese and U.S. federal regulations and AUB policies designed to protect the rights and welfare of participants in research.
Do you have any questions about the above information? Do you wish your child to participate in this study?

J. Future Contact

Would you like to be contacted for future research? Yes _____ No _____
Please notify that the investigator has the right to end subject's participation in this study.

Participant Consent:
Signing the consent form
I have read (or someone has read to me) this form and I am aware that I am being asked to give permission for my minor child (or child under my guardianship) to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to give permission for my child/child under my guardianship to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

_____
Printed name of subject

_____

Printed name of person authorized to give permission for minor subject/participant

_____
Signature of person authorized to give permission for minor subject/participant (when applicable)

_____ _____ AM/PM
Relationship to the subject Date and time

Investigator/Research Staff

I have explained the research to the parent or legal guardian of the child subject/participant before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the parent/legal guardian of the child participant/subject.

_____ _____

Printed name of person obtaining permission Signature of person obtaining permission

_____ AM/PM
Date and time

APPENDIX VI

ENGLISH CONSENT FORM

عنوان البحث: تقييم حالة اليود في البول عند الأطفال في لبنان

إسم الباحث: د. عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

الباحثين المساعدين: د. هلا غطاس/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

منسقي البحث: دارين شاتيلا

المطلوب منك هو مشاركتك في هذه الدراسة البحثية. هذه الأبحاث تجري عادة لايجاد طرق جديدة و فعالة لمعالجة الناس أو لفهم بطريقة أفضل كيف يفكر الأطفال في بعض الاشياء أو كيف يتصرف الاطفال و الراشدين في بعض الاوقات.

هذا البيان سوف يخبرك أكثر عن هذه الدراسة لمساعدتك في تحديد ما اذا كنت تود المشاركة . يرجى طرح أي أسئلة قبل أن تقرر. بإمكانك أن تفكر و تناقش الموضوع مع أهلك أو أصدقائك قبل أن تقرر.

من الممكن أن تقول "لا" في حال كنت لا تود المشاركة في هذه الدراسة.و اذا قلت "نعم" يمكنك أن تغير رأيك و تنسحب من هذه الدراسة في أي وقت و دون مشاكل.

اذا قررت أن تكون في هذه الدراسة، يجب أن تحصل على موافقة من شخص راشد (عادة من الاهل) كي تشارك.

١- عن ماذا تدور حول هذه الدراسة؟

هذه الدراسة هي عن معدن اليود وهو معدن مهم من اجل نمو الاطفال. يتواجد اليود في المأكولات البحرية و يضاف الى الملح. سوف نقوم بقياسه في البول لنتأكد أن جميع الاطفال يحصلون عليه بشكل كاف و بالتالي ينمون بشكل طبيعي.

٢- ماذا سأفعل اذا كنت مشاركا في هذه الدراسة؟

اذا كنت ستشارك يجب الحصول على موافقة الاهل اولا و من ثم اعطاء عينة من البول في هذا الانبوب المخصص و سنأخذ طولك ووزنك.

٣- كم من الوقت سوف أكون في هذه الدراسة؟

سوف نقوم بزيارتكم مرتين. المرة الاولى لتوزيع بيان الموافقة . فور الحصول على موافقة مشاركتكم سوف نقوم بالزيارة الثانية التي ستستغرق ٣٠ دقيقة فقط.

٤- هل بإمكاني التوقف عن مشاركتي في هذه الدراسة؟

بإمكانك التوقف عن المشاركة في أي وقت. اذا كان هناك تجاوزات يمكنك التوقف عن اكمال هذه الدراسة في أي وقت و لكن عليك لزوم مقعدك حتى نهاية وقت البحث.

٥- ما هي المخاطر التي قد اتعرض لها اذا كنت مشاركا في هذا البحث؟

لا توجد أية مخاطر قد تتعرض لها.

٦-ما هي الامور الجيدة التي ستحصل من جراء مشاركتي في هذا البحث؟

هناك العديد من الامور الجيدة منها انك ستساعدنا كثيرا باعطائنا معلومات مهمة لمساعدة أصدقائك الذين لا ينمون بشكل جيد.

