



# Malnutrition in school-aged children and adolescents in Senegal and Cambodia : public health issues and interventions

Marion Fiorentino

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# THÈSE

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**Institut de Recherche pour le Développement**

Spécialité : **Nutrition Santé**

Présentée par **Marion Fiorentino**

**Malnutrition in school-aged children and  
adolescents in Senegal and Cambodia: public  
health issues and interventions**

**Malnutrition chez les enfants d'âge scolaire et les adolescents  
au Sénégal et au Cambodge : problèmes de santé publique et  
interventions**

Soutenue le 18 décembre 2015 devant le jury composé de

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## Context and objectives of the research

According to FAO, 870 million people were undernourished in 2010 and 2012. Albeit a global reduction in almost all regions during the previous 2 decades, the reduction rate globally slowed since 2007-2008, consequently to the global financial, economic and food price crisis in 2008, failing to meet the MDG1 by 2015 <sup>1</sup>. Undernourishment indicates insufficient caloric intake, based on food availability of countries, which does not necessarily reflect malnutrition, while malnutrition is measured by epidemiological studies conducted on representative samples of populations using anthropometric and biological indicators<sup>2</sup>.

Malnutrition represents a public health issue worldwide. Retardation in linear growth leading to stunting affected 165 million or one third of under-5 children worldwide in 2011, with higher rates found in SEA. In Africa alone <sup>3</sup>, 52 million children were wasted, meaning having a body weight too low for their height. Undernutrition among pregnant women increases the risk of intrauterine growth restriction (IUGR) and low birth weight, which are associated to neonatal mortality and stunting. Suboptimum breastfeeding increases the risk for mortality. Stunting is associated with impaired physical and cognitive development of children. Vitamin A, zinc, iodine and iron deficiency account for 12% of preschool children deaths <sup>4</sup>. Iodine deficiency is responsible for mental retardation and impaired cognitive function <sup>5</sup>. Iron deficiency leads to microcytic anemia, which impairs immune and endocrine function, and decrease work capacity <sup>6</sup>. Zinc deficiency contributes to stunting and to increased morbidity <sup>7</sup>. Vitamin A deficiency is the leading cause of preventable pediatric blindness and increase morbidity <sup>8,9</sup>. Folic acid deficiency in pregnant women can lead to neural tubes defects <sup>10</sup>. Undernutrition, including intrauterine growth restriction, suboptimum breastfeeding, stunting, wasting, and micronutrient deficiencies is the major contributor to disease burden among preschool years old. It is estimated to be responsible for the death of 3.1 million child each year, corresponding to half of all child death <sup>3,4</sup> mostly in the developing world. Although prevalence of undernutrition was reduced in the previous 2 decades, in SEA and Africa malnutrition is still a major concern, as prevalence of stunting is increasing in Africa, while the highest absolute number of stunted children is found in SEA <sup>4,11</sup>. Thus, undernutrition among children preschool is widely prevalent and its severe consequences on health and development have hindered the economic development and human capital <sup>10</sup>. Maternal nutritional deficiencies contribute to the intergenerational cycle of malnutrition and poverty, and it is widely acknowledged that the critical period to break the cycle of malnutrition is during the first 1000 days of life: from the conception to 2 years. Therefore, nutrition programs target in priority preschool children and pregnant and lactating women, with most efforts and funding given to interventions such as promotion of early and exclusive breastfeeding, micronutrient supplementation of pregnant women, vitamin A supplementation of preschool children, or promotion of dietary diversity and complementary feeding <sup>12</sup>. WHO recommended early child development programs as the most cost-effective interventions in developing countries, regarding child health as well as poverty and inequalities reduction <sup>13</sup>.

However, there is a growing interest about improving nutrition among school-aged-children (SAC) and adolescents. In opposition to under 5 years old children (U5) U5<sup>1</sup>, , children aged 5-9.9 y will be designated here as "School-aged children" (SAC). WHO defines adolescent as individuals between 10 and 19 years, with the cut-off between early (EA) and late adolescence

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<sup>11</sup> « School children » will be used as a generic term to refer to both school-aged children and adolescents studying in primary schools

(LA) at 15 years <sup>14</sup>. In 1996, a symposium already questioned “Adolescent nutrition: are we doing enough?”, arguing that world interest in adolescent health regarding pregnancy and HIV had grown in the previous decade, but that nutrition stayed neglected <sup>15</sup> although elevated prevalence of anemia, stunting and thinness were observed among adolescent. In 2006, WHO published a review about adolescent nutrition in South-East-Asia which reported a large percentage of adolescents suffering from nutritional deficiencies and a prevalence of anemia between 12% and 100% across the countries <sup>16</sup>. A review published in 2010 by Best et al <sup>17</sup> summarized the data of nutritional status of children 5-12 y worldwide. Prevalence of severe or moderate anemia, as well as prevalence of iron, iodine, zinc, and vitamin A deficiencies ranged from 20% to 30%, suggesting an important public health issue among SAC, especially from Africa and SEA. The researchers warned about the lack of data. UNICEF published a report in 2011 “Adolescence: an age of opportunity” asking for more efforts to be done in favor of adolescent health, especially in nutrition, and in adolescent girls.

Malnutrition among SAC also inhibits physical and mental development. Short height and low BMI for age at school age are associated with future reduced work capacity and obstetric complications <sup>18,19</sup>. Micronutrient deficiencies and consequent anemia among SAC may impair their growth, vision, concentration, cognitive performance and immune defenses. Recent evidence highlighted the possibility to improve growth, cognition and health outcomes in SAC by improving nutrition <sup>20-24</sup>. It is known that adequate nutrition and health supports development, growth and cognitive achievement among SAC <sup>25</sup>.

Early adolescence is characterized by a growth spurt and physical changes associated with sexual maturation <sup>26</sup>. Recent neurological research brought to light the tremendous development of the brain during early adolescence. The neuronal network is drastically reorganized and the number of brain cells can double within one year <sup>26</sup>. Although most part of the brain are developed during fetal life and infancy, the frontal lobe which control reasoning and executive functions achieves a growth spurt during early adolescence. Therefore, nutritional deprivation occurring during adolescence not only perpetuates the stunting process but it can seriously impair cognitive functions and jeopardize schooling achievement. Moreover, lateness of puberty will extend the adolescence, maintaining individuals in a critical situation of high nutrient requirements. The brain and skeletal growth spurt as well as the appearance of sexual characteristics usually occur earlier in girls compared to boys. Nutrition during adolescence should be particularly monitored because of the risk of pregnancy in developing countries where early marriage is common <sup>27</sup>. Adolescent pregnant girls bear the double burden of their own plus their babies’ growth and development. Therefore, even if the main focus remains to improve nutrition of preschool children, investing in late childhood and adolescence could be an effective way to reinforce the global gains achieved in health and nutrition of young children since 1990 <sup>3</sup>. Children 5-14 y represent 1.1 billion individuals worldwide, of which 90% live in Low or Middle Income Countries (LMIC) <sup>28\*</sup>. Schools could be an effective platform to deliver health and nutrition interventions such as school feeding and deworming programs.

School feeding programs have been widely implemented with 68 million children receiving daily breakfast, lunch or snacks <sup>29</sup>. But large discrepancies remain between countries or within countries. School feeding programs globally increased school attendance in developing countries, but benefits on nutrition, health and schooling outcomes lack evidence and could be improved using cost-effectiveness tools such as staple food fortification.

During the past century, research about indicators to diagnose malnutrition in human populations has been tremendous. One strategy is to evaluate the adequacy of dietary and

nutrient intake through food consumption surveys. In order to compare intake with requirements, this implicates to establish nutrient requirements adapted to the population's needs and to collect accurate data about food consumption. This method does not take into account nutrient absorption, retention and utilization. A more direct strategy consists in evaluating the nutritional status by measuring anthropometry and body composition, micronutrient status using biomarkers, most of involving biochemical analysis on blood, urine, hair or sweat samples, or by functional indicators or even clinical evaluation. Ideally, the biomarkers should reflect functional outcomes, and provide information on the level of severity of deficiency or the level of stores. The selection of indicator is crucial and international recommendations are needed for researchers to adequately evaluate nutritional status and dietary patterns between different populations. Beyond the choice of indicator, the establishment of adequate cut-offs to define deficiency is compelling. Indeed, nutritional status does not only depend on food intake but it is influenced by many individual and environmental factors such age, gender, pregnancy, inflammatory status, parasite infestation or genetics. Internationally recommended indicators of nutritional deficiencies and cut-offs adapted to SAC and adolescents are lacking. The growth reference for 5-19 years children of WHO was updated in 2006 but this latest growth reference was questioned as a standard <sup>30</sup>). Due to poor hygiene and sanitation in the developing world, SAC are particularly at risk for infections. Inflammation raises nutrient requirements, but also affects concentrations of several biomarkers. Ferritin concentrations for example are widely known to be increased by the inflammatory response and some tools were established to adjust prevalence of iron deficiency for inflammation. But one may question the impact of inflammation on transferrin soluble receptor, which is the other indicator of iron status widely used in epidemiological studies, and so far, very little research about this subject is available. Retinol and RBP are influenced by inflammation too. No internationally recommended cut-off to define inflammation is available. Moreover, plasma/serum retinol cut-offs to determine vitamin A deficiency and marginal vitamin A status currently used for SAC and adolescents were determined for young children.

## Development of the research

This thesis focuses on the nutrition of school-aged children and adolescents from developing countries. The thesis is based on studies successively conducted in Senegal and in Cambodia, whose the common main objectives were to define their nutritional status and assess the impact of nutritional interventions on micronutrient deficiencies, health and development in school-aged children (SAC) and adolescents.

Consequently the thesis will first review in Chapter 1 the epidemiologic situation of malnutrition in the world and the steps to define sustainable intervention strategies adapted to the specific context of countries that are: i) assessment of the nature and the magnitude of nutritional issues in the target populations ii) evaluation of their determinants iii) definition of potential interventions iv) assessment of their efficacy, their effectiveness and their impact.

The Chapter 2 will present the first study carried out in Senegal (2 publications). This study was part of a project of two NGOs (Enda-Graf Sahel and GRET) and a French foundation (Danone Communities) aiming at developing a specific food complement for urban Senegalese schooled children. The expertise of IRD was requested for the micronutrient formulation of the food complement. However, the absence of epidemiologic data regarding the target population led us to propose to conduct a cross-sectional study to determine the nutritional status of children from a representative sample of state schools in Dakar and to evaluate their dietary nutrient intake. The results of this study will be presented in chapter 2 (2 publications).

The second study was conducted by IRD in school children in Cambodia, in collaboration with an American NGO (PATH) and an international organization (World Food Program)<sup>2</sup>. The main objective of this study was to assess the effectiveness of introducing 3 different micronutrient-fortified rices in school breakfasts on improving nutritional and health outcomes. The results of this study are described in chapter 3 (baseline characteristics of the school children, 2 publications) and chapter 4 (impact of the intervention, 2 publications).

Data produced by these 2 studies brought us to an overall brainstorming about public health nutrition issues in school-aged children and adolescents, especially indicators currently used to determine them. Consequently, we reanalyzed the combined data from these 2 studies and the results of these analyses are presented in chapter 5 (2 publications).

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<sup>2</sup> The author declares no conflicts of interest with any of organizations involved in the projects in Cambodia and Senegal



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*Chapter 1.    Nutrition among school-  
aged children and adolescents in  
developing countries: Literature review*

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### **1.1. Growth, brain growth, sexual maturation and early pregnancy during school age and adolescence leads to specific nutritional requirements: **what are the consequences of malnutrition in school-aged children and adolescents?****

The growth period arising in adolescence is the second most important in life after the one occurring during the first year of life. This growth spurt dramatically increases nutrient requirements. Adequate intake of energy, protein, calcium, iron, zinc and folate is therefore crucial, especially during the peak of velocity of growth when nutritional requirements may be up to twice those of the remaining period of adolescence <sup>31,32</sup>. Due to different body composition and biological changes, and to earlier puberty timing in girls, nutrient requirements start to diverge between boys and girls at puberty. At menarche, iron needs are tremendously increased in girls due to blood losses <sup>33</sup>. School age and adolescence is also a critical period for neurological development. Even though neurulation, the formation of the receptive language, seeing and hearing brain areas occur during fetal life and before the age of 5 years, synaptogenesis and neurogenesis continues up to the end of adolescence <sup>34</sup>. Peaks of brain development, especially frontal lobes have been found in the first 2 years, 7 to 9 years and in the mid-teenage years <sup>35,36</sup>. Therefore, the development of the full range of executive functions is suggested to occur in late childhood and adolescence as myelination of the frontal lobes proceeds <sup>37</sup>. Peaks of brain development occur at different timing according to the brain area : frontal and parietal gray matter growth peaks at 12 for boys and 10 for girls, while white matter volume increase linearly, and peaks of temporal gray matter growing occur at the end of adolescence <sup>38</sup>.

#### *1.1.1. Consequences of acute and chronic malnutrition during school age and adolescence*

Stunting was found to predispose to chronic metabolic diseases obesity or overweight in late childhood or in adulthood, probably because the rate of lipid oxidation is lower in stunted children, leading to more central fat accumulation <sup>39</sup>. Stunting is also associated with long-term adverse effects such as impaired cognitive performance. Stunting is mainly the result of chronic nutritional deprivation that occurs before the age of 3 years, which is hard to recover later in life. However, some research suggests that health and nutrition interventions can slow down or even reverse the stunting process in SAC <sup>21,24,40</sup>. Stunting had adverse consequences at adult age. Low women height is associated with small pelvic size, which increases risk of obstetric complications during delivery. Reduced height was also found to impair work capacity <sup>18</sup>. Thinness reflects recent nutritional deprivation <sup>17</sup>. Thinness during school-age and adolescence may delay pubertal maturation. Thinness is a particular concern among adolescent girls which are more likely to give low birth weight babies. Muscular strength and work capacity is more likely to be reduced in individuals who were thin during school age <sup>18</sup>. Obese or overweight SAC are more at risk for type 2 diabetes, high blood pressure, metabolic syndrome and psychological disorders <sup>41</sup>. Dyslipidemia and hyperinsulinemia associated with the metabolic syndrome track throughout the lifetime and are associated with cardiovascular disease <sup>42,43</sup>.

### *1.1.2. Consequences of micronutrient deficiencies during school age and adolescence*

#### *1.1.2.1. Iron role and deficiency*

The main role of iron is oxygen transportation, as a component of hemoglobin. Worldwide, iron deficiency is the main cause of anemia, which symptoms as fatigue, breathlessness and headaches that can impair physical activity and concentration among SAC <sup>44,45</sup>. Moreover, iron deficiency and iron deficiency anemia was showed to increase the risk of infection as well as cognitive impairment <sup>45</sup>. Therefore the implication of iron deficiency in anemia leads to increased morbidity, absenteeism, and indirectly to impaired school achievement. Including SAC in epidemiological studies concerning anemia has been recommended<sup>46</sup>, but is often not done. One major concern about iron deficiency among SAC and adolescents is its adverse effects on cognitive functions. Iron deficiency and iron deficiency anemia are consistently associated with impaired cognitive function and lower school performance in SAC <sup>47-51</sup>. A study among 12000 Taiwanese SAC and adolescents showed increased risk for mood disorders, autism spectrum disorder, attention deficit hyperactivity disorder, and developmental disorders associated with iron deficiency anemia <sup>52</sup>. The lower scores in cognitive functions with ID or IDA could be the result of hematologic impairment during early childhood, which adverse effects on cognition perpetuates at school age. However, studies reported improved cognitive scores or educational achievement or improved motor development in response to iron supplementation among SAC with ID, IDA or normal iron status <sup>22,45,53-55</sup>. As an example, a meta-analysis of randomized controlled trials of daily iron supplementation in children 5-12 years reported improvement of height, global cognitive scores, measures of attention and concentration. Among anemic children, daily iron supplementation improved intelligence quotient and weight <sup>56</sup>. This indicates that some of the cognitive deficit resulting from iron deficiency at school age can be reversed, In contrast to infants, where long-terms effects of iron deficiency may be permanent <sup>57,58</sup>. The adverse effects on cognitive and educational performance of IDA in school-age children appear more transitory than in infants, where cognitive impairment is less reversible<sup>45</sup>. Iron supplementation was also found to improve the beneficial effect of deworming on school attendance <sup>59</sup>. The brain is sensitive to iron deficiency because iron plays a role in neurotransmission, especially in the dopamine pathway, and in homeostatis regulatory mechanisms <sup>36,60</sup>. Iron deficiency alters myelination, neurotransmitter synthesis and hippocamp energy metabolism <sup>61</sup>. Therefore, iron is a key nutrient in the development of executive functions <sup>36</sup>.

#### *1.1.2.2. Iodine role and deficiency*

Iodine is crucial for cerebral growth and development because it is required for the production of the thyroid hormones triiodothyronine (T3) and thyroxine (T4). Long-term and severe iodine deficiency causes cretinism and goiter. However, even at less severe stage, iodine deficiency can be responsible for lower cognitive performance, deaf-mutism, or birth defects. Iodine deficiency among pregnant women and infants is the 1<sup>st</sup> cause of preventable mental retardation in children <sup>44</sup>. A meta-analysis reported that SAC living in iodine-deficient areas have intelligence quotient 13 points lower than children from iodine-sufficient regions (<sup>62,63</sup>. Some studies report positive correlation between cognitive performance and urinary iodine <sup>64,65</sup>, with also evidence of iodine supplementation improving cognitive functions in SAC <sup>23,64,66-68</sup>. Iodine deficiency has more serious consequences at fetal age, but cognitive functions can be



affected across all ages as hypothyroidism as an impact on neuronal development, structure and function<sup>36</sup>. Therefore, some of the cognitive impairment resulting from iodine deficiency still can be addressed at school age.

#### 1.1.2.3. Vitamin A role and deficiency

Vitamin A is a component of the retina of the eye, and plays a crucial role in vision. Severe vitamin A deficiency leads to corneal destruction and blindness, but this condition called xerophthalmia usually peaks at 2 to 3 years of age <sup>17,44</sup>. However, chronic vitamin A deprivation persisting at school age may increase the prevalence of xerophthalmia beyond 5 years <sup>69</sup>. Vitamin A is also involved in the immune response. Because vitamin A deficiency is associated with increased morbidity and mortality in infants and young children, efforts to control vitamin A deficiency are concentrated on pregnant women and young children, thus vitamin A supplementation is not usually recommended in SAC <sup>17,44</sup>. However, studies in Thailand and Colombia revealed that vitamin A deficiency, even mild, increase the risk of respiratory diseases and of diarrhea in SAC <sup>70,71</sup>. Some research suggest a role of vitamin A in growth during later childhood and in sexual maturation, making adolescent particularly vulnerable to vitamin A deficiency <sup>72,73</sup>. Plus, vitamin A deficiency can have adverse effects on iron status, with vitamin A supplementation contributing in the prevention of anemia <sup>73</sup>. A study on anemic Tanzanian SAC showed improvement of growth and hemoglobin status with vitamin A supplementation <sup>74</sup>. Therefore, vitamin A status should be monitored among SAC and adolescent, because of the potential adverse consequences on health and schooling outcomes linked to the risk for eye disorders, increased morbidity and ceased growth. But overall, data about the impact of vitamin A deficiency or the benefits of vitamin A interventions on health outcomes such as growth velocity and eye functioning among SAC and adolescents is lacking.

#### 1.1.2.4. Zinc role and deficiency

Zinc plays a role in multiple aspects of metabolism, and zinc deficiency results in reduced growth rather than in specific clinical signs <sup>75</sup>. Therefore, due to the growth spurt during adolescence, zinc status is particularly worrisome among this age range. Indeed, zinc supplementation improved growth velocity among stunted children and adolescents in 3 studies <sup>76-78</sup>. Zinc deficiency is also associated with impaired immune defenses and increased prevalence of diarrhea <sup>44</sup>. The beneficial effect of zinc supplementation to prevent acute diarrhea and pneumonia and to reduce mortality was demonstrated among under 5 years old children, but such evidence among SAC or adolescent is scarce <sup>79-81</sup>. However, zinc supplementation improved cognitive performance and taste acuity among adolescent Indian girls <sup>82</sup>.

#### 1.1.2.5. Other micronutrients role and deficiencies

Iron, iodine, zinc and vitamin A are the most studied micronutrients <sup>17</sup> as deficiencies are very prevalent around the world, and their adverse effects on health and development on children are well described. However, in the present review of literature, I will also focus on less considered vitamins and minerals such as thiamine, vitamin E and copper.

Thiamin (Vitamin B<sub>1</sub>) is found in vegetal and animal sources, mainly in the form and outer coats of unrefined cereals, but also in green vegetables, fish, meat, fruit and milk. It is highly water soluble thus rice should not be washed <sup>44</sup>. Thiamin diphosphate is cofactor for some enzymes involved in glucose and carbohydrates metabolism. Thiamin triphosphate has functional properties in the neuronal membrane. Thus, thiamin has a crucial role in the development and the maintenance of neuronal functions <sup>83</sup> especially in frontal lobes and basal ganglia <sup>84</sup>. Thiamin deficiency results in neuronal losses related to brain energy deficit due to lower activity of thiamine dependent enzymes and oxidative stress affecting carbohydrate metabolism. The most known consequence of thiamin deficiency is the neurological and cardiovascular disease beriberi. The dry beriberi is associated with motor and sensory disorders while the wet beriberi symptoms include edema, mental confusion and heart disorders. Infantile beriberi occurs in children breastfed by thiamin deficient mothers and cause cardiac disorders, aphonia and sudden death <sup>(44,85)</sup>. Other signs of thiamin deficiency are weakness, fatigue, psychosis <sup>44</sup>. Thiamin deficiency during infancy results in long lasting language disorders <sup>86</sup>. Low thiamin intake in adolescent results in increased risk of aggressiveness and delinquency <sup>87</sup>. Some research suggest a possible role of thiamin deficiency in the apparition of tumors <sup>88</sup>. A study reported that low intake of vitamin B1, B2, B3, B5, B6 is associated with aggressiveness and delinquency while low intake of vitamin B6 and folate is associated with withdrawnness and depression.

Copper is mainly found in seafood, organ meats, nuts, beans, dried fruits and vegetables, black pepper, cocoa, and dark green leafy vegetables. Intestinal absorption (30-40%) is regulated according to the intake. Clinical features of copper are mostly related to cuproenzymes activities (metalloproteins). Copper is involved in metabolism, angiogenesis, oxygen transport, and antioxidant protection, including copper zinc superoxide dismutase <sup>89</sup>. Copper deficiency main clinical manifestations are hematologic, with lower white blood cells, which reduces immune response, and neutropenia. Mechanism of reduction of neutrophils is not clear, but hypotheses include destruction of cells in the bone marrow or in the circulation, impaired cell differentiation, proliferation, or increased cellular destruction. Both high and low serum copper levels were found to be associated with unexplained anemia <sup>90</sup>. Women have generally intake below their requirements, which may have harmful effect during pregnancy such as spontaneous abortion, bone, immune and neurological abnormalities or increased risk for cardiovascular disease in later life <sup>89,91</sup>. Nervous damages, hardly distinguishable from vitamin B12 neurological manifestations, such as neuropathies, or myelopathies can occur at severe stage of copper deficiency <sup>92</sup>. Copper deficiency has 2 adverse effects on iron metabolism. When iron and copper deficiencies are concomitant, it causes hypochromia which continues even after treatment with copper alone. As a later effect, even after iron stores are repleted, erythropoiesis will stay impaired <sup>93</sup>. Increased severe respiratory infection prevalence was observed in copper-

deficient infants. Following hematological manifestations as anemia and neutropenia, copper deficiency can cause osteoporosis, damage nerve cords and result in spasticity.

Vitamin E is exclusively provided by the diet, and is the major lipid-soluble antioxidant in the cell antioxidant protection system. Its most interesting form from a nutritional point of view is alpha-tocopherol. Vitamin E is essential to protect cell membranes and low-density lipoprotein from oxidation by free radicals <sup>85</sup>. But some research suggests a wider biological role for alpha-tocopherol, including regulation of gene expression and coordinated movement through cerebellar cells. However more research is needed. Early signs of vitamin E deficiency include leakage of muscle enzymes and increase erythrocyte hemolysis <sup>85</sup> which can cause anemia <sup>94</sup> In human and animal models, vitamin E deficiency was shown to induce myopathies, neuropathies and retinal damage <sup>85,94,95</sup>. Neuropathy and anemia may be caused by excessive free radical damage to the axons of sensory neurons and to the red blood cell membrane <sup>94</sup>. Prolonged vitamin E deficiency during infancy impair cognitive functions <sup>96</sup>. Some research highlights the protective role of vitamin E against heart disease but evidence remains inconsistent <sup>85,97</sup>. Some research showed that in adolescents and in children, poor vitamin E status is associated with stunting <sup>94,98</sup>.

Effects of micronutrient deficiencies on cognitive functions are described in sections 3.1 and 4.2.

### *1.1.3. Focus on adolescent girls, a vulnerable population due to the risk of pregnancy*

#### *1.1.3.1. Magnitude of childbearing by adolescent girls in the developing world*

The number of adolescents (10-19y) living in developing countries is estimated to be 1.1 billion. They represent 88% of the total population of adolescents and 19% of the population of developing countries. Half of them live in South Asia, East Asia of pacific region <sup>26</sup>. Although marriage during adolescence had declined in the past decades, especially in Latin America and in North Africa <sup>99</sup> still 21% of girls aged 15-19y are currently married in the developing world, with the rate reaching 30% in the least developed countries <sup>26</sup>. The highest rates of marriage of girls before 15y are found in South East Asia, India and sub-Saharan Africa, with respectively 53%, 38% and 35% of girls being married before 15 y in Bangladesh, in Niger and in Chad respectively <sup>27</sup>. As marriage is generally followed by childbearing, 20% of young women gave birth before the age of 18 in developing countries<sup>26</sup> this proportion reaching 66% in Sub-Saharan Africa <sup>100</sup>. With 55 births per 1000 girls 15-19 y, the rate of birth among adolescents is twice higher in the developing countries compared to industrialized countries <sup>26</sup>. 16 million girls 15-19 y and 1 million girls <15y give birth every year, 95% of these births occurring in low and middle income countries <sup>101</sup>.

#### *1.1.3.2. Multifactorial early marriage and pregnancy*

Poverty and cultural traditions are powerful factors driving early marriage (figure 1). Marrying off a girl results in one less mouth to feed and ensure that she will be supported. In some cultures, families receive a “bride price” in the form of cattles or money <sup>27</sup>. In insecure environments, marriage of young girls could be seen as a strategy to protect them from rapes

and dishonor. In areas where girls education is not valued, marriage and procreation is often the only option for girls <sup>27</sup>. Uneducated girls are more likely to have few alternatives for their future, few skills and self-confidence to become economically dependent. But premature marriage is not only practiced in underprivileged families. The high social value of virginity conduct families to marry their daughters immediately after puberty or even before, in order to protect them from promiscuity and the risk of pregnancy outside of marriage. The social pressure to become pregnant within 1 year after the wedding is high in the developing countries, even if their own physical growth is not terminated <sup>27</sup>. For example, in Cambodia, where 10% of 15-19y girls are married, 8% of them already experienced a pregnancy, and only 15% of married adolescent girls use a modern birth-control method <sup>102</sup>.

### 1.1.3.3. Health consequences of early pregnancies

Pregnancy in adolescents can have adverse consequences on the fetus and the infant. In low and middle income countries, infants born to mothers under 20 y are at 1.5 - 2 times higher risk to be stillborn or to die before the age of 5 years <sup>101,102</sup>. Reasons for this are numerous.

First of all, adolescents in developing countries are likely to enter pregnancy with poor nutritional status. Rates of anemia among girls 15-19y are above 40% in India and some sub-Saharan countries, which indicates a severe national public health issue. Prevalence of underweight in girls 15-19y ranged from 25 to 47% in Sub-Saharan Africa, India and Bangladesh <sup>26</sup>. Nutritional deficiencies in pregnant or lactating women are known to be risk factors with poor pregnancy outcomes and poor nutritional infant status. Maternal iron deficiency and iron deficiency anemia for example are associated with low birth weight <sup>103</sup>. Vitamin A deficiency in lactating women is associated with low vitamin A breastmilk concentration and hence to vitamin A deficiency in their infants <sup>104</sup>. Poor micronutrient status in pregnant women was also found to be related to altered metabolism, organ growth and function, and to elevated risk of chronic disease in their children later in life<sup>105</sup>.

Secondly, besides pre-pregnancy nutritional status, pregnancy during adolescence is associated with poor pregnancy outcomes compared to pregnancy in adults even in developed countries. A study in Gabon reported odds ratio >2 for the risk for low birth weight and less frequent antenatal care visits in adolescent pregnant girls compared to adult counterparts but no information about their nutritional status was available <sup>106</sup>. Animal studies reported that initial low maternal BMI as well both overnourishment and undernourishment during pregnancy in adolescents resulted in lower birth weight and placental weight <sup>107</sup>.

Because of irregular menstruations and the absence of planning of the pregnancy common among adolescents, adolescent pregnancies are often undetected for late into pregnancy, preventing early start of folic acid supplementation, recommended prior to conception and until the 12<sup>th</sup> week of pregnancy. Folate deficiency is a particular concern in pregnancies because deficiency increases the risk for neural tubes defects in the fetus.<sup>108</sup> It was also associated with a higher risk for Small-Gestational-Age (SGA) delivery in pregnant UK adolescents.<sup>109</sup> Another concern is calcium status in adolescent pregnancy. The growth spurt during adolescence may deplete calcium stores. The skeleton is still increasing its density during adolescence and demineralization of bone during this period could be harmful <sup>108,110</sup>. Suboptimal calcium status in pregnant adolescents can both impair maternal as fetal bone growth<sup>110</sup>.

Pregnancy during adolescence has not only harmful effects on infants but also on mothers. Adolescent girls contribute to 11% of births worldwide, but because their bodies are not ready for childbearing, they account for 23% of the global burden of disease related to pregnancy and childbirth<sup>102</sup>. Indeed, girls aged 10-14 years have 5 time higher risk to die from pregnancy and childbirth complications than adult women<sup>102</sup>. Complications related to pregnancy or childbirth such as hemorrhage, sepsis, preeclampsia, eclampsia, are the leading cause of mortality among 15-19 y girls<sup>27,100</sup>. Among the 16 million of adolescent girls giving birth every year, 50,000 die<sup>100</sup>, or 31.3/10,000 deliveries.

Maternal-fetal competition for nutrients was first observed in adult women. While well-nourished mothers provide unlimited access to nutrients for their fetuses, undernourished women tend to retain their own tissue nutrient stores at the expense of the fetal growth<sup>111</sup>. In adolescent girls, pregnancy superimposes nutrient requirements for fetal growth on those for maternal growth. Therefore, there is a competition between the mother and the fetus to meet simultaneously and adequately the nutrient requirements for the growth of both mother and fetus. Indeed, even if it was generally admitted that the growth spurt occur before menarche<sup>112,113</sup>, it was showed that growth can continue during years following menarche, especially in undernourished populations where adolescence can be extended, offering an opportunity to catch up for stunting<sup>114-116</sup>.

The nutrition partitioning between mother and fetus is modified according to the nutritional status of the mother<sup>108 108</sup>. Some researchers suggest that in case of severe deficiencies, maternal nutrition is favored, possibly through immature placental development,<sup>108</sup> while in moderately malnourished or normally nourished mothers, the fetal compartment is favored<sup>108,117</sup>. A prospective study in rural Bangladesh revealed that pregnancy and lactation among malnourished adolescents resulted in weight loss and depletion of fat and lean body mass. The linear growth was hampered between 0.6 to 2.7 cm in pregnant girls compared to non-pregnant girls. This phenomenon is suggested to only occur in malnourished girls. The negative effect of pregnancy on anthropometric outcomes was larger among adolescents who became pregnant less than 2y after their menarche. 2 hypotheses were suggested. Impairment of growth and mass gain may be related to the competition for nutrients between the teenage mothers and the fetus related to insufficient nutrient intake. Or among adolescent, further elevation of oestrogen level during pregnancy may accelerate the epiphyseal closure, therefore terminating bone growth<sup>118</sup>.

Other research reported that nutrient partitioning is regulated by maternal, placental and fetal endocrine hormones, and is altered in pregnant adolescents where maternal growth is promoted at the expense of the increasing nutrient requirements of fetal and mammary gland development<sup>119</sup>. In normal pregnancies, fat stores usually increase during the 1st two trimesters, but decrease during the third trimester and immediate postpartum<sup>120</sup>. One study reported increasing maternal fat stores late in pregnancy among adolescent mothers still experiencing growth, which was related to lower birth weights. Even though they were accumulating more fat in late pregnancy and had larger gestational gains compared to non-growing pregnant adolescent or to pregnant adults, they gave births to lower birth weight infants. Moreover, birth weight was correlated to maternal energy intake only in still-growing mothers, suggesting a higher reliance of the fetus on the maternal diet than on maternal fat lipolysis for energy supply. Although weight gain and accumulation of nutrient stores seemed sufficient, mobilization of stores in late pregnancy was suggested to be reserved to maternal

growth instead of fetal growth. No data about their nutritional status were available but around 20% of them had insufficient energy intake <sup>121</sup>.

Hyperinsulinemia is known to restrict placental blood flow and to diminish lipolysis by reducing blood flow to adipose tissue. Researchers suggested that nutrient competition among pregnant adolescents may be regulated through hyperinsulinemia and insulin resistance to reduce maternal tissue uptake of glucose and to maintain adequate maternal glucose concentrations <sup>121</sup>. Also, oxidation of maternal glucose which provides fetal energy may be different if the mother is adolescent or adult. Adolescents may increase glucose production primarily through glycogenolysis while their adult counterparts do so through gluconeogenesis. Plus, it appears that pregnant adolescents do not increase glucose production by the same magnitude as pregnant adults <sup>122</sup>. Over- nourishment of pregnant adolescents may also adversely impact pregnancy outcomes. Animal studies reported a significant reduction of placental mass, birth weight, duration of gestation, quality and quantity of colostrum, and increased occurrence of spontaneous abortion in over nourished pregnant adolescents compared to normally nourished counterparts. This negative effect of overnourishment is probably caused by reduced placental growth <sup>119</sup>. Impairment of pregnancy outcomes in overnourished adolescents is related in early pregnancy to impaired proliferation of fetal cells, uteroplacental blood flows and angiogenesis. In later stages of pregnancy, placental mass is reduced in overnourished adolescents compared to normally nourished adolescents, resulting in reduced fetal nutrient uptakes. Similar animal models suggest that in pregnant undernourished adolescent, fetal growth is also reduced despite normal placenta mass, while maternal stores and transplacental glucose gradient are diminished <sup>123</sup>. Nutrient partitioning orientation in pregnant adolescents depends on maternal nutritional status and evidence remains unclear. However pregnant adolescents in developing countries have to support the triple burden of delayed growth, poor nutrient intake and childbearing, which can have adverse effect on the mother, the child or both.

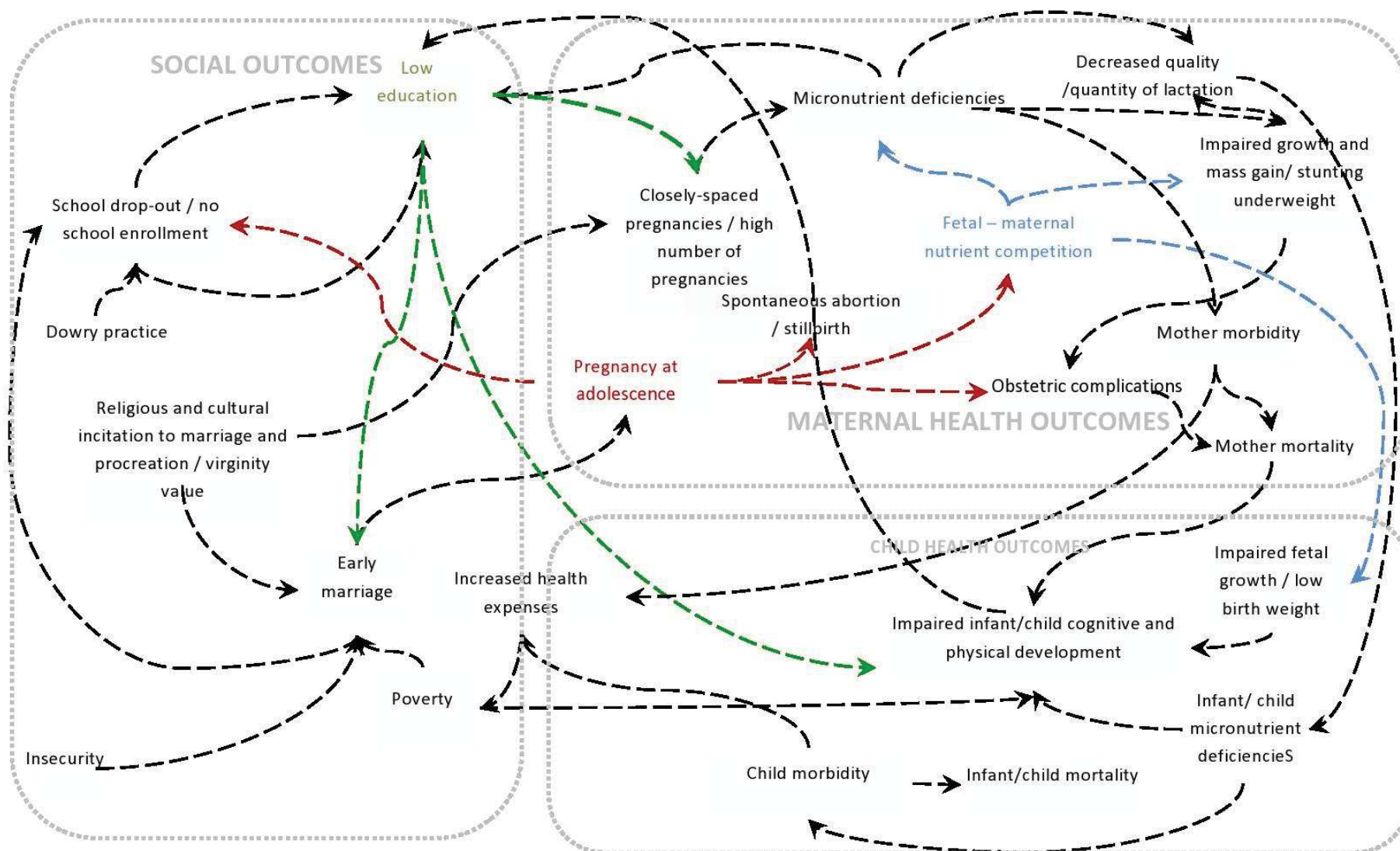
#### 1.1.3.4. Social Consequences of early pregnancies

Early marriage and childbearing may also have dramatic social consequences. Indeed, married adolescents usually drop out school and do not go back to school as they are expected to be at home to take care of the house, husband and children. Because of their poor education and the sexual past of their husbands they are also more exposed to HIV and sexually transmitted disease compared to their non-married counterparts which are likely to no have started their sex life. Because of their inexperience and their poor ability to manage a home and a family, young brides and mothers are also more likely to be divorced and abandoned, increasing their financial and social insecurity <sup>27</sup>. Premature marriage and childbearing therefore dramatically contributes to the intergenerational cycle of malnutrition and poverty.