٧-هل ساحصل على أي شئ لانني شاركت في هذه الدراسة؟

كلا لن تحصل علي اي شئ.

٨- مع من يمكنني التحدث عن هذه الدراسة؟

لأي أسئلة حول البحث، يمكنك الاتصال بالباحثين، أهلك، و اساتذتك.

للحصول على أية معلومات مع أشخاص لا علاقة لهم في هذا البحث يرجى الاتصال بالمكتب التالي في الجامعة الأمريكية في بيروت:مجلس المراجعة المؤسسية

الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان 350000 (01) مقسم 5445 أو 5440، email: irb@aub.edu.lb

الباحثون:

لقد شرحت كل التفاصيل التي تتعلق بهذا البحث للطفل المشارك قبل الحصول على امضاء الأخير. لا يوجد فراغات في هذه الوثيقة. و قد تم اعطاء نسخة لأهل الطفل المشارك أو للوصي الشرعي.

الإسم المطبوع للشخص المأذون للموافقة من أجل الشخص:_____

إمضاء الشخص المأذون للموافقة:_____ التاريخ و الوقت:_____

APPENDIX V

ENGLISH ASSENT FORM

Child Assent Form

Study Title: The assessment of urinary iodine status of children in Lebanon
Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut
Co-Investigator: Dr. Hala Ghattas/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut
Researchers: Dareen Shatila

- **You are being asked to be in a research study. Studies are done to find better ways to treat people or to better understand how kids think about things or how kids and adults may behave at different times.**
- **This form will tell you about the study to help you decide whether or not you want to participate.**
- **You should ask any questions you have before making up your mind. You can think about it and discuss it with your family or friends before you decide.**
- **It is okay to say "No" if you don't want to be in the study. If you say "Yes" you can change your mind and quit being in the study at any time without getting in trouble.**
- **If you decide you want to be in the study, an adult (usually a parent) will also need to give permission for you to be in the study.**

1. What is this study about?
This study is about iodine, a mineral needed for all children to grow. Iodine is mainly derived from marine sources and is added to salt. We want to measure its level in the urine to check if children are getting enough of this mineral and are growing properly.

2. What will I need to do if I am in this study?
If you want to be in this study, you need to take your parent's permission first, and then fill this tube with urine, and then we will take your height and weight.

3. How long will I be in the study?
We will visit you two times, the first time to distribute the consent forms to ask for your parents' permission. Once we get your parents' permission we will come for the second visit and be with you for 30 minutes only.

4. Can I stop being in the study?
You may stop being in the study at any time. If there are limitations, such as you may discontinue completing the test/survey at any time, but you must remain at your desk in this room until the survey period ends.

5. What bad things might happen to me if I am in the study?
No health risks will happen to you.

6. What good things might happen to me if I am in the study?
The good things is that you will help us a lot in providing good information needed to help your friends who are not growing well.

7. Will I be given anything for being in this study?
No you will not be given any reward.

8. Who can I talk to about the study?
For questions about the study you may contact **us the researchers, your parents, and your teachers.**

To discuss other study-related questions with someone who is not part of the research team, you may contact the AUB Institution Review Board at 961-1-350000 or oo01@aub.edu.lb