Multifactorial health and social causes and consequences of premature pregnancies in developing countries are summarized on figure 1.



**FIGURE 1 DETERMINANTS AND CONSEQUENCES OF ADOLESCENT PREGNANCIES IN THE DEVELOPING WORLD: CONCEPTUAL FRAMEWORK**



### 1.1.3.5. Interventions to prevent early marriage and early childbearing

Consequently, specific nutrient intakes requirements for pregnant and married adolescent girls, taking needs for both adolescence and pregnancy into account, should be established<sup>100</sup>. Nutritional interventions for pregnancy and pre-pregnancy such as micronutrient supplementation should focus in priority on married adolescents. Social interventions to delay age at marriage and to promote family planning should be implemented among adolescents.

## 1.2. Prevalence of malnutrition worldwide: is nutrition a public health issue among school-aged children and adolescents?

### 1.2.1 Chronic and acute malnutrition

A review about literature from 2002 to 2009 reported prevalence of malnutrition among children aged 6 to 12 years from Latin America, Africa and Asia. In average, prevalence of stunting was 22% in Africa, 29% in SEA, and 16% in Latin America. Stunting was above 20%, indicating a public health issue, in rural school-aged children in India, Nepal, and Laos. High prevalence of stunting, between 30% and 74% were reported at national level in Guatemala, North Korea, Madagascar, Malawi and Vietnam. The review indicates average prevalence of thinness of 35% in Africa and SEA, and 6% in Latin America. According to national surveys, between 35% and 50% of SAC were classified as thin in Sri Lanka, Vietnam, Madagascar and Uganda. Severe prevalence of thinness, ranging from 77% to 90% was observed in disadvantaged settings of eastern India, Bangladesh and rural South Africa.

**TABLE 1 PUBLIC HEALTH (PH) SIGNIFICANCE OF NUTRITIONAL ISSUES** <sup>124-129</sup>

Nutrition issue	Indicator	Prevalence	Public health significance
<b>Stunting</b>	low height for age z-scores	≥20%	PH issue
<b>Vitamin A deficiency</b>	low serum/plasma retinol level	10-20%	moderate PH issue
		≥20%	severe PH issue
		≥5%	mild PH issue
	night blindness	1-4.9 %	moderate PH issue
		0.01-0.99%	severe public health problem
<b>Anemia</b>	low Hb level	5-20%	mild PH issue
		20-40%	moderate PH issue
		≥40%	Severe PH issue
<b>Vitamin B12 deficiency</b>	low serum vB12	≥5%	public health issue
<b>Iron deficiency</b>	low ferritin and high TFR	low ferritin <20% and high TFR < 10%	ID is not prevalent
		low ferritin < 20% and high TFR ≥ 10%	ID and inflammation are prevalent
		low ferritin ≥ 20% and high TFR < 10%	ID is prevalent
		low ferritin ≥ 20% and high TFR ≥ 10%	ID is prevalent



### *1.2.2 Iron, vitamin A, zinc, and iodine deficiencies*

Only the prevalence of vitamin A, zinc, iodine and iron deficiency and anemia were reported in the review by Best et al due to lack of studies available reporting other micronutrient deficiencies. Most studies on malnutrition of SAC and adolescents reports anemia and iodine deficiency <sup>17</sup>. Prevalence of iron deficiency and iron deficiency anemia was in average respectively 29% and 19% in Africa, 20% and 4% in SEA, 14% and 9% in Latin America. Iron deficiency was very high in Cote d'Ivoire, with 59% of rural SAC being iron deficient. More data are available about anemia, which was in average 29% in Africa, 32% in SEA and 14% in Latin America. In Africa, prevalence of anemia was below 10% in Rwanda and Ethiopia at national levels. But in some regions of Cote d'Ivoire, Kenya and Mali, prevalence of anemia was above 40%, indicating a severe public health issue, which was similar in rural areas of India, Philippines, Vietnam and Cambodia and in some urban and rural areas of India. National surveys reported prevalence between 23% and 38% in Thailand, the Philippines, Nicaragua and Colombia <sup>17</sup>. However, in some Asian settings, hemoglobinopathies was a greater contributor to anemia than iron deficiency <sup>46</sup>. Thus, the etiology of anemia should be carefully examined before designing any iron-containing intervention to reduce the prevalence of anemia.

The review does not report median urinary iodine concentrations (UI), which is used as criteria to assess the magnitude of IDD in population of SAC. However, prevalence of iodine deficiency defined as children below 100 µg/L was on average 33% in SEA and in Africa, and 14% in Latin America. Prevalence between 60% and 90% were reported in several African and Asian countries at national level. National surveys in Latin America reported prevalence below 10% in most cases, but in some areas of Guatemala, Bolivia and Mexico, prevalence ranged from 40% to 80%. These findings indicate that iodine deficiency is prevalent in SAC from developing countries, and is not necessarily limited to children from poor settings <sup>17</sup>. Prevalence of vitamin A deficiency among SAC was in average 32% in Africa, 17% in SEA, and 9% in Latin America. Prevalence of vitamin A deficiency ranging from 50% to 90% indicating severe public health issue was observed at national level in the Philippines, and in subpopulations of SAC of Botswana, South Africa, Kenya, Burkina Faso and India. Data about zinc deficiency are scarce. The review of Best et al suggested an average prevalence of zinc deficiency around 54% in Africa, 49% in Latin America, and 29% in SEA. Zinc deficiency ranged from 19% to 57% in most studies conducted in Africa, Latin America and SEA <sup>17</sup>.

### *1.2.3 Other micronutrient deficiencies*

Beriberi is known to be still prevalent in rural Asia because of the habit of polishing of rice, removing the outer core which contains vitamin B1. It affects principally infants and alcoholic individuals. However, prevalence of thiamine deficiency of 90% was reported in Indian SAC <sup>130</sup>. A national survey in Taiwan reported around 10% of marginal thiamin status and 8% of thiamin deficiency in SAC <sup>131</sup>. Hookworm infection, which is common in developing countries <sup>132</sup>, is a risk factor for thiamine deficiency <sup>85</sup>. Figures about thiamine status in SAC and adolescents from developing countries are very scarce. Thiamin deficiency was common among sick infants in Lao <sup>133</sup>. Thiamine fortification of staple foods such as flour was shown to be an effective simple and safe strategy to improve thiamine status <sup>134</sup>.

### **1.3. What are the structural and conjectural determinants of malnutrition in school-aged children and adolescents from the developing world?**

#### *1.3.1 Impoverished children living in urban areas are also vulnerable to nutritional deficiencies*

It is widely recognized that undernourishment is more prevalent in rural areas compared to urban areas. A review of evidence from 36 developing countries in 2005 reported consistently lower stunting and wasting prevalence in urban compared to rural areas, with HAZ being 0.5 higher, and prevalence of stunting significantly lower in most cases. Socioeconomic determinants of nutritional status were similar in urban and rural areas. In average, children in urban areas benefit from higher women education, better availability of water and sanitary facilities and higher socioeconomic status which all have a positive impact on their growth, and to a lesser extent on their weight gain. However, the authors also highlighted the enormous gap within urban areas, arguing that children from poor urban areas may be just as vulnerable as rural children <sup>135</sup>. Furthermore, a study in 2005 among 15 sub-Saharan countries reported that the discrepancy between rural and urban areas has tended narrow in the past 2 decades, due to an increase in the prevalence of urban malnutrition, and that this urban-rural difference became negligible after adjusting for SES status <sup>136</sup>. Indeed, even if health and sanitation service are more available in urban areas, differences between socioeconomic groups within urban areas are tremendous, with the poorest households living in slums at high health risks. This may explain, at least partly, similar stunting rates between the poorest in urban areas and the poorest in rural areas <sup>137</sup>. Living in urban areas is characterized by a greater reliance on cash income for food and non-food purchase, making urban residents more dependent on employment and labor. Poor urban households become heavily vulnerable to price and income changes. In Africa, urban populations tends to consume more rice and wheat, which are internationally traded, at the expenses of traditional cereals and roots <sup>138</sup>. This makes poor urban residents particularly exposed to the international market variations. Breastfeeding was reported to be 4-6 months shorter in urban areas than in rural areas <sup>138</sup> and overall, research suggests that mothers in poor urban settings combine their income-generating activities with their child care responsibilities. Their efficiency depends on their options for alternative childcare. The limited family network in urban areas may make women more isolated. The poorest will then take their child to their places of work, which can have adverse consequences on their income<sup>69</sup>. Consequently to urban lifestyle and increased female employment, home-prepared food consumption decrease at the expense of street food or processed food which are usually of poor nutritional and sanitary quality <sup>139</sup>. <sup>138</sup>. One of the coping strategies of poor urban households is urban agriculture. A positive association was found between engagement in urban agriculture and dietary adequacy indicators <sup>140</sup>as it can be an importance source of micronutrient rich foods for the family if it is consumed rather than sold <sup>138</sup>. However, urban agriculture is currently a negligible part of agricultural production and income, although it could play a substantial role in urban poverty and food insecurity reduction<sup>141</sup>.

### *1.3.2 The food price crisis and the global economic crisis intensified food insecurity and malnutrition among under-privileged populations, especially in urban areas*

The world witnessed a fuel and food price crisis in the 2000's, followed by a global financial and economic crisis in 2008, which had a worldwide impact on food security and nutrition. In 2008, the price of the food basket was 48% higher (20% in West and Central Africa, 30% in Latin America, 45% in Asia, 68% in East and East and Southern Africa) compared to the 5 previous years <sup>142</sup>. Despite the decrease of food prices in 2009 following the global financial crisis, all developing countries suffered from a decline in energy consumption between 2005 and 2010, resulting in potential 450,000 more people affected by hunger <sup>142</sup>. These numbers do not account for coping strategies <sup>142</sup>. It is known that vulnerable households first reduce the diversity and the quality of food before diminishing their energy intake <sup>143,144</sup>. For example, in the context of the food price crisis of 2008, street food was increasingly preferred because of their cheap price resulting from economies of scale <sup>145</sup>. Therefore, the price food crisis may not have resulted in lower energy intake but in lower micronutrient intake. In both cases, it is likely that the food prices crisis had increased malnutrition rates in the developing world, especially among urban poorest households <sup>142</sup>. A study in Bangladesh reported an increase in the prevalence of wasting in 24-59 months children of 5.5% in rural areas, and of 6.7% in urban areas, with a stronger effect among the poorest households <sup>146</sup>. Another study suggested that economic crises impaired the vitamin A status of mothers and young children in vulnerable populations, and that, the prevalence of vitamin A deficiency and night blindness may not have declined even after food prices declined after 2008 <sup>147</sup>. The recent food price crisis pushed many poor households to put unemployed mothers or children on the job market, which impaired child care and school attendance <sup>148</sup>. Consequently, the food price crisis has increased the vulnerability of poor urban households to food insecurity, while the global crisis caused unemployment and decreased incomes, leading to global increase of poverty and malnutrition. The nutritional status of SAC and adolescents living in poor urban settings may have been affected also but data is scarce.

### *1.3.3 The transition between preschool age and school age*

The effect on nutritional status of the transition from preschool age to school age is not well described. One can assume that under 5 years old children spend more time under the supervision of their mother or caretaker. Under 5 years old children are more likely to benefit from maternal feeding and health care than SAC. Indeed in Senegal, at the time children are enrolled to school, mothers often give a small amount of money, for their breakfast or snacks <sup>145</sup>. SAC consumed less food prepared at home than their younger counterparts. This could explain a degradation of their nutritional status at school age, which is aggravated with age, as their autonomy grows. This phenomenon could be characterized by a low prevalence of chronic malnutrition and a high prevalence of acute malnutrition. Indeed, street food or processed food that children and adolescents buy with their small allowance money often present poor sanitary and nutritional value <sup>149,150</sup>.

### *1.3.4 Double burden of malnutrition among school-aged children and adolescents from developing countries*

The prevalence of overweight was on average lower in SEA and Africa compared to Latin America with prevalence of overweight including obesity being respectively 7%, 13% and 26%. Most studies in Latin America were conducted in urban settings, but at the national level, the prevalence of overweight was between 20% and 30% in Mexico and in Brazil, and the prevalence of obesity was 9% in Mexico, and 21% in Chile, suggesting that overnutrition is not restricted to urban populations in Latin America. In urban China and India, overweight was found around 25% and obesity between 12% and 21% <sup>17</sup>.

In the past decades, many developing countries have experienced a nutrition transition related to massive urbanization and globalization and characterized with increasing consumption of energy, saturated fats, refined sugars and salt, increasing the risk of obesity and associated chronic diseases <sup>138</sup>. In 2006, Popkin reported that dietary changes, activity patterns, and large-scale decreases in food prices have contributed to the emergence of non-communicable diseases in urban areas of developing countries. New diets are characterized by higher intakes of animal protein and partially hydrogenated fats and lower intakes of fiber <sup>141</sup>. As seen above, the food price crisis and the global crisis may have blocked this tendency of increasing overnourishment. Overweight is much more prevalent in Latin America compared to SEA and Africa. Furthermore, it may be a concern only among higher states of urban societies <sup>151</sup>. A study in China reported that high SES and urban location are positively associated with frequency of intake of high-energy foods <sup>152</sup>.

To conclude, malnutrition, encompassing both under- and over-nutrition, can have serious adverse effects on the physical and development of SAC and adolescents. The prevalence of stunting and acute malnutrition, as well as iron, iodine, zinc and vitamin A deficiencies are high among SAC and adolescents in the developing world, especially in Africa and SEA. Structural and conjectural factors such as the transition between the preschool age to the school age, the urbanization, and the food prices and economic crisis in the past decade may explain poor food habits and nutritional status among SAC and adolescents in the developing world. Four publications in this thesis will illustrate these findings. Results of a cross-sectional survey among children from primary state schools of Dakar are presented in 2 papers, one about the anthropometric and micronutrient status, and the second about nutrient intake derived from 24-hours recall. In addition, 2 papers will describe the determinants of malnutrition in Cambodian school children.

## **1.4. How to evaluate malnutrition in school-aged children and adolescents worldwide?**

### *1.4.1 Anthropometry*

Child growth standards are needed to rank children relatively to others of the same age and sex and to evaluate their growth status. A growth standard represents a recommended pattern of growth associated with specific health outcomes. It should be designed to describe how children should grow in optimal conditions and should not reflect the growth pattern of a defined population in a particular time and place. Although growth pattern is highly influenced by environmental conditions during childhood such as nutrition and infection, genetics specificities

and secular trends may also have an impact on growth observed in populations. Indeed, genetics determinants may influence height, timing of puberty, and in a lesser extent, weight, fat mass and fat distribution<sup>153</sup>. Therefore one may question whether an international growth standard for SAC and adolescents would be representative for specific populations.

The need for the development of a new international growth standard of SAC and adolescents was raised because of the outbreak of childhood obesity and the release of a new international growth standard for children <5 y. Indeed the previous reference NCHS/WHO reference, the CDC2000 reference and the IOTF cutoffs are skewed which leads to underestimate obesity in SAC and adolescents <sup>154</sup>. Even if decades ago it was admitted that growth patterns were similar among different subpopulations for children 5 y when they are exposed to the same external growth factors <sup>155</sup>, the difference of the genetic impact on growth, maturation and puberty during school age and adolescence across populations remains unknown and needs to be investigated. In research comparing children, even healthy and privileged, from Africa, East Asia, South Asia, West Asia, and Europe to the NCHS/WHO reference, it was observed that secular trends in linear growth still may be occurring in some of the regions. Although African children and adolescents of upper socioeconomic status and African American achieved similar heights to the NCHS/WHO reference, children from Asia had lower heights. Finally heights of these privileged children vary less than 4cm from 7y to the beginning of the puberty, and afterwards they are around 5-6cm below NCHS/WHO reference (except for children from Northern Europe which are above the reference) <sup>156</sup>. These findings challenge the legitimacy of applying a growth reference based on a single population and of combining subpopulations to establish an international growth standard given the possible genetic differences in growth potential. Therefore, the sampling method for the development of an international growth standard for SAC and adolescents should *at minima* include multi ethnic sampling strategies designed to capture the variation in human growth patterns <sup>154</sup>. But facing the methodological heterogeneousness of datasets from various countries, WHO smoothed the 1977 NCHS/WHO reference using the original sample of well-nourished non-obese American children <sup>157</sup> which resulted in a new international reference of Height-for-Age and BMI-for-Age for children and adolescents aged 5-19 y. One may question the applicability of this reference to children and adolescents from developing countries.

Moreover, BMI is related to lean mass and fat mass but it is not able to differentiate between them, so BMI is limited to evaluate body composition, which give precious information to assess nutritional status. Assessing obesity and eating disorders using weight for height or BMI gives poor sensibility for monitoring response to treatment. Knowledge on the distribution of body fat data is needed to prevent and diagnose cardiovascular disease hypertension and type 2 diabetes which are known to start during childhood and adolescence <sup>30</sup>. In LMIC countries, data of body composition are useful to understand the tissue accretion patterns among malnourished children as well as the risks for chronic disease in populations <sup>30</sup>. The “4-component model” is suggested by Wells to be the gold standard to measure body composition <sup>30</sup>. However, it consists in collecting data on body weight, body volume, total body water, and bone mineral mass, which is expensive and complicated in field epidemiological surveys.

Cheap and easy measurements such as skinfold thicknesses and circumferences were used to estimate calorie reserve in the form of fat, and protein reserve in the form of muscle <sup>158,159</sup>. However, their use to evaluate undernourishment and body composition among children and adolescents from developing countries is limited. First, only few data of TSF and MUAC or derived indicators are available as reference for SAC and adolescents of LMIC. In the past, Frisancho developed reference for MUAC, TSF and fat area for SAC and adolescent, by gender

and age range of one year. He also established reference for muscle area by gender and height. However, these references were based on white individuals from the NCHS reference. Some references were established in only a few countries <sup>160,161</sup>. Secondly, skinfolds thicknesses do not reflect the total amount of fat in the body, thus data on skinfold thickness may poorly predict lean mass <sup>162</sup>. Between-population variability in body composition may be partly influenced by genetic factors and therefore a universal body composition standard may not be possible or relevant <sup>30</sup>. A study on infants born to European, Indian, and mixed couples living in UK revealed that both fathers and mothers contributed to ethnic differences in fetal growth and birth weight, which suggest genetic influences or a long-term nutritional consequence over generations <sup>163</sup>.

Consequently, the choice of the methodology to collect data and the absence of consensus about body composition patterns are 2 main issues in the assessment of body composition of children and adolescents from LMIC. Therefore the new international reference of WHO 2007 of height for age and BMI for age is challenged as an international standard.

#### *The example of Cambodian SAC vs. Senegalese SAC*

In this thesis, anthropometric status of SAC from Cambodian and Senegalese samples according to various references is presented in table 2. Height and weight were lower in Cambodian children, which were younger than Senegalese children. Cambodian children were more stunted than Senegalese children. HAZ, BAZ, WAZ were significantly lower in Cambodian children. Undernourishment was significantly more prevalent in Cambodian children compared to Senegalese children according to prevalence of low BMI, low BMI, low TSF, low MUAC, low muscle area, low muscle circumference and low fat area. However, ratio of muscle-arm area and ratio muscle-fat area were significantly higher in Cambodian children than in Senegalese children, suggesting relatively more lean mass and less fat mass. These findings may result from different nutritional past in the 2 populations, secular trends, or genetic determinants.

#### *1.4.1 Micronutrient indicators*

WHO recommends indicators to assess folate, iron, vitamin A and iodine deficiency in populations (table 3). However, only for iodine deficiency cut-offs were established based on populations of SAC. No official indicator of other micronutrient status are available, therefore cut-offs and indicators used in SAC and adolescents vary across studies.



**TABLE 2 EVALUATION OF ANTHROPOMETRIC STATUS OF CAMBODIAN AND SENEGALESE SCHOOL-AGED CHILDREN AND ADOLESCENTS**

	N	Mean value	SD	N	Mean value	SD	P-value*	Cut-offs and interpretation	Prevalence (%)		P-value**	Range	Reference
	<i>Cambodia</i>			<i>Senegal</i>					<i>Cambodia</i>	<i>Senegal</i>			
Height (cm)	2549	<b>124.5</b>	11.4	602	<b>138.7</b>	14.3	<0.001	-					
Height for Age (z-scores)	2549	<b>-1.8</b>	1.1	602	<b>-0.1</b>	1.2	<0.001	<-3 z-scores : severe stunting	<b>12.3</b>	<b>0.7</b>	<0.001	5-19y	NCHS (National Center for Health Statistics) 1977
							<0.001	[-3,-2[ z-scores : moderate stunting	<b>29.1</b>	<b>4.2</b>			
Weight (kg)	2550	<b>22.7</b>	5.6	602	<b>30.1</b>	9.6	<0.001	-					
Weight for Age (z-scores)	1483	<b>-1.8</b>	0.9	292	<b>-0.6</b>	1.2	<0.001	<-3 z-scores : severe wasting	<b>11.4</b>	<b>1.0</b>	<0.001	5-10y	NCHS (National Center for Health Statistics) 1977
								[-3,-2[ z-scores : moderate wasting	<b>30.3</b>	<b>5.8</b>			
BMI (kg/m <sup>2</sup> )	2549	<b>14.4</b>	1.3	602	<b>15.2</b>	2.3	<0.001	BMI corresponding to adult BMI <16 :thinness grade 3	<b>5.9</b>	<b>6.5</b>	<0.001	2 - 18y	pooled national surveys (Brazil, Great Britain, Hong Kong the Netherlands, Singapore, USA)
								BMI corresponding to adult BMI [16;17.5[: thinness grade 2	<b>15</b>	<b>9.1</b>			
								BMI corresponding to adult BMI [17;18.5[: thinness grade 1	<b>39.5</b>	<b>32.7</b>			
								<-3 z-scores : severe thinness	<b>5.0</b>	<b>5.8</b>			
BMI for Age (z-scores)	2541	<b>-1.5</b>	0.9	602	<b>-1.1</b>	1.1	<0.001	[-3,-2[ z-scores : moderate thinness	<b>20.6</b>	<b>12.8</b>	<0.001	5-19y	NCHS (National Center for Health Statistics) 1977
								]1;2] z-scores : overweight	<b>0.1</b>	<b>0.0</b>			
								>2 z-scores : obesity	<b>0.0</b>	<b>0.3</b>			

\* T-test

\*\*  $\chi^2$  test

**TABLE 2 (CONTINUED) EVALUATION OF ANTHROPOMETRIC STATUS OF CAMBODIAN AND SENEGALESE SCHOOL-AGED CHILDREN AND ADOLESCENTS**

	N	Mean value	SD	N	Mean value	SD	P-value*	Cut-offs and interpretation	Prevalence (%)		P-value**	Range	Reference
	<i>Cambodia</i>			<i>Senegal</i>					<i>Cambodia</i>	<i>Senegal</i>			
MUAC (cm)	2550	<b>16.8</b>	1.7	601	<b>18.5</b>	2.4	<0.001	<5th percentile (for age range 1 y)	<b>66.6</b>	<b>38.8</b>	<0.001	1-75 y	
TSF (mm)	2549	<b>6.4</b>	2.0	602	<b>7.6</b>	3.2	<0.001	<5th percentile (for age range 1 y)	<b>47.1</b>	<b>32.9</b>	<0.001	0-44 y	
								<9mm (girls), 7mm (boys) : underweight (corresponding to 15% fat and 7%fat)	<b>58.4</b>	<b>44.9</b>	<0.001	10-17 y	
Muscle area (mm <sup>2</sup> )	2549	<b>1770</b>	367	601	<b>2096</b>	500	<0.001	<5th percentile (for age range 1 y)	<b>43.1</b>	<b>21.1</b>	<0.001	1-75 y	US health and nut examination survey 1971-74 - whites
								<5th percentile (for height range 2 cm)	<b>18.6</b>	<b>25.8</b>	<0.001	84-180 cm	
Muscle circumference (mm)	2549	<b>148</b>	15	601	<b>161</b>	19		<5th percentile (for age range 1 y)	<b>43.0</b>	<b>20.8</b>	<0.001	1-75 y	
Fat area (mm2)	2549	<b>513</b>	196	601	<b>669</b>	355	<0.001	<5th percentile (for age range 1 y)	<b>61.0</b>	<b>41.8</b>	<0.001	1-75 y	
Proportion muscle / arm area (%)	2549	<b>77.7</b>	5.4	601.0	<b>76.7</b>	6.1	<0.001						
Ratio muscle area/fat area (%)	2549	<b>3.7</b>	1.1	601	<b>3.5</b>	1.1	<0.001						
Age (years)	2549	<b>9.5</b>	2.4	602	<b>10.2</b>	2.4	<0.001						

\* T-test

\*\*  $\chi^2$  test



**TABLE 3 INDICATORS OF MICRONUTRIENT STATUS AT POPULATION LEVELS AS DEFINED BY WHO**

<i>Micronutrient</i>	<i>Indicator</i>	<i>Cut-off</i>	<i>Interpretation</i>	<i>Conditions</i>	<i>Based on</i>	<i>apply on</i>
<b>Iron</b>	ferritin	< 15 ug/L	depleted iron stores	known as sensitive to inflammation (cf <b>Chapter 5.1</b> )	based on studies examining ferritin and microcytic anemia, or response to iron, or bone marrow	≥5 y except pregnant women)
		< 30 ug/L	depleted iron stores in the presence of inflammation			<5 y
		> 200 ug/L	severe risk of iron overload			adults
	transferrin soluble receptor	> 5 - 8 mg /L	tissue iron deficiency	observed as sensitive to inflammation (cf <b>Chapter 5.1</b> )	depends upon the commercial assay used	-
<b>Iodine</b>	urinary iodine	median < 20 ug/L	insufficient iodine intake, severe iodine deficiency	single urine sample	studies on populations of SAC (≥6 y)	all age groups, except pregnant and lactating women
		median 20-49 ug/L	insufficient iodine intake, moderate iodine deficiency			
		median 50-99 ug/L	insufficient iodine intake, mild iodine deficiency			
		median 100-199 ug/L	adequate iodine intake and nutrition			
		median 200-299 ug/L	iodine intake above requirements, risk of more than adequate iodine intake			
		≥ 300 ug/L	excessive iodine intake, risk of iodine-induced hyperthyroidism, autoimmune thyroid disease			
<b>Vitamin A</b>	serum/plasma retinol	<0.35 umol/L	severe vitamin A deficiency			6-71 months
		0.35-0.7 umol/L	moderate vitamin A deficiency			24-71 months
<b>Folate</b>	serum/plasma folate levels	< 4 ng/mL	folate deficiency		based on homocysteine concentrations as metabolic indicator, in american men and women aged 30y and older	all age groups, except pregnant women and WRA (in which red blood cell folate level < 400 ng/mL indicates folate insufficient to prevent NTDs)
	red blood cell folate level	< 151 ng/mL				

## **1.5. Review of coverage and cost-effectiveness of school feeding programs and staple food fortification: how to improve nutrition among school-aged children and adolescents?**

### *1.5.1 School feeding programs (SMP)*

#### *1.5.1.1 Progress of coverage of school feeding program*

WFP evaluated the worldwide situation of school feeding in 2013 through the analysis of 169 countries. 368 millions of children receive a meal every school day, of which 30 million in Africa and 168 million in SEA and Pacific. 25 million of these daily meals are distributed by WFP. In low income countries only 18% of children receive free school meals, as school feeding is usually only available in some areas selected for their vulnerability.<sup>29</sup> Between 2008 and 2013, 39 countries have scaled-up school feeding in response to a crisis with respectively 13, 12, 8, and 5 countries regarding to the food crisis, an armed conflict, a natural disaster or the financial crisis respectively<sup>29</sup>. For example, the number of beneficiaries in Haiti has been tripled following the recent earthquake. In Togo, responding to the food crisis, 20,000 children living in food-deprived rural areas started in 2010 to be part of the school feeding program<sup>164</sup>. School feeding consists in providing school meals or snacks to be eaten during school hours or in distributing dry take home food rations to children at the end of each month if they attended school regularly<sup>29</sup>.

#### *1.5.1.2 Benefits: decreasing hunger, incentive for school attendance, improvement of nutritional status*

Research shows that providing school meals, snacks, and take-home rations through school feeding programs can alleviate short-term hunger, increase children's abilities to concentrate, learn, perform specific tasks, and school meals have been linked to an increase in the enrolment of girls. These effects seem to be greater among children who are also chronically undernourished, usually the poorest children<sup>164,165</sup>. Attendance is increased by 2 to 17 days per year depending on studies<sup>165</sup>. For example, improvement of cognition scores was observed in Jamaica, with a stronger effect in undernourished children<sup>166</sup>, and in Kenya<sup>20</sup>. Math scores, physical activity, behaviors, and muscle mass were also improved in Kenyan children as well as height gain in those who were stunted<sup>20</sup>. School feeding even had a beneficial impact on weight gain in younger siblings of beneficiaries of take home rations in Burkina Faso<sup>167</sup>. In Bangladesh, the implementation of school meals increased the attendance by 6% and the enrollment by 14%<sup>11</sup>. Therefore, school feeding interventions can have a positive impact on health and schooling outcomes, even if the food is not fortified. Local food supply for school feeding, often known as "Home Grown School Feeding" (HGSF), can be a strategy to offer more dietary diversity and fresh products<sup>29</sup>. HGSF programs conducted in Brazil and Chili<sup>29</sup> were successful to stimulate the economy, which could have long-term beneficial effects on reduction of poverty and nutrition in the community.

### 1.5.1.3 Costs of school feeding

According to WFP, school feeding worldwide represents an annual investment of 75 million USD. In low income countries, school feeding is estimated by WFP to cost on average 56 USD per child per year <sup>29</sup>. A cost-effectiveness study estimated the costs and the benefits of unfortified school meals in 6 countries of Sub-Saharan Africa <sup>165</sup>. Costs were standardized for 200-day school year and a 700 kcal ratio and took into account pipeline breaks. It varied between 28 and 68 USD per child and per year. The cost of school feeding varies according to location, geography, type and amount of commodities, number of children. Cost is higher for example in landlocked countries due to higher transport costs. A larger number of children allow economies of scale. Commodities accounted for more than half of the costs. The rest consists in transport, storage, handling and direct operational costs. Cost of school feeding per outcome were estimated in the review : 18 USD per IQ point, 33 USD per point on math achievement or aptitude, 2-8 USD per extra day of attendance, 27-160 USD per additional cm of height and 55-160 USD per additional kg of weight. Effects appear significant but small, but they may have been underestimated. Effect of school feeding program on IQ, weight gain and attendance are similar to those of other school health and nutrition interventions, such as iron supplementation, deworming or malaria prevention, which cost less than 4USD per child and per year. However, these costs may not have been calculated as accurately as the cost of school feeding program.

### 1.5.1.4 Challenges and perspectives of school feeding in developing countries

Given the drastic increase of school meals distribution in the past decade <sup>29</sup>, the challenge is not any longer to demonstrate the need to implement school feeding programs but to determine how to improve the efficiency of these programs. Food basket, size of rations and nutritional value of meals are highly variable among countries <sup>29</sup>. Tracks to diminish costs and improve the health benefits of school feeding program should be explored. Distributing fortified biscuits, which cost 11-12 USD per child per year, may reduce the cost as well as the substitution effect (children less fed at home when they are fed at school) <sup>165</sup>. Targeting undernourished children, according to areas, the times of year when poverty is highest and the attendance lowest would increase the effect and reduce the cost of feeding all children all year along. Home grown school feeding program (HGSF) is a new concept and only a few countries implement it on a national scale. However, these programs are becoming popular among governments, partners and donors, giving the benefits of HGSF on local production and increasing availability of funding for school feeding <sup>168</sup>.

Local procurement of food is a possible means to achieve sustainable programs, to reduce costs and at the same time to stimulate local agricultural production<sup>164,165</sup>. It is critical that long-term sustainability is incorporated into programs from their inception, and that programs are continuously revisited as they evolve. As more and more governments seek to expand these programs in their countries, it is important to have more opportunities for knowledge sharing among developing countries that focus on ways to improve the procurement of locally available nutritious foods and compare best practices <sup>164</sup>. Combining SFP with other interventions such as micronutrient fortification or deworming is another strategy to enlarge the impact on health and schooling outcomes <sup>165</sup>. School feeding program can integrate food fortification,

deworming, nutrition education and access to clean drinking water. Galloway et al recommend to implement accurate cost-effectiveness studies of school health and nutrition interventions to identify the most cost-effective package of interventions <sup>165</sup>.

### *1.5.2 Rice fortification, a promising strategy to prevent micronutrient deficiencies*

#### *1.5.2.1 Why choosing rice as a vehicle for fortification?*

Food fortification such as iodization of salt, fortification of milk with vitamin D, and enrichment of flour and bread with vitamins B and iron has significantly contributed to the disappearance of goiter, rickets, beriberi, and pellagra, respectively caused by iodine, vitamin D, vitamin B1, and vitamin B3 deficiency in the United States since the early 20<sup>th</sup> century <sup>169,170</sup>. More recent trials have demonstrated beneficial impact of fortified foods on nutritional outcomes : in Vietnam, fortified fish sauce improved iron status and reduced anemia, fortification of monosodium glutamate in the Philippines, of margarine in Denmark the Philippines, and of sugar in Central America diminished vitamin A deficiency prevalence, and fortified milk reduced vitamin D deficiency in US <sup>171,172</sup>.

Micronutrient deficiencies are associated with polished rice consumption in many developing countries. Although paddy rice is a natural good source of thiamine, riboflavin and niacin, hulling and milling turn polished rice into a poor source of micronutrients. Rice represents one to two thirds of caloric intake in 17 countries of Asia and the Pacific and in 6 Sub-Saharan countries, indicating diets lacking in diversity. Prevalence of anemia, stunting and thiamin, riboflavin and vitamin A deficiencies are generally higher in populations where polished rice is consumed <sup>173</sup>.

#### *1.5.2.2 Current knowledge about the efficiency of fortified rice*

Gains on health and schooling outcomes of fortified rice interventions among SAC were reported by several studies (table 4). Plus, compared to other interventions aiming at increasing micronutrient intake, food fortification is cheaper and has a high cost-benefit ratio <sup>174</sup>.

**TABLE 4 EFFECTIVENESS OF RICE FORTIFICATION AMONG SCHOOL CHILDREN IN DEVELOPING COUNTRIES**

Country / Year	Sample	Study method	Study results	Fortified rice
India 2007- 2008 <sup>175</sup>	140 children 5-11 participating into the MD meal program (at BL, around 40% of anemia, 30 % ID, 15% of IDA in both groups)	double blind placebo controlled trial 8 months (166 days of consumption) FR-MDM (fortified rice-Midday Meal) vs. UFR-MDM unfortified rice-Midday meal	improvement of ferritin $8.2 \pm 2.1 \mu\text{g/L}$ in FR-MDM group vs. decrease in control group ( $P<0.001$ ). decrease of ID prevalence (-19% in FR-MDM group $P<0.05$ ) increase in control group +6%	Extruded Iron Ultra Rice (9.6 mg FE /g blended with natural rice 1.6:98.4 blend ratio  corresponded to 19mg iron/d in MFPP (Micronized ferric pyrophosphate)  <b>15 mg FE /100 g blended raw rice</b>  meant to meet the gap to fulfill iron RDA for Indian children
India 2006 <sup>176</sup>	184 children with depleted iron stores 6-13 y participating into a school lunch program	double blind randomized placebo controlled trial 7 months iron fortified rice vs. non fortified rice in lunch meal	larger improvement of ferritin $+9.5 \mu\text{g/L}$ in iron group vs. $+2.3 \mu\text{g/L}$ in control group ( $P<0.05$ )  decrease of ID/IDA prevalence -53%/-15% in iron group vs. -30%/-1% in control group ( $P<0.001$ )  no effect on anthropometry and morbidity	Extruded iron-fortified rice (10 mg FE/g as MGFP 1:50 blend ratio corresponding to 20 mg iron/d in MGFP (Micronized ground ferric pyrophosphate)  200-300 g cooked rice daily  <b>20 mg FE /100 g raw blended rice</b>

Country / Year	Sample	Study method	Study results	Fortified rice
Thailand 2008 <sup>177</sup>	203 children with low zinc status 7-12 receiving lunch meals and daily milk (but not during the summer before the study)	double blind randomized placebo controlled trial 5 months iron-vitamin A-zinc fortified rice vs. non fortified rice in lunch meal	Larger improvement of serum Zn in the fortified rice group +11.3 µmol/L vs. +10.6 in the control group (P<005). No effect on FE or VA status (not deficient)	Hot-extruded fortified rice (10mg FE, 9 mg Zn, 1050 µg VA/g, 1:50 blend ratio)  assumption : increase of intake by 97.5%, 10% of bioavailability of Fe, moderate bioavailability of Zinc, 40% of loss of vitamin A  <b>20 mg FE, 18 mg zn, 2100 µg VA /100 g or raw blended rice</b>
India 2009-2010 <sup>130</sup>	258 children 6-12 y (prevalence of ID, VAD and ZnD<10%)	double blind randomized placebo controlled trial 6 months low-iron fortified rice vs. high iron fortified rice vs. unfortified rice in lunch meal	in high-iron and low iron fortified rice group VS control, larger improvement of plasma vitamin B12 (+92 pmol/L and +105 pmol/L vs. 20 pmol/L P<0.001), plasma homocystein (-2.6 and -2.3 µmol/L vs. 0 µmol/L P<0.05 and physical performance (respectively – no effect on iron, zinc, vitamin A status, or cognition)	DSM fortified rice  <b>low-iron rice 6.25 mg FE/ 100 g blended raw rice</b>  <b>high iron rice 12.5 mg FE/100 g blended raw rice</b>  Micronized ground ferric pyrophosphate  Assumptions: to provide 40-50% of RNI for vitamin A, thumaine, niacin, vitamin B6, folate, iron, zinc for 7-9y

### 1.5.2.3 Technologies to fortify rice

The four technologies currently available to produce fortified kernels assigned to be blend to unfortified rice are described in table 5.

**TABLE 5 TECHNOLOGIES TO PRODUCE FORTIFIED RICE KERNELS** <sup>178</sup>

Technology	Description
hot extrusion	Dough made of rice flour, the micronutrient mix and water passes through an extruder which cut it into grain like structures, at high temperatures (70-110°C)
cold extrusion	Similarly, rice-shaped kernels are produced through an extruder, but at temperatures below 70°C. Cold-extruded kernels are easier to distinguish from real rice than hot extruded kernels
coating	The fortificant is mixed with waxes and gum, and then sprayed on the surface of rice kernels in several layers,, which are afterwards mixed with unfortified rice
dusting	Similarly to flour fortification, micronutrient powder is simply dusted on rice grains and sticks to it because of electrostatic forces.

The choice of the technology depends on investment capacities and the level of consumer preferences : the fortified rice can be homogenous or on the contrary, fortified rice can be colored to be distinct from non-fortified rice, indicating higher nutritional value <sup>178</sup>. Dusting technology is cheap as no blending is needed, but it is unsuitable for developing countries where rice is washed before cooking. Coated rice usually leads to changes in color, smell and taste. The extrusion technology is considered as the most adapted to areas where populations wash or rinse the rice and it produces acceptable rice for taste and appearance <sup>178</sup>. Several studies had demonstrated the acceptability of extruded fortified rice <sup>179,176,175</sup>. With hot extrusion, kernels are almost undistinguishable from natural rice (transparency, sheen, consistency, and flavor) while cold extrusion produces more opaque kernels. However, hot extrusion implies high investment costs therefore coating and cold extrusion are more appropriate for small or pilot programs while hot extrusion is more suitable for long-term rice fortification programs <sup>178</sup>.

### 1.5.2.4 Selection of fortificants and fortification levels regarding to desired impact and toxicity

It is recommended to choose a condiment or staple food as vehicles for food fortification. Condiments are usually fortified with only one or 2 nutrients while several minerals and vitamins can be added to cereals without modifying the taste and the color. Combining iron-fortification of 2 foods may be useful to minimize the alteration of taste and color <sup>173</sup>. Vitamin A and iron deficiencies are widely spread in the developing world and were shown to be reduced



using fortification and supplementation. For example, flour fortification with folic acid drastically diminished the incidence of NTDs in the USA. In addition, zinc supplementation can reduce mortality and diarrhea prevalence and might improve growth. Vitamin B12 is only present in animal source foods which are usually hardly consumed in non-affluent populations due to the relative high price. Therefore iron, vitamin A, folic acid, zinc and vitamin B12 are recommended as rice fortificants <sup>180</sup>. Recent studies revealed insufficient intake of vitamin B6, niacin and thiamin in several Asian subpopulations. Also, beriberi was shown to be prevalent in SEA. Therefore, thiamin, niacin and vitamin B6 are recommend additions too <sup>180</sup>. Vitamin B2 (riboflavin), vitamin C, iron as ferrous sulfate and beta carotene can change the color of the fortified rice kernels <sup>173</sup>, perhaps reducing acceptability. Currently, ferric pyrophosphate is the preferred form of iron as fortificant in rice, although bioavailability is lower than from for example iron sulphate <sup>176,178</sup>. Retinyl esters are an alternative form to vitamin A which does not affect the color of fortified kernels. Dry forms of vitamin E, vitamin B1, niacin, folic acid, vitamin B12, zinc as zinc oxide and selenium are all compatible with rice fortification. Vitamin K, biotin, pantothenic acid, vitamin B6 can also be added to rice without any impact on taste or color <sup>173</sup>. Ideally, the WHO/FAO recommend to set fortification level following : measurement of dietary micronutrient intake among populations at risk for deficiency followed by estimation of the bioavailability of zinc and iron in the local diet in order to compare the absorbed amount to requirements <sup>171</sup>. But such data are often unavailable in developing countries. Therefore, based on flour fortification level and on the same rationale linked to rice fortification, de Pee proposed recommended levels (mg /100g) to achieve intake that meets the estimated average requirements of adults <sup>180</sup> (table 6).

**TABLE 6 RECOMMENDED NUTRIENTS AND NUTRIENT LEVELS (MG/100 G OF RICE) FOR RICE FORTIFICATION WHERE RICE CONSUMPTION IS 150-300 G PER CAPITA PER DAY BY DE PEE**

Iron	Folic acid	Vitamin B12	Vitamin A	Zinc	Thiamin	Niacin	Vitamin B6
7	0.13	0.001	0.15	6	0.5	7	0.6

Bioavailability of iron and zinc related to phytate content of polished rice were accounted. Folic acid fortification level is established to meet the RNI for anyone older than 14 y while levels for vitamin B12, B6 and B3 meets the EAR <sup>180</sup>. Toxicity level of some micronutrients should be carefully taken into account when fortificant levels are established. Vitamin A toxicity can result in liver toxicity, loss of appetite ophthalmological and dermatological disorders. Vitamin D toxicity is associated with hypocalcaemia and associated disorders. Excessive iron results in gastrointestinal damage, liver necrosis and formation of free radicals <sup>181</sup>. Consequently, iron, vitamin A (retinol) and, vitamin D, are classified as nutrients with a small safety margin, with upper intake levels less than 5 times superior to the recommended intake for which fortification level should be carefully considered <sup>181</sup>. Indeed, the RNI for vitamin A is close to upper intake, which raises concern when considering vitamin A fortification, so recommended levels correspond to 25% of the RNI of women preschool and SAC <sup>180</sup>. This limitation is questioned as it was set for affluent populations and could conflict with the need to increase intake in less developed countries. Furthermore, vitamin A capsules distributed to young children contain a much higher dose that the upper intake level. Therefore vitamin A level could be increased <sup>182</sup>. In order to stay below the upper intake level for niacin of 35 mg/day, recommendation for niacin level should be carefully adapted to the prevalence of inadequate



intake and the consumption range of fortified rice. As for vitamin A, much higher doses of niacin, up to several grams/d are given as medication to lower LDL concentrations. Vitamin E, B6, B12 and C are classified in intermediate category, while vitamin K, thiamine, riboflavin, pantothenic and biotin are harmless even at 100 times the recommendation<sup>181</sup>. There is no upper limit for vitamin B12, thiamin and vitamin B6. However, because levels of fortification are determined as function of the dietary needs of healthy adults, vulnerable subgroups with high requirements such as pregnant and lactating women and infants may need additional specific interventions. In the case of SAC, fortification for adolescent girls at risk to be pregnant may not be sufficient to meet their high nutrient requirements. For example Iron-folate tablets will be needed for pregnant women.

#### 1.5.2.5 Costs of rice fortification programs

Implementing fortification of rice may be useful if the population consume at least 100g of rice daily (36 kg/year)<sup>178</sup>. Costs linked to production and distribution of fortified rice consists in production and transportation of fortified kernels, in blending of fortified kernels with unfortified rice, and in distribution or sales of rice<sup>174</sup>. However, costs associated to pre and post-implementation research, and costs to support and monitor the rice fortification program have to be taken into account too.

Roks analyzed the cost of rice fortification at each step<sup>174</sup>. Installing respectively a hot or a cold extrusion facility cost around \$4 million (which covers 1.5 to 8 million people, depending on annual consumption) and \$0.5 million (which covers 0.3 to 2.5 million people) respectively, and start to be profitable from an estimated yearly demand of 1500 MT or 300 MT of fortified kernels<sup>178</sup> respectively. The method to dry extruded fortified kernels also has an impact on the overall cost: natural dry may be cheaper than using a dryer system but it can affect the quality and hygiene of kernels<sup>174</sup>. The cost of investment can be supported by milling industry, e.g based on perceived market potential or corporate social responsibility, but external support may be needed too. Broken rice is a by-product of the rice milling industry and can be used to produce rice powder intended to be mixed with micronutrients to produce fortified rice kernels. Thus, fortification of rice allows to add value to broken rice<sup>174</sup>. Cost to produce fortified kernels vary according to several parameters<sup>174</sup>. A lower mixing ratio associated with higher content of micronutrient in kernels would be more cost effective but could be a problem for taste or look of fortified rice. Blended ratio is usually between 1:200 (0.5%) and 1:50 (2%)<sup>174</sup>. The cost usually decreases when the order size increase. Rice fortification is practical to implement in mills who product more than 5 MT per hour or 15000 MT / year<sup>174</sup>. The overall cost of production is \$1.89 to \$6/kg of fortified kernels, 2-2.5 /kg on average, of which ~50% (\$1 - \$1.4) is allocated to the actual production of the fortified kernel<sup>174</sup>. The higher overall cost of rice fortification compared to other food fortification is associated with the synthesis and blending of artificial kernels, thus the price of the micronutrient premix has only a small impact on the cost of fortified kernels production (26% to 33%), and hence a small impact on the overall cost of rice fortification program. Therefore the choice of micronutrients and their amount is supposed not to be limited for economic reasons, but to be guided also by nutrient requirements and safety (see above)<sup>174</sup>. As an example, if producing fortified kernels cost 3USD/kg (of which 1 USD/kg represents the fortificant premix cost), the blending ratio is 1:100, and the price of normal rice is USD 400/MT, the cost to fortify 1MT of rice will be:

$400 \times 99\% + 3000 \times 1\% = 426$  USD, therefore fortified rice will be 6% more expensive than unfortified rice. For populations consuming between 100 and 300 g of uncooked rice per day, the additional cost of fortified rice compared to unfortified rice is estimated to be between \$0.22 and \$2.18 per year and per person <sup>178</sup>. For example, a fortified rice providing more than 100% of EAR of vitamin B12, folic acid, vitamin B1, between 50% and 76% of vitamin A, niacin and zinc EAR, and 18% of iron EAR to women consuming 200 g of rice per day would only cost an additional 0.22\$ per woman per year<sup>178</sup>. This only includes the price to produce fortified kernels, not the additional program costs.

Overall, more research about costs of rice fortification is needed. The fortified kernels cost could be supported by the consumer in commercial settings. When distributed, the additional cost could be covered by the party who operates the existing rice distribution, as national government or WFP, supported by donors. Fortified rice expires one year after blending, due to decrease micronutrient content and product acceptability rather than harmful effects <sup>174</sup>. To our knowledge, facilities to produce hot or cold extruded fortified kernels are only available in Asia (China, India, the Philippines) and in Latin America (Colombia, the Dominican Republic, Brazil, Costa Rica). <sup>174</sup>. Consequently, the cost of transportation of fortified rice kernels have to account transportation from abroad to the country, which vary according to the mode of transport, quantity and market price, and importation fees and customs clearance fees. Then, costs of transportation inside the country depend on distance, complexity of the supply chain. In order to blend fortified kernels with unfortified rice, an accurate proportioning device has to be added at the end of the processing line in sophisticated rice milling facilities, which usually cost up to \$20,000, but which will not need extra labor cost once installed.

Otherwise simple manual mixers cost approximately \$200 but need extra labor cost <sup>174</sup>. If the rice milling is centralized there will be less logistic difficulties and the cost efficiency of rice fortification would be higher <sup>174</sup>. Fortified food are meant to be distributed through the existing sales or distribution tracks for unfortified food, therefore no additional cost related to distribution or sales should be input to food fortification. However, in case fortified rice is to sell, social marketing expenses may be needed <sup>174</sup>. Scale-up the distribution of fortified rice will allow economy scales on fortified kernels production and transportation costs.

#### 1.5.2.6 Key success factors and challenges of rice fortification

To ensure efficiency of rice fortification, sensory properties of rice as taste, smell and texture should be similar to those of unfortified rice, fortificants have to be stable and bioavailable, fortification has to be regulated by authorities and accepted by the target population <sup>174</sup>. Keys success factors for implementation of rice fortification were identified by Piccoli et al <sup>173</sup>:

- adequate per capita rice consumption : 70-100 g uncooked fortified rice/ person/day or 100-200 g cooked fortified rice /person /day
- high level of micronutrient deficiencies
- Existence of government-sponsored or manager safety nets: India, Indonesia and Bangladesh implemented public safety net programs distributing free or highly subsidized rice, targeting low income households, adolescent girls, under 5 years old children, pregnant and lactating women or SAC. When it is possible, WFP use fortified food in its program in disaster-stricken areas and its school feeding program.
- Large market size and awareness of consumers for market-driven fortification: If consumers are aware of the benefits of fortified rice, they may be willing to pay the

small additional price linked to rice fortification, which will be as reduced as the market is large, due to scale economies.

- Consumer awareness and/or acceptance: as rice is a staple food in many countries, the type of rice with specific shine, color or taste can have social connotations rooted in traditions. Therefore, if they are aware of the benefits of fortification, they may be willing to pay for and to consume fortified rice, which is not likely to happen among poor and uneducated populations. This could be addressed by safety net or subsidy strategies.
- Adapted technologies: acceptance etc. In countries where soaking and washing are practiced, coating and dusting technologies are not relevant
- adequate capacity in the rice processing industry : fortification of rice programs would be more effective if there are a few large mills with appropriate technology rather than many smaller mills
- No or limited restrictions on rice trade : subsidies to increase fortified rice consumption and production, and the reduction of import barriers for fortified rice kernels
- ease of doing business : rice fortification will be more easily implemented in countries less affected by corruption and bureaucracy

To conclude, school feeding and rice fortification are promising nutrition interventions likely to improve nutritional status of SAC in developing countries. School feeding is being widely implemented in the world, including developing countries, in order to improve school attendance and concentration but cost-effectiveness and nutritional benefits could be improved through food fortification. Fortification of rice is promising for bridging the nutrient gap in poor settings. Combining rice fortification with school feeding program could be a cost-effective way to increase micronutrient intake. Effects on the use of rice fortified with multiple micronutrients on micronutrient status and cognitive performance are reported in this thesis in 2 different papers (chapter 4).

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***Chapter 2. Nutritional status and  
its dietary determinants of urban  
African school-aged children and  
adolescents: Case study in Senegal***

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## **2.1. Anthropometric and micronutrient status of school-children in an urban West Africa setting: a cross-sectional study in Dakar, Senegal (Published paper)**

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### *Introduction*

The prevalence of food insecurity in Sub-Saharan Africa is the highest in the world, with rates as high as 30% of the population being undernourished <sup>183</sup>. For instance, 26% of the population in Senegal is undernourished, which ranks it 155<sup>th</sup> over 187 countries in the 2011 Human Development Index <sup>184</sup>. Due to considerable rural migration and urbanization during the last decades, 42% of the Senegalese population now live in urban areas <sup>185</sup>. Many households in the Dakar area are therefore without basic infrastructures, while simultaneously vulnerable to food insecurity<sup>186</sup>. Moreover, albeit the general assumption that urban populations have access to more diversified foods, studies in West Africa have shown that micronutrient status can be low in urban areas <sup>187</sup>.

Nutrition interventions generally neglect school children despite their high prevalence of malnutrition and micronutrient deficiency <sup>17</sup>. Iodine, iron and folic acid micronutrient deficiencies affect the development of the brain and cognitive functions of school children <sup>36</sup>. Iodine deficiency, even mild, could impede full intellectual potential <sup>188</sup> with differences in intellect as large as 10 – 15% between iodine deficient and non-deficient populations. On the other hand, deficiencies of vitamin A and zinc are associated with different scenarios affecting school performance, such as absenteeism due to increased morbidity <sup>189,190</sup>.

Data on nutritional status of school children in Senegal are scarce and recent data on school children living in urban areas are lacking. A study in 1994 conducted on a representative sample of 774 children (aged from 5 to 15 y) of state primary schools of the Dakar department showed that 34% of pupils were anemic, 10% underweight, 5% stunted and 11% wasted <sup>191</sup>. More recent, a study on Senegalese food practices and nutrition was conducted to identify strategies to reduce malnutrition <sup>145</sup>. New food practices are emerging in urban areas with food prepared at home decreasing, while street foods purchases increased. Furthermore, when a child reaches school age, it can be observed that mothers take less care of their children's diet. The rarity of school canteens in urban state school further reduces meal opportunities for children <sup>145</sup>. While school children consume less meals with adults, an increasing number of households can't afford to spend money on snacks for their children. Many children declare having difficulties to concentrate in class due to hunger <sup>186</sup>.

It is therefore likely that the nutritional intake of foods from school-aged children in Senegalese urban areas is inadequate. The main objective of the study lies in the assessment of the anthropometric and micronutrient status of school children attending state primary schools in Dakar and suburbs. The Dakar region was selected because it has the highest population density (4513 people/km<sup>2</sup>) and schooling rate (90%) of the country <sup>192</sup>.

## Participants and methods

### Study area and population

The Dakar region, including the capital Dakar and its suburbs, is located on the Cap-Vert peninsula. It represents 0.3% of the Senegal area and 21% of its population. The study was conducted in February-March 2010. The target population, children from state primary schools of Dakar region, was estimated to be over 200,000 children, distributed among 370 state schools.

### Study design

The study was a cross-sectional survey. A two-stage cluster sampling method was chosen with schools considered as primary sampling units. Within randomly selected schools, and without criteria for age and gender, children were randomized as final sampling units. The required number of participants was calculated following the formula  $n = (1.64^2 \times P \times (1-P)) / m^2$ ; where the prevalence  $P$  was estimated equal to 0.5 and the expected precision  $m$  for this prevalence to be 0.05. Moreover, a design effect equal to 2 was chosen<sup>193</sup> leading to a required sampling size of 538 children. Thirty schools were randomly selected, with 20 children per school, giving a final sample size of 600 children. The regional education authority provided the list of schools, while children were randomly selected from lists provided by the school directors.

### Data collection

Mothers or caretakers of children were surveyed at home for socioeconomic characteristics of the households. Birth dates of children were recorded from the school lists and checked with birth certificate or identity card. When official documents were missing, children's mothers or caretakers were questioned using a local events calendar. Children were defined as participants less than 10 y, and adolescents as participants from 10 y and above, according to the World Health Organization (WHO) definition<sup>14</sup>.

The nutritional assessment period lasted 6 weeks between February to March 2010, with one school visited each day. Blood, urine samples and anthropometric measurements were collected at schools in the morning between 8 and 10 AM. They were then verified for their identity. In order to define their fasting status, children were also asked the last time they consumed food. Weight and height were measured without footwear and wearing minimal clothes. To avoid between-measurers variability, all anthropometric measurements were performed by only one trained anthropometrist. The accuracy of the scale and the stadiometer was checked every day using a set of 2 calibration weights and one calibration tape. Height was measured twice to the nearest 0.1 cm on a Seca 214 stadiometer and mean values were used. When differences between two measures of height for the same child exceeded 0.5 cm, measurements were repeated. Weight was measured once to the nearest 100 g on a Pespe T125 Terraillon scale. Height-for-age (HAZ) and BMI-for-age z-scores (BAZ) were calculated according to the WHO 2006 reference<sup>157</sup>. Stunting was defined by HAZ < -2 z-scores. Overweight was defined by BAZ between 1 z-scores and 2 z-scores and obesity by BAZ > 2 z-scores. Two growth international references were used to classify thinness: the WHO reference defining mild, moderate and severe thinness respectively by z-scores between -2 and -1, between -3 and -2 and < -3<sup>157</sup>; the



IS reference defining grades 1, 2, 3 of thinness corresponding to WHO cut-off for BMI of 16, 17, and 18.5 respectively at age 18 <sup>194</sup>

Four (4) ml of venous blood were collected in a Terumo heparin Venosafe vacutainer with heparin by an experienced phlebotomist (using sterile single-use material). Urine was taken in a sterile container. Urine and blood samples were stored immediately in an icebox containing ice-packs and transported to the laboratory within a maximum of 3 hours after the first sample withdrawal.

Hemoglobin concentration (Hb) was measured at arrival at the laboratory of Pasteur Institute in Dakar on whole blood with HemoCue® Hb 201+ and HemoCue controls (Hemotrol low, medium, high, HemoCue®). Moreover 5% of blood samples were measured by hematology analyzer Cell-dyn® as an external control. Blood samples were centrifuged at 4000 x g for 10 minutes at -4°C, plasma aliquoted in 4 eppendorf tubes and stored at -20°C for 6 weeks until completion of the field work and sent with dry ice to the Nutripass laboratory of the Institut de Recherche pour le Développement (IRD, Montpellier, France) for zinc and iodine measurements and to the CBS laboratory (Willstaett, Germany) for determination of retinol-binding protein (RBP), C-reactive protein (CRP), ferritin (FER), soluble transferrin receptor (TFR) and  $\alpha$ 1-acid-glycoprotein (AGP). RBP, FER, TFR, CRP, AGP were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) technique <sup>195</sup>. Plasma zinc was measured by flame atomic absorption spectrophotometry (AAS), using trace-elements free procedures and urinary iodine (UIC) was measured using an ammonium persulfate method <sup>196</sup>

Inflammation was determined by elevated CRP (>5 mg/L) and/or elevated AGP (> 1 g/L) allowing differentiation between incubation phase (high CRP), convalescence phase (both AGP and CRP elevated) and late convalescence phase (elevated AGP only) <sup>197</sup>. Anemia was defined by Hb below cut-offs depending on age and gender: 115 g/L for participants <12y; 120 g/L for adolescents between 12 and 15 y and girls  $\geq$ 15 y; 130 g/L for boys  $\geq$ 15 y ); severe anemia was defined as Hb<70 g/L; depleted iron stores were defined by corrected FER <15  $\mu$ g/L<sup>198</sup>. Correction factors of FER were 0.77, 0.53 and 0.75 for participants respectively in incubation, early convalescence, and late convalescence phases <sup>197</sup>. Iron tissue deficiency was defined by TFR>8.3 mg/L. Low FER and high TFR are both considered as indicators of iron deficiency (ID) <sup>198</sup> so ID was defined by iron stores depleted (low FER) and/or iron tissue deficiency (high TFR). Body iron was calculated according to the formula of Cook : body iron (mg/kg) = - (log (TFR/FER ratio)-2.8229)/0.1207 <sup>199</sup>. "Body iron deficiency" was defined by body iron <0. Vitamin A status was measured by RBP concentration which reflects plasma retinol concentration because RBP occurs in a 1:1:1 complex with retinol and transthyretin<sup>200</sup> RBP concentrations were corrected in participants with inflammation by factors 1.15, 1.32, 1.12 respectively for incubation, early convalescence and late convalescence <sup>201</sup> . Vitamin A deficiency (VAD) was defined by corrected RBP <0.7  $\mu$ mol/L and  $\geq$ 0.35  $\mu$ mol/L and severe VAD was defined by corrected RBP <0.35  $\mu$ mol/L<sup>200</sup>. Marginal VAD was defined for corrected RBP values  $\geq$ 0.7  $\mu$ mol/L and <1.05  $\mu$ mol/L <sup>202</sup>.

Zinc deficiency (ZnD) was defined by plasma zinc concentration <0.65 mg/L for participants <10y independently of their fasting status and for participants >10 y, cut-offs are 0.66 mg/L for non fasting girls, 0.70 mg/L for fasting girls and non fasting boys, and 0.74 mg/L for fasting boys<sup>203</sup>.



Iodine deficiency (IDD) was defined by a median UIC below 100 µg/L and/or a proportion of participants below 50 µg/L higher than 20%. Mild IDD was defined by a median UIC between 50 and 99 µg/l, moderate IDD by a median IUC between 20 and 49 µg/l and severe IDD by a median UIC below 20 µg/l, iodine nutrition above requirements by a median UIC between 200 and 299 µg/l, and excessive iodine nutrition by a median UIC equal or above 300 µg/l <sup>124</sup>

## Ethics

The protocol was approved by the ethical committee of the National Health Research of Senegal. The school directors informed parents of the selected children on the purpose and proceedings of the study. Written informed consent was obtained from all parents at the beginning of the study. Severe anemic participants received iron supplementation as treatment.

## Data management and statistical analysis

Data entry, including quality checks and validation by double entry of questionnaires, was performed with EpiData version 3.1 (EpiData Association, Odense, Denmark). Data management and analyses were performed with the SAS software version 9.2 (SAS, V9.2; SAS Institute, Cary, NC).

All analyses took into account characteristics of the cluster sampling design using the appropriate survey procedures of SAS. Categorical variables were expressed as percentages and standard errors of prevalence (surveyfreq procedure). Interval variables were expressed as arithmetic means and standard errors of means (surveymeans procedure), except ferritin and transferrin whose distributions were not normal and which were also expressed as geometric means and standard errors of means.

Associations between prevalence and gender or age group were assessed by prevalence OR using logistic regression models (survey logistic procedure). Comparisons of means between gender and age groups were done through ANOVA (surveyreg procedure). Thus regression models included relevant cofounders (gender, age group or interaction according to models) to estimate adjusted ORs and differences.

## *Results*

In total, 604 children aged from 5 - 17 years participated in the study, 317 girls (52.5%) and 287 boys (47.5%, Table 7). About half of the participants were adolescents (n=287, 47.6%) and others were children (n=317, 52.4%). Of the children, 26% were schooled in Dakar, 57% in nearby suburbs of Dakar (Pikine, Thiaroye, Guediawaye, KeurMassar) and 17% in far suburbs (Rufisque and surroundings). Of the children, 5.7% of the subjects had an elevated CRP and 10.6% an elevated AGP. Prevalences of incubation (high CRP and low AGP), convalescence (high CRP and high AGP), and late convalescences were (low CRP and high AGP) 1.5%, 4.2% and 6.4% respectively.

Whereas half of the mothers had a job (54%), only 26% finished primary school. Most (93 %) households had electricity, but only 41% had a fridge.

Six hundred and four school children participated to the study and were measured for anthropometry, with 596 blood samples being obtained. 12 samples are missing, due to the refusal of 3 children and insufficient blood collection from 9 others. Hb was measured on all blood samples whereas CRP, AGP, FER, TFR were measured on 594 samples and Zn on only 584 due to insufficient blood volume. A total of six hundred urine samples were collected with only 4 children refusing. Due to the loss of labels during the transfer of samples, it was decided that 4 would not be measures for iodine.

**TABLE 7 ANTHROPOMETRIC AND BIOCHEMICAL STATUS OF PARTICIPANTS FOR ALL AND DISAGGREGATED FOR CHILDREN (<10Y) AND ADOLESCENTS (≥10Y)**

	All			Children			Adolescents			
	n	Mean / Prevalence	SE*	n	Mean / Prevalence	SE*	n	Mean / Prevalence	SE*	p
BMI (kg/cm <sup>2</sup> )	604	15.23	0.12	287	14.51	0.14	317	15.87	0.15	<0,0001
Thinness grade 1 <sup>a</sup> (%)		33.6%	2.1%		30.7%	2.8%		36.3%	2.7%	NS
Thinness grade 2 <sup>b</sup> (%)		10.4%	1.2%		10.8%	1.7%		10.1%	1.4%	NS
Thinness grade 3 <sup>c</sup> (%)		6.5%	0.9%		5.9%	1.5%		6.9%	1.5%	NS
BAZ	602	-1.14	0.05	286	-1.04	0.07	316	-1.22	0.06	NS
Mild thinness <sup>d</sup>		36.9%	2.2%		34.6%	3.2%	316	38.9%	3.0%	NS
Moderate thinness <sup>e</sup> (%) scores		12.8%	1.3%		12.2%	1.8%		13.3%	1.9%	NS
Severe thinness <sup>f</sup> (%)		5.6%	0.8%		3.8%	1.3%	316	7.3%	1.5%	NS
HAZ	595	-0.13	0.06	279	0.10	0.09	316	-0.33	0.07	0.02
Stunting <sup>g</sup> (%)		4.9%	0.9%		3.2%	1.1%		6.3%	1.5%	NS
Plasma retinol (μmol/l)	594	1.14	0.01	279	1.09	0.01	315	1.18	0.01	0.001
Vitamin A deficiency <sup>h</sup> (%)		3.0%	0.8%		4.3%	1.2%		1.9%	0.9%	NS
Vitamin A marginal status <sup>i</sup> (%)		35.9%	2.0%		45.5%	3.2%		27.3%	2.5%	0.00
Plasma zinc (μmol/l)	584	0.75	0.01	269	0.75	0.02		0.75	0.01	NS
Zinc deficiency <sup>j</sup> (%)		25.9%	3.7%		23.4%	4.4%		27.9%	3.9%	NS
Iodine (μg/l)	600	146.67	7.09	283	145.33	8.57	317	147.87	7.19	NS
Iodine < 20 μg/l (%)		1.3%	0.47%		0.7%	0.5%		1.9%	0.9%	NS
Iodine ≥ 20 μg/l and <50 μg/l (%)		6.0%	1.7%		6.0%	2.2%		6.0%	1.9%	NS
Iodine ≥50 μg/l and <100 μg/l (%)		25.5%	2.9%		26.5%	3.5%		24.6%	3.3%	NS
Iodine ≥100 μg/l and <200 μg/l (%)		44.8%	2.8%		46.3%	3.4%		43.5%	3.4%	NS
Iodine ≥200 μg/l and <300 μg/l (%)		17.0%	2.5%		15.2%	3.3%		18.6%	2.4%	0.07
Iodine ≥ 300 μg/l		5.3%	1.3%		5.3%	1.6%		5.4%	1.6%	NS
Hb (g/l)	596	12.58	0.05	279	12.51	0.08		12.65	0.09	NS
Anemia <sup>k</sup> (%)		14.4%	1.5%		13.3%	1.9%		15.5%	2.3%	0.07
Ferritin (μg/l)**	594	24.86	0.64		22.81	0.64	315	25.05	0.64	NS
Low ferritin <sup>l</sup> (%)		21.4%	1.8%		23.3%	2.5%		19.7%	2.0%	NS
Transferrin receptor (mg/l)**		8.76	0.50		7.94	0.50		7.67	0.50	NS
High transferrin receptor <sup>m</sup> (%)		33.3%	2.1%		35.5%	2.9%		31.4%	3.0%	NS
Body iron (mg/l)		2.56	0.13		2.32	0.16		2.77	0.19	NS
Negative body iron deficiency (%)		17.7%	1.6%		20.4%	2.4%		15.2%	1.6%	NS
ID according to FER and sTFR(%)		39.1%	2.4%		41.2%	3.1%		37.1%	3.3%	NS
IDA according to FER, sTFR and Hb(%)		10.6%	1.4%		10.0%	1.8%		11.1%	2.0%	0.21
IDA according to BodyIron and Hb(%)		7.1%	1.1%		6.8%	1.4%		7.3%	1.5%	NS

\*standard error ; \*\*geometric means NS non significant

<sup>a</sup> consistent with WHO adult 17 ≤ BMI < 18.5 (IS reference); <sup>b</sup> consistent with WHO adult 16 ≤ BMI < 17 (IS reference); <sup>c</sup> consistent with WHO adult BMI < 16 (IS reference); <sup>d</sup> BAZ< -1 z-scores and ≥- 2 z-scores (WHO reference); <sup>e</sup> BAZ < -2 z-scores and ≥- 3 z-scores (WHO reference) ; <sup>f</sup> BAZ < -3 z-scores (WHO reference); <sup>g</sup> HAZ < -2 z-scores (WHO reference); <sup>h</sup> retinol<0.7 μmol/L; <sup>i</sup> retinol<1.05 and ≥ 0.7 μmol/L; <sup>j</sup> zinc<0.65 mg/L for children<10y, zinc<0.70 mg/L for fasting girls>10y and non fasting boys>10y, zinc <0.66 mg/L for non fasting girls >10y, zinc <0.74 μg/L for fasting boys>10y; <sup>k</sup> hb<11.5 g/dL (<12y), hb <12.0 g/dL (children<15y and girls>15y), hb<13.0 g/dL (boys>15y); <sup>l</sup> corrected ferritin <12 μg/L; <sup>m</sup> sTFR>8.3 mg/L

Anthropometric characteristics and micronutrient status of participants are shown in table 7, for all and disaggregated by age group. Less than 5% of the participants were stunted. Mean HAZ was significantly lower in adolescents than in children ( $P=0.02$ ) and had a tendency to be lower in boys than in girls ( $P=0.08$ ). Cumulate moderate and severe thinness measured with BAZ was much more prevalent, affecting almost 20% of participants without any statistical difference between boys and girls or age groups. Both severe thinness ( $BAZ < -3$  z-scores) and grade 3 of thinness (equal to adult BMI  $< 16$  kg/cm<sup>2</sup>) were found in around 6% of children. Prevalence of moderate thinness ( $BAZ \geq -3$  z-scores and  $< -2$  z-scores) was 13% while prevalence of grade 2 of thinness was 10% (equal to adult BMI between 17 and 16 kg/cm<sup>2</sup>). Mild thinness ( $BAZ \geq -2$  z-scores and  $< -1$  z-scores) was 37% and slightly higher than thinness grade 1 which was 34% (equal to adult BMI between 16 and 18.5 kg/cm<sup>2</sup>). Only 3.0% of participants were overweight and two participants were obese (0.3%).

Fourteen percent of the participants were anemic. No significant difference was observed between gender groups but adolescents had a tendency to be more affected by anemia than children ( $P=0.07$ ). Only 3 participants had severe anemia. Prevalence of low FER was 21% and prevalence of high TFR was 33%. Mean TFR was significantly higher in boys than in girls ( $P=0.02$ ) as well as percentage of high TFR ( $P=0.03$ ) whereas there was no significant difference between children and adolescents. Prevalence of ID was found in approximately one third of participants, without any difference between age or gender groups. Prevalence of negative body iron content was 18%. Iron deficiency anemia was 11% when ID was defined by high TFR and/or low FER and 7% when ID was defined by negative body iron. While VAD was present in 3% of the participants, none had severe vitamin A deficiency. In contrast, approximately 40% of the participants had marginal VAD. Mean RBP was significantly lower in boys ( $P=0.01$ ) than in girls and in children compared to adolescents ( $P=0.001$ ). Moreover boys were significantly more affected by marginal VAD than girls ( $P=0.003$ ; figure 2) and children were more affected than adolescents ( $P<0.0001$ ). ZnD was highly prevalent at 26% of all participants and affected boys more than girls ( $P=0.02$ , figure 2). Median UIC was 137 µg/L with 7% of the participants having UIC  $< 50$  µg/L and 26% having UIC  $\geq 50$  µg/L and  $< 100$  µg/L. Only 1% had IUC  $< 20$  µg/L. Mean UIC was significantly higher in boys ( $P=0.04$ ) and the prevalence of very high UIC ( $> 300$  µg/L) tended to be higher in boys ( $P=0.07$ , figure 2). 22% of participants had elevated UIC, 17% between 200 and 299 µg/L and 5% above 300 µg/L. The table 8 reports the public health significance of each nutritional disorder reported in the current paper according to international references <sup>124-128</sup>.

No association was found between micronutrient deficiency and thinness/stunting. Multiple micronutrient deficiency was not prevalent. No association has been found between iron deficiency, zinc deficiency, thinness, stunting, and socioeconomic characteristics (education level of the mother, socioeconomic status of the mother and the household head).

**TABLE 8 PUBLIC HEALTH SIGNIFICANCE OF NUTRITIONAL DISORDERS IN CHILDREN FROM PRIMARY STATE SCHOOLS OF DAKAR**

Indicators	Prevalence	Public health significance
Vitamin A deficiency	3.0%	mild
Zinc deficiency	25.9%	mild
Iodine deficiency disorders	UI <50 µg/L 7%	no
	median UIC 136.8 µg/L	
Anemia	14.4%	mild
Iron deficiency	lowFer 21.4% highTfR 33.3%	yes
Thinness	50.6% (corresponding to BMI<18.5 kg/cm <sup>2</sup> in adults)	high prevalence (serious situation)
Stunting	prevalence 4.9%	low prevalence

### Discussion

The present study, which was carried out in a representative sample of school children attending state primary schools in Dakar, showed multiple nutritional problems. Prevalence of thinness, anemia and deficiencies of iron and zinc were high. In contrast, overweight and vitamin A deficiency were less prevalent. More than 50% of the children had evidence for inadequate (too low or too high) iodine intakes.

In the present study, both references from WHO and IS have been used because they are relatively new and under-used: in 376 previous studies on school-children, neither references were used <sup>17</sup>. The comparison between the 2 references shows only slight differences. Prevalence of grade 3 thinness as defined by Cole was similar to severe thinness defined by WHO, which is consistent with a recent study carried out in Seychelles <sup>204</sup>. Prevalence of grade 2 and 1 thinness (Cole) were slightly lower than moderate and mild thinness respectively as defined by WHO. As reported earlier, the WHO reference gives higher prevalence of especially mild thinness, regardless of age of the subjects.

Many factors underline the high prevalence of thinness observed in the present study. For instance, infections or communicable diseases could have contributed to thinness. In our study, 12% of children had signs of inflammation as indicated by the elevated concentrations of acute phase proteins. Malaria, estimated to affect 8% of children, or diarrhea, are major causes of disease in Senegal. Other infections such as HIV/AIDS or tuberculosis are less prevalent in Senegal, with 0.7% of adults and 0.4% of people aged 15-24 y being HIV-positive <sup>205</sup>. and 0.2% of adult population is affected by tuberculosis <sup>206</sup>One might therefore assume that thinness is closely related to low dietary intake. It has been demonstrated that whereas malnutrition in infancy and young childhood is strongly related to stunting, thinness is an indicator of malnutrition in all age groups and suggest recent undernutrition<sup>207</sup>. The low prevalence of stunting (5%) would indicate that nutritional intake in the first years of life was probably adequate, with under nutrition only appearing later in childhood.

A study on food practices and nutrition in urban regions of Senegal identified several reasons for food insecurity and undernutrition in urban school-aged children<sup>145</sup>. These are mainly related to changing dietary habits. For instance, urban populations tend to decrease domestic foods in favor of street foods, with only one meal being home-cooked and consumed in the afternoon. For breakfast and dinner, families tend to increasingly buy snacks in the street. Worryingly, children attending urban state schools do not have lunch at home, while simultaneously not having access to canteens and thus school meals. During school days, they buy cheap foods from street sellers, which most often lack in proteins and micronutrients. This is highlighted by the finding that in the present study that found almost 6% of the participants were categorized as severe malnourished (BAZ <-3 while only 1.9% of children under 5 y living in urban areas of Senegal have weight for height <-3 z-score<sup>185</sup>.