Signing the assent form

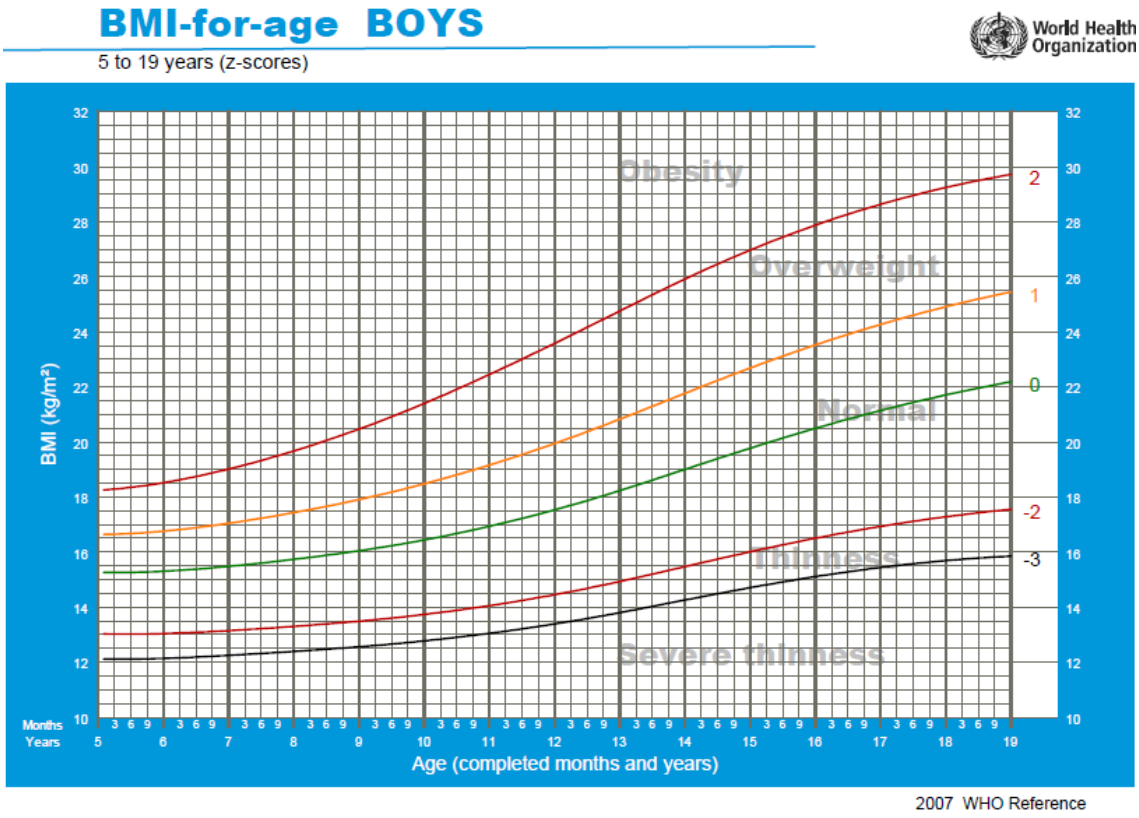
Investigator/Research Staff

I have explained the research to the participant before requesting the signature above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

Printed name of person obtaining assent	**Signature of person obtaining assent**
	AM/PM
	Date and time