Advocacy and more importantly actions for intervention improving the nutritional status of school children in Senegal are thus vital. Offering affordable and healthy meals in school canteens is but only one option available.

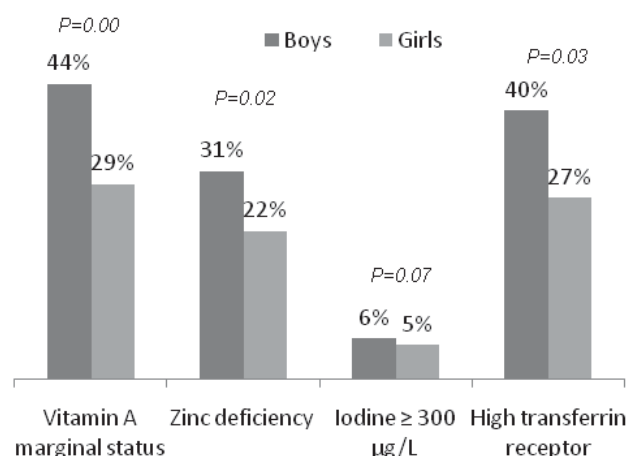
The prevalence of anemia (14%) still represents a public health problem<sup>208</sup>. This prevalence was less than half of the prevalence of anemia reported in school children in Dakar 18 years ago (35%)<sup>191</sup>. Another study carried out in Dakar in 2003 on a small sample of seven years old children indicated a prevalence of anemia of 39%<sup>209</sup>. These combined data suggest that anemia has decreased among Senegalese school children in Dakar over the last decade. Compared to other African countries, the prevalence of anemia found in the present study was much lower than the prevalence of ~40% recently found in 2 studies among school children in Burkina Faso and Cote d'Ivoire<sup>210</sup>. In contrast, a recent study carried out in 6-16 y old school children in rural Kenitra, Morocco found a similar anemia prevalence of 12%<sup>211</sup>.

Prevalence of ID defined either by low ferritin and/or by high transferrin receptor concentrations was high (39%). However, in this study more participants had elevated TFR (33%) than low ferritin (21%) or negative body iron (18%). Both indicators measure different stages of iron deficiency, with low ferritin indicating the depletion of iron stores and elevated TFR related to the iron-deficient erythropoiesis, indicating a later stage of iron deficiency<sup>210</sup>. To our knowledge, it is the first time that ID in a population was more related to abnormal TFR values than to low ferritin. However, a study carried out in 5-15 y old children in Cote d'Ivoire recommends the use of higher cut-offs for TFR i.e. 9.4 mg/L for African populations (versus 8.3 mg/L used in our study) to improve its performance in defining iron status in children. However, the authors concluded that TFR had only a modest sensitivity and specificity in identifying iron deficiency, regardless of the diagnostic cutoffs chosen<sup>210</sup>. When applying this cut-off, the prevalence of high TFR decreased from 33 to 20 %, which was very close to the 18% of children with a negative total body iron. This study suggests that a cut-off of 9.4 mg/L for TFR might be better in these settings. Even with this higher cut-off, the prevalence of ID defined by either abnormal ferritin or TFR remained high at 29%.

ID prevalence was more than double the prevalence of anemia demonstrating that anemia prevalence rates cannot be used as a proxy indicator for ID<sup>198</sup>. To allow correction of ferritin concentrations in the presence of inflammation, iron status indicators have to be included in epidemiologic studies combined with indicators of inflammation. In addition, the prevalence of iron deficiency anemia (IDA) was low and only represented half of the anemia prevalence, indicating that other factors contributed to anemia. Malaria infection and hemoglobinopathies

such as sickle cell are likely contributors with prevalences of 8% and 10% respectively <sup>212,213</sup>. The prevalence of vitamin A deficiency, which can contribute to anemia, was low. However, other micronutrients such as vitamins B12 and folic acid were not measured. These deficiencies could have played a role in the etiology of the non-ID anemia found in the present study <sup>214</sup>.

In Burkina Faso, 40% of school children aged 7-14 y in Ouagadougou are vitamin A deficient <sup>215,216</sup>, whereas 59% of children aged 6-9 y are vitamin A deficient and 8% have severe VAD in rural Northern Ethiopia<sup>217</sup>. Both studies used retinol concentrations as an indicator while the current study uses RBP concentrations. However, at low plasma retinol concentrations, RBP concentrations is a less sensitive indicator of vitamin A status, as more unbound RBP appears in the circulation <sup>104,218</sup>. Using RBP concentrations may therefore have underestimated the prevalence of vitamin A deficiency in the current study. Mean RBP concentration was significantly lower and prevalence of marginal vitamin A status higher in boys and in children. Although the study notes the differences in vitamin A deficiencies between boys and girls, with higher risks for boys, it does not provide a clear answer for this variance. However, similar sex differences for other micronutrients such as iron and zinc have been reported before <sup>219</sup>.



**FIGURE 2 GENDER-RELATED DIFFERENCES IN PREVALENCE OF ABNORMAL STATUS**

Zinc deficiency (ZnD) was highly prevalent with almost 1/4 of all school children affected representing a significant higher rate than the cut-off of 20% indicating a public health problem <sup>126</sup>. Furthermore, in consensus with similar studies, boys were significantly more affected than girls. The higher requirements of zinc for boys generally suggest that boys are more sensitive to ZnD<sup>220-223</sup>. Indeed, boys have higher proportion of muscles per kilogram body weight, which contains a higher content of zinc than fat, and the growth rate of boys is higher than girls <sup>203</sup>.

According to both the median UIC (136.8 µg/L) and proportion of children with UI<50 µg/L (7%), iodine deficiency is not a major public health issue in school children in Dakar. Iodine nutrition in Dakar is supposed to be good, as Senegal introduced iodized salt in 1995. According to a national survey in 2006, 70.5% of households consume adequate iodized salt (>15 ppm) <sup>185</sup>. Moreover, Dakar's geographical location near the sea ensures adequate intake from seafood <sup>185</sup>. Nonetheless, 33% of children had insufficient iodine intake (UIC below 100 µg/L), and although this is lower than WHO estimates for Africa in general (41% of children 6-12 years having UIC<100 µg/L <sup>5</sup> it is still a considerable percentage of children. Worrying, only 45% of the



children had adequate iodine intake (UIC between 100 and 199 µg/L), with 22% of the children being at risk for iodine-induced hyperthyroidism (UIC above 200 µg/L) and 5% of children risk adverse health consequences (UIC above 300 µg/L) like iodine-induced hyperthyroidism or autoimmune thyroid disease)<sup>124</sup> Two studies in non coastal countries Lesotho and Angola on children from rural schools reported respectively 94% and 78% of children with UIC<50 µg/L and only 0% and 2% children with UIC≥200 µg/L. As iodine deficient populations seem to be more sensitive to health consequences of excessive iodine intakes <sup>5</sup>, urgent attention is needed for iodine nutrition in school children in Dakar.

Apart from gender and age related differences, it was not possible to identify a specific vulnerable socioeconomic group of children, or a group of children more affected by malnutrition. Nutritional issues seem to randomly affect the whole population of the study.

Not including non-school attendees is a limitation of the study, as they might be the most vulnerable to malnutrition. However, one of the objectives of the study was to provide information to the education ministry in order to illustrate nutritional disorders in urban school attendees. The goal was to reorient the strategy of school-feeding programs towards broader coverage, as only rural state schools received school meals. The entire population of urban school-aged children and adolescents should be taken into account by nutrition policy.

### *Conclusion*

To conclude, many school-aged children in urban Senegal have a poor nutritional status, as the high prevalence of iron and zinc deficiency illustrate. Furthermore, iodine intake as indicated by urinary iodine concentrations was either too low or too high in over half of the children. Although the low rate of stunting in the population suggests adequate nutrition during the first years of life, the prevalence of thinness going up to almost 20% remains alarming. The study highlighted that the transition from home meals in the preschool period to self-catering at school is most likely the basis for these multiplex of nutritional problems. The study therefore stresses the need for nutritional interventions to improve dietary quality and quantity of school children in Senegal.

## **2.2. Nutrient intake is insufficient among African urban school-aged children and adolescents: results from two 24-hours recall in primary state schools in Dakar, Senegal (Manuscript, drafted)**

*To be submitted*

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### *Introduction*

Malnutrition is estimated to be highly prevalent in African school-aged children, with 20% of stunting, 30% of thinness, anemia, iron, iodine or vitamin A deficiency, 50% of zinc deficiency and 10% of overweight <sup>17</sup>. Due to growth, cognition and educational achievements, nutrient requirements of school-aged children (5 – 9 years (y) ) are high <sup>17</sup>. Because of late school enrollment often occurring in low and middle income countries (LMIC) primary school age also includes early adolescence, a period when skeleton and brain growth spurts and sexual maturation dramatically increase nutrient requirements <sup>17</sup>. Early marriage, still common in the developing world <sup>26</sup>, makes adolescent girls particularly vulnerable to undernutrition in case of childbearing <sup>108,117</sup>. Thus, nutrient deficiencies may be especially harmful for cognitive and physical development during school age and early adolescence (10 - 14 y). Although physiological status and health conditions like menarche or parasitosis have an influence on nutritional status, nutritional status is mainly related to nutrient intake. Thus, dietary intake data are crucial to understand the role of the diet in nutritional deficiencies, and eventually to design effective nutritional interventions.

In LMIC, diet of school-aged children and adolescents is usually poor in fruits, vegetables and animal products, leading to inadequate protein and micronutrient intake <sup>151</sup>. Moreover, urban new lifestyles increase sedentary activities and consumption of high fat and sugar snacks and beverages that are often energy dense but micronutrient poor <sup>151</sup>. Although living in urban areas was known to improve dietary diversity and therefore nutritional status <sup>224</sup>, some evidence showed that in poor urban settings micronutrient deficiencies are still prevalent <sup>215</sup> and can coexist with increasing overweight. Indeed, in African urban areas, school-aged children spend more time out-of-home away from their caretaker supervision and consume more street food compared to their younger counterparts <sup>145</sup>. Thus, changes in food patterns due to the transition from preschool to school age can affect nutrient intakes especially when no school feeding programs are available. Consequently, populations of school-aged children and adolescents living in African urban areas may be at risk for double burden of malnutrition associated with inadequate dietary intake.

Recent data on nutrient intake adequacy and its implication for nutrition in primary school children living in African urban settings is scarce. No data on dietary intake of school-aged children and adolescents from Senegal were available <sup>151</sup>. The authors conducted a cross-sectional study investigating anthropometry, micronutrient status and dietary intake among a representative sample of 600 children attending primary state schools in Dakar, Senegal. Results from anthropometric and biochemical data were published elsewhere <sup>225</sup>. Chronic malnutrition was low but acute malnutrition was prevalent, with less than 5% of the children

being stunted and 18% of children having BMI-for-age  $<-2$  z-scores. Overweight and obesity were not prevalent in that population. Micronutrient status was poor with 36% of children suffering from marginal vitamin A status, 26% being zinc deficient and 39% being iron deficient. The objectives of the present article were i) to estimate nutrient intake ii) to report prevalence of insufficient/excessive nutrient intake – for all and by age and gender groups iii) to evaluate inadequate nutrient intake as risk factors for micronutrient deficiencies measured on blood samples.

### *Participants and methods*

#### *Study design and participants*

The study was a representative cross-sectional survey targeting children registered in primary state schools in the area of Dakar. 600 school children were randomly selected through a two-stage random cluster sampling of children attending primary state schools in the Dakar area (30 schools  $\times$  20 children). Details of study design and sampling are provided elsewhere <sup>225</sup>. Adolescents were defined as equal or above 10 y <sup>14</sup>.

#### *Data collection*

The study was conducted over 30 school-days in February-March 2010 by trained interviewers. Two quantitative 24-hours recalls (24-hr) were conducted, at school, with three school days between repeated recalls. Children were asked to provide details on all foods and beverages consumed during the previous day. Rice and cereal gruels were quantified by weighting salted replicas to the nearest gram, using an electronic scale Terraillon®. The other foods were quantified using pictures of different portion size <sup>226</sup>, household measures, standard units or purchase price. During the afternoon following the 24-hr, mothers or caretakers were asked at home about recipes of each home-cooked food or beverage mentioned by the child during the 24-hr. The amount of each ingredient was estimated in grams, household measures or price. Similarly, recipes of foods (snacks and beverages) sold inside or outside schools were recorded by interviewing vendors. During the second 24-hr, anthropometric measurements (height and weight), blood and urine samples were collected to measure vitamin A, zinc, iron and iodine status.

#### *Data management and analysis*

Data entry, including quality checks and validation by double entry of questionnaires, was performed with epidata entry version 3.1 (epidata Association, Odense, Denmark). A food composition table compiled from different food composition tables <sup>227-230</sup> was specifically developed for the study. Data management was performed using epidata Analysis version 2.2 (epidata Association, Odense, Denmark). Energy and nutrient intakes from repeated 24-hr were adjusted for intra individual variability to obtain estimated usual intakes, using PC-Side (PC Software for Intake Distribution Estimation, ISU, 1997, Chicago) <sup>231</sup>. Estimated energy requirement (EER) were calculated using Schofield equations taking into account height, weight and age, Physical Activity Level (PAL) for active and low active children <sup>232</sup>. Cut-offs used to estimate insufficient and excessive intakes are summarized in the table 9 <sup>233-235</sup>. Prevalence of insufficient and excessive nutrient intake was estimated using PC-Side. Prevalence of

insufficient nutrient intake was secondly calculated based on nutrient intake not adjusted for day-to-day variability and presented in the supplementary material 2. Crude odds ratios (OR) and odds ratios adjusted for age group and gender were calculated to measure the association between insufficient nutrient intake and micronutrient deficiencies.

### Ethics

The protocol was approved by the ethical committee of the National Health Research of Senegal. The school directors informed parents of the selected children on the purpose and proceedings of the study. Written informed consent was obtained from all parents at the beginning of the study. Severe anemic participants received iron supplementation as treatment.

**TABLE 9 RECOMMENDATIONS OF DAILY INTAKE OF MACRONUTRIENTS AND MICRONUTRIENTS FOR POPULATIONS OF CHILDREN 4-18 Y**

	Vitamin A	Folic acid	Vitamin C	Zinc	Iron	Calcium	Carbohydrate		Protein		Lipid	SFA	MUFA	PUFA	Fiber
Units	µg	µg	mg	mg	mg	mg	g	%EI	g/kg	%EI	%EI	%EI	%EI	%EI	g
<i>Insufficient intake</i>															
Cut-off	EAR	EAR	EAR	EAR	EAR	AI	EAR	AMDR	EAR	AMDR	AMDR	-	-	-	AI
4-8y	275	160	22	4	4.1	800	100		0.76						25
9-13y boys	445	250	39	7	5.9	1300	100		0.76						31
9-13y girls	420	250	39	7	5.7	1300	100	45	0.76	10	25	-	-	5*	26
14-18y boys	630	330	63	8.5	7.7	1300	100		0.73						38
14-18y girls	485	330	56	7.3	7.9	1300	100		0.71						26
<i>Excessive intake</i>															
Cut-off	UL	UL	UL	UL	UL	UL	-	AMDR	-	AMDR	AMDR	-	-	-	-
4-8y	900	400	650	12	40	2500									
9-13y	1700	600	1200	23	40	2500	-	65	-	30	35	10*	within limits of total fat*	15*	-
14-18y	2800	800	1800	34	45	2500									

*These cut-offs are recommended in the essential guide to nutrient requirements of the Institute of Medicine (IOM) <sup>233</sup> except <sup>\*234</sup>*

SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; PUFA : Polyunsaturated Fatty Acid; %EI : % of energy intake; EAR : Estimated Average Requirement; AI : Adequate Intake; AMDR : Acceptable Macronutrient Distribution Ranges; UL : Upper Limit

NB : In the present study, 167 (31%) participants were <9 y, 160 (29%) participants were boys aged from 9 to 13 years, 182 (33%) participants were girls aged from 9 to 13 y, 19 (3%) participants were boys ≥14 y, 17 (3%) participants were girls ≥14 y

## Results

Dietary intake was obtained for 599 participants aged 5 to 17 y. Fifty four children identified as misreporters were excluded, leading to a final sample of 545 children. 52.6% were girls and 45.1% were <10 y, resulting in 116 boys<10 y, 130 girls<10 y, 142 adolescent boys and 157 adolescent girls.

Most of children had insufficient energy intake, 7% had insufficient protein intake and 88% had insufficient fiber intake. None of them had carbohydrates intake below recommendation. Mean contribution to energy from carbohydrates, proteins, lipids, PUFAs and SFAs were within the AMDRs but the contribution of proteins and carbohydrates to energy was insufficient in 31% and 5% of children, respectively. No children had excessive contribution of protein and carbohydrates to energy, but respectively 32%, 21% and 14% of children had excessive contribution of lipids, SFAs, and PUFAs (Table 10). Iron and vitamin C intakes were insufficient for half of the children (Table 11) and zinc and vitamin A intakes were insufficient for more than two thirds. Calcium and folic acid intakes were insufficient in all children. No micronutrient was consumed above their respective Upper Limits.

Prevalence of inadequate nutrient intake and contribution of macronutrients to energy were different between age groups. Prevalence of insufficient contribution of carbohydrates to energy was higher in adolescents compared to children <10 y in both genders (Figure 3). Protein intake was lower in adolescents compared to children <10 y, among boys ( $1.17 \pm 0.06$  g/kg vs.  $1.49 \pm 0.08$  g/kg,  $P < 0.001$ ) and among girls ( $1.05 \pm 0.05$  g/kg vs.  $1.41 \pm 0.08$  g/kg  $P < 0.001$ ). Insufficient protein intake was more prevalent among adolescent boys compared to boys <10 y ( $8 \pm 5\%$  vs.  $1 \pm 2\%$ ,  $P < 0.05$ ). PUFAs' contribution to energy was higher in adolescents compared to children <10 y, in boys ( $13.3\%$  vs.  $12.3\%$ ,  $P < 0.001$ ) and in girls ( $12.6\%$  vs.  $11.6\%$ ,  $P < 0.001$ ), and so was prevalence of excessive contribution of lipid and PUFA to energy (Figure 3). Prevalence of excessive contribution of SFAs to energy was higher in girls <10y compared to adolescent girls. Insufficient zinc intake was more prevalent in adolescents compared to children <10 y in both genders (Figure 4). Among girls, prevalence of insufficient vitamin C intake was higher in adolescents compared to girls <10y (Figure 4). Among boys, prevalence of insufficient vitamin A intake was higher in adolescents compared to boys <10y.

Differences in prevalence of inadequate intake according to gender were also observed. Intakes were higher in adolescent boys compared to adolescent girls in energy ( $1507 \pm 45$  kcal vs.  $1399 \pm 38$  kcal,  $P < 0.001$ ), in carbohydrates ( $193 \pm 6$  g vs.  $183 \pm 6$  g,  $P < 0.05$ ), in proteins ( $39 \pm 2$ g vs.  $36 \pm 1$ g,  $1.17 \pm 0.06$ g/kg vs.  $1.05 \pm 0.05$ g/kg,  $P < 0.01$  for both), in fibers ( $20 \pm 1$ g vs.  $18 \pm 1$ g,  $P < 0.001$ ), in lipids ( $58 \pm 2$  g vs.  $53 \pm 2$  g,  $P < 0.001$ ), in SFAs ( $14 \pm 1$ g vs.  $13 \pm 1$  g,  $P < 0.05$ ), and in PUFAs ( $23 \pm 1$ g vs.  $20 \pm 1$ g,  $P < 0.001$ ). Proportion of daily energy coming from PUFAs was higher in boys compared to girls, among children <10y ( $12.3 \pm 0.4\%$  vs.  $11.6 \pm 0.3\%$ ,  $P < 0.001$ ) and among adolescents ( $13.3 \pm 0.4\%$  vs.  $12.6 \pm 0.5\%$ ,  $P < 0.05$ ). Excessive proportion of PUFAs in total energy was more prevalent in boys<10y compared to girls<10y (Figure 3). Mean vitamin C intake was lower in boys<10y compared to girls <10y ( $24 \pm 2$  mg vs.  $34 \pm 2$  mg,  $P < 0.001$ ). Mean folic acid and zinc intake were also lower in boys <10y compared to girls <10y ( $40 \pm 2$  µg vs.  $47 \pm 3$  µg,  $P < 0.001$ ;  $4.6 \pm 0.3$  mg vs.  $5.1 \pm 0.3$  mg,  $P < 0.05$ ). Mean folic acid intake was higher in adolescent boys compared to adolescent girls ( $60 \pm 3$  µg vs.  $51 \pm 3$  µg,  $P < 0.001$ ). Prevalence of insufficient zinc intake was significantly higher in adolescent girls compared to adolescent boys (Figure 4).

The risk for iron deficiency was higher when intake of iron and protein were under EAR and when intake were high in energy from lipids and carbohydrates (Table 12). Zinc deficiency risk was higher when zinc intake was insufficient, when contribution of protein to energy intake was insufficient,

when contribution of lipid and SFA was excessive and it was lower when fiber intake was below EAR. Risk for vitamin A marginal status was higher when iron intake, vitamin C intake and contribution of protein to energy intake was insufficient, and lower when energy intake from lipid was high.

**TABLE 10 MEAN ENERGY AND MACRONUTRIENT DAILY INTAKE (ADJUSTED FOR WITHIN-PERSON VARIABILITY) AND PREVALENCE OF INSUFFICIENT AND EXCESSIVE MACRONUTRIENT DAILY INTAKE**

	mean intake/ proportion of inadequate intake	±
Energy (kcal)	1365	23
Energy/EER 1	0.65	0.01
Children with energy intake below EE1 (%)	99	1
Energy/EER 2	0.75	0.01
Children with energy intake below EE2 (%)	93	2
Protein (g)	36	1
Protein/body weight (g/Kg)	1.25	0.04
Children with protein intake /body weight below EAR (%)	7%	2%
% Energy from protein	11%	0%
Children with % energy intake from protein below AMDR (%)	31%	4%
Children with % energy intake from protein above AMDR (%)	0%	0%
Carbohydrates (g)	176	3
Children with fiber intake below EAR (%)	0%	1%
% Energy from carbohydrates	53%	0.4%
Children with % energy intake from carbohydrates below AMDR (%)	5%	1.8%
Children with % energy intake from carbohydrates above AMDR (%)	0%	0.3%
Dietary fiber (g)	19	1
Children with fiber intake below AI (%)	88%	3%
Lipid (g)	52	1
% Energy from lipid	33%	0.3%
Children with % energy intake from lipid below AMDR (%)	1%	0.8%
Children with % energy intake from lipid above AMDR (%)	32%	3.9%
SFA (g)	13	0
% Energy from SFA	8%	0%
Children with % energy intake from SFA above 10%	21%	3%
MUFA (g)	15	0
% Energy from MUFA	9%	0.1%
PUFA (g)	19	0
% Energy from PUFA	12%	0.2%
Children with % energy intake from PUFA below 5% (%)	0%	0.0%
Children with % energy intake from PUFA above 15%	14%	2.9%

AMDR: Acceptable Macronutrient Distribution Ranges; AI : Adequate Intake; EAR : Estimated Average Requirements; EER1 : Estimated Energy Requirement for active children ; EER2 : Estimated Energy Requirement for low active children; SFA : Saturated Fatty Acids; MUFA : Mono-unsaturated Fatty Acids; PUFA : Poly-unsaturated Fatty Acids



**TABLE 11 MEAN MICRONUTRIENT DAILY INTAKE (ADJUSTED FOR WITHIN-PERSON VARIABILITY) AND PREVALENCE OF INSUFFICIENT AND EXCESSIVE MICRONUTRIENT DAILY INTAKE**

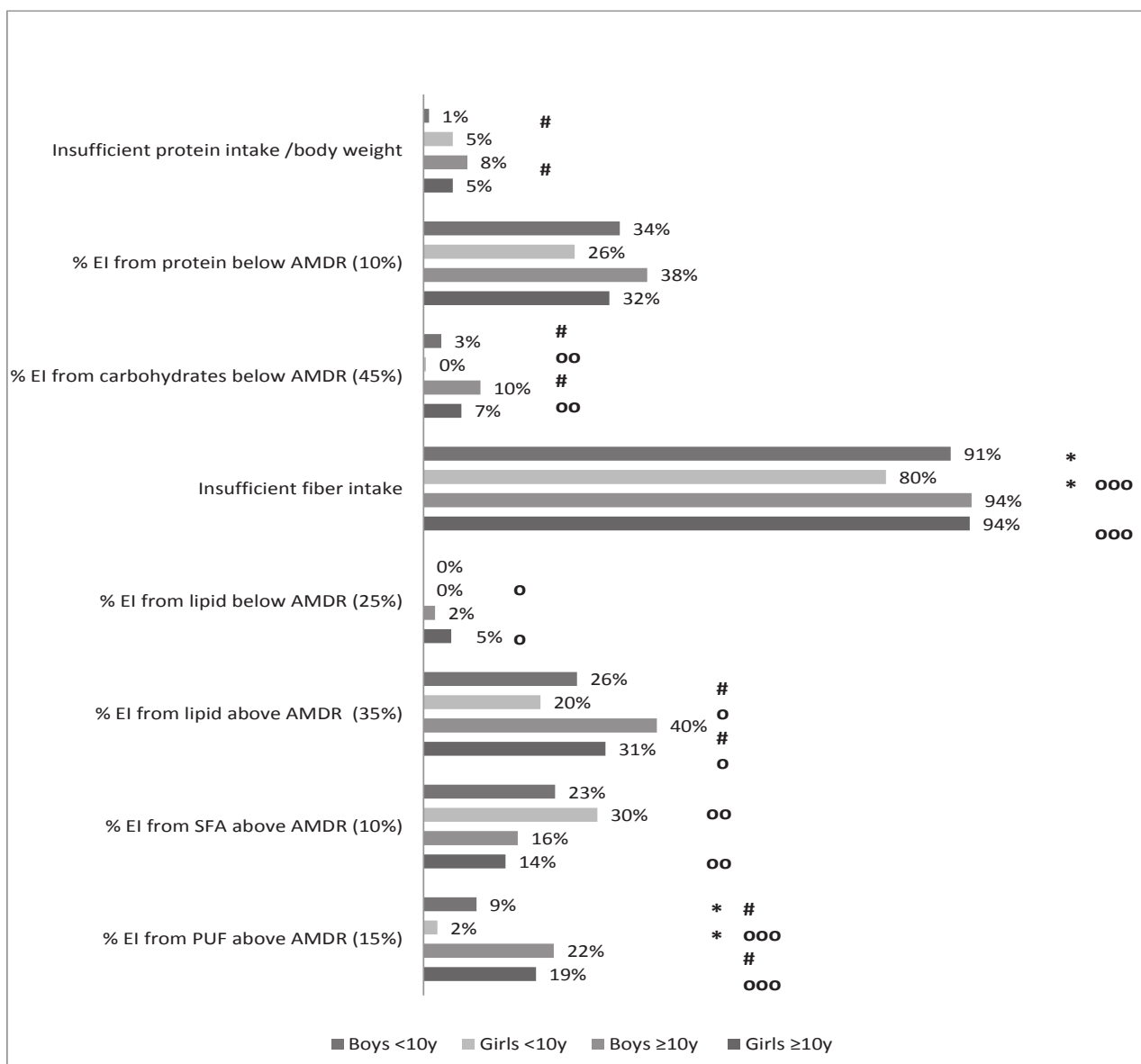
	mean intake/ proportion of inadequate intake	±
Iron (mg)	5.6	0.1
Children with iron intake below EAR (%)	46%	4%
Children with iron intake above UL (%)	0%	0%
Zinc (mg)	5.3	0.2
Children with zinc intake below EAR (%)	69%	4%
Children with zinc intake above UL (%)	1%	1%
Calcium (mg)	268	8
Children with calcium intake below AI (%)	100%	0%
Children with calcium intake above UL (%)	0%	0%
Vitamin C (mg)	38	2
Children with vitamin C intake below EAR (%)	53%	4%
Children with vitamin C intake above UL (%)	0%	0%
Folic acid (µg)	50	2
Children with folic acid intake below EAR (%)	100%	0%
Children with folic acid intake above UL (%)	0%	0%
Vitamin A (µg)	309	17
Children with vitamin A intake below EAR (%)	79%	3%
Children with vitamin A intake above UL (%)	0%	0%
AI: Adequate Intake; EAR: Estimated Average Requirement; UL: Upper Limit		

**TABLE 12 CRUDE AND AGE-GENDER ADJUSTED ODDS RATIOS (OR) AND CONFIDENCE INTERVAL (CI) FOR INSUFFICIENT NUTRIENT INTAKE ASSOCIATED WITH MICRONUTRIENT DEFICIENCIES**

		Iron deficiency		Marginal vitamin A status		Zinc deficiency	
		OR	95% CI	OR	95% CI	OR	95% CI
Iron intake below EAR	Crude	1.32	[ 0.93 ; 1.88 ]	1.84	[ 1.26 ; 2.68 ]	1.05	[ 0.74 ; 1.48 ]
	Adjusted *	1.47	[ 1.02 ; 2.13 ]	1.80	[ 1.22 ; 2.65 ]	1.35	[ 0.93 ; 1.96 ]
Zinc intake below EAR	Crude	1.24	[ 0.83 ; 1.84 ]	1.53	[ 1 ; 2.33 ]	1.24	[ 0.84 ; 1.82 ]
	Adjusted *	1.46	[ 0.95 ; 2.26 ]	1.27	[ 0.8 ; 2.02 ]	1.76	[ 1.13 ; 2.72 ]
Vitamin C intake below EAR	Crude	0.74	[ 0.52 ; 1.06 ]	1.93	[ 1.32 ; 2.83 ]	1.24	[ 0.87 ; 1.78 ]
	Adjusted *	0.83	[ 0.55 ; 1.24 ]	1.80	[ 1.21 ; 2.68 ]	1.00	[ 0.53 ; 1.87 ]
Fiber intake below AI	Crude	0.68	[ 0.39 ; 1.16 ]	0.88	[ 0.51 ; 1.5 ]	0.09	[ 0.05 ; 0.14 ]
	Adjusted *	0.59	[ 0.32 ; 1.11 ]	1.13	[ 0.64 ; 1.98 ]	0.39	[ 0.17 ; 0.88 ]
Vitamin A intake below EAR	Crude	0.87	[ 0.56 ; 1.35 ]	1.73	[ 1.07 ; 2.81 ]	1.12	[ 0.73 ; 1.72 ]
	Adjusted *	0.72	[ 0.43 ; 1.18 ]	1.16	[ 0.68 ; 1.96 ]	1.20	[ 0.73 ; 1.98 ]
Protein per kg of body weight below EAR	Crude	2.20	[ 1.17 ; 4.13 ]	0.30	[ 0.13 ; 0.67 ]	0.36	[ 0.12 ; 1.07 ]
	Adjusted *	2.22	[ 1.14 ; 4.33 ]	0.44	[ 0.19 ; 1.03 ]	0.61	[ 0.19 ; 2.02 ]
% of energy coming from protein below AMDR	Crude	0.25	[ 0.16 ; 0.39 ]	4.58	[ 3.03 ; 6.92 ]	2.91	[ 1.98 ; 4.29 ]
	Adjusted *	0.34	[ 0.22 ; 0.54 ]	3.28	[ 2.07 ; 5.19 ]	3.00	[ 1.91 ; 4.69 ]
% of energy coming from carbohydrates above AMDR	Crude	0.17	[ 0.08 ; 0.36 ]	2.10	[ 0.84 ; 5.3 ]	4.26	[ 2.47 ; 7.35 ]
	Adjusted *	0.21	[ 0.1 ; 0.45 ]	1.87	[ 0.69 ; 5.05 ]	1.36	[ 0.66 ; 2.81 ]
% of energy coming from lipid above AMDR	Crude	2.04	[ 1.41 ; 2.95 ]	0.48	[ 0.31 ; 0.75 ]	1.93	[ 1.33 ; 2.8 ]
	Adjusted *	2.02	[ 1.39 ; 2.93 ]	0.47	[ 0.29 ; 0.74 ]	1.67	[ 1.09 ; 2.56 ]
% of energy coming from SFA above AMDR	Crude	1.44	[ 0.91 ; 2.29 ]	0.53	[ 0.31 ; 0.92 ]	3.31	[ 2.04 ; 5.38 ]
	Adjusted *	1.43	[ 0.9 ; 2.28 ]	0.57	[ 0.32 ; 1 ]	2.89	[ 1.7 ; 4.91 ]
% of energy coming from PUFA above AMDR	Crude	2.90	[ 1.79 ; 4.68 ]	0.37	[ 0.19 ; 0.69 ]	0.93	[ 0.58 ; 1.49 ]
	Adjusted *	3.02	[ 1.85 ; 4.91 ]	0.51	[ 0.26 ; 1 ]	0.99	[ 0.59 ; 1.66 ]

\*Adjusted for age group and gender

AI : Adequate Intake; AMDR : Acceptable Macronutrient Distribution Ranges; EAR : Estimated Average Requirement; PUFA: Polyunsaturated Fatty Acid; SFA: Saturated Fatty Acid



**FIGURE 3 PREVALENCE OF INSUFFICIENT MACRONUTRIENT INTAKE ACCORDING TO AGE AND GENDER GROUP**

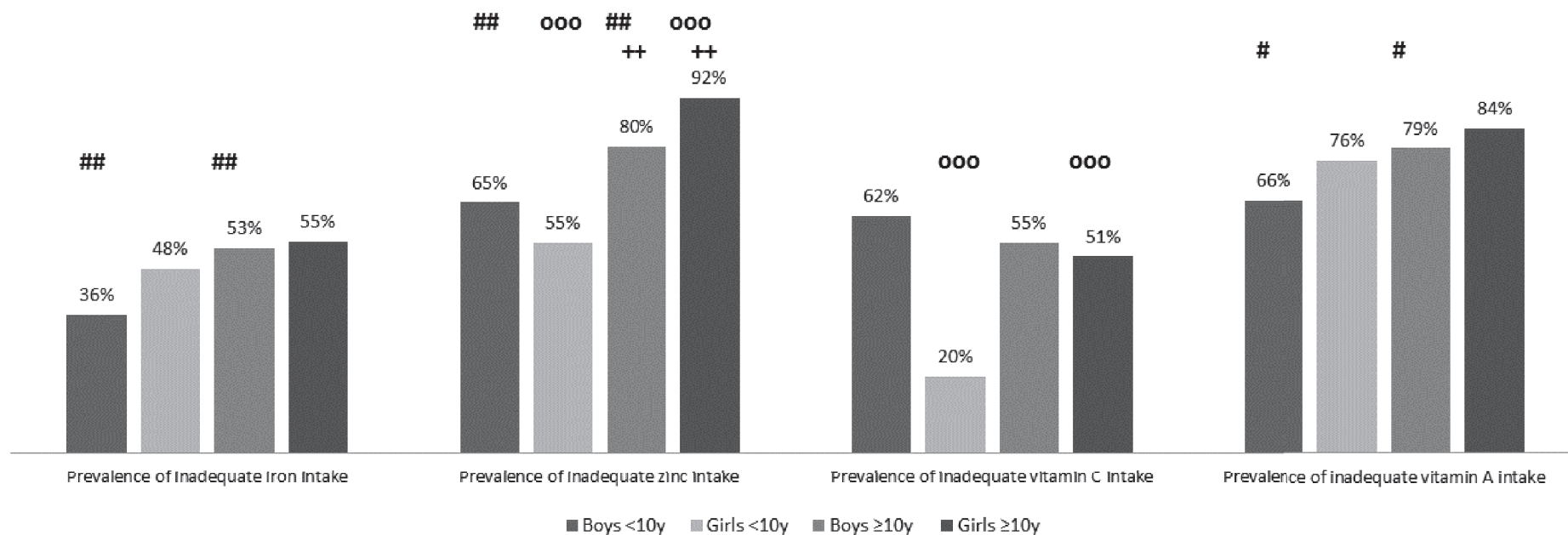
%EI: percent of energy intake; AMDR : Acceptable Macronutrient Distribution Range

P-value for  $\chi^2$ -test between boys <10y and girls <10y : \*\*\*: P-value<0.001; \*\*: P-value<0.01; \* P-value <0.05

P-value for  $\chi^2$ -test between boys ≥10y and girls ≥10y : +++: P-value<0.001; ++: P-value<0.01; + P-value <0.05

P-value for  $\chi^2$ -test between boys <10y and boys ≥10y : ###: P-value<0.001; ##: P-value<0.01; # P-value <0.05

P-value for  $\chi^2$ -test between girls <10y and girls ≥10y: ooo: P-value<0.001; oo: P-value<0.01; o P-value <0.05



**FIGURE 4 PREVALENCE OF INSUFFICIENT MICRONUTRIENT INTAKE ACCORDING TO AGE AND GENDER GROUP**

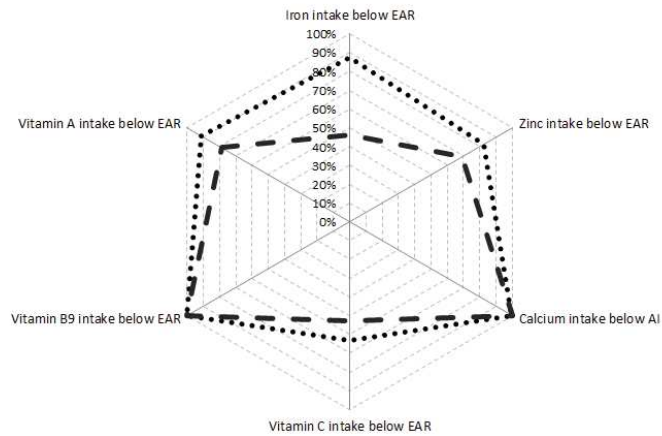
P-value for  $\chi^2$ -test between boys <10y and girls <10y : \*\*\*: P-value<0.001; \*\*: P-value<0.01; \* P-value <0.05

P-value for  $\chi^2$ -test between boys ≥10y and girls ≥10y : +++: P-value<0.001; ++: P-value<0.01; + P-value <0.05

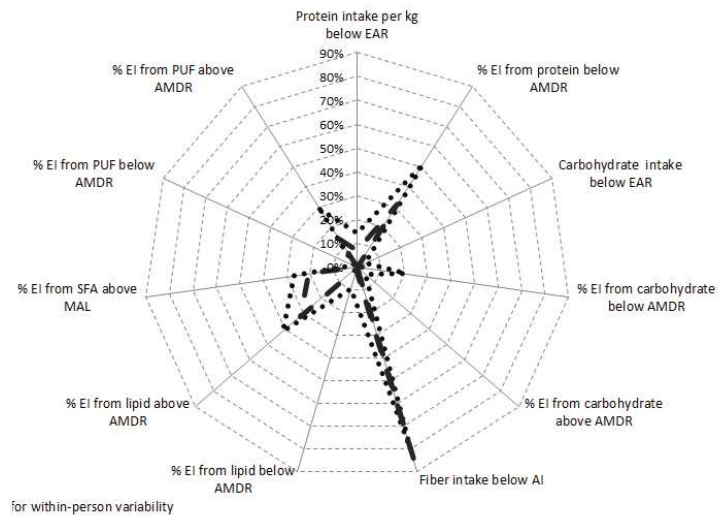
P-value for  $\chi^2$ -test between boys <10y and boys ≥10y : ####: P-value<0.001; ##: P-value<0.01; # P-value <0.05

P-value for  $\chi^2$ -test between girls <10y and girls ≥10y: ooo: P-value<0.001; oo: P-value<0.01; o P-value <0.05

### 3a. Micronutrients



### 3b. Macronutrients



..... Unadjusted — Adjusted for within-person variability

**FIGURE 5 UNADJUSTED AND ADJUSTED PREVALENCE OF INSUFFICIENT AND EXCESSIVE INTAKE**

%EI : % of energy intake; AI : Adequate Intake; AMDR : Acceptable Macronutrient Distribution Ranges; EAR : Estimated Average Requirement; PUFA : Polyunsaturated Fatty Acid; SFA: Saturated Fatty Acid; UL : Upper Limit

## Discussion

### Energy, macronutrients and fiber intake

Energy intake was insufficient in most children and the distribution of macronutrient was unbalanced with excessive lipid intake, especially from SFA, and insufficient protein intake for one third of the children. Few data on energy and macronutrient intake are available for African children but a study carried out in Cameroonian urban adolescents also indicated unbalanced diets with excessive carbohydrates and insufficient protein contributions to energy <sup>236</sup>. In the current study, lipid and SFA excessively contributed to energy intake and it contrasts with a study in Ghanaian rural school children where energy intake from carbohydrates and lipids were respectively higher and lower compared to the present study <sup>237</sup>.

Most children had fiber intake below the Adequate Intake (AI). Even if fibers are not essential nutrients of which insufficient intakes lead to biochemical or clinical deficiency symptoms AI has been established to define fiber intake providing the greatest protection against coronary heart disease <sup>238</sup>. Moreover, dietary fiber is considered as protective against inflammatory bowel disease and colorectal cancer <sup>239,240</sup>, of which excessive fat consumption, especially SFA, is suspected to be a risk factor <sup>241</sup>.

Insufficient protein intake and contribution of protein to energy intake were respectively risk factors for iron and zinc deficiency. This is not surprising as food rich in zinc and iron are usually also rich in protein (meat, shellfish, legumes), thus diets lacking of these products may lead to concomitant protein, iron and zinc poor status <sup>242</sup>. In the present study, excessive energy coming from lipids and from SFA were also risk factors for zinc deficiency, which is notable as some research suggests that the type of dietary fat influences the effects of zinc deficiency on fatty acid status and especially lipid concentrations in the liver <sup>243</sup>. We also observed that excessive lipid and PUFA intake was positively associated with iron deficiency. This could compensate the PUFA status decline induced by iron deficiency which is suggested in other research<sup>244</sup>. Indeed, Smuts et al assumed a possible disruption of PUFA metabolism in iron deficient children when they observed higher SFA status and lower PUFA status among iron deficient school children compared to children with normal iron status. Not surprisingly, in our study insufficient fiber intake was a protective factor for zinc deficiency, as dietary fiber is known to impair zinc absorption <sup>238</sup>.

Even if energy intake increased with age, insufficient fiber, protein and micronutrient intake and inadequate contribution of macronutrients to total energy intake were higher among adolescent compared to children<10y, which suggest poorer nutrition in adolescents, where mean height for age was lower in the present study <sup>225</sup>. A research conducted in Cameroonian urban adolescents also reported a high prevalence of insufficient contribution of proteins to energy intake <sup>236</sup>. These findings suggest that in African urban areas, the increase of macronutrient intake with age does not compensate drastically increased requirements at adolescence. Adolescent girls had the lowest ratio of energy intake by estimated energy requirement. Macronutrient intake and nutritional status of adolescent girls is particularly worrisome, as thinness of adolescent mothers is associated with low birth weight of their babies

## Iron intake

In the present study, iron intake was poor similarly to what was reported in Ghanaian<sup>237</sup> and Kenyan school children<sup>245</sup>. Because of high consumption of tea and low meat intake among African urban children<sup>246</sup> the absorption of iron in the present population is probably low<sup>85</sup> and therefore prevalence of insufficient iron intake based on EARs may be underestimated. Although peers reported that iron intake does not predict well serum ferritin concentrations<sup>247</sup>, in our study, insufficient iron intake was a risk to be iron deficient. Consequently, iron deficiency which affects 39% of school children of Dakar is likely to result from insufficient iron intake. By causing anemia and reducing physical and cognitive capacities, iron deficiency may impair school attendance and performance in the studied population<sup>17</sup>. Iron requirements are very high in adolescents especially in girls where menarche usually follows the growth spurt<sup>85</sup>. Insufficient iron intake was a risk factor for marginal vitamin A status. Some research suggested that iron deficiency may induce vitamin A deficiency by inhibiting mobilization of vitamin A store in rats<sup>248</sup> but the impact of iron deficiency on vitamin A metabolism remains unclear.

## Zinc intake

Insufficient zinc intake was highly prevalent in children, as reported in other studies in school children and adolescents from urban Cameroon<sup>236</sup>, rural Ghana<sup>237,249</sup> and Kenya<sup>237</sup>. As reported in the literature<sup>250</sup>, in the present study, insufficient zinc intake was a good predictor of serum zinc concentrations and therefore a good predictor of risk of zinc deficiency. Poor zinc intake and zinc deficiency may disturb growth, immune system and appetite and increase morbidity from diarrhea and acute respiratory infections<sup>85</sup>. Albeit higher zinc intake, insufficient zinc intake was more prevalent in the adolescent groups compared to children < 10 y, probably due to much higher requirements associated with growth spurt<sup>85</sup>, where zinc has a central role<sup>85</sup>.

## Calcium intake

In the present study, mean dietary calcium was below recommendations<sup>251,252,238</sup> and all children had insufficient calcium intake. Very high proportions of insufficient calcium intake were also reported in Cameroonian and Moroccan urban adolescents<sup>236,253</sup>, and in school children in Ghana<sup>237</sup>. Dietary calcium has been shown to be correlated to serum calcium levels in African school children and adolescents<sup>254</sup> and low dietary calcium is associated with hypocalcaemia<sup>255</sup> and decreased bone mineral density<sup>254</sup>. So it is assumed that school children of Dakar are at high risk for calcium deficiency. Although rickets mostly affect infants, it can also occur during late childhood and adolescence if diet is poor in calcium or in vitamin D and/or high in phytates<sup>256</sup>. Therefore, although cut-offs used in the present study may be considered as too high, in other research on osteomalacia, mean levels of calcium intake in school children and adolescents suffering from rickets ranged from 150 to 300 mg, which is similar to the present study<sup>256-258</sup>. Hence the population in the present study is at high risk for calcium deficiency, and therefore for rickets and osteomalacia<sup>259</sup>. The insufficient intake of calcium in the present study may be surprising, as consumption of dairy products is culturally part of the Senegalese diet<sup>260</sup>. However their high price in urban areas reduces their accessibility.



## Vitamin C intake

Half of children had insufficient vitamin C intake. Elevated proportion of poor vitamin C intake was also observed in Kenyan and Ghanaian school children <sup>237,245</sup> as well as among Cameroonian and Nigerian adolescents <sup>236,261</sup>. As vitamin C intake was shown to be a good predictor of vitamin C serum values <sup>250,262</sup>, school children from Dakar are likely to be at risk for vitamin C deficiency. A diet low in fruits rich in both vitamin A and vitamin C could explain why insufficient vitamin C intake was associated with poor vitamin A status in the present study <sup>263</sup>. Vitamin C deficiency results in skeletal pain, increased susceptibility to infections, impaired wound healing at a moderate stage, and scurvy at a severe stage <sup>85</sup>. Vitamin C has antioxidant properties <sup>264-266</sup> and is known to improve immune response and to reduce the incidence of infectious diseases in children from developing countries <sup>267</sup>. It also stimulates iron absorption and metabolism, especially for non-heme iron <sup>268,269</sup>. High cost and instability during storage are the major obstacles to use vitamin C in nutritional intervention programs <sup>270,271</sup> but distributing fruits through school feeding programs in African urban areas may improve vitamin C, iron, and vitamin A status, especially in vegetables based diets <sup>237</sup>.

## Folic acid intake

In the present study, all children had folic acid insufficient intake and mean folic acid intake was lower than what was reported in rural Kenyan and Ghanaian school children <sup>237,245</sup> although diets in these populations were already considered as folic-acid deficient. No recent data on folic acid status in Senegalese populations was available but a high risk of folic acid deficiency is assumed in this population as folic acid intake predicts well serum folic acid concentrations <sup>250,272</sup>. Insufficient folic acid intake and folic acid deficiency may result in leucopenia, intestinal malabsorption, blood coagulation impairment, increased sensibility to infections <sup>85</sup>, and macrocytic anemia, which is the second most common type of nutritional anemia, after iron deficiency <sup>44</sup>. Among girls from the present sample, folic acid intake did not increase at adolescence, oppositely to most of other micronutrients, and is lower than in boys. Similarly, insufficient folic acid intake was found in more than three quarters of urban adolescents in studies in Cameroon and Morocco <sup>236,253</sup>. Thus, low folic acid intake in adolescent girls from African urban areas is worrisome, due to its serious adverse consequences during pregnancy on the development of the foetus <sup>85</sup>.

## Vitamin A intake

Children from the present sample had poor vitamin A intake. Vitamin A intake is highly dependent on seasons <sup>273</sup>. In Senegal, mangos are only available from April to August, while the present study was conducted in February-March. A high proportion of children with insufficient vitamin A intake was also reported outside the mango season in a study conducted in Ghana<sup>237</sup>. Only 3% of children in the current study were vitamin A deficient, which is considered as mild public health issue and negligible compared to other urban school children from West Africa <sup>215</sup>. However, both prevalence of marginal vitamin A status <sup>225</sup> and insufficient vitamin A intake suggested a risk for vitamin A deficiency, which is involved in morbidity linked to diarrhea and measles, in growth retardation <sup>85</sup> and in a large part of blindness or severe visual impairment <sup>274</sup>. No significant difference between boys and girls in the proportion of insufficient vitamin A intake was observed but boys were more affected by marginal vitamin A status than girls <sup>225</sup>,

which is consistent with previous research <sup>275</sup>. As growth rates under 10 y are higher in boys than in girls <sup>85</sup>, the higher requirement of vitamin A in boys may not be adequately taken into account in EARs before adolescence. Risk for vitamin A poor status is probably a condition appearing at school age, as more than 90% of Senegalese children under 5 y receive yearly vitamin A supplementation <sup>276</sup>. Percentage EI coming from lipid above AMDR was a protective factor for marginal vitamin A status. Indeed, an increased intake of dietary fat is likely to improve the vitamin A absorption <sup>85</sup>.

### Dietary and environment determinants

African urban school children and adolescents are supposed to be more at risk of overweight <sup>17</sup> <sup>151</sup> and to consume more meat, vegetables, cereals, milk products than their rural counterparts <sup>224</sup>. However, in our study, children had insufficient micronutrients and energy intake, especially from protein, and acute malnutrition and micronutrient deficiencies were prevalent, while no overweight and low chronic malnutrition were observed, which suggests adequate care and diet in early childhood and degradation of diet and nutritional status when reaching school age <sup>225</sup>. Thus, nutrient intake data as well as biochemical and anthropometric data from this study indicated recent energy, protein and micronutrients deprivation in children and adolescents from primary state schools of Dakar, most likely due to deficient and inadequate diet, lacking of quantity and diversity with minimum consumptions of animal foods and fruits and vegetables. Consequently, fat and SFA excessively contributes to the total dietary intake, partly because of consumption of processed food and street food rich in low-quality lipids or sugar and poor in protein<sup>151</sup>. Indeed, it is known that urban lifestyle added to the food price crisis and global crisis in the 2000's led to reduce the number of meals consumed at home in favor of street food in urban settings <sup>145</sup>. Research suggests that in low-income households, meat is the food category most restricted in the children's diet <sup>277</sup>. Moreover, reduced supervision by mothers and caretakers at school age, and increased autonomy for feeding may also partly explain the degradation of dietary intake and nutritional status all along late childhood and adolescence. Indeed, without any public school feeding program in urban areas, children reaching the school age are mostly self-catered, which may cause poor food habits. As in many developing countries, school lunches in Senegal are limited to areas of high food insecurity, in rural areas, or where the World Food Program provides school meals.

### Possible interventions

Because of its implication in impaired physical and cognitive development during school age and adolescence<sup>17</sup>, undernutrition associated with insufficient energy, protein and micronutrients intake in children from Senegalese urban schools should be addressed. School may be an efficient framework for nutritional and food interventions such as school feeding, nutrition education, or supplementation. A non-negligible part of the foods consumed by children and adolescent in urban settings is street food, therefore nutritional education programs should encourage the purchase of fruits instead of low-quality snacks <sup>145</sup>. Accessible and cheap dark green leafy vegetables and legumes are sufficient to ensure adequate folic acid status in poor populations <sup>278</sup>. Although bioavailability of vitamin A is much higher in animal products, these are also less affordable so carotenoids are usually the main source of vitamin A for economically deprived populations <sup>85</sup>. Consequently, traditional Senegalese recipes

containing unrefined palm oil and green leafy vegetables should be promoted among mothers in order to improve vitamin A and folic acid status. In African settings, school feeding program was shown to improve intake of energy <sup>279</sup> and micronutrients <sup>151,237</sup>, such as calcium, vitamin C and vitamin A through the distribution of fruits or dairy products. Beneficial effects of fortified food distribution through school feeding programs on micronutrient status were reported <sup>175,280</sup>. Calcium supplementation showed positive effects on calcium bone acquisition of children accustomed to low-calcium diets <sup>281,282</sup> and on long-term bone mass growth<sup>283</sup>.

### Strengths and limitations

24-hours recall can be as efficient as weighted records <sup>284</sup> and it is more accurate than food frequency questionnaire to evaluate nutrient intake among school age children as reported in a review <sup>285</sup>, especially when it is repeated at least once <sup>286</sup>. It is statistically more efficient to increase the number of individuals than the number of days of recall, but at least more than one day is needed to estimate the intra-individual variability <sup>287</sup>. It is admitted that children from 7-8 years are able to recall food consumed during the previous 24-hours recall but that for children under 7 years, parental help is needed <sup>288</sup>. However, dietary recalls collected from mothers can be inaccurate as out-of-home food intake is often omitted from mother's recall <sup>289</sup>. Plus, in the present study, it was not possible to interview children along with their mothers because of their out-door morning activities but they were asked to check the recall of their child when the interviewers came to the household to collect recipes. Among low-income households, fluctuations in food supplies especially low before paydays makes diets highly variable <sup>277</sup>, which was taken into account in the present study conducted over 30 days. In Senegal, home-cooked meals are usually eaten in a common plate which complicates the estimation of individual's consumption of staple food. The use of salted replicas to estimate rice portions in the present study was likely to be more accurate than the number of handfuls, which was reported as not satisfactory in Senegalese young children <sup>290</sup>. For other food, the use of handfuls, households measures and photographs may have lacked of accuracy <sup>288</sup>. But authors assume that the purchase price was a good indicator for children and mothers with limited budgets, likely to remember well the prices of street food and ingredients. The food composition table was derived from 5 tables, in which composition of prepared meals or snacks in Senegal was not available, so we gathered quantitative recipes for all households and street vendors. Home-cooked meals composition was specific to each child for each day, and the composition of snacks sold in the school or in the surroundings was specific to each school. Therefore, one of the strengths of the study is that nutrient composition of food was not approximated and that inter-households, intra-areas and day-to-day variability were taken into account.

Albeit its infrequent use, the EAR <sup>291</sup> is still considered by some peers as the most relevant Dietary Reference Intake to assess risk of insufficient nutrient intake at population level, which takes into account the variability for both the requirement and the usual intake <sup>233</sup>. This method limits over- and underestimation of the prevalence <sup>292</sup> as it is observed in the present study and in other research <sup>291</sup>. However, the EAR cut-point may not be adapted to energy intake and to iron intake, due to the high correlation between intakes and energy requirements, and to the high skewness of iron requirement distribution in menstruating women <sup>293</sup>. But only 38 girls in the present sample had already reached menarche, representing 7% of the whole sample. Overall, the main recommendations for using the EAR cut-point method to evaluate adequacy of nutrient intake in a group was followed: minimum 100 individuals, to adjust for day-to-day variability and to exclude under-reporters <sup>294</sup>. One may object that EAR, AI and UL used in the

current study were based on the diet of healthy individuals from North America, which may not be adapted to African populations. However, the poor micronutrient status indicated by the biochemical data supports high prevalence of insufficient nutrient intake found in the present paper.

### *Conclusion*

Diets were energy-deficient and unbalanced between poor protein and fiber and excessive fat and SFA. For all micronutrients, at least half of the children had insufficient intake, suggesting a diet poor in dairy products, meat, fruit and vegetables, with a special concern for zinc, vitamin A, folic acid and calcium. Nutrient intake inadequacy increases with age. These findings are in accordance with anthropometric and biochemical data indicating high rates of thinness and micronutrient deficiencies. Similarly to what was observed in other African rural and urban populations of school-aged children and adolescents, nutrition of school-aged children and adolescents from primary state schools of Dakar is worrisome, because of high nutrient needs related to growth spurt, puberty, school achievement or early pregnancies in adolescent girls. Reduction of familial care, increase of street food consumption, conjectural food prices crisis may explain a poorer diet at school age compared to infancy, which seems to be aggravated at adolescence. The findings of the present study highlight the need of nutritional interventions in Senegalese urban schools such as school feeding program and nutrition education.



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*Chapter 3. Evaluation of  
determinants and consequences of  
malnutrition among school  
children: data from Cambodia case*

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### **3.1. Stunting, poor iron status and parasite infection are significant risk factors for lower cognitive performance in Cambodian school children (Published paper)**

*Published in Plos One (2014)*

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#### *Introduction*

Worldwide, undernutrition and micronutrient deficiencies substantially impair human health and socio-economic development. Both developed and developing countries are concerned by the burden of micronutrient deficiencies disorders, although the highest prevalence exist in Sub-Saharan Africa and South Asia <sup>3</sup>. In Cambodia, despite a considerable reduction in national poverty since the mid-1990s, undernutrition remains a major problem, and approximately one fifth of the population was still living on less than \$1.25 per day in 2009. According to the most recent Cambodia Demographic and Health Survey (CDHS 2010) , 40% of children are stunted, 11% are wasted, and 55% of children of 6-59 months are anemic <sup>295</sup>.

Nutrition is one of many key factors affecting mental development of children. Deficiencies of critical micronutrients such as iron, folate or iodine can lead to impaired cognitive functions due to their decisive role in brain development. For example, iron deficiency (ID) has been estimated to impair the optimal mental development of 40% to 60% of the developing world's infants <sup>296</sup>. Indirectly, deficiencies of other micronutrients like vitamin A or zinc can increase the risk of morbidity thereby increasing school absenteeism and reducing learning abilities and school performance.

Iron deficiency anemia (IDA), the most severe degree of ID, has been associated with poorer cognitive performance but it is still unclear if malnutrition has detrimental effects on cognitive performance independent of anemia in school-aged children <sup>51</sup>. Some recent systematic reviews based on randomized controlled trials provided evidence for a positive effect of iron supplementation on different measures of cognition in anemic and non-anemic children >5y <sup>55</sup>, on attention and concentration in adolescents and women <sup>297</sup> , and beneficial effect of micronutrient interventions (food-based or supplementation) on short term memory <sup>298</sup>. In addition to IDA, stunting is a known risk factor for impaired child development <sup>299</sup>. Despite a general decrease of the global prevalence, stunting still affects one third of the children under 5 y in the developing world <sup>300</sup>. Several studies have documented the relationship between cognitive ability and stunting in young children <sup>301,302</sup> but few have explored it within school-aged children <sup>17,303,304</sup>.

School-aged children are often omitted from public health research. Early child development programs typically focus on the nutritional status and milestones of children under 5 y. Thus, our knowledge regarding the prevalence and impact of micronutrient deficiencies on children >5 y remains scarce. Yet, some areas of the brain and higher cognitive functions continue to develop throughout childhood and adolescence <sup>36</sup>. The myelination of frontal lobes which are thought to be responsible for executive, "higher-order" cognitive activities, starts around 6mo and continues until adulthood. Changes in the volumes of cortical gray and white matters, significantly correlated to children's performance on a verbal learning task, occur during childhood and adolescence <sup>305</sup>. As during infancy, this ongoing brain development throughout



childhood is likely to be affected by detrimental effect of poor nutritional status, hence warranting further research on the impact of nutritional status on cognitive performance of school-aged children. In Cambodia, there is currently no data available concerning the nutritional status of children >5y, nor how malnutrition potentially affects their mental development.

The present study aimed to evaluate the anthropometric and micronutrient status (iron, vitamin A, zinc and iodine) of Cambodian children aged 6-16 y and to determine if these outcomes are associated with cognitive performance.

### *Subjects and methods*

#### *Study population and design*

Data were collected as part of a randomized placebo-controlled trial investigating the impact of multi-micronutrient fortified rices on health and development of Cambodian schoolchildren (FORISCA UltraRice+NutriRice study, FOrtifiedRIce for School meals in CAMbodia). Baseline data collection was conducted in November 2012 in 20 primary schools from 5 districts of Kampong Speu province in Cambodia. Kampong Speu is one of the 23 provinces of Cambodia, situated 60 km west of the capital Phnom Penh. Agriculture is predominant, with rice farming as the main occupation and income source. The schools were randomly selected from primary schools participating in school meal or take-home ration programs of the UN World Food Program (WFP). Children attending the selected schools were eligible to be part of the study if they were between 6-16 y of age, had written informed consent from parents/caregivers and did not have any mental or severe physical handicap. In each school, 132 children were randomly selected after stratification by sex and grade, hence 2640 children. 197 children were not recruited because they were absent on the day of data collection or refused to participate. Hence, a total of 2443 schoolchildren aged 6-16 y participated in the study. The study was approved by the National Ethic Committee for Health Research (NECHR) of the Ministry of Health, Phnom Penh, Cambodia, the Ministry of Education, Youth and Sports, Phnom Penh, Cambodia, and the Research Ethics Committee of PATH, Seattle, USA.

#### *Anthropometric measurements*

Height and weight were measured using standardized procedures <sup>18,159</sup>. Children wore minimum clothing and no shoes. Height was measured in duplicate to the nearest 0.1 cm with a wooden stadiometer. Weight was measured once, to the nearest 0.1 kilogram using an electronic scale (Seca, 881 U, Germany). Z-scores for height-for-age (HAZ) were calculated with AnthroPlus software version 1.0.4 using the WHO 2007 standards. Moderate and severe stunting were respectively defined as  $-3 < \text{HAZ} < -2$  and  $\text{HAZ} < -3$  respectively.

#### *Cognitive performance tests*

The cognitive performance evaluation included three tests: the Raven's Colored Progressive Matrices test (RCPM), and two standardized tests from the Wechsler Intelligence Scale for Children (WISC III): block design and picture completion. RCPM, the coloured form of Raven's Progressive Matrices test for use with children, is a widely used nonverbal test of intelligence which was designed as a measure of overall intellectual ability <sup>37,306</sup>. The WISC III, designed for

children aged 6-16 y, is one of the most widely used tests of the intelligence of children <sup>307</sup>. Block design is a measure of problem solving to assess executive functions <sup>307</sup>. Picture completion evaluates alertness to detail and visual discrimination. The cognitive tests were conducted by 25 students from Psychology department of Royal University of Phnom Penh. The interviewers were trained in a 1 week workshop to ensure standardization in the assessment and scoring procedures. Each child was tested individually using standardized test protocols translated into Khmer language. Since norms are not available for Cambodia, interpretations of the scores of cognitive test were used as raw scores. For all cognitive tests, higher scores indicate better performance.

### Blood and urine samples collection

Blood samples (5ml) were collected by venipuncture and aliquoted in a trace-element free vacutainers with no anticoagulant (Vacurette, Greiner Bio One, Austria). Urine samples were collected from the children in a sterile plastic container. Blood and urine samples were then stored in cool-boxes containing ice-packs and transported to Phnom Penh within 5h of collection. The blood samples were centrifuged at 2700 rpm (1300g) for 10 min at room temperature. Serum and urine were then aliquoted in capped Eppendorf tubes and stored at -30°C until transfer for analysis.

### Hemoglobin concentration

Hemoglobin concentrations were determined immediately after blood taking using the HemoCue (301+ system, HemoCueAngholm, Sweden). Anemia was defined as hemoglobin concentration <115 g/L for children between the ages 6 and 11 y, <120 g/L for children between the ages 12 and 14 y and girls aged 15 y and older and <130 g/L for boys aged 15 y and older according to WHO guidelines <sup>308</sup>.

### Laboratory analysis

*Ferritin (FER), soluble transferrin Receptor (TFR), retinol-binding protein (RBP), C-reactive protein (CRP),  $\alpha$ 1-acid-glycoprotein (AGP) and zinc serum concentrations*

Serum samples were sent on dry ice to the VitMin laboratory (Willstaett, Germany) for determination of retinol-binding protein (RBP), C-reactive protein (CRP), ferritin (FER), soluble transferrin receptor (TFR) and  $\alpha$ 1-acid-glycoprotein (AGP), and to National Institute of Nutrition (Hanoi, Vietnam) for zinc analysis. RBP, FER, TFR, CRP, AGP were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) technique <sup>195</sup>. Zinc concentration was measured using a flame atomic absorption spectrophotometer (GBC, Avanta+) using trace element-free procedures. Inflammation was defined as high CRP (>5mg/L) and/or high AGP concentrations (>1g/L). Inflammation status was then categorized in four groups based on CRP and AGP levels: no inflammation (normal CRP and AGP), incubation (high CRP and normal AGP), early convalescence (high CRP and AGP), and late convalescence (normal CRP and high AGP) <sup>197</sup>. FER is affected by presence of infection or inflammation, therefore FER concentrations were adjusted using correction factors published by Thurnham et al., namely 0.77, 0.53 and 0.75 for children respectively in incubation, early convalescence, and late convalescence phases. Low FER (corrected value <15  $\mu$ g/L) was used as indicator of depleted iron stores <sup>308</sup>, and high TFR (>8.3mg/L) as indicator of tissue iron deficiency <sup>210,309</sup>. Iron deficiency was defined using both

FER and TFR indicators, i.e. by depleted iron stores or tissue iron deficiency. Total body iron (BI) was calculated from FER corrected for inflammation and TFR as described by Cook *et al.* <sup>199</sup>. Serum retinol is bound to RBP in a 1-to-1 complex, hence RBP concentrations were used to evaluate vitamin A status <sup>200</sup>. RBP concentrations were adjusted for the presence of inflammation using correction factors of 1.15, 1.32 and 1.12 for incubation, early convalescence and late convalescence phases respectively <sup>201</sup>. Corrected RBP cut-offs were used to define marginal vitamin A status ( $0.70 \mu\text{mol/L} \leq \text{corrected RBP} < 1.05 \mu\text{mol/L}$ ), vitamin A deficiency ( $< 0.70 \mu\text{mol/L}$ ) and severe vitamin A deficiency ( $< 0.35 \mu\text{mol/L}$ ) respectively <sup>200,310</sup>. Zinc deficiency was defined using the following cut-offs: serum zinc concentration  $< 0.66 \text{ mg/L}$  for girls  $\geq 10 \text{ y}$ ,  $< 0.70 \text{ mg/L}$  for boys  $\geq 10 \text{ y}$ , and  $< 0.65 \text{ mg/L}$  for boys and girls  $< 10 \text{ y}$  <sup>311</sup>.

#### *Urinary iodine concentration*

Urine samples were sent on dry ice to the Provincial Preventive Medicine Center (Thai Nguyen, Vietnam) for determination of urinary iodine concentration (UIC) using spectrophotometric methods <sup>312</sup>. Iodine deficiency (IDD) was defined by a median UIC below  $100 \mu\text{g/L}$  and/or a proportion of participants below  $50 \mu\text{g/L}$  higher than 20%, and iodine nutrition above requirements by a median UIC above  $200 \mu\text{g/L}$  <sup>313</sup>.

#### *Parasite infestation*

Plastic containers and instructions for fecal sample collection were distributed to the children on the day of data collection and requested to be returned with fecal sample to the school the following day. Samples were then stored in a cool box, transported to the National Malaria Center (CNM, Phnom Penh, Cambodia) and stored at  $4^\circ\text{C}$  until analysis. Quantitative parasite egg counts were performed by CNM using the Kato-Katz method <sup>314</sup>. The egg output was expressed as eggs per gram feces (epg).

#### *Socio-economic survey*

Socio-economic information was collected on a sub-sample ( $n = 616$  children, FORISCA-NutriRice study) by trained interviewers during household visits. Questionnaires were answered by parents or caretaker and included information about household characteristics, caretaker's level of education and amount and source of household income.

#### *Data management and Statistical analysis*

Data entry, including quality checks and validation by double entry of questionnaires, was performed with EpiData version 3.1 (EpiData Association, Odense, Denmark). Data management and analyses were performed using SPSS version 20.0 software (SPSS, Inc., Chicago, IL). Normality of distributions was evaluated using Kolmogorov-Smirnoff test. Not normally distributed data were considered to be reasonably close to normality to allow parametric tests when skewness and kurtosis values ranged between  $-1.0$  and  $+1.0$ . Continuous variables that were not normally distributed were log-transformed. Block design score was categorized as score below and above median value. All analyses took into account characteristics of the cluster sampling design (school clusters). The main effects of explanatory variables on cognitive tests scores were first assessed in univariate analysis using ANCOVA for RCPM and Picture completion tests, and logistic regression for Block design test, with all analysis including age as covariate. Multivariate analyses were then performed to evaluate the associations between

cognitive tests scores and variables assessing nutritional status (stunting, anemia and micronutrient status) while taking into account the effect modification or confounding of other variables (age, gender, parasite infection, socio-economic status). Any variable having a p-value <0.25 in the univariate test was considered for the multivariate analysis. Interactions were tested and if significant at the 0.05 level, analyses were run separately at each level of the variable modifying the effect. Multiple comparisons were conducted by using the Bonferroni post-hoc test.

## *Results*

### *Characteristics of the studied sample*

A total of 2443 children from grades 1-6 participated in the study. Characteristics of the participants are presented in Table 13. The mean  $\pm$  SD age of children was  $9.6 \pm 2.3$  y and half (49.9%) were girls. The prevalence of anemia was 15.7% including 0.1% severe anemia. There was no significant difference in anemia prevalence between boys and girls, or age groups; however, prevalence varied between 4.8% and 30.0% across the schools. The overall prevalence of stunting (HAZ<-2SD) was 40.0%, including 10.9% severe stunting (HAZ<-3SD). Children  $\geq 10$  y were significantly more affected by stunting (54.0% and 29.6% respectively) or severe stunting (18.5% and 5.3% respectively) than children <10 y, but no difference was found between gender. Inflammation (CRP>5 mg/L and/or AGP>1 g/L) was found in more than one third of the children (39.5%), with boys being more affected than girls. Parasite infection was found in 18% of the children, boys being more affected than girls ( $p<0.05$ ). Only 1.5% of the children had depleted iron stores (FER<15  $\mu$ g/L) whereas 50.1% had tissue iron deficiency (TFR>8.3 mg/L). There was no significant difference between age groups or genders for both indicators. Prevalence of iron deficiency, as defined by low FER and/or high TFR, was 51.2% without any difference between age groups or gender. Only 2% of the children had negative body iron stores but marginal body iron stores (total body iron<4 mg/kg body weight) were prevalent (13.9%). Prevalence of iron-deficiency anemia was 10.0% with no difference between boys and girls. Prevalence of vitamin A deficiency (VAD) was 0.7%, with no severe VAD. However, 7.9% of the children had marginal VA status ( $0.7 \leq \text{corrected RBP} < 1.05$   $\mu$ mol/L). Children <10 y were significantly more affected by marginal VA status (9.4% and 6% respectively) than children >10 y ( $p<0.05$ ). Prevalence of marginal VA status was also higher in boys than in girls (9.2% and 6.6% respectively,  $p<0.05$ ). Most children (93%) exhibited low serum zinc concentrations, indicative of zinc deficiency. Approximately one fifth (17%) of the children were iodine deficient while half (50.2%) had iodine intake above requirements.

**TABLE 13 CHARACTERISTICS OF SCHOOL-CHILDREN PARTICIPATING IN THE STUDY**

	BOYS	GIRLS	ALL	p-value
n	1223	1220	2443	
Age	9.75 ± 2.34	9.54 ± 2.17	9.65 ± 2.26	p<0.05
% inflammation	41.6 (n=491)	37.5 (n=446)	39.5 (n=937)	p<0.05
% parasite infection	20.0 (n=177)	16.1 (n=148)	18.0 (n=325)	p<0.05
<b>ANTHROPOMETRY</b>				
HAZ	-1.80 ± 1.00 (n=1219)	-1.71 ± 1.06 (n=1216)	-1.75 ± 1.03 (n=2435)	p<0.05
% HAZ<-2SD	41.6(n=507)	38.5 (n=468)	40.0 (n=965)	NS
% HAZ<-3SD	11.1(n=135)	10.8 (n=131)	10.9 (n=266)	NS
<b>IRON STATUS</b>				
Hb (g/L)	123.8 ± 9.9 (n=1203)	124.5 ± 9.5 (n=1206)	124.2 ± 9.7 (n=2409)	NS
% anemia	16.8 (n=202)	14.7 (n=177)	15.7 (n=379)	NS
% severe anemia	0.1 (n=1)	0.2 (n=2)	0.1 (n=3)	
FER <sup>1,2</sup> (µg/L)	66.28 ± 1.68 (n=1181)	68.63 ± 1.68 (n=1190)	67.45 ± 1.68 (n=2371)	NS
% FER <sup>1</sup> <15 µg/L	1.9 (n=22)	1.2 (n=14)	1.5 (n=36)	NS
TfR <sup>2</sup> (mg/L)	8.56 ± 1.32 (n=1181)	8.41 ± 1.30 (n=1190)	8.48 ± 1.31 (n=2371)	NS
% TfR >8.3 mg/L	51.8 (n=612)	50.3 (n=598)	51.0 (n=1210)	NS
% ID <sup>3</sup> total	52.2 (n=616)	50.3 (n=599)	51.2 (n=1215)	NS
% ID <sup>3</sup> with anemia	10.8 (n=127)	9.3 (n=111)	10.0 (n=238)	NS
Body iron (mg/kg)	5.90 ± 2.27 (n=1181)	6.09 ± 2.21 (n=1190)	5.99 ± 2.24 (n=2371)	p<0.05
% body iron <0	2.5 (n=29)	1.6 (n=19)	2.0 (n=48)	NS
% body iron <2	5.0 (n=59)	5.0 (n=59)	5.0 (n=118)	NS
% body iron <4	14.5 (n=171)	13.3 (n=158)	13.9 (n=329)	NS
<b>VITAMIN A STATUS</b>				
RBP <sup>1</sup> (µmol/L)	1.54 ± 0.41 (n=1181)	1.62 ± 0.45 (n=1190)	1.58 ± 0.43 (n=2371)	p<0.01
% marginal VA status <sup>4</sup>	9.2 (n=109)	6.6 (n=79)	7.9 (n=188)	p<0.05
% VAD <sup>5</sup>	0.8 (n=10)	0.6 (n=7)	0.7 (n=17)	NS
<b>IODINE STATUS</b>				
% iodine deficiency <sup>6</sup>	14.8 (n=175)	19.8 (n=233)	17.3 (n=408)	p<0.05
% above requirements <sup>7</sup>	52.5 (n=620)	47.9 (n=564)	50.2 (n=1184)	p<0.05
<b>ZINC STATUS</b>				
% zinc deficiency <sup>8</sup>	93.7 (n=931)	91.9 (n=907)	92.8 (n=1838)	NS
<b>SOCIO-ECONOMIC STATUS (on a sub-group n=616)</b>				
Income <sup>9</sup> (\$/year)	1995 (495;4291)	1825 (500;4050)	1935 (500;4150)	
Caretaker's level of education:				
% no or informal schooling	11.9 (n=35)	14.8 (n=42)	13.3 (n=77)	NS
% primary school	65.3 (n=192)	59.0 (n=167)	62.2 (n=359)	NS
% secondary school	22.8 (n=67)	26.1 (n=74)	24.4 (n=141)	NS

Results are mean ± SD unless stated, <sup>1</sup>corrected for inflammation, <sup>2</sup>geometric mean ± SD, <sup>3</sup>based on FER<sup>1</sup> < 15 µg/L and/or TfR > 8.3 mg/L, <sup>4</sup>0.7 ≤ RBP<sup>1</sup> < 1.05 µmol/L, <sup>5</sup>RBP<sup>1</sup> < 0.7 µmol/L, <sup>6</sup>UIC < 100 µg/L, <sup>7</sup>UIC ≥ 200 µg/L, <sup>8</sup>zinc < 0.66 mg/L for girls ≥ 10 y, < 0.70 mg/L for boys ≥ 10 y, and < 0.65 mg/L for boys and girls < 10 y, <sup>9</sup>median (10th;90th ), HAZ: height-for-age z-scores, Hb: hemoglobin, FER: ferritin, TfR: transferrin receptors, ID: iron-deficiency, NS: not significant, RBP: retinol binding protein, VA: vitamin A, VAD: vitamin A deficiency

## Factors associated with cognitive performance

Cognitive scores were associated with stunting and micronutrient status (Table 14 (RCPM test), Table 15 (Picture completion test) and Table 16 (Block design test)). Children with severe stunting scored significantly lower than non stunted children in all tests ( $p < 0.001$  for all), with reduction of score reaching up to -2 points in RCPM test (12% of mean score), or -1.7 points in picture completion test (22% of mean score) after adjustment on all variables. Children with moderate stunting scored significantly lower (Picture completion and Block design tests, both  $p < 0.001$ ) or tend to score lower (RCPM test,  $p = 0.053$ ) when compared with non-stunted children. For the block design test, severely stunted children were 2.5 times more likely to score below the median value than children with normal height status ( $OR = 2.53$ ,  $p < 0.001$ ).