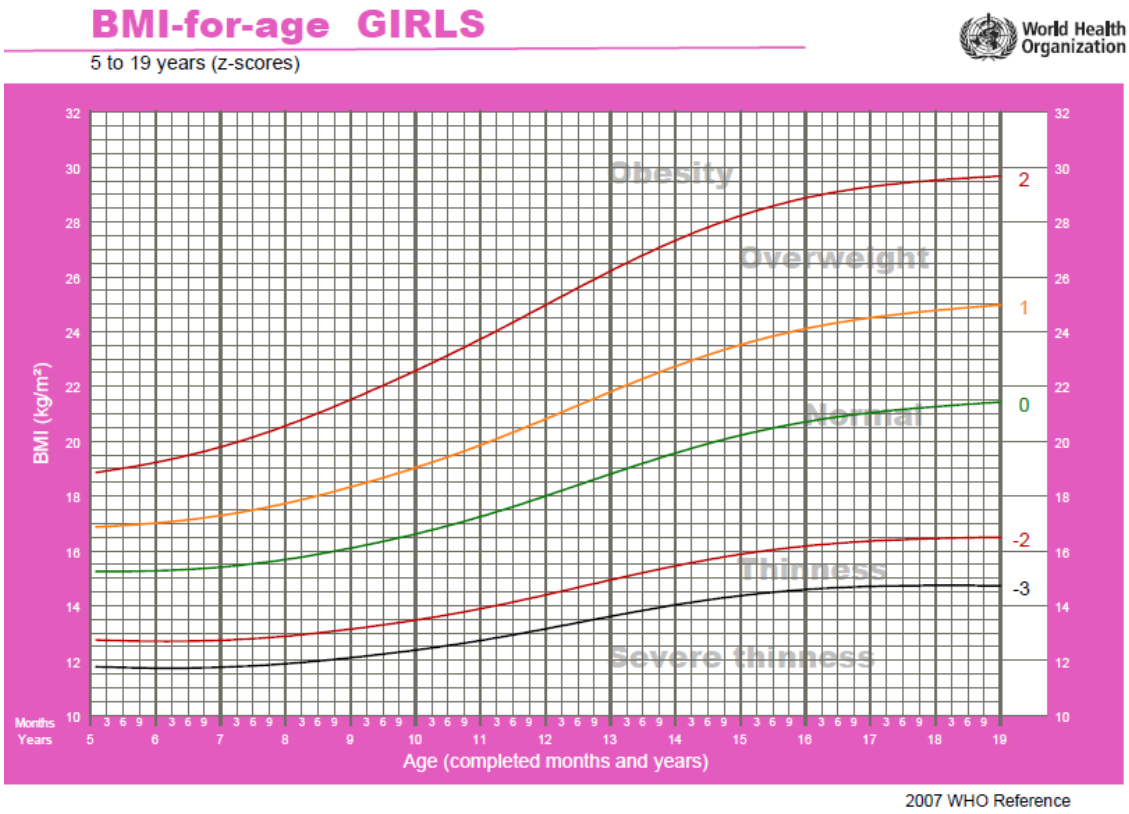
APPENDIX VI

GROWTH CHART BMI FOR AGE-BOYS



APPENDIX VII

GROWTH CHART BMI FOR AGE-GIRLS



APPENDIX VIII

DEFINITION OF STATISTICAL VARIABLES

Name of variable	Description
Student ID	Continuous variable
School Type	1: Public 2: Private 3: Private Free
District	1: Akkar 2: Beirut 3: Bekaa 4: Hermel 5: Mount Lebanon 6: North 7: Nabatiyeh 8: South
Gender	1: Male 2: Female
Age (years)	Continuous variable
Class	1: Grade 1 2: Grade 2 3: Grade 3 4: Grade 4 5: Grade5
Height (cm)	Continuous variable
Weight (kg)	Continuous variable
BMI (kg/m^2)	Continuous variable calculated from weight and height
Percentile	Continuous variable obtained from the WHO growth charts
Nutritional Status	1: Sever Thinness 2: Thinness 3: Normal 4: Overweight 5: Obese
Creatinine (mg/dl)	Continuous variables measured using Vitros 350
Predicted Creatinine (mg/d)	Continuous variable calculated as suggested by Remer, Neubert, & Maser-Gluth (2002)
Ca (mg/dl)	Continuous variables measured using Vitros 350
Mg (mg/dl)	Continuous variables measured using Vitros 350
P (mg/dl)	Continuous variables measured using Vitros 350
Ca/Crea (mg/mg)	Continuous variable calculated from Ca/Crea
Mg/Crea (mg/mg)	Continuous variable calculated from Mg/Crea
P/Crea (mg/mg)	Continuous variable calculated from P/Crea
Predicted 24 h Ca excretion (mg/d)	Continuous variable calculated from the cross multiplication using the predicted creatinine values: $\frac{Ca \times Predicted\ 24\ hour\ Cr}{Cr}$

Predicted 24 h Mg excretion (mg/d)	Continuous variable calculated from the cross multiplication using the predicted creatinine values: $\frac{Mg \times Predicted\ 24\ hour\ Cr}{Cr}$
Predicted 24 h P excretion (mg/d)	Continuous variable calculated from the cross multiplication using the predicted creatinine values: $\frac{P \times Predicted\ 24\ hour\ Cr}{Cr}$
Predicted 24 h Ca excretion (mg/kg/d)	Continuous variable calculated by dividing the predicted 24 h Ca excretion by weight
Predicted 24 h Mg excretion (mg/kg/d)	Continuous variable calculated by dividing the predicted 24 h Mg excretion by weight
Predicted 24 h P excretion (mg/kg/d)	Continuous variable calculated by dividing the predicted 24 h P excretion by weight
Ca/P	Continuous variable calculated from Ca/P
Date of collection	Date (dd-Mon-yy)

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