Univariate analysis showed that positive but marginal body iron stores ( $0 < \text{body iron} \leq 4 \text{mg/kg}$ ) were associated with significantly lower scores in RCPM and picture completion tests (both  $p < 0.05$ ). After adjustment on all variables, boys with iron-deficiency anemia (IDA) scored significantly lower than children with normal iron status in RCPM test (-1.46;  $p < 0.05$ ). Score difference was not significant for girls with IDA. For the picture completion test, children with normal iron status tended to score higher than iron-deficient children with anemia (-0.81;  $p = 0.067$ ) or without anemia (-0.49;  $p = 0.064$ ), for both genders. Vitamin A deficiency, iodine disorders (deficiency or excessive intake) and zinc deficiency were not significantly associated with cognitive performance.

Girls scored significantly lower than boys in the RCPM test (-0.72;  $p < 0.01$ ) and were more likely ( $OR = 1.27$ ;  $p < 0.05$ ) to score below the median value in the block design test. Parasite infection was associated with an increased risk of scoring below median value for the block design test ( $OR = 1.62$ ;  $p < 0.05$ ), and with lower scores in RCPM and picture completion tests, but only for girls (both  $p < 0.05$ ).

In the sub-sample of school children for which data were available ( $n = 616$ ), socio-economic status (caretaker's level of education and household's income) was significantly associated with scores in RCPM test, but not in Picture completion and Block design tests. In RCPM test, higher household's income and caretaker's level of education were both associated with higher test score (both  $p < 0.05$ , Table 17). Results of multivariate analysis of association between RCPM scores and explanatory variables before and after adjustment on socio-economic status are given in Table 17. The results obtained for this sub-group were similar to those obtained on all children, with lower statistical significance as a consequence of smaller number of subjects. Adjustment on socio-economic status did not change the results: severe stunting and parasite infection were associated with lower scores in RCPM test, as well as IDA for boys.



**TABLE 14 FACTORS ASSOCIATED WITH COGNITIVE PERFORMANCE IN RCPM TEST AMONG PARTICIPATING SCHOOL CHILDREN**

Variable	n	Estimated mean <sup>1</sup> ± SE	Estimated mean difference <sup>1</sup> (95% CI)	p-value		Estimated mean difference (95% CI) adjusted for all variables <sup>9</sup>	p-value
<b>Gender</b>							
male	1201	16.88 ± 0.14					
female	1224	16.44 ± 0.14	-0.44 (-0.77; -0.12)	0.008		-0.72 (-1.12; -0.33)	p<0.001
<b>Stunting</b>							
normal status	1500	17.03 ± 0.13					
-3 < HAZ < -2	677	16.41 ± 0.26	-0.62 (-1.32; 0.08)	0.10		-0.56 (-1.12; 0.006)	0.053
HAZ < -3	244	15.12 ± 0.29	-1.91 (-2.70; -1.12)	p<0.001		-1.97 (-2.81; -1.12)	p<0.001
<b>Parasite infection</b>							
not infected	1471	17.49 ± 0.11					
infected	324	17.00 ± 0.28	-0.49 (-1.09; 0.11)	0.11	M	0.29 (-0.49; 1.08)	0.47
					F	-1.43 (-2.18; -0.68)	0.016
<b>Iron deficiency<sup>2</sup></b>							
no ID	1148	17.21 ± 0.13					
ID without anemia	968	16.93 ± 0.14	-0.28 (-0.73; 0.18)	0.43	M	-0.31 (-1.10; 0.48)	1.00
					F	-0.31 (-1.01; 0.38)	0.84
ID with anemia	236	16.41 ± 0.35	-0.81 (-1.70; 0.086)	0.092	M	-1.46 (-2.73; -0.18)	0.019
					F	-0.36 (-1.56; 0.84)	1.00
<b>FER<sup>3</sup> (mg/L)</b>							
≥50	1811	17.19 ± 0.10					
15-50	507	16.69 ± 0.20	-0.50 (-1.03; 0.02)	0.068			
<15	34	16.50 ± 1.07	-0.69 (-3.26; 1.87)	1.00			
<b>TfR (mg/L)</b>							
≤8.3	1152	17.21 ± 0.13					
>8.3	1200	16.86 ± 0.12	-0.348 (-0.698; 0.01)	0.052			
<b>Body iron (mg/kg)</b>							
>4	2028	17.16 ± 0.09					
0-4	278	16.42 ± 0.28	-0.75 (-1.44; -0.06)	0.029			
<0	46	15.85 ± 0.82	-1.32 (-3.29; 0.65)	0.33			
<b>VA status</b>							
normal VA status	2170	16.69 ± 0.12					
marginal VA status <sup>4</sup>	173	16.48 ± 0.42	-0.21 (-1.06; 0.65)	0.63			
VAD <sup>5</sup>	15	16.15 ± 1.26	-0.54 (-3.02; 1.95)	0.67			
<b>Iodine status</b>							
adequate	763	17.21 ± 0.18					
iodine deficiency <sup>6</sup>	406	16.72 ± 0.33	-0.49 (-1.39; 0.41)	0.57			
above requirements <sup>7</sup>	1171	17.33 ± 0.14	1.12 (-0.42; 0.65)	1.00			
<b>Zinc status</b>							
normal status	140	17.62 ± 0.51					
zinc deficiency <sup>8</sup>	1827	17.08 ± 0.11	-0.54 (-1.57; 0.49)	0.30			

<sup>1</sup>adjusted for age, <sup>2</sup>defined by FER<sup>3</sup>< 15 µg/L and/or TfR > 8.3 mg/L, <sup>3</sup>corrected for inflammation, <sup>4</sup>0.7 ≤ RBP<sup>3</sup>< 1.05 µmol/L, <sup>5</sup>RBP<sup>3</sup>< 0.7 µmol/L, <sup>6</sup>UIC<100mg/L, <sup>7</sup>UIC ≥ 200mg/L, <sup>8</sup>zinc < 0.66 mg/L for girls ≥ 10 y, <0.70 mg/L for boys ≥ 10 y, and <0.65 mg/L for boys and girls <10 y, <sup>9</sup>significant interactions between gender and parasite, and gender and iron deficiency, ID: iron deficiency, FER: ferritin, TfR: transferrin receptors, VA: vitamin A, VAD: VA deficiency, M: male, F: female, RCPM: Raven's colored progressive matrices



**TABLE 15 FACTORS ASSOCIATED WITH COGNITIVE PERFORMANCE IN PICTURE COMPLETION TEST AMONG PARTICIPATING SCHOOL CHILDREN**

Variable	n	Estimated mean <sup>1</sup> ± SE	Estimated mean difference <sup>1</sup> (95% CI)	p-value		Estimated mean difference (95% CI) adjusted for all variables <sup>9</sup>	p-value
<b>Gender</b>							
male	1201	7.38 ± 0.13					
female	1224	7.29 ± 0.14	-0.09 (-0.41; 0.23)	0.60			
<b>Stunting</b>							
normal status	1500	7.80 ± 0.13					
-3 < HAZ < -2	677	6.69 ± 0.25	-1.11 (-1.79; -0.43)	p<0.001		-1.07 (-1.62; -0.52)	p<0.001
HAZ < -3	244	5.99 ± 0.29	-1.81 (-2.58; -1.04)	p<0.001		-1.68 (-2.50; -0.86)	p<0.001
<b>Parasite infection</b>							
not infected	1485	7.69 ± 0.18					
infected	304	7.09 ± 0.31	-0.60 (-1.30; 0.10)	0.095	M	0.02 (-0.73; 0.77)	0.96
					F	-0.85 (-1.61; -0.09)	0.028
<b>Iron deficiency<sup>2</sup></b>							
no ID	1148	7.43 ± 0.17					
ID without anemia	968	7.24 ± 0.18	-0.19 (-0.78; 0.40)	1.00		-0.49 (-1.01; 0.02)	0.064
ID with anemia	236	7.00 ± 0.36	-0.43 (-1.39; 0.52)	0.82		-0.81 (-1.66; 0.04)	0.067
<b>FER<sup>3</sup> (mg/L)</b>							
≥50	1811	7.89 ± 0.10					
15-50	507	7.53 ± 0.19	-0.36 (-0.88; 0.15)	0.27			
<15	34	8.11 ± 1.05	0.22 (-2.31; 2.74)	1.00			
<b>TfR (mg/L)</b>							
≤8.3	1152	7.98 ± 0.12					
>8.3	1200	7.64 ± 0.12	-0.35 (-0.69; -0.01)	0.048			
<b>Body iron (mg/kg)</b>							
>4	2028	7.90 ± 0.09					
0-4	278	7.13 ± 0.27	-0.77 (-1.45; -0.10)	0.019			
<0	46	7.33 ± 0.80	-0.57 (-2.51; 1.36)	1.00			
<b>VA status</b>							
normal VA status	2170	7.36 ± 0.11					
marginal VA status <sup>4</sup>	173	7.56 ± 0.41	0.19 (-0.82; 1.21)	1.00		0.08 (-0.84; 0.99)	1.00
VAD <sup>5</sup>	15	4.79 ± 1.22	-2.57 (-5.52; 0.37)	0.11		-0.86 (-3.43; 1.71)	1.00
<b>Iodine status</b>							
adequate	763	7.73 ± 0.17					
iodine deficiency <sup>6</sup>	406	7.72 ± 0.33	-0.01 (-0.90; 0.88)	1.00			
above requirements <sup>7</sup>	1171	8.03 ± 0.14	0.30 (-0.23; 0.83)	0.51			
<b>Zinc status</b>							
normal status	140	8.21 ± 0.50					
zinc deficiency <sup>8</sup>	1827	7.83 ± 0.10	-0.38 (-1.38; 0.62)	0.45			

<sup>1</sup>adjusted for age, <sup>2</sup>defined by FER<sup>3</sup>< 15 µg/L and/or TFR > 8.3 mg/L, <sup>3</sup>corrected for inflammation, <sup>4</sup>0.7 ≤ RBP<sup>3</sup>< 1.05 µmol/L, <sup>5</sup>RBP<sup>3</sup>< 0.7 µmol/L, <sup>6</sup>UIC<100mg/L, <sup>7</sup>UIC ≥ 200mg/L, <sup>8</sup>zinc < 0.66 mg/L for girls ≥ 10 y, <0.70 mg/L for boys ≥ 10 y, and <0.65 mg/L for boys and girls <10 y, <sup>9</sup>significant interactions between gender and parasite, ID: iron deficiency, FER: ferritin, TFR: transferrin receptors, VA: vitamin A, VAD: vitamin A deficiency, M: male, F: female

**TABLE 16 UNIVARIATE AND MULTIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH POOR PERFORMANCE IN BLOCK DESIGN TEST AMONG PARTICIPATING SCHOOLCHILDREN**

Variable	n	odds-ratio <sup>1</sup> (95% CI)	p-value	odds-ratio adjusted for all variables (95% CI)	p-value
<b>Gender</b>					
male	1208	1		1	
female	1213	1.28 (1.06; 1.55)	0.009	1.27 (0.99; 1.63)	0.058
<b>Stunting</b>					
normal status	1446	1		1	
-3 < HAZ < -2	706	1.88 (1.50; 2.37)	p<0.001	1.73 (1.29; 2.33)	p<0.001
HAZ < -3	263	3.67 (2.56; 5.26)	p<0.001	2.35 (1.47; 3.75)	p<0.001
<b>Parasite infection</b>					
not infected	1471	1		1	
infected	324	1.64 (1.21; 2.24)	0.002	1.73 (1.24; 2.42)	0.001
<b>Iron deficiency<sup>2</sup></b>					
no ID	1148	1		1	
ID without anemia	968	0.95 (0.77; 1.17)	0.62	1.09 (0.83; 1.43)	0.52
ID with anemia	236	1.32 (0.95; 1.85)	0.10	1.17 (0.74; 1.85)	0.26
<b>FER<sup>3</sup> (mg/L)</b>					
≥50	1811	1			
15-50	507	1.11 (0.88; 1.40)	0.39		
<15	34	1.31 (0.60; 2.90)	0.50		
<b>TfR (mg/L)</b>					
≤8.3	1152	1			
>8.3	1200	1.01 (0.83; 1.23)	0.93		
<b>Body iron (mg/kg)</b>					
>4	2028	1			
0-4	278	1.23 (0.92; 1.66)	0.17		
<0	46	1.24 (0.62; 2.49)	0.54		
<b>VA status</b>					
normal VA status	2149	1			
marginal VA status <sup>4</sup>	186	0.91 (0.64; 1.30)	0.60		
VAD <sup>5</sup>	17	1.67 (0.48; 5.89)	0.42		
<b>Iodine status</b>					
adequate	763	1			
iodine deficiency <sup>6</sup>	406	0.91 (0.68; 1.21)	0.52		
above requirements <sup>7</sup>	1171	0.94 (0.74; 1.19)	0.60		
<b>Zinc status</b>					
normal status	140	1		1	
zinc deficiency <sup>8</sup>	1827	1.43 (0.95; 2.15)	0.091	1.32 (0.82; 2.13)	0.26

<sup>1</sup>adjusted for age, <sup>2</sup>defined by FER<sup>3</sup>< 15 µg/L and/or TFR > 8.3 mg/L, <sup>3</sup>corrected for inflammation, <sup>4</sup>0.7 ≤ RBP<sup>3</sup>< 1.05 µmol/L, <sup>5</sup>RBP<sup>3</sup>< 0.7 µmol/L, <sup>6</sup>UIC<100mg/L, <sup>7</sup>UIC ≥ 200mg/L, <sup>8</sup>zinc < 0.66 mg/L for girls ≥ 10 y, <0.70 mg/L for boys ≥ 10 y, and <0.65 mg/L for boys and girls <10 y, ID: iron deficiency, FER: ferritin, TFR: transferrin receptors, VA: vitamin A, VAD: vitamin A deficiency, M: male, F: female

**TABLE 17 FACTORS ASSOCIATED WITH COGNITIVE PERFORMANCE IN RCPM TEST BEFORE AND AFTER ADJUSTMENT ON SOCIO-ECONOMIC STATUS IN A SUB-SAMPLE OF SCHOOL CHILDREN (N=616)**

Variable	n	mean difference <sup>1</sup> (95% CI)	p-value	mean difference <sup>2,3</sup> (95% CI) after adjustment for SES	p-value
<b>Gender</b>					
male	311				
female	305	-0.29 (-1.06; 0.48)	0.47	-0.47 (-1.27; 0.33)	0.25
<b>Stunting</b>					
normal status	367				
-3 < HAZ < -2	176	-0.33 (-1.42; 0.75)	1.00	-0.55 (-1.67; 0.57)	0.71
HAZ < -3	67	-1.68 (-3.33; -0.04)	0.044	-1.60 (-3.29; 0.08)	0.067
<b>Parasite infection</b>					
not infected	387				
infected	48	M -0.09 (-1.85; 1.66)	0.92	M 0.20 (-1.63; 2.03)	0.83
		F -2.32 (-4.24; -0.40)	0.018	F -2.27 (-4.25; -0.29)	0.025
<b>Iron deficiency<sup>4</sup></b>					
no ID	261				
ID without anemia	262	M 0.13 (-1.49; 1.74)	1.00	M -0.01 (-1.63; 1.65)	1.00
		F -0.25 (-1.75; 1.24)	1.00	F -0.26 (-1.77; 1.25)	1.00
ID with anemia	65	M -2.09 (-4.60; 0.42)	0.14	M -2.23 (-4.78; 0.32)	0.11
		F -1.05 (-3.25; 1.15)	0.76	F -0.95 (-3.31; 1.41)	1.00
<b>Socio-economic status</b>					
Household income <sup>5</sup>	616			0.03 (0.00; 0.05)	0.048
Caretaker's education <sup>6</sup>	616			0.18 (0.02; 0.34)	0.024

<sup>1</sup>variables in the model: age, gender, stunting, parasite infection, iron deficiency, <sup>2</sup>variables in the model: age, gender, stunting, parasite infection, iron deficiency, caretaker's level of education, income, <sup>3</sup>interaction between gender and parasite infection, and gender and iron-deficiency, <sup>4</sup>defined by FER(corrected for inflammation) < 15 µg/L and/or TFR > 8.3 mg/L, <sup>5</sup>x100 US\$/year, <sup>6</sup>years of schooling, ID: iron deficiency, SES: socio-economic status, M: male, F: female

## Discussion

Within this large cross-sectional survey of Cambodian school children, both long-term nutritional deficits as reflected by stunting and current micronutrient status, especially iron status, significantly affected cognitive performance. According to the WHO classification system, the observed prevalence of anemia (15.7%) indicated a mild public health problem in Cambodia, with only 3 cases of severe anemia. The prevalence of depleted iron stores (1.5%) or negative body iron (2%) were both very low. In contrast, more than half of the children had high TFR concentrations. TFR reflects tissue iron needs but may have also been increased by factors like hemoglobinopathy or inflammation <sup>315,316</sup>. TFR levels were actually higher in children showing abnormal Hb type (HbE) or inflammation, but prevalence of high TFR was still important in the sub-sample of children with normal Hb profile (48.5%) or without inflammation (42.1%). Iron stores being adequate for most of the children (as indicated by the high ferritin concentrations), high TFR concentration suggests a functional tissue iron deficiency due to either an impaired release of iron from stores or impaired systems for transporting iron to target tissues.

The prevalence of iron-deficiency anemia (10%) represented about two-thirds of the overall anemia prevalence. Also, approximately one tenth (9%) of the children exhibited low VA status,

with 0.7% being deficient, and >90% had low serum zinc concentrations, indicating a very high prevalence of zinc deficiency in this population. Hence, this population of school children exhibited concurrent deficiencies of micronutrients, as reported previously for younger children in SE Asia <sup>104,223</sup>.

Poor iron status was associated with lower cognitive ability, however the effect was modified by gender: children with IDA, especially boys, scored lower than iron-replete children. Interestingly, ID was associated with lower scores in the picture completion test even without anemia. Also, lower scores in two of the three tests were found for children with positive but marginal total body iron stores (0-4 mg/kg) as compared to children with replete stores. Thus, the findings from this study indicate that marginal iron status may have impaired cognitive performance before the onset of anemia. This evidence suggests that interventions are needed to improve iron status in school children even in the absence of IDA.

Stunting is an indicator of early-life chronic malnutrition, with most of the growth deficit occurring in the first 2 y of life <sup>317</sup>. In the present study, stunting was a high risk factor for lower scores in all tests after controlling for age, gender and micronutrient status (iron, vitamin A, zinc, iodine), and also for socio-economic status in the sub-sample of children. Furthermore, this study showed that the prevalence of stunting, both moderate and severe, was higher in children  $\geq 10$  y than in children  $< 10$  y. One possible explanation could be that nutritional status of young children has improved considerably over the last decade in this area, but the national data reported a consistent 40% of stunting among children  $< 5$  y in 2005 and 2010 <sup>295,318</sup>. Another explanation could be that growth faltering continues after 5 y of age. Thus, considering also the extremely high prevalence of zinc deficiency found in the studied 6-16 y children (93%), interventions like zinc fortification could possibly help to limit worsening of stunting and, as a consequence, of cognitive performance impairment.

Children infected by parasites, especially girls, exhibited poorer cognitive performance than non-infested children. Possible links between parasite infection and cognitive outcomes are reduced school attendance due to illness, loss of concentration, or through nutrition by affecting absorption of micronutrients such as iron and iodine <sup>319,320</sup>. In the present study, parasite infections remained a highly significant factor in the multivariate analysis which included anthropometry and micronutrient status indicators, suggesting an effect of parasite infection on cognition independent from nutrition. Therefore, the negative effect of parasite infection on cognition might be associated to higher absenteeism from school due to illness. Regardless of the underlying mechanisms, deworming programs could benefit school performance in Cambodian school children.

Although not available for all studied children, socio-economic status was included in analysis for a sub-sample of children (n=616). The results obtained from this sub-group were similar to the whole sample and remained unchanged after correcting for confounding effect of caretaker's level of education and household's income. Thus, we can reasonably assume that associations between cognitive outcomes and variables reflecting nutritional status found for the 2443 children are not due to confounding effects of socio-economic status.

Thus, the present study showed that poor cognitive performance was multi-factorial, with nutritional factors as stunting and iron deficiency and non-nutritional factors such as parasite infection being implicated. These results are consistent with recent studies showing a relationship between cognitive abilities below average and low HAZ in school children in South East Asia <sup>304</sup>, and a beneficial effect of iron supplementation on attention, concentration and different measures of cognition <sup>55,297</sup>. Hence, as stunting and iron deficiency in school-aged

children reflect long-term and current nutritional status respectively, interventions at different periods of age could have a beneficial effect on cognitive abilities. Nutritional programs in early life to ensure good nutrition and prevent stunting before start of schooling are crucial. In addition, strategies at school-age like deworming, surveys for early recognition and prevention of iron deficiency before the onset of anemia, or interventions to prevent worsening of growth faltering should be considered to contribute to optimal cognitive development. Combining nutrition specific interventions, such as food fortification, with nutrition sensitive interventions, such as deworming, might have synergistic benefits.

### 3.2. Height, zinc and soil-transmitted helminthes infections in school children: a study in Cuba and Cambodia (Published paper)

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#### *Introduction*

Height for age, expressed as z-scores of internationally accepted reference curves, is recommended by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) and Food and Agriculture Organization (FAO) as an indicator of chronic undernutrition<sup>321</sup>. Undernutrition can be caused by insufficient intake of macronutrients, micronutrients or both. Poor growth has been associated with insufficient intake and/or absorption of micronutrients<sup>300</sup>. An important micronutrient deficiency prevalent in many middle- and low-income countries is zinc deficiency, for which over 20% of the world's population is estimated to be at risk<sup>3</sup>. Zinc, a trace metal micronutrient, influences many physiological functions, among which growth<sup>322,323</sup>. Deficiency in zinc is recognized as a major cause of morbidity and mortality in developing countries<sup>203,324</sup>. Though generally accepted as a public health concern, documentation on zinc deficiency at the population level remains challenging, as there is no gold standard for the measurement of zinc levels<sup>325,326</sup>. To date, plasma/serum zinc concentration, dietary intake, and stunting prevalence are the best-known indicators of zinc deficiency<sup>203</sup>.

Infections with soil-transmitted helminths (STH) such as *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm affect approximately a quarter of the world's population, and the vast majority of these populations live in middle- and low-income countries in (sub)tropical regions<sup>327</sup>. STH infections have been associated with reduced height for age and stunting, and are strongly related to poverty<sup>328,329</sup>. Populations of these endemic regions often show a poor nutritional status<sup>330</sup>. Zinc deficiency and STH infections are thus likely to coexist in these areas. Moreover, several studies have suggested a role for zinc in susceptibility to STH infections<sup>331,332</sup>. Although the effects of zinc deficiency and STH infections on growth have both been widely studied, data on the association between zinc, STH infection and growth are scarce.

Poor nutritional status and STH infection are intricately linked, whereby STH infection can lead to malnutrition and malnutrition may increase susceptibility to STH infection<sup>332</sup>. Likewise, STH infections and poor nutritional status can affect growth, either independently or in combination. Economic development, population nutritional status, as well as STH species distributions vary greatly between STH endemic countries. For example, Cambodia remains a low-income country with a high prevalence of stunting despite considerable economic development and significant improvement in its population health conditions since the end of the civil war. Food insecurity is still a reality for many of its inhabitants, and, additionally, a high prevalence of STH infection has been reported, mostly by hookworm and *A. lumbricoides*<sup>333</sup>. In contrast, Cuba, which is also an STH endemic country, has a high development index and is categorized as an upper middle-income country. In Cuba, the epidemiological transition has firmly settled in and overweight

rather than underweight is currently a public health concern<sup>334</sup>. Estimates of zinc deficiency prevalence are not available for these countries. The present paper aimed at assessing the associations between height for age, zinc status and STH infections in school-aged children in these two different populations.

## Methods

### Study Population Cuba

A cross-sectional study within school-aged children was performed in 2009 in San Juan y Martínez, Pinar del Río, a municipality in the West of Cuba. The municipality is situated in a rural mountainous area, which is endemic for STHs<sup>335</sup>. From 13 randomly selected schools, 1389 children were included in the study. Written informed consent was obtained from the parents or caretakers of each child. The study was approved by the ethical committees of the Institute of Tropical Medicine in Antwerp (Belgium), the Pedro Kourí Institute of Tropical Medicine and the National Institute for Hygiene, Epidemiology and Microbiology in Havana (Cuba).

### Study Population Cambodia

Data from the baseline measurements of a randomized controlled trial on the effects of multiple-micronutrient-fortified rice on child nutrition and morbidity were used. The trial was conducted in rural Kampong Speu province, Cambodia, in November 2012. Children from 20 randomly selected schools were included ( $N = 2471$ ). All parents or caretakers were asked to sign an informed consent form. Ethical approval was obtained from the Cambodian Ministry of Health, Education and Planning and the Ethical Review board of PATH, USA.

### Height for Age

Height measurements were performed to the nearest 0.1 cm by trained investigators using standard procedures. Age in months was calculated from the children's birth date, retrieved via interviews and verified by school records and birth certificates (Cambodia). Height for age z-scores were calculated according to the WHO 2007 reference curves, using the WHO macro for SPSS<sup>157</sup>. Stunting was defined as height for age z-score below  $-2$  SD. For analyses where age or height for age as continuous covariates were not linearly associated with the dependent variable, data were categorized. Cutoffs were chosen so that three categories of approximately equal group size were made. Because age and height for age ranges differed between both populations, the categories were defined differently per population. In the Cuban data, age was categorized as 4 to 7,  $\geq 7$  to 10 and  $\geq 10$  to 13 years old. Cuban height for age z-scores were categorized as  $<0$ ,  $0-1$  and  $>1$  SD. In the Cambodian data, age was categorized as 5 to 10,  $\geq 10$  to 13 and  $\geq 13$  to 17 years old. Here, height for age z-scores were categorized as  $\leq -2$ ,  $-2$  to  $0$  and  $>0$  SD.

### Parasitology and Treatment

In both countries, one fresh stool sample was collected from each child. Stools were examined by the Kato-Katz technique (duplicate 25 mg smears) according to standard procedures to detect *A. lumbricoides*, *T. trichiura*, and/or hookworm<sup>336</sup>. Infection intensity was recorded as eggs per gram feces (epg) and classified according to WHO guidelines. STH positive children



received anthelmintic treatment: in Cuba, one single dose of 500 mg mebendazole, which has been evaluated and is the treatment of choice in Cuba<sup>337</sup> and in Cambodia, one single dose of 400 mg albendazole was given<sup>338</sup>.

### Plasma Zinc and Inflammation

In Cambodia, zinc was measured in plasma. C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP) were measured alongside plasma zinc, in order to adjust for the effects of inflammation on plasma zinc concentrations. Plasma zinc and CRP and AGP were measured in 5 mL of venous blood, obtained from participants by venipuncture. Plasma zinc concentration was measured by flame atomic absorption spectrophotometry and verified against reference material at the National Institute for Nutrition in Hanoi, Vietnam. Deficiency was defined as plasma zinc below 9.9  $\mu\text{mol/L}$  for children below the age of 10, below 10.1  $\mu\text{mol/L}$  for girls age 10 and older and plasma zinc below 10.7  $\mu\text{mol/L}$  for boys age 10 and older<sup>203</sup>. In 100  $\mu\text{L}$  plasma aliquots, CRP and AGP were measured by sandwich enzyme-linked immunosorbent (ELISA) techniques (VitMin Laboratories, Germany)<sup>195</sup>. Inflammation categories were defined as elevated CRP only, elevated AGP only, both CRP and AGP elevated or no elevated CRP or AGP. Elevated CRP was defined as values above 5 mg/L, elevated AGP was defined as  $> 1 \text{ g/L}$ <sup>339</sup>.

### Hair Zinc

In Cuba, zinc was measured in hair. Two months before the measurements, parents or guardians of the participating children were asked not to cut the hair of their children. Approximately 200–500 mg of hair was collected with the use of stainless steel scissors in the nape or (lower) occipital region of the head approximately 1.5 cm away the scalp. The distal ends of the hair were cut from the samples, leaving a specimen of approximately 2 cm in length. Samples were stored in plastic bags at  $-20^\circ\text{C}$  until the determination of the zinc content. In the laboratory, the samples were analyzed for zinc content by spectrometry. In order to assure the quality of the zinc measurements taken, samples first underwent a washing procedure, to remove exogenous zinc without removing endogenous zinc. Ultra-pure reagents and pretested vials were used. Zinc analysis was done according to the protocol of D'Haese *et al.*<sup>340</sup>. A cutoff value of 70  $\mu\text{g/g}$  wet weight was used to define zinc deficiency<sup>203,341</sup>. Due to funding restraints, hair zinc was measured in a subset of 230 Cuban children.

### Statistical Analysis

Analyses were done using SPSS software version 21 (IBM, NY, USA). Hair zinc followed a skewed distribution, therefore the data for this variable were natural log-transformed for regression analysis and expressed as median and interquartile range for descriptive analysis. The variable STH infection refers to the presence of any STH infection, 'zinc' refers to zinc concentration and 'height for age' refers to height for age z-score in all analyses. For statistical testing, linear regression analysis was performed with height for age z-scores, plasma zinc or the natural logarithm of hair zinc as continuous dependent variables. Covariates of each analysis are specified in the table footnotes. In the analyses of associations between zinc and STH infection with height for age, age was included as a continuous covariate and inflammation categories were included as categorical covariate for the plasma zinc data. In the analysis of associations between zinc and STH infection, covariates age and height for age z-scores were included as categorical variables, created from age and height for age categories. Sex was added

as binary covariate in all analyses. Statistical significance was defined as a *p* value below 0.05, for variables as well as interaction terms.

## Results

### Characteristics of the Study Populations

The mean height for age z-score (0.06) of the Cuban children was significantly higher than the median of the reference population (z-score = 0) (*p* 0.03). Only 21 (1.6%) of the Cuban children presented with stunting (Table 18). In the Cambodian children, mean height for age z-scores were significantly lower than 0 (*p* < 0.001) and stunting was common (42.9%). Zinc deficiency was highly prevalent in Cambodia (92.8%), whereas zinc deficiency was found in only 12.2% of the Cuban children. Prevalence of STH infections was 8.4% and 16.8% for Cuba and Cambodia, respectively. In the Cuban study, the most common STH infections were *A. lumbricoides* (61.4%) and *T. trichiura* 36.8%), while hookworm (97.0%) was the predominant STH infection in Cambodia. In both populations, most STH infections were of light intensity (Table 18).

**TABLE 18 CHARACTERISTICS OF THE STUDY POPULATIONS.**

	<b>Cuba (N = 1389)</b>	<b>Cambodia (N = 2471)</b>
	<i>n</i> (%) or mean ± sd	<i>n</i> (%) or mean ± sd
<b>Age (years)</b>	8.14 ± 2.07	9.68 ± 2.27
<b>Sex (female)</b>	640 (47.0%)	1236 (50.0%)
<b>Height for age z score</b>	0.06 ± 1.04	-1.81 ± 1.05
<b>Stunted</b>	21 (1.6%)	1056 (42.9%)
<b>STH infection <sup>a</sup></b>	114 (8.4%)	302 (16.8%)
<b><i>Ascarislumbricoides</i></b>	70 (5.2%)	5 (0.3%)
<b>Light (&lt;5.000 epg)</b>	55 (4.1%)	5 (0.3%)
<b>Moderate (5.000–50.000 epg)</b>	15 (1.1%)	0
<b>Heavy (&gt;50.000 epg)</b>	0	0
<b><i>Trichuristrichiura</i></b>	42 (3.1%)	6 (0.3%)
<b>Light (&lt;1.000 epg)</b>	38 (2.8%)	6 (0.3%)
<b>Moderate (1.000–10.000 epg)</b>	2 (0.1%)	0
<b>Heavy (&gt;10.000 epg)</b>	2 (0.1%)	0
<b>Hookworm</b>	15 (1.1%)	293 (16.3%)
<b>Light (&lt;2.000 epg)</b>	13 (1.0%)	283 (15.8%)
<b>Moderate (2.000–4.000 epg)</b>	0	9 (0.5%)
<b>Heavy (&gt;4.000 epg)</b>	2 (0.1%)	1 (0.1%)
<b>Hair zinc (µg/g)</b>	113 (91-137) <sup>b</sup>	--
<b>Zinc deficiency <sup>c</sup></b>	28 (12.2%)	--
<b>Plasma zinc <sup>d</sup> (µmol/L)</b>	--	7.65 ± 1.69
<b>Zinc deficiency <sup>e</sup></b>	--	1884 (92.8%)
<b>Inflammation</b>		
<b>No inflammation</b>	--	1450 (60.5%)
<b>Only CRP elevated</b>	--	8 (0.3%)
<b>Only AGP elevated</b>	--	816 (34.1%)
<b>CRP &amp; AGP elevated</b>	--	122 (5.1%)

<sup>a</sup>: N= 1353 (Cuba) or N = 1795 (Cambodia);

<sup>b</sup>: median (IQR), N =230;

<sup>c</sup>: hair zinc < 70 µg/g;

<sup>d</sup>:N=2112;

<sup>e</sup>: age 4–9: plasma zinc < 9.9 µmol/L; girls age 10 and up: plasma zinc < 10.1 µmol/L boys age 10 and up: plasma zinc < 10.7 µmol/L, N = 2030.

## Associations between Height for Age, Zinc and STH Infection

STH infected Cuban children had on average lower height for age compared to their uninfected peers (Table 19), and regression analysis showed a significant negative association between STH infection and height for age (Table 20). The association between hair zinc and height for age was not significant but did show a positive trend. In Cambodia, plasma zinc, but not STH infection, was significantly associated with height for age (Table 20). In both populations, STH x zinc interaction terms were not statistically significant. However, when stratifying for STH infection, in the uninfected Cuban children a significant, positive association (aB 0.471,  $p = 0.033$ ) was found between hair zinc and height for age.

**TABLE 19 ZINC AND HEIGHT FOR AGE IN STH INFECTED AND UNINFECTED CHILDREN.**

		N	Zinc concentration	N	Height for age z score (mean $\pm$ sd)
<b>Cuba</b>	<b>STH uninfected</b>	160	112.55 (88.3–136.0) <sup>a</sup>	1251	0.11 $\pm$ 0.97
	<b>STH infected</b>	70	113.35 (94.4–143.7) <sup>a</sup>	117	–0.31 $\pm$ 1.16
<b>Cambodia</b>	<b>STH uninfected</b>	1239	7.74 $\pm$ 1.70 <sup>b</sup>	1450	–1.81 $\pm$ 1.05
	<b>STH infected</b>	254	7.52 $\pm$ 1.70 <sup>b</sup>	296	–1.84 $\pm$ 1.09

<sup>a</sup>. Hair zinc in  $\mu\text{g/g}$ , median (IQR);

<sup>b</sup>. Plasma zinc in  $\mu\text{mol/L}$ , mean  $\pm$  sd.;

**TABLE 20 LINEAR REGRESSION MODELS OF HEIGHT FOR AGE BY STH INFECTION AND ZINC.**

	independent variable	N	aB <sup>a</sup>	p
<b>Cuba<sup>b</sup></b>	STH infection	226	–0.483	0.001
	Zinc		0.335	0.082
<b>Cambodia<sup>c</sup></b>	STH infection	1448	–0.008	0.902
	Zinc		0.033	0.029

<sup>a</sup>: regression coefficient;

<sup>b</sup>: adjusted for sex and age in months;

<sup>c</sup>: adjusted for sex, age in months and inflammation categories

In the Cuban study, the median hair zinc concentration was slightly higher in STH infected than in uninfected children (Table 19), but the result of the regression analysis was not statistically significant (Table 21). In contrast, STH infected children in the Cambodian study had on average lower plasma zinc concentrations than their uninfected peers (Table 20). This association was borderline significant (Table 21).

**TABLE 21 LINEAR REGRESSION MODELS OF ZINC BY STH INFECTION.**

	<b>Variable</b>	<b>N</b>	<b>aB</b>	<b>p value</b>
<b>Cuba <sup>a</sup></b>	STH infection	230	0.068	0.206
<b>Cambodia <sup>b</sup></b>	STH infection	1795	-0.233	0.051

<sup>a</sup>: adjusted for sex, age categories and height for age categories;

<sup>b</sup>: adjusted for inflammation categories, sex, age categories and height for age categories

### Discussion

The present study showed different associations between height for age, STH infection and zinc in Cuban and Cambodian schoolchildren. In the Cuban study population STH infection was significantly associated with lower height for age, while hair zinc concentrations were not. Conversely, in the Cambodian study population plasma zinc, but not STH infection, was significantly associated with higher height for age.

The two populations were markedly different in mean height for age. The Cuban schoolchildren were on average taller than the reference population<sup>157</sup> and stunting was rare. These characteristics generally indicate an adequate zinc status at population level<sup>203</sup> and this was confirmed by the observed hair zinc values. STH infection appeared to have a stronger effect than zinc on height for age in Cuban children. Because stunting was rare in the Cuban study population, the associations occurred in children of normal height. The Cambodian schoolchildren included in the study had a low mean height for age compared to the reference population<sup>157</sup> and stunting was common. The observed stunting suggested a zinc deficient population<sup>203</sup>, which was indeed corroborated by the observed plasma zinc values. In these children, STH infection was not associated with height for age.

This study also examined the relation between zinc and STH infection. Plasma zinc concentrations were lower in STH infected Cambodian children than in their uninfected peers. This association was borderline significant. Few other studies have addressed associations between zinc and STH infection. In 2009, Rosado *et al.* found that while zinc supplementation increased height for age in Mexican infants, this effect was diminished by *Ascaris* infection<sup>342</sup>. Kongsbak *et al.* found *T. trichiura* to be a significant predictor of serum zinc in a Bangladeshi population where stunting was common<sup>331</sup>. In this study, *T. trichiura* had a larger effect on serum zinc than did *A. lumbricoides*, suggesting species-specific differences. Osei *et al.* did not find serum zinc to differ significantly between STH infected and uninfected Indian children<sup>343</sup>. Two recent meta-analyses found no significant effect of zinc supplementation on STH (re-) infection rate<sup>344,345</sup>. The present study did not distinguish between the effects of the different STH species. In our Cambodian study, children carried almost exclusively hookworm infections. Hence, STH species-specific effects on zinc could not be determined in this population. Likewise, a comparison between zinc deficient and zinc sufficient children in STH infection was not possible, since almost all of the Cambodian children were zinc deficient.

The different associations between STH and stunting found in the two populations might reflect the difference in predominating STH species. In the present study, the Cuban children were

more often infected with *A. lumbricoides* or *T. trichiura*, while hookworm was the prevailing STH infection in Cambodia. These species have distinct life cycles and might therefore have quite different effects on nutritional status<sup>320,330</sup>. Recently, in a study conducted in children in the Philippines, Papier *et al.* showed that the proportion of stunted children was significantly higher among children infected with hookworm than among children infected with *A. lumbricoides*, and *T. trichiura*<sup>346</sup>. These findings are corroborated by the results of this study.

This study has some limitations, warranting caution in its interpretation. Since the present study is cross-sectional, causality cannot be inferred. STH infections and zinc deficiency are often put forward as important causes of child stunting<sup>203,330</sup>. However, reduced height for age might also reflect a generally poor nutritional status, which can influence both zinc uptake and susceptibility to infections. Stunting is also strongly related to poverty, as are STH infections and zinc status<sup>329,347</sup>. Moreover, observed associations between height, zinc and STH might all be explained in the context of 'environmental enteropathy'; repeated exposure to intestinal pathogens resulting in inflammation and remodeling of the mucosa, causing widespread malabsorption<sup>348</sup>.

Associations between zinc and helminths can also be interpreted in various ways. STH infection might damage or block the intestinal mucosa, resulting in reduced uptake of nutrients<sup>330</sup>. Additionally, the STH might compete with the host for essential elements. Inflammation resulting from infection can also lead to reduced micronutrient levels in plasma, induced by the acute phase response<sup>349</sup>. For this reason, inflammation was taken into account in the present analysis. On the other hand, zinc status can influence susceptibility to infection by its effects on immune function<sup>203</sup>.

While the importance of assessing zinc levels has been recognized for many years, a reliable and representative method to measure zinc remains a challenge. Serum or plasma zinc is considered the best available biomarker of zinc deficiency in populations<sup>203</sup>. It has been shown that plasma zinc reflects dietary zinc intake and that it responds consistently to zinc supplementation<sup>203,350</sup>. However, the timing of blood collection and fasting status influence the zinc concentrations measured in plasma<sup>351</sup>. Moreover, zinc is considered a 'type-II' nutrient, meaning that no real stores exist, and that growth faltering is one of the key features of deficiency<sup>352</sup>. Associations between low zinc concentration in hair and poor growth have been documented<sup>203</sup>. Hair zinc has been shown to increase after supplementation<sup>351</sup>. However, it has been argued that zinc in hair reflects a more extended period of exposure than plasma zinc<sup>203</sup>. It cannot be excluded that differences observed in the present study might be (partly) due to the use of different methods of zinc measurement. Presently, there are no reliable data on the correlation between hair zinc values and plasma or serum zinc values. Moreover, although the effects of the acute phase response on plasma zinc levels are widely recognized, there is currently no standard method of accounting for this in school-age children<sup>339,353</sup>.

Based on the results of this study, we recommend that STH infection and zinc status at population level should be taken into account when assessing the potential factors contributing to stunting. It is essential to define a standard and reliable method of measuring zinc and accounting for inflammation effects in order to further elucidate associations between zinc, STH infection and growth. In populations living in STH endemic areas, a possible association between zinc and STH should be considered. This will improve (the evidence base for) interventions on child growth, for instance by pairing zinc supplementation with helminth control strategies.

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*Chapter 4.    Effectiveness of multi-  
micronutrient fortified rice through a  
school feeding program:  
Case study in Cambodia*

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#### **4.1. Impact of multi-micronutrient fortified rice on hemoglobin, iron and vitamin A status of Cambodian school-aged children: a double-blind randomized controlled trial** (Published paper)

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##### *Introduction*

Micronutrient deficiencies, also known as hidden hunger, remain a critical public health problem affecting a third of the world's population <sup>296</sup>. Iron deficiency (ID), the primary cause of anemia, has adverse effects on both human health and socio-economic development, with increased susceptibility to infections, elevated risk of maternal and child mortality, impaired cognitive and physical development of children and lower work productivity of adults <sup>208,354</sup>. Like ID, vitamin A deficiency (VAD) ranks among the 15 leading causes of the global burden of disease and was estimated to be responsible for 0.6 million deaths in children under 5 y of age<sup>300</sup>. VAD can cause xerophthalmia and impairs the immune system thereby increasing the severity and mortality risk of infectious diseases such as measles and diarrheal disease <sup>85</sup>.

The 2011 estimates suggest anemia affects around 800 million children and women worldwide <sup>354</sup>. Anemia is primarily caused by iron deficiency but also by other micronutrient deficiencies such as vitamins B2, folate, and B12. Also vitamin A, selenium and copper have been associated with anemia <sup>208</sup>. Non-nutritional causes of anemia include acute and chronic diseases like malaria, HIV, and tuberculosis, or heavy blood loss such as that associated with intestinal parasite infections <sup>208</sup>. Hemoglobinopathies, one of the most common human genetic disorders <sup>355</sup>, must also be considered a factor of anemia, especially in South-East Asia where thalassemias are common <sup>356</sup>.

Both women of reproductive age and children are the populations most at risk for anemia and micronutrient deficiencies. Approximately 273 million of children (43%), 32million pregnant women (38%), and 496million non-pregnant women (29%) were estimated to be anemic in 2011 <sup>354</sup>. In Cambodia, undernutrition remains a major problem as large segments of the child population (6-59 months) are affected by stunting (40%), wasting (11% ), and anemia (55%) <sup>295</sup>. Micronutrient deficiencies and malnutrition are also widely spread in school-children: it is estimated that iron and vitamin A deficiencies affect 20% of school-aged children in South-East Asia, while 30% are zinc or iodine deficient <sup>17</sup>. Micronutrient deficiencies during school years can impair physical and mental development and reduces school attendance by increasing morbidity. Some studies reported that it is still possible to improve cognition at school age by improving micronutrient status <sup>53,68,357</sup> as well as positive effects on morbidity and growth, but the overall effects on these outcomes were equivocal and more evidence is required from studies in different contexts.

The inclusion of micronutrient rich foods in the daily diet, like meat and a variety of vegetables and fruits, is often not affordable for populations living under conditions of poverty in both developed and developing countries. Food fortification is a cost-effective alternative to food-based approaches for controlling and preventing micronutrient deficiencies, and could improve the nutritional status of populations at risk. The Copenhagen Consensus 2008 actually ranked micronutrient fortification among the top three international development priorities using a cost-benefit analysis <sup>358</sup>. The fortification of staple foods is advantageous because it does not require the target population to change their dietary habits and allows fortification with multiple micronutrients since deficiencies often occur concurrently <sup>69</sup>. Many studies carried out in Latin America, Africa and India showed that rice fortification is safe and effective in improving micronutrient status, with impact depending on the micronutrient content of the fortified rice <sup>130,176,359,360</sup>. In rice consuming countries such as Cambodia, multi-micronutrient fortified rice

could be a promising strategy to address micronutrient deficiencies. However, evidence of impact is needed by the Cambodian government and WFP to support including fortified rice in food-based social safety net programmes or as a potential vehicle in the government's proposed national food fortification guidelines.

Consequently, the objective of the FORISCA UltraRice+NutriRice study, a large scale cluster-randomized, double-blinded, placebo-controlled trial, was to evaluate the impact of three different types of multi-micronutrient fortified rice distributed through the WFP school-meal program (SMP) on micronutrient status, health, and cognition of Cambodian schoolchildren. This paper examines the impact of fortified rice on hemoglobin, iron and vitamin A status.

## *Subjects and Methods*

### *Study site*

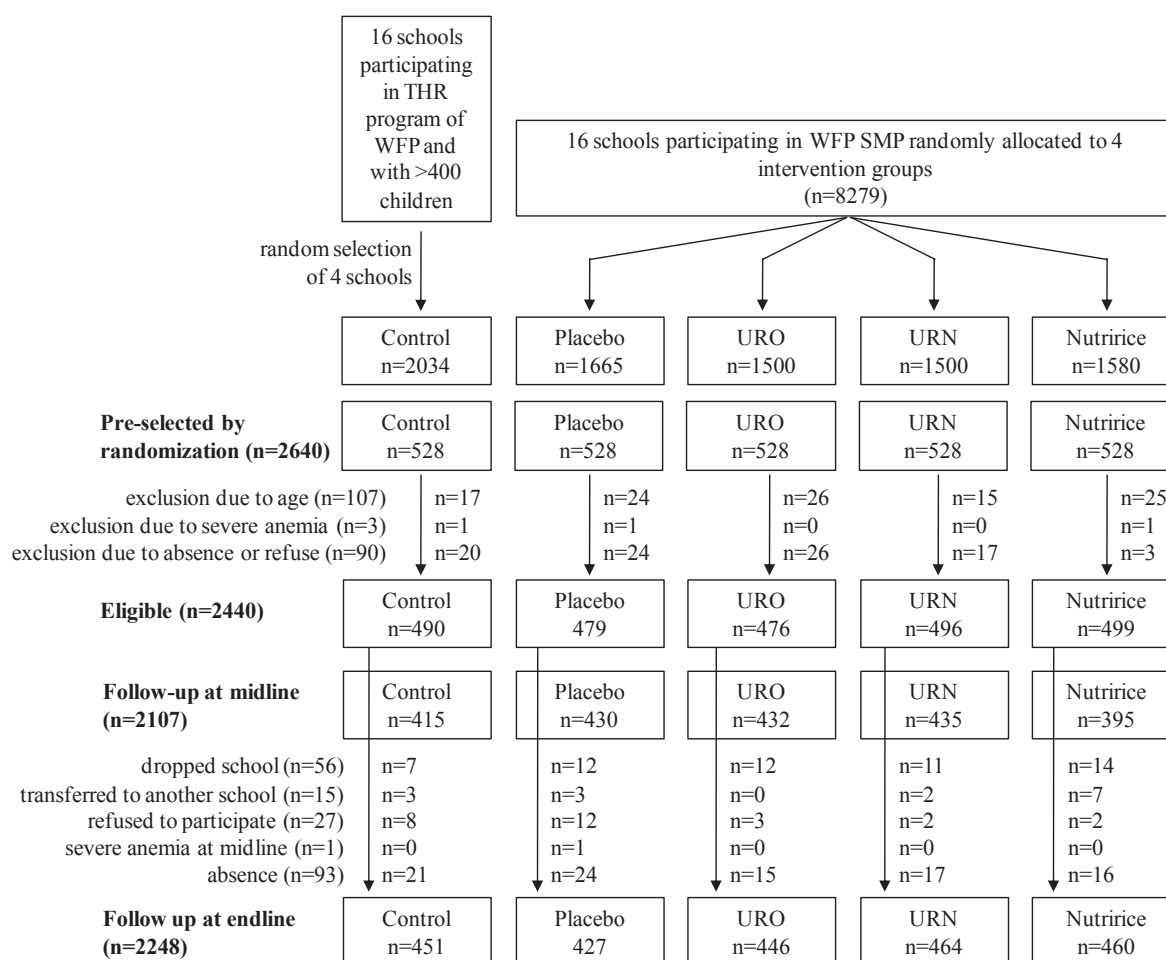
The study was conducted between November 2012 and July 2013 in 20 primary schools from 5 districts of Kampong Speu province in Cambodia. Kampong Speu is one of Cambodia's 23 provinces, situated 60 km west of Phnom Penh, the capital city. Agriculture is predominant, with rice farming as the main occupation and income source.

### *Study design*

A total of 20 primary schools were selected to constitute four intervention groups (including placebo) and a control group. Sixteen schools (intervention groups and placebo) were selected from the primary schools participating in the World Food Programme (WFP) school meal program. This program provides children with a daily breakfast consisting of rice, beans, canned fish, iodine-fortified salt, and vegetable oil enriched with vitamins A and D. The 16 selected schools were randomly allocated to 1 of the 4 intervention groups using a computer generated list with predefined criteria of group size. Randomization was done by one of the researchers (MAD) not involved in the field work and the codes were not known by any researchers or field staff during implementation, thus assuring the study was double-blinded. The four intervention groups were: 1) fortified cold-extruded rice UltraRice original formulation (URO), 2) fortified hot-extruded rice UltraRice new formulation (URN), 3) fortified hot-extruded rice Nutrice and 4) non-fortified rice (placebo). Four schools were randomly selected from sixteen primary schools participating in another program of WFP (take-home ration program) but not receiving a school meal so as to constitute the control group to assess the impact of the normal school meal program, and the additional benefits of including fortified rice.

Prior to the study, all parents of children from the 20 schools were invited to attend a meeting at which the study procedures were explained. Written informed consent was obtained from the parents as was verbal assent from the participating children. Children attending the selected schools were eligible to be part of the study if they were 6-16 y of age, written informed consent was obtained from parent/caregiver, and the child did not have a mental or severe physical handicap. Children with severe anemia (defined as hemoglobin concentration <70 g/L) were excluded, but received multiple micronutrient supplements for 2 months, after which hemoglobin concentrations were re-assessed.

A sample size of 500 children per group was calculated to detect a difference in Hb concentration of 4 g/L, assuming an average Hb concentration of 110g/L<sup>361</sup>. Other outcomes such as changes in FER, TfR and RBP concentrations all needed smaller sample sizes. In each school, 132 children were randomly selected after stratification by sex and grade, hence 528 children per group and a total of 2640 children. Two hundred children were not recruited because of absence on the day of data collection or refusal of participation (n=90), age outside age criteria (n=107) or severe anemia (n=3). A total of 2440 schoolchildren aged 6-16 y participated in the study. Figure 6 shows the subject selection scheme of the study.



**FIGURE 6 TRIAL PROFILE. THR: TAKE-HOME RATION, URO: ULTRARICE ORIGINAL FORMULATION, URN: ULTRARICE NEW FORMULATION, WFP-SMP: WORLD FOOD PROGRAMME SCHOOL-MEAL PROGRAM**

The primary outcomes evaluated in the FORISCA UltraRice+NutriRice study were the prevalence of anemia and micronutrient deficiencies, anthropometry, health and general well-being, and cognitive function. Prevalence of helminth infection, gut flora, and immune function were evaluated as secondary outcomes. This paper focuses on the impact of the intervention on the prevalence of anemia, evaluated using hemoglobin concentration, and iron and vitamin A deficiencies, respectively evaluated using FER and TfR and RBP plasma concentrations.

The study was approved by the National Ethic Committee for Health Research (NECHR) of the Ministry of Health, Phnom Penh, Cambodia, the Ministry of Education, Youth and Sports, Phnom Penh, Cambodia, and the Research Ethical committee (REC) of PATH, Seattle, USA. Written informed consent was collected from all parents/caregivers of children prior to the enrolment in the study.

## Intervention

The standard WFP-SMP breakfast consists of 115 g of (uncooked) rice, 15 g of canned fish, 15 g of yellow split peas, 5 g of oil (fortified with vitamin A and vitamin D) and 3 g of salt (iodized). Breakfast was distributed 6 days/week during 6 months. Fortified 'kernels', produced by extrusion, were provided by PATH (UltraRice technology) and DSM (Nutririce). The UltraRice original (URO) was produced by cold extrusion and the UltraRice new (URN) and Nutririce by hot extrusion. The fortified rice for consumption was then obtained by blending the kernels at a ratio of 1/100 with the same local unfortified rice used for placebo group. Blending was done under supervision of WFP at a local food factory in Phnom Penh. Rice

was packaged in bags containing a letter (A – H) according to allocation to intervention group, with 2 letters per intervention group to strengthen blinding. The micronutrient contents of the three different types of fortified rice (URO, URN and Nutririce) are given in Table 22. A previous study conducted in primary schools located in the same region in Cambodia showed good acceptability of fortified rice by parents and children<sup>179</sup>. Participant micronutrient status was evaluated at baseline and after 3 and 6 months of the intervention. Children were dewormed using mebendazole just after baseline and endline, according to the standard procedures of the Ministry of Health, Cambodia.

**TABLE 22 MICRONUTRIENT CONTENTS OF THE FORTIFIED RICES PER 100G OF UNCOOKED BLENDED RICE. URO: ULTRA RICE ORIGINAL FORMULATION, URN: ULTRA RICE NEW FORMULATION.**

<b>Micronutrients</b>	<b>URO</b>	<b>URN</b>	<b>NutriRice</b>
Iron(mg)	10.67	7.55	7.46
Zinc (mg)	3.04	2.02	3.68
Vitamin B1 (mg)	1.06	1.43	0.69
Folic acid (mg)	0.17	0.28	0.14
Vitamin A (IU)	-	2140	960
Vitamin B3 (mg)	-	12.57	7.98
Vitamin B12 (µg)	-	3.8	1.26
Vitamin B6 (mg)	-	-	0.92

#### Blood samples collection

Blood samples (5ml) were collected by venipuncture and aliquoted into trace-element free vacutainers with no anticoagulant (Vacurette, Greiner Bio One) and into EDTA tubes (2ml) for hemoglobinopathy analysis. Samples were then stored in a cool box containing ice-packs and transported to Phnom Penh within 5 h of blood collection. The blood samples were centrifuged at 2700 rpm (1300g) for 10 min at room temperature. Serum was aliquoted in capped Eppendorf tubes and stored at -30°C until transfer for analysis. The anticoagulated blood samples were transported to the Institut Pasteur du Cambodge for hemoglobinopathies analysis by electrophoresis (MINICAP System).

#### Hemoglobin concentration

Hemoglobin concentrations were measured immediately after blood taking using the HemoCue (301+ system, HemoCue Angholm, Sweden). The HemoCue system was controlled on each day of blood collection using three levels of blood controls (HemoTrol®). Anemia was defined as hemoglobin concentration <115 g/L for children <12 y, <120 g/L for children between 12 and 15 y and girls ≥15 y, and <130 g/L for boys ≥ 15 y according to WHO guidelines<sup>308</sup>.

#### Blood samples analysis: markers of iron, vitamin A and inflammation status

Serum samples were transported on dry ice to the VitMin laboratory (Willstaett, Germany) for determination of retinol-binding protein (RBP), ferritin (FER), soluble transferrin receptors (TfR), C-reactive protein (CRP), and α1-acid-glycoprotein (AGP) concentrations. RBP, FER, TfR, CRP, AGP were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) technique<sup>195</sup>. Inflammation was defined as high CRP (>5mg/L) and/or high AGP concentrations (>1g/L), and categorized in four groups based on inflammation markers levels: no inflammation (normal CRP and AGP), incubation phase (high CRP and normal AGP), early convalescence phase (both CRP and AGP elevated), and late convalescence phase (high AGP only)<sup>197</sup>. Serum FER levels can be affected by infection or inflammation, therefore FER concentration was adjusted by multiplying values by correction factors published by Thurnham *et al.*, namely 0.77, 0.53 and 0.75 for children respectively in incubation, early convalescence, and late convalescence phases (19). While ferritin is a positive acute phase protein that is elevated in the presence

of inflammation, TfR is not significantly affected by infection or inflammatory processes <sup>308</sup>. Depleted iron stores were defined by low FER (corrected value <15 µg/L) <sup>308</sup>, and tissue iron deficiency by high TfR (>8.3mg/L) <sup>210,362</sup>. Iron deficiency was defined by depleted iron stores and/or tissue iron deficiency. Total body iron (BI) was calculated from FER corrected for inflammation and TfR as described by Cook *et al.* <sup>199</sup>. A cut-off of 4mg/kg of body weight was used to define marginal body iron stores. Serum retinol is bound with RBP in a 1-to-1 complex <sup>200</sup>, hence RBP concentrations were used as a proxy for more conventional circulating retinol concentrations to evaluate vitamin A status. RBP concentrations were adjusted for the presence of inflammation by multiplying values by correction factors of 1.15, 1.32 and 1.12 for incubation, early convalescence and late convalescence phases respectively <sup>201</sup>. Vitamin A deficiency (VAD) was defined by corrected RBP < 0.70 µmol/L, severe vitamin A deficiency by corrected RBP <0.35 µmol/L and marginal vitamin A status by corrected RBP values ≥0.7 and < 1.05 µmol/L <sup>200,310</sup>.

### *Parasite infestation*

On the day of data collection, children received a plastic container and instructions for fecal sample collection and were requested to return a fecal sample to the school the following day. Samples were then stored at 4°C and analyzed by the National Malaria Center (CNM, Phnom Penh, Cambodia) using the Kato-Katz method <sup>314</sup>. The parasite egg output was recorded as eggs per gram feces (epg).

### *Data management and Statistical analysis*

Data entry and validation by double entry of questionnaires was performed using EpiData version 3.1 software (EpiData Association, Odense, Denmark). Data management and analyses were performed using SPSS version 20.0 software (SPSS, Inc., Chicago, IL). Normality of distributions was evaluated using Kolmogorov-Smirnoff test. Not normally distributed data were considered to be reasonably close to normality to allow parametric tests when skewness and kurtosis values ranged between -1.0 and +1.0 <sup>363</sup>. Continuous variables that were not normally distributed were log-transformed. Baseline characteristics were compared between intervention groups using ANOVA and Pearson's chi-square tests. Risk factors for anemia were analysed using binary logistic regression. Generalized mixed models (linear or binary logistic regression) were used to evaluate the effects of time, group and time x group interaction on Hb, FER, TfR and prevalence of VAD, while taking into account random effects of individuals and school clusters. Primary analysis were performed including age, gender, hemoglobinopathy and baseline characteristics (inflammation, parasite infection, Hb, iron and VA status) in the model. Final models were adjusted on variables identified as having a significant fixed effect in the primary analysis. Multiple comparisons were conducted by using the Bonferroni post-hoc test. The significance level was set at 5 % (p < 0.05) for all tests.

### *Results*

Of the 2440 children included at baseline, 192 did not complete the study (control: n=39, placebo: n=52, URO: n=30, URN: n=32, Nutrice: n=39) due to absence on the day of data collection (n=93), dropping out of school (n=56), transfer to another school (n=15) or refusal to cooperate (n=27) (Figure 6). One child received treatment for severe anemia at midline and was consequently excluded from follow-up.

Baseline characteristics of participants in each group are presented in Table 23. There were no significant differences in age and gender between the study groups. The mean ± SD age of children at baseline was 9.6±2.3 y and half (49.9%) were girls. Placebo and MMFR groups did not differ in baseline characteristics for Hb, body iron, prevalence of anemia, ID, and iron deficiency anemia (IDA). However, despite the randomization, the prevalence of marginal VA status, FER and TfR levels, inflammation, parasite infection and hemoglobinopathy significantly differed between groups. Furthermore, the control group was significantly different from the placebo and intervention groups for most indicators. This difference was



expected as schools in the control group were not selected to be part of the WFP school meal program precisely because of their better status according to poverty, food insecurity and education indicators. Inflammation (CRP>5 mg/L and/or AGP>1 g/L) was found in more than one third of the children (39.5%) and parasite infection in 18% of the children. Only 1.4% of the children had depleted iron stores (FER<15 µg/L) whereas 51% had tissue iron deficiency (TfR>8.3 mg/L). Only 2% of the children had negative body iron stores but marginal body iron stores (total body iron<4 mg/kg body weight) was more prevalent (13.9%). Prevalence of iron deficiency, as defined by low FER and/or high TfR, was 51.2%, including 10% of iron-deficiency anemia. Prevalence of vitamin A deficiency (VAD) was 0.7%, with no severe VAD, whereas 7.9% of the children had marginal VA status ( $0.7 \leq \text{corrected RBP} < 1.05 \mu\text{mol/L}$ ). Using a higher cut-off of  $0.725 \mu\text{mol/L}$  as suggested by Hix *et al.*<sup>364</sup> led to higher though similar prevalence: 1.2% of VAD and 10.2% of marginal VA status. At baseline, the prevalence of anemia in all schools was 15.6%. Anemia was multi-factorial with hemoglobinopathy, VAD and depleted iron stores being the strongest risk factors (all  $p < 0.01$ ) (**Table 24**).

The intervention had a significant effect on Hb and iron status when compared with the placebo group (interaction effect:  $p < 0.001$  for all) (**Table 25**). After 3 months, Hb significantly increased by 0.8 g/L for children receiving URN rice when compared with children receiving unfortified rice ( $p = 0.048$ ), but at the end of the intervention, no significant differences remained between the groups. The FER concentration significantly increased by 8 and 10 µg/L in Nutririce and URN groups after 6 months of the intervention ( $p < 0.001$ ). TfR concentrations also increased in those two groups, after 3 ( $p < 0.05$ ) and 6 months ( $p < 0.001$ ). No significant difference was found for the group receiving URO, although Hb and TfR tended to decrease in the first 3 months. The intervention had no effect on total body iron.

Inflammation status functioned as a significant effect modifier of the intervention on Hb and iron status (**Table 26**). For children with no inflammation (both CRP<5mg/L and AGP <1g/L) at baseline, midline and endline, Hb concentration significantly increased by 2.1 g/L after 3 months in URN group when compared to the placebo group ( $p < 0.01$ ). The increase was still significant after 6 months in this group (+1.8 g/L,  $p = 0.015$ ). Although not statistically significant, Hb also tended to increase after 6 months in the two other groups receiving fortified rice, URO and Nutririce ( $p = 0.054$  and  $p = 0.095$ , respectively) for this sub-sample of children with no inflammation. TfR concentrations were significantly increased after 6 months in both URN and Nutririce groups ( $p < 0.001$ ). Increase of FER was significant in URN group when compared with placebo ( $p < 0.001$ ), and there was a trend for higher FER in the children receiving Nutririce ( $p = 0.07$ ). No significant difference was found in prevalence of anemia.

The intervention had a significant impact on vitamin A status, with a lower prevalence of marginal vitamin A status in children receiving fortified rice including vitamin A i.e. URN and Nutririce (**Table 27**). After 6 months, these children had respectively 4 times (OR=0.24,  $p < 0.001$ ) and 5 times (OR=0.20,  $p < 0.001$ ) less risk of marginal VA status than children in the placebo group. Risk was reduced by almost 50% (OR=0.52,  $p < 0.05$ ) after 3 months for children in Nutririce group.

**TABLE 23 BASELINE CHARACTERISTICS OF ALL CHILDREN PARTICIPATING IN THE STUDY AND FOR EACH INTERVENTION GROUP**

	ALL	CONTROL	PLACEBO	URO	URN	NUTRIRICE	P-value <sup>5</sup>
N	2440	490	479	476	496	499	
Age (y)	9.65 ± 2.26	9.82 ± 2.30	9.61 ± 2.28	9.55 ± 2.14	9.64 ± 2.22	9.60 ± 2.35	NS
% girls	49.9 (n=1218)	50.6 (n=248)	49.9 (n=239)	49.6 (n=236)	50.6 (n=251)	48.9 (n=244)	NS
% inflammation	39.5 (n=935)	45.6 <sup>a</sup> (n=215)	43.5 <sup>a</sup> (n=201)	42.3 <sup>a,b</sup> (n=199)	32.3 <sup>c</sup> (n=158)	34.0 <sup>b,c</sup> (n=162)	<0.05
% parasite infection	17.9 (n=323)	9.4 <sup>a</sup> (n=33)	22.9 <sup>b</sup> (n=83)	23.9 <sup>b</sup> (n=89)	19.5 <sup>b,c</sup> (n=74)	12.9 <sup>a,c</sup> (n=44)	<0.05
Hemoglobinopathy (% HbE)							
Hb E ≤5%	54.1 (n=1130)	57.4 <sup>a,b</sup> (n=236)	60.1 <sup>b</sup> (n=256)	52.7 <sup>a,b</sup> (n=225)	50.7 <sup>a,b</sup> (n=218)	49.5 <sup>a</sup> (n=195)	<0.05
Hb E 5-80%	37.8 (n=789)	36.0 <sup>a,b</sup> (n=148)	31.7 <sup>b</sup> (n=135)	41.2 <sup>a</sup> (n=176)	39.5 <sup>a,b</sup> (n=170)	40.6 <sup>a,b</sup> (n=160)	<0.05
HbE>80%	8.1 (n=169)	6.6 (n=27)	8.2 (n=35)	6.1 (n=26)	9.8 (n=42)	9.9 (n=39)	NS
<b>ANEMIA and IRON STATUS</b>							
Hb (g/L)	124.2 ± 0.2	125.6 ± 0.4 <sup>a</sup>	123.6 ± 0.4 <sup>b</sup>	124.3 ± 0.4 <sup>a,b</sup>	123.6 ± 0.4 <sup>b</sup>	124.1 ± 0.4 <sup>a,b</sup>	<0.05
% anemia	15.6 (n=376)	9.8 <sup>a</sup> (n=48)	18.9 <sup>b</sup> (n=89)	15.3 <sup>a,b</sup> (n=72)	17.8 <sup>b</sup> (n=88)	16.4 <sup>b</sup> (n=79)	<0.05
FER <sup>1</sup> (μg/L)	76.2 ± 36.9 (n=2368)	83.0 ± 35.8 <sup>a</sup> (n=471)	77.0 ± 36.5 <sup>a,b</sup> (n=462)	79.7 ± 38.8 <sup>a</sup> (n=470)	69.7 ± 36.3 <sup>c</sup> (n=489)	71.9 ± 35.3 <sup>b,c</sup> (n=476)	<0.05
% FER <sup>1</sup> <15 μg/L	1.4 (n=34)	0.2 <sup>a</sup> (n=1)	0.4 <sup>a</sup> (n=2)	0.9 <sup>a</sup> (n=4)	3.7 <sup>b</sup> (n=18)	1.9 <sup>a,b</sup> (n=9)	<0.05
TFR (mg/L)	8.8 ± 2.5 (n=2368)	8.5 ± 2.1 <sup>a</sup> (n=471)	9.0 ± 2.4 <sup>b</sup> (n=462)	9.1 ± 2.5 <sup>b</sup> (n=470)	8.4 ± 2.5 <sup>a</sup> (n=489)	8.9 ± 3.0 <sup>a,b</sup> (n=476)	<0.05
% TFR > 8.3 mg/L	51.0 (n=1207)	46.7 <sup>a</sup> (n=220)	55.6 <sup>a,b</sup> (n=257)	56.6 <sup>b</sup> (n=266)	47.0 <sup>a</sup> (n=230)	49.2 <sup>a,b</sup> (n=234)	<0.05
% ID <sup>2</sup> total	51.2 (n=1212)	46.7 <sup>a</sup> (n=220)	55.6 <sup>a,b</sup> (n=257)	56.6 <sup>b</sup> (n=266)	47.6 <sup>a,b</sup> (n=233)	49.6 <sup>a,b</sup> (n=236)	<0.05
% ID <sup>2</sup> with anemia	9.9 (n=235)	6.4 <sup>a</sup> (n=30)	12.1 <sup>b</sup> (n=56)	10.4 <sup>a,b</sup> (n=49)	10.6 <sup>a,b</sup> (n=52)	10.1 <sup>a,b</sup> (n=48)	<0.05
Body iron (mg/kg)	6.0 ± 2.2 (n=2368)	6.5 ± 1.8 <sup>a</sup> (n=471)	6.0 ± 2.0 <sup>b</sup> (n=462)	6.0 ± 2.2 <sup>b</sup> (n=470)	5.8 ± 2.4 <sup>b</sup> (n=489)	5.8 ± 2.4 <sup>b</sup> (n=476)	<0.05
% BI ≥ 4 mg/kg	86.2 (n=2042)	92.6% <sup>a</sup> (n=436)	87.2 <sup>a,b</sup> (n=403)	86.2 <sup>b</sup> (n=405)	83.8 <sup>b</sup> (n=410)	81.5 <sup>b</sup> (n=388)	<0.05
% BI 0-4 mg/kg	11.9 (n=281)	6.8% <sup>a</sup> (n=32)	11.7 <sup>a,b</sup> (n=54)	12.6 <sup>b</sup> (n=59)	12.7 <sup>b</sup> (n=62)	15.5 <sup>b</sup> (n=74)	<0.05
% BI < 0 mg/kg	1.9 (n=45)	0.6 <sup>a</sup> (n=3)	1.1 <sup>a,b</sup> (n=5)	1.3 <sup>a,b</sup> (n=6)	3.5 <sup>b</sup> (n=17)	2.9 <sup>a,b</sup> (n=14)	<0.05



VITAMIN A STATUS							
RBP <sup>1</sup> (μmol/L)	1.58 ± 0.43 (n=2368)	1.60 ± 0.39 <sup>a,d</sup> (n=471)	1.62 ± 0.43 <sup>a, b</sup> (n=462)	1.69 ± 0.43 <sup>b</sup> (n=470)	1.48 ± 0.43 <sup>c</sup> (n=489)	1.52 ± 0.44 <sup>c,d</sup> (n=476)	<0.05
% marginal VA status <sup>3</sup>	7.9 (n=188)	5.3 <sup>a</sup> (n=25)	6.9 <sup>a,b</sup> (n=32)	3.2 <sup>a</sup> (n=15)	12.9 <sup>c</sup> (n=63)	11.1 <sup>b,c</sup> (n=53)	<0.05
% VAD <sup>4</sup>	0.7 (n=17)	0.2 (n=1)	0.4 (n=2)	0.2 (n=1)	1.2 (n=6)	1.5 (n=7)	

Results are mean ± SD unless stated, <sup>1</sup>corrected for inflammation using multiplier correction factors published by Thurnham *et al.* (19,24), <sup>2</sup>based on FER<sup>1</sup> < 15 μg/L and/or TfR > 8.3 mg/L, <sup>3</sup>0.7 ≤ RBP<sup>1</sup> < 1.05 μmol/L, <sup>4</sup>RBP<sup>1</sup> < 0.7 μmol/L, <sup>5</sup>from ANOVA test. Groups in the same subset (a, b or c) do not differ significantly from each other's at the 0.05 level (Bonferroni post-hoc test). BI: body iron, Hb: hemoglobin, FER: ferritin, TfR: transferrin receptors, ID: iron-deficiency, NS: not significant, URO: UltraRice original formula, URN: UltraRice new formula, RBP: retinol binding protein, VA: vitamin A, VAD: vitamin A deficiency

TABLE 24 RISK FACTORS FOR ANEMIA AT BASELINE

Factors	Adjusted OR <sup>2</sup> (95% CI)	p-value
<b>Gender (reference: male)</b>	0.86 (0.62; 1.19)	0.355
<b>Age</b>	1.03 (0.96; 1.12)	0.378
<b>Parasite infection (reference: no infection)</b>	1.63 (1.10; 2.42)	0.016
<b>Inflammation (reference: no inflammation)</b>		
Incubation	1.95 (0.26; 14.58)	0.514
early convalescence	2.20 (1.08; 4.48)	0.029
late convalescence	1.18 (0.82; 1.69)	0.375
<b>Hemoglobinopathy (reference: HbE &lt; 5%)</b>		
5% ≤ HbE < 80%	1.87 (1.30; 2.69)	0.001
HbE ≥ 80%	24.10 (15.09; 38.49)	<0.001
<b>VA status (reference: normal VA status)</b>		
marginal VA status (0.7 < RBP <sup>1</sup> < 1.05 μmol/L)	1.57 (0.91; 2.72)	0.106
VAD (RBP <sup>1</sup> < 0.7 μmol/L)	8.56 (2.30; 31.89)	0.001
<b>Depleted iron stores (FER<sup>1</sup> &lt; 15 μg/L)</b>	52.97 (11.43; 245.55)	<0.001
<b>Tissue iron deficiency (TfR &gt; 8.3 mg/L)</b>	0.99 (0.70; 1.42)	0.979

<sup>1</sup>corrected for inflammation, <sup>2</sup>from binary logistic regression, adjusted for age, gender, parasite infection, inflammation, hemoglobinopathy, VA and iron status. Hb: hemoglobin, FER: ferritin, TfR: transferrin receptors, VA: vitamin A, VAD: vitamin A deficiency.

**TABLE 25 BIOCHEMICAL OUTCOMES AND EFFECT SIZES AFTER 3 AND 6 MONTHS OF INTERVENTION FOR ALL PARTICIPATING CHILDREN**

		Hb (g/L)					FER <sup>1</sup> (µg/L)				
		n	Mean	SE	interaction term <sup>2</sup>		n	mean	SE	interaction term <sup>2</sup>	
					β coefficient (95% CI)	p-value				β coefficient (95% CI)	p-value
B	Placebo	470	123.7	1.3	-		462	77.5	3.5	-	
	URO	471	124.7	1.3	-		470	79.9	3.5	-	
	URN	494	123.7	1.3	-		489	70.0	3.5	-	
	Nutririce	482	124.4	1.3	-		476	72.1	3.5	-	
M	Placebo	428	123.3	1.3	-		426	69.0	3.5	-	
	URO	428	123.5	1.3	-0.74 (-1.54; 0.06)	0.068	428	68.4	3.6	-3.08 (-7.22; 1.06)	0.144
	URN	434	124.1	1.3	0.80 (0.01; 1.59)	0.048	347	64.2	3.6	2.72 (-1.57; 7.02)	0.214
	Nutririce	394	124.4	1.3	0.50 (-0.31; 1.31)	0.230	393	66.5	3.6	2.88 (-1.33; 7.09)	0.180
E	Placebo	425	122.6	1.3	-		421	71.6	3.6	-	
	URO	445	124.1	1.3	0.51 (-0.28; 1.30)	0.207	443	72.6	3.5	-1.46 (-5.58; 2.65)	0.486
	URN	464	123.0	1.3	0.36 (-0.42; 1.14)	0.368	463	74.8	3.5	10.70 (6.62; 14.78)	<0.001
	Nutririce	454	123.5	1.3	0.19 (-0.60; 0.98)	0.633	450	74.5	3.5	8.32 (4.19; 12.44)	<0.001
		TfR (mg/L)					Body iron (mg/kg)				
		n	mean	SE	interaction term <sup>2</sup>		n	mean	SE	interaction term <sup>2</sup>	
					β coefficient (95% CI)	p-value				β coefficient (95% CI)	p-value
B	Placebo	462	8.98	0.24	-		462	6.01	0.27	-	
	URO	470	9.11	0.24	-		470	6.05	0.27	-	
	URN	489	8.42	0.24	-		489	5.79	0.27	-	
	Nutririce	476	8.87	0.24	-		476	5.78	0.27	-	
M	Placebo	426	8.11	0.24	-		426	5.99	0.27	-	
	URO	428	7.98	0.24	-0.26 (-0.53; 0.01)	0.059	428	5.94	0.27	-0.09 (-0.30; 0.13)	0.427
	URN	347	7.90	0.24	0.34 (0.06; 0.62)	0.017	347	5.75	0.27	-0.01 (-0.23; 0.21)	0.928
	Nutririce	393	8.49	0.24	0.49 (0.21; 0.76)	0.001	393	5.62	0.27	-0.13 (-0.35; 0.08)	0.227
E	Placebo	421	8.18	0.24	-		421	6.11	0.27	-	
	URO	443	8.08	0.24	-0.24 (-0.50; 0.04)	0.088	443	6.09	0.27	-0.06 (-0.27; 0.15)	0.582
	URN	463	8.51	0.24	0.89 (0.62; 1.15)	<0.001	463	6.00	0.27	0.11 (-0.09; 0.32)	0.284
	Nutririce	450	8.74	0.24	0.66 (0.39; 0.93)	<0.001	450	5.87	0.27	-0.01 (-0.22; 0.20)	0.947

Results are mean ± SE unless stated, <sup>1</sup>corrected for inflammation, <sup>2</sup>Generalized linear mixed models adjusted for age, gender and baseline characteristics were used to evaluate the effects of time x group interaction term, B: baseline, M: midline, E: endline, Hb: hemoglobin, FER: ferritin, TfR: transferrin receptors, URO: UltraRice original formula, URN: UltraRice new formula.

**TABLE 26 BIOCHEMICAL OUTCOMES AND EFFECT SIZES AFTER 3 AND 6 MONTHS OF INTERVENTION FOR THE SUB-SAMPLE OF CHILDREN WITH NO INFLAMMATION AT BASELINE, MIDLINE AND ENDLINE**

	Hb (g/L)					FER (mg/L)					TfR (mg/L)				
	n	mean	SE	interaction term <sup>2</sup> β coefficient (95% CI)	p-value	n	mean	SE	interaction term <sup>2</sup> β coefficient (95% CI)	p-value	n	mean	SE	interaction term <sup>2</sup> β coefficient (95% CI)	p-value
Placebo	125	124.7	1.6	-		125	84.5	5.0	-		125	8.3	0.3	-	
URO	142	125.3	1.6	-		142	81.8	4.8	-		142	8.9	0.3	-	
URN	136	124.7	1.7	-		136	68.5	5.0	-		136	7.9	0.3	-	
Nutririce	125	124.8	1.6	-		125	71.6	4.9	-		125	8.3	0.3	-	
Placebo	125	123.9	1.6	-		125	78.0	5.0	-		125	7.8	0.3	-	
URO	142	124.2	1.6	-0.3 (-1.7; 1.1)	0.688	142	73.1	4.8	-2.2 (-9.3; 5.0)	0.550	142	7.8	0.3	-0.6 (-1.1; -0.2)	0.004
URN	135	126.0	1.7	2.1 (0.7; 3.5)	0.004	136	63.0	5.0	1.0 (-6.2; 8.3)	0.781	136	7.5	0.3	0.1 (-0.4; 0.5)	0.747
Nutririce	125	125.1	1.6	1.1 (-0.4; 2.5)	0.147	125	66.6	4.9	1.6 (-5.8; 9.0)	0.669	125	7.8	0.3	0.0 (-0.4; 0.5)	0.936
Placebo	125	123.0	1.6	-		125	79.2	5.0	-		125	7.5	0.3	-	
URO	142	124.9	1.6	1.4 (-0.0; 2.8)	0.054	142	77.9	4.8	1.4 (-5.7; 8.6)	0.697	142	8.0	0.3	-0.1 (-0.5; 0.3)	0.646
URN	136	124.7	1.7	1.8 (0.3; 3.2)	0.015	136	75.9	5.0	12.7 (5.5; 20.0)	0.001	136	8.2	0.3	1.1 (0.6; 1.5)	<0.001
Nutririce	125	124.3	1.6	1.2 (-0.2; 2.7)	0.095	125	73.1	4.9	6.8 (-0.5; 14.2)	0.070	125	8.2	0.3	0.7 (0.3; 1.2)	0.001

Results are mean ± SE unless stated,<sup>2</sup>Generalized linear mixed models adjusted for age, gender and baseline characteristics were used to evaluate the effects of time x group interaction term, B: baseline, M: midline, E: endline, Hb: hemoglobin, FER: ferritin, TfR: transferrin receptors, URO: UltraRice original formula, URN: UltraRice new formula.

**TABLE 27 PREVALENCE OF MARGINAL VA STATUS AFTER 3 AND 6 MONTHS OF INTERVENTION AMONG ALL CHILDREN.**

All children					
		n	% (95% CI)	interaction term	
				Adjusted OR <sup>1</sup> (95% CI)	p-value
B	Placebo	462	5.4 (2.9; 9.9)	-	
	URO	470	2.6 (1.3; 5.4)	-	
	URN	489	12.3 (7.2; 20.3)	-	
	Nutririce	476	11.0 (6.4; 18.4)	-	
M	Placebo	426	11.0 (6.3; 18.6)	-	
	URO	428	13.0 (7.6; 21.4)	2.55 (1.22; 5.33)	0.012
	URN	347	15.4 (9.0; 25.1)	0.60 (0.33; 1.10)	0.101
	Nutririce	393	12.2 (7.0; 20.4)	0.52 (0.28; 0.96)	0.036
E	Placebo	421	12.4 (7.1; 20.6)	-	
	URO	443	8.3 (4.7; 14.5)	1.37 (0.65; 2.91)	0.410
	URN	463	6.3 (3.4; 11.4)	0.20 (0.10; 0.37)	<0.001
	Nutririce	450	6.8 (3.7; 12.1)	0.24 (0.13; 0.45)	<0.001

<sup>1</sup>mixed logistic regression model adjusted for age, gender and baseline characteristics was used to evaluate the effect of time x group interaction term. B: baseline, M: midline, E: endline, Hb: hemoglobin, FER: ferritin, TfR: transferrin receptors, URO: UltraRice original formula, URN: UltraRice new formula.

## Discussion

This study is the largest to date to test the effectiveness of three types of MMFR to improve micronutrient status and reduce deficiencies among schoolchildren. Over the intervention period, consumption of fortified rice had a significant effect on iron and VA status when compared with the placebo group receiving normal rice. However, there was no overall impact after 6 months on hemoglobin concentrations, with Hb concentrations only 0.2 – 0.5 g/L higher in the fortified rice groups as compared to placebo. There was no impact on anemia prevalence either, which according to WHO classification, represented only a mild public health problem (15.7%) in this population <sup>208</sup>. The lack of impact on anemia prevalence might be explained by the multifactorial nature of anemia which was associated with low FER and VAD, but also with non-nutritional factors like parasite infection, inflammation and hemoglobinopathy.

Hence, several factors could underlie this lack of impact on hemoglobin concentrations. First, the high prevalence of hemoglobinopathies in the study population may have blunted the effect of fortified rice on Hb concentrations, as it has been reported that women with thalassemia had a reduced iron incorporation after iron supplementation <sup>365</sup>. However, in our study, there was no difference between children with normal hemoglobin and hemoglobinopathies in terms of response to the intervention in hemoglobin concentrations, perhaps because the majority of the hemoglobinopathies in the present study consisted of HbE. Second, inflammation increases hepcidin concentrations, which reduces iron absorption from the gut <sup>366</sup>. Indeed, in our study population, the prevalence of sub-clinical inflammation was high and a significant effect modifier. In children without inflammation, all the 3 types of MMFR increased or tended to increase Hb concentrations, whereas there was no impact of fortified rice on hemoglobin concentrations in children with inflammation. The increase in hemoglobin concentrations in children without inflammation over the 6 mo intervention (1.2 – 1.8 g/L) was however small considering that 6 to 10 mg of iron was provided (dependent on MMFR), 6 days per week for 6 months. Third, the form of the iron used, ferric pyrophosphate (FePP), is known to have a lower bioavailability than ferrous sulfate <sup>367</sup>, but FePP is preferred however because of its superior organoleptic qualities (i.e. color, taste, smell). However, other studies using rice fortified with only FePP (and not other micronutrients) have significantly improved hemoglobin concentrations<sup>359,360</sup>. Finally, iron status at baseline might have been an important factor in the overall response in hemoglobin concentrations to the intervention. Actually, in the present study, the baseline prevalence of depleted iron stores, as reflected by FER concentration, was very low(1.4%) whether it was estimated using a cut-off of 15 µg/L with FER values corrected for inflammation (98.6%), or a higher cut-off of 30 µg/L with uncorrected values (95%)<sup>308</sup>.

Surprisingly and in contrast to FER, >50% of the children had high TfR concentrations suggesting functional iron deficiency. Iron-deficient erythropoiesis (IDE) is actually the most common cause of elevated TfR <sup>199</sup>. The sequential process of development of iron deficiency generally starts with depletion of iron stores (low FER) leading to a lack of iron from the tissue (high TfR), IDE and finally to IDA. Although inconsistent with this general pattern of iron status biomarkers, the observed discrepancy between FER and TfR levels has already been reported in malaria and non-malaria environments <sup>225,368-370</sup>. Functional tissue iron deficiency can also occur despite normal or even increased storage iron, due to impaired release of iron from stores or impaired physiological systems for transporting iron to target tissues <sup>198,315</sup>. Moreover, TfR concentration depends both on the number of TfR per cell, a function of the iron status of the cell, and on the number of erythroid precursors in the bone marrow <sup>371</sup>. Thus, TfR reflects the

tissue iron needs but also the intensity of erythropoiesis. Some diseases common in developing countries, including thalassemia, megaloblastic anemia due to folate deficiency, or hemolysis due to malaria, may increase erythropoiesis and TfR independently of iron status <sup>321</sup>. Malaria may not be considered as a significant cause in our study since prevalence is very low in the study area. Hemoglobinopathy, on the other hand, was highly prevalent (45.9% children with abnormal Hb types >5%) and could thus be a potential explanation for the high TfR. Indeed, in our study population, TfR concentrations were significantly higher for children with >80% of abnormal Hb type, mainly HbE, than children with a normal Hb profile (+2.04 mg/L, 95%CI: 1.63; 2.45,  $p < 0.001$ ). However, there was no difference in TfR concentrations between children with normal Hb type (HbA >95%) and HbA levels between 20% and 95%, indicating that for example in children with heterozygote HbE, TfR concentrations were not significantly increased. In addition, in children with normal Hb, TfR was increased in 49% of the children, showing that in children without hemoglobinopathy, there was also a major discrepancy between iron stores and tissue iron needs, and thus indicating other causes for the elevated TfR. This discrepancy could also have been caused by sub-clinical inflammation which was highly prevalent (39.5%). Cytokines released during inflammation induce the production of hepcidin<sup>198</sup>, which then inhibits macrophages iron release and intestinal iron absorption<sup>366</sup>. This hypothesis is supported by the bigger effect of fortified rice on Hb concentration observed for children without any inflammation over the intervention period. However, simultaneous high FER and high TfR concentrations was also prevalent in the children without inflammation: in this sub-sample (n=1434), 42% of the children had an elevated TfR. TfR is thought to be less affected by inflammation than FER (38), but in the present study, children with inflammation had TfR levels significantly higher than children without inflammation (+1.15 mg/L, 95CI: 0.94; 1.37,  $p < 0.001$ ). Thus, inflammation alone could not explain the high prevalence of elevated TfR in the present study. This high prevalence might be the consequence of using an inappropriate cut-off: a cut-off of 8.3 mg/L might actually be too low, as suggested for African populations, for whom a higher cut-off of 9.4 mg/L has been proposed <sup>210</sup>. Yet, even with this higher cut-off, 33% of the school children in the present study had an elevated TfR at baseline.

Vitamin A fortified rice was very effective in improving vitamin A status. After 6 months of intervention, whereas the prevalence of low vitamin A status increased in the placebo and URO groups who received VA only through the fortified oil included in all types of school meals, it declined in both groups receiving school meals with rice fortified with VA (URN and Nutririce groups). Consumption of Nutririce and URN reduced by 76% and 80% respectively the risk of having marginal VA status when compared with the placebo group receiving unfortified rice.

Vitamin A status was also an important predictor of anemia, with the prevalence of anemia almost twice higher for children with marginal VA status or VAD (corrected RBP <1.05  $\mu\text{mol/L}$ ) than children with normal VA levels (24.5% vs. 15.0%). Interestingly, there appears to be an effect of the vitamin A containing rice on iron status also, as the increase in FER is found only in the URN and Nutririce groups. Indeed, although URO rice contained the highest concentration of iron, there was no increase in iron stores, whereas the highest increase in FER was found in the rice with the highest vitamin A content (URN). Vitamin A has been shown to increase iron mobilization from stores <sup>372-374</sup> and to improve erythropoiesis. However, it appears that in the present study, erythropoiesis was increased without mobilization of additional iron from stores, given the higher TfR concentrations in the 2 VA containing fortified rice groups. In addition, the VA could have enhanced iron absorption from the gut<sup>375,376</sup>

Hence, this study showed that a multi-micronutrient fortified rice containing VA was very effective in improving VA status of school children. However, the effectiveness in improving



hemoglobin concentrations and iron status was limited, partly by sub-clinical inflammation. Most of the children had repleted iron stores, yet half of them had elevated TfR. This suggested functional ID and impairment in mobilization or transport of iron from stores to the cells, possibly due to inflammation or other concurrent micronutrient deficiencies like vitamin A, B12 or folate. This study also demonstrates that tackling anemia and micronutrient deficiencies might be optimized by combining fortification strategy with non-nutritional approaches which address infections and inflammation. The impact of this intervention study on anthropometry, cognitive outcomes and zinc and iodine status will be addressed in separate publications.

## **4.2. Effect of fortified rice on cognitive performance in Cambodian school-aged children depends on premix composition and cognitive function tested** (Manuscript, drafted)

*To be submitted*

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### *Introduction*

In South-East Asia, micronutrient deficiencies remain highly prevalent <sup>3</sup>. In addition to the most vulnerable groups (i.e. pregnant and lactating women, and young children), the prevalence of micronutrient deficiencies is also high among school-aged children. Over 30% of school-aged children in South-East Asia are affected by zinc deficiency, while 20% of school-aged children are iron or vitamin A deficient <sup>17</sup>. During the primary school years, anemia and deficiencies of iron, zinc, iodine, vitamin A, vitamin B12, vitamin B6 or vitamin B9 can impair concentration and cognitive function and reduce school attendance by increasing morbidity <sup>17,51,377,378</sup>. Therefore, micronutrient deficiencies are detrimental to optimal schooling, while at the same time education is recognized as a prime opportunity to break the cycle of poverty and undernutrition <sup>379,380</sup>.

Although the '1000-days-window' concept rightly highlights that micronutrient deficiencies in fetal life or early childhood can permanently damage cognitive function and development <sup>381</sup>, it is important to realize that it is still possible to improve cognition among primary school-aged children by improving micronutrient status. Several studies have clearly shown a beneficial impact of micronutrient supplementation or food fortification on morbidity and cognitive performance <sup>53,68,378,382,383</sup> with multiple micronutrients appearing to be more efficient than single or double micronutrient interventions <sup>384</sup>. However, the overall evidence remains equivocal <sup>298,357</sup>. In South East Asia, a region where refined rice typically provides 70% of the dietary energy intake, rice fortification could be a cost-effective strategy to reduce micronutrient deficiencies and anemia <sup>130,175,176,359,360,385</sup> and therefore possibly improve cognitive performance in school-aged children.

The school meal program (SMP) of the UN World Food Program (WFP) reached almost 26 million children worldwide in 2011 <sup>29</sup>. The primary objective of the WFP SMP is to increase school enrollment and attendance, however, it can also serve to improve child nutritional status at the same time <sup>29</sup>. Fortification of the school meal with micronutrients could help reduce the prevalence of micronutrient deficiency at very little additional cost. For example, depending on technologies, to fortify rice in school meals would only add 0.50 -2.00 US dollar per child/year <sup>178</sup>.

In Cambodia, malnutrition among children under five years of age is highly prevalent, with 40% stunting and 11% wasting prevalence <sup>386</sup>. Unfortunately, data on the nutritional status of primary school-aged children are scarce in Cambodia. The WFP distributes school meals to more than 500,000 school-aged children in rural Cambodia <sup>29</sup>. The standard school meal, eaten as breakfast, consists of rice cooked with chick peas, served with canned tomato sauce with fish. Rice is the main component of the program and is also the main vehicle in many other food-

based social safety net programs in Cambodia as well as in Asia. Therefore, evidence for the effectiveness of rice fortification would be of immediate importance to these programs as well as for the development of national fortification guidelines in the whole region. The FORISCA project (Fortified Rice for School children in Cambodia) aimed to assess the effect of fortifying the rice in the WFP-SMP with multiple micronutrients on micronutrient status and functional outcomes. The present paper reports on the effects of consuming rice fortified with multiple micronutrients using 3 different fortification formulations, on cognitive performance based on specific cognitive functions in primary school-aged Cambodian children.

### *Subjects and methods*

#### *Study site*

The study was conducted in Cambodia, in 16 primary schools from Kampong Speu province, around 60 km south-west of the capital Phnom Penh. Kampong Speu province can be characterized as rural, with most families involved in rice farming. In 2010, prevalence of stunting and underweight in pre-school children in Kampong Speu province was 44% and 9% respectively <sup>386</sup> and in 2005 more than 60% of pre-school children were anemic in Kampong Speu province <sup>387</sup>. Representative data on school children were not available.

#### *Study design, selection of participants and ethics*

The study period was November 2012 to June 2013, with a rolling recruitment of 1 month and a 6 month intervention period. The study was a double-blind, cluster-randomized, placebo-controlled feeding trial, conducted in 16 schools selected from all primary schools participating in the SMP of WFP (N=18) in Kampong Speu province that gave daily breakfast at school, but did not give Take Home Rations (THR). The sixteen schools were randomly allocated to 1) placebo (normal rice) 2) UltraRice®Original 3) UltraRice®New and 4) NutriRice®. To ensure blinding, the intervention groups were coded with letters (A – H), and 2 schools were allocated to each intervention letter code. Randomization of schools was done by a researcher not involved in the study implementation (MAD), and codes were only known to the researcher and the head of logistics department of WFP, responsible for the distribution of the rice to the schools. Codes were broken only after data collection was completed, and all biochemical data analysis had been done. Before the study commenced, all parents of children of the selected schools were invited to attend a meeting at which objectives and proceedings of the study were explained, as well as their right to refuse to participate in or to continue the study. Only children for whom written informed parental consent was obtained were eligible for randomization. In each school, 132 children were randomly selected from lists provided by the school director, with stratification by grade and gender, giving a total number of 2112 eligible children. Age of each child was calculated using the birthdates provided by the registration book at school, itself based on birth certificates. Exclusion criteria were age < 6 years and severe anemia (hemoglobin concentration <70 g/L <sup>198</sup>). Severely anemic children received appropriate treatment.

The study was approved by the National Ethic Committee for Health Research (NECHR) of the Ministry of Health (Phnom Penh, Cambodia), and the Research Ethics Committee (REC) of PATH (Program for Appropriate Technology in Health, Seattle, USA). The trial was registered at [linicalTrials.gov](http://linicalTrials.gov) (Identifier: NCT01706419).

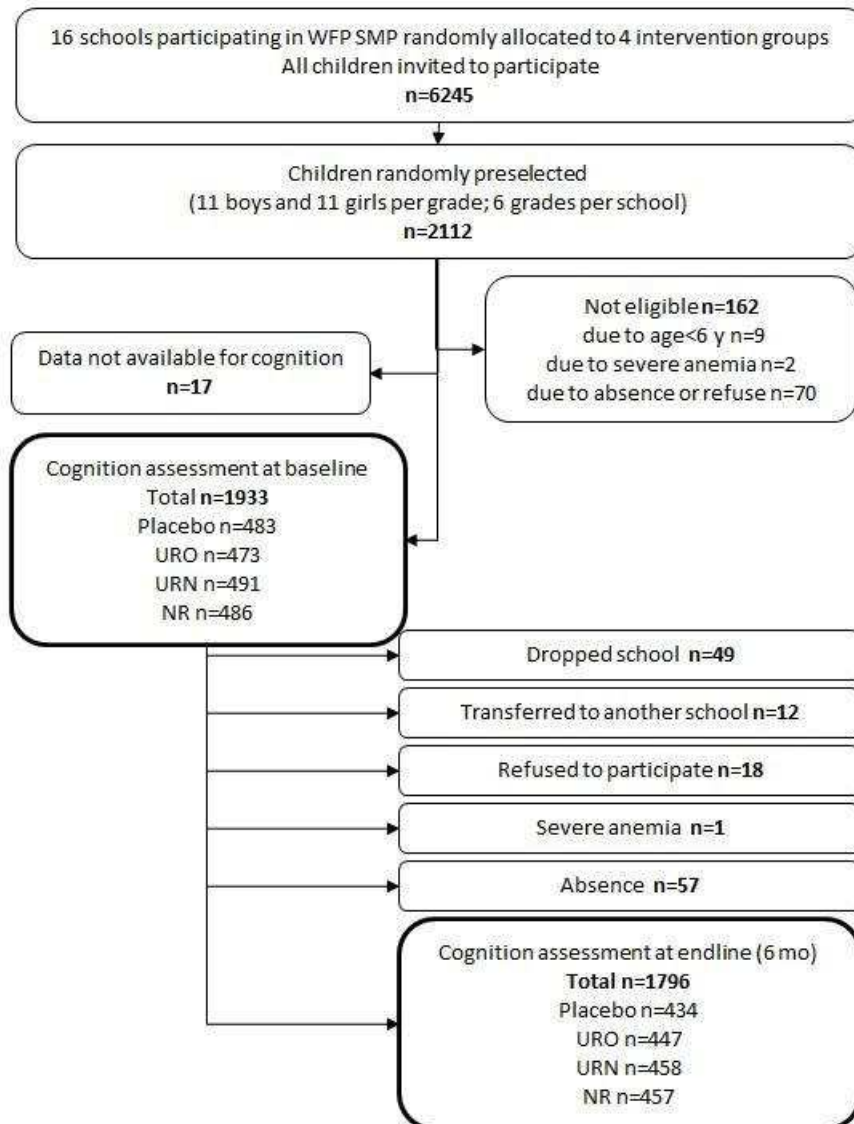


FIGURE 7 STUDY DESIGN

## Intervention

The meal was composed of rice (115 g uncooked) and yellow split peas (15 g uncooked) cooked together with a sauce made from canned fish (15 g), vegetable oil fortified with vitamin A and vitamin D (5 g) and iodized salt (3 g). Fortified rice kernels were produced from fortified rice flour by extrusion, using cold extrusion techniques for UltraRice®Original, warm extrusion techniques for UltraRice®New, and hot extrusion techniques for NutriRice® (DSM). Kernels were blended at a ratio of 1:100 with local normal rice to produce fortified rice. The same rice was used for the placebo intervention. The micronutrient composition of the UltraRice®Original (URO), UltraRice®New (URN), and NutriRice® (NR) is provided in Table 28. The meal was cooked in the school kitchen and served as breakfast at the beginning of the school day. The school meal was distributed for at least 6 months during school days (6 days

per week, except during national holidays). An earlier study had shown that fortified rice (UltraRice®Original and NutriRice®) were highly accepted by Cambodian school-aged children <sup>179</sup>. Children were dewormed using mebendazole after baseline and endline sample collection. Cognitive performance, anthropometry, parasite infestation and micronutrient status were evaluated at baseline and after 6 months of intervention

**TABLE 28 MICRONUTRIENT COMPOSITION OF UNCOOKED RICE PER 100G OF BLENDED RICE**

	Iron (mg)	Zinc (mg)	Vitamin A (IU)	Vitamin B6 (mg)	Vitamin B12 (ug)	Vitamin B1 (mg)	Vitamin B9 (mg)	Vitamin B3 (mg)
UltraRice®original	10.7	3.0	0.0	0.0	0.0	1.1	0.2	0.0
UltraRice®new	7.6	2.0	2140.0	0.0	3.8	1.4	0.3	12.6
NutriRice®	7.5	3.7	960.0	0.9	1.3	0.7	0.1	8.0

### Data collection

Blood samples and anthropometric measurements were collected and cognitive tests performed at schools, after breakfast between 7:30 am and 10:00 am.

### Cognitive tests

Cognitive tools generally suffer from intercultural variability <sup>298,388</sup> : in order to avoid bias linked to translation in Khmer language, tests not involving oral items and with predominantly pictorial content have been chosen in the present study <sup>389</sup>. Three tests were performed to assess cognitive performance: the Raven's Colored Progressive Matrices (RCPM), a common non-verbal test of overall intellectual ability in children  $\geq 5$  y <sup>37,306,390</sup>, and two standardized tests from the Wechsler Intelligence Scale for Children (WISC III) widely used to evaluate the intelligence of children aged 6-16 y <sup>307</sup>: block design and picture completion.. RCPM and WISC III tests were previously used in Vietnamese school children in nutritional intervention trials <sup>391</sup>. RCPM consists of selecting the correct piece (choice of 6 different patterns) that completes an illustrated pattern. The Block design test requires constructing a design with blocks to match a given design on a picture. The Picture completion test consists of identifying the missing detail in a picture. Higher scores indicate better performance. Fluid intelligence underlies reasoning functions whereas crystallized intelligence is related to experience, education and culture as it requires acquired skills and knowledge <sup>383</sup>. Block design and RCPM tests are associated with fluid intelligence <sup>392-394</sup> while picture completion is related to crystallized intelligence <sup>395</sup>.

Each child was tested individually. The cognitive tests were administered by a team of 25 students from the Psychology Department of Royal University of Phnom Penh. The team was specifically trained during a one week workshop prior to the start of the intervention to ensure standardization of proceedings and scoring.

### Anthropometric measurements

Weight and height were measured without footwear and wearing minimal clothing, using standardized procedures <sup>159</sup>. Weight was measured once to the nearest 100 g (Seca 881 U scale, Germany). The accuracy of the scales was checked every day using a set of 2 calibration weights. Height was measured twice to the nearest 0.1 cm on a wooden stadiometer and mean values were used. When differences between two measures of height for the same child exceeded 0.5 cm, measurements were repeated. Height-for-age (HAZ) and BMI-for-age z-scores (BAZ) were

calculated according to the WHO 2006 reference <sup>157</sup>. Stunting and severe stunting were defined as HAZ<-2 and HAZ<-3, respectively. Thinness and severe thinness were defined as BAZ<-2 and BAZ<-3, respectively. Overweight was defined by  $1 < \text{BAZ} \leq 2$  and obesity by  $\text{BAZ} > 2$  z-scores. Anthropometric measurements were performed by 3 teams who were trained and standardized before baseline and again before endline<sup>159</sup>.

#### *Blood and urine sample collection and hemoglobin concentration measurements*

Five mL of venous blood were collected by experienced phlebotomists in a Vacuette (Greiner Bio One) trace-element free vacutainer without anticoagulant. Urine was taken in a sterile container. Urine and blood samples were stored immediately at 4 °C in an icebox containing ice-packs and transported to the laboratory within a maximum of 5 hours after the first sample was obtained. Blood samples were centrifuged at 2700 G for 10 minutes at room temperature. Serum and urine were then aliquoted in Eppendorf tubes and stored at -30°C. Hemoglobin concentration (Hb) was measured on site in whole blood immediately after blood taking, using HemoCue® R 301+ and HemoCue controls (Hemotrol low, medium, high, HemoCue ®).

#### *Parasite infestation*

Plastic containers and instructions for stool sample collection were given to the child on the day of data collection and requested to be returned to the school the following day. Stool samples were then stored in a cool box, transferred to the National Malaria Center (CNM, Phnom Penh, Cambodia) and stored at 4°C until analysis. Quantitative parasite eggs counts were performed by CNM using the Kato-Katz method <sup>314</sup>.

### Laboratory analysis

#### *Ferritin (FER), soluble transferrin Receptor (TfR), retinol-binding protein (RBP), C-reactive protein (CRP), $\alpha$ 1-acid-glycoprotein (AGP) serum concentrations*

Serum samples were sent on dry ice to the VitMin laboratory (Willstaett, Germany) for determination of RBP, CRP, FER, TfR, and AGP concentrations. All these proteins were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) technique <sup>195</sup>. Inflammation was defined as an elevated CRP (>5 mg/L) and/or elevated AGP (>1 g/L) allowing differentiation between incubation phase (high CRP), convalescence phase (both AGP and CRP elevated) and late convalescence phase (elevated AGP only)<sup>197</sup>. Anemia was defined by Hb below cut-offs depending on age and gender: 115 g/L for participants <12 y; 120 g/L for adolescents between 12 and 15 y and girls  $\geq 15$  y; 130 g/L for boys  $\geq 15$  y; severe anemia was defined as Hb<70 g/L; depleted iron stores were defined by inflammation-corrected FER<15  $\mu$ g/L <sup>198</sup>. Correction factors of FER were 0.77, 0.53 and 0.75 for participants respectively in incubation, early convalescence, and late convalescence phases, after Thurnham et al <sup>197</sup>. Tissue iron deficiency was defined by TfR>8.3 mg/L <sup>195</sup>. Low FER and high TfR concentrations are both considered indicators of iron deficiency (ID) <sup>198</sup> so ID was defined by depleted iron stores (low FER) and/or iron tissue deficiency (high TfR). Body iron was calculated according to the formula of Cook<sup>199</sup>: body iron (mg/kg) =  $2(\log(\text{TfR}/\text{FER ratio})22.8229)/0.1207$ , using FER corrected for inflammation. Body iron was considered low when < 4 mg/kg <sup>396</sup>. Vitamin A status was measured using RBP concentration which reflects serum retinol concentration as RBP occurs in a 1:1:1 complex with retinol and transthyretin <sup>200</sup>. RBP concentrations were corrected in participants with inflammation by factors 1.15, 1.32, 1.12 respectively for incubation, early convalescence and late convalescence <sup>201</sup>. Vitamin A deficiency (VAD) was defined by corrected



RBP < 0.7  $\mu$ mol/L<sup>200</sup> and marginal VAD by corrected RBP values  $\geq$  0.7  $\mu$ mol/L and < 1.05  $\mu$ mol/L<sup>202</sup>.

#### *Serum zinc concentrations*

Serum samples were sent on dry ice to the National Institute of Nutrition (Hanoi, Vietnam). Serum zinc was measured by flame atomic absorption spectrophotometry (AAS), using trace-element free procedures. Considering that none of the children were fasting due to the school breakfast, zinc deficiency was defined by serum zinc concentration < 0.65 mg/L for participants < 10 y; for participants  $\geq$  10 y, cut-offs are 0.66 mg/L for girls and 0.70 mg/L for boys<sup>203</sup>. Severe zinc deficiency was defined as serum zinc concentration < 0.5 mg/L<sup>397</sup>.

#### *Urinary iodine concentration*

Urine samples were sent on dry ice to the National Institute of Nutrition (Hanoi, Vietnam). Urinary iodine was measured using an ammonium persulfate method at the Thai Nguyen Provincial Hospital, Vietnam, which is one of the 3 reference laboratories for iodine determination in Vietnam<sup>196</sup>. Iodine deficiency and iodine status above requirements were respectively defined by urinary iodine concentration (UIC) below 50  $\mu$ g/L and above 200  $\mu$ g/L<sup>124</sup>.

#### *Rice sample analysis*

Composition of the fortified kernels for iron, zinc, vitamin A, B6, B12, B9 and B3 was analyzed after production by Silliker laboratories using standard methods. The final composition of blended fortified rice was calculated taking into account a mixing ratio of 1:100. The composition of the fortified rice kernels is given in Table 28.

#### *Data management and statistical analysis*

Data entry, including quality checks and validation by double entry of questionnaires, was performed with EpiData version 3.1 (EpiData Association, Odense, Denmark). Data management and analyses were performed using SPSS version 20.0 software (SPSS, Inc., Chicago, USA). Normality of data was checked before analysis with the Kolmogorov-Smirnov test. In addition, absolute values of skewness and kurtosis of the distribution curves of < 2 and < 7 were used to indicate that the distribution of continuous variables was close to normal<sup>398</sup>. Baseline characteristics were compared between intervention groups using ANOVA for continuous variables, and using Pearson  $\chi^2$  test for categorical variables.

*Primary analysis:* Generalized linear mixed models including interventions groups, visit (baseline, endline), intervention groups x visit, age, gender and clustering (school within group) as fixed factors were used to assess the impact of intervention on the raw scores of the cognitive tests compared to the placebo group. P-value < 0.05 was considered as significant and P-value between 0.1 and 0.05 as a tendency.

*Secondary analysis:* Low iron status, helminthes infection and stunting were identified earlier as risk factors for low cognitive scores in children participating in the FORISCA study<sup>399</sup>. Inflammation was shown to significantly affect the effect of the FORISCA intervention on micronutrient status<sup>400</sup>. In order to assess if these covariates had an impact on the effect of the interventions on cognitive scores in this study, interactions of body iron below 4 mg/kg, helminthes infection, stunting and inflammation were included as fixed factors in the previous generalized mixed models.



## Results

### Baseline characteristics

In total cognition data were available for 1933 children at baseline and 1796 children at endline (Figure 7). Biochemical, physiologic and anthropometric characteristics of the 1933 children at baseline are presented in Table 29.

Mean age at enrollment was 9.7 years old, with participants' age ranging from 6 years to 16 years. Due to late school enrollment and repeating of class, 44% of the participants were  $\geq 10$  years, defined as adolescent according to the WHO definition<sup>401</sup>, but in this paper all participants will be referred to children. Stunting was highly prevalent in the children participating in the FORISCA study (43 %) whereas 26% classified as 'thin'. Iron stores were adequate ( $\text{FER} > 15 \mu\text{g/L}$ ) in most children (99%), whereas paradoxically, tissue iron deficiency was highly prevalent (48% of participants with  $\text{TfR} > 8.3 \text{ mg/L}$ ). Accordingly, half of the children were classified as iron deficient, but this was almost exclusively related to high TfR. Almost all of the children were zinc deficient (>90%) while less than 1% of the children were vitamin A deficient. Prevalence of anemia was 17%.

### Primary analysis

All cognitive scores improved over the 6 months intervention ( $P < 0.001$ , Table 30). On average, the scores increased by 7 (54%), 3 (20%) and 3 (38%) points for block design, RCPM, and picture completion tests respectively. The intervention had a significant overall impact on block design scores ( $P = 0.003$ ). Improvement of block design scores were significantly higher comparatively to the placebo group only in children consuming UltraRice®original ( $\beta = 1.17$ ,  $P = 0.03$ ). No significant difference in RCPM scores or picture completion scores was found between the intervention groups.

### Secondary analysis

For block design scores, only stunting had an influence on the impact of the intervention ( $P = 0.006$  for overall interaction, Table 31) while intestinal parasite infection, inflammation and low body iron did not. The increase in block design score over time was higher in non-stunted children receiving NutriRice® compared to stunted children receiving NutriRice® (difference of scores -0.69,  $P = 0.05$ ).

For RCPM scores, parasite infection had a negative effect on the intervention ( $P = 0.045$  for overall interaction). Among children receiving UltraRice®Original, RCPM scores increased more in children without parasites than in children with parasites (difference of scores -0.08,  $P = 0.010$ ). Low body iron, stunting and inflammation did not affect the impact of the intervention with regard to RCPM scores.

For picture completion scores, both stunting and low body iron had a strong effect on the impact of the intervention ( $P < 0.001$  and  $P = 0.001$  respectively). In children receiving UltraRice®New, those with low body iron increased less in their picture completion scores compared to children with body iron  $\geq 4 \text{ mg/kg}$  (difference of scores 0.51,  $P = 0.001$ ). Similarly the scores of stunted children increased less compared to non-stunted children receiving UltraRice®Original (difference of scores -0.51,  $P = 0.015$ ).

**TABLE 29 CHARACTERISTICS OF CHILDREN AT BASELINE (WITH AVAILABLE COGNITION DATA)**

	n	ALL	Placebo	URO	URN	NR	P-value
% girls	1933	<b>50.3</b>	50.7	50.1	50.7	49.8	NS
age (years)		<b>9.7 ± 2.2</b>	9.6 ± 2.3	9.6 ± 2.2	9.7 ± 2.2	9.8 ± 2.4	NS
% stunted children HAZ < - 2 z-score	1927	<b>42.6</b>	42.9	39.3	45.0	45.1	NS
% thin children BAZ < - 2 z-score		<b>25.8</b>	24.9	26.7	29.8	21.9	0.040
% children infected by helminthes	1442	<b>20.3</b>	23.0	24.9	19.8	12.9	0.000
% children lightly infected by hookworms		<b>17.8</b>	20.0	22.2	17.4	11.1	0.001
% children with inflammation (CRP> 5 mg/L or AGP > 1g/L)	1882	<b>37.8</b>	43.2	42.8	32.8	32.7	0.000
hemoglobin (g/dL)	1903	<b>12.4 ± 1</b>	12.4 ± 1	12.4 ± 1	12.4 ± 0.9	12.4 ± 1.1	NS
% anemic children *	1903	<b>17.1</b>	18.4	15.4	16.9	17.6	NS
ferritin (mg/L) **		<b>74.8 ± 37.3</b>	77.5 ± 38	79.8 ± 36.6	70.1 ± 35.2	71.9 ± 37.3	0.000
% children with low ferritin (ferritin corrected for inflammation <15 µg/L)		<b>1.7</b>	0.4	0.6	3.7	1.9	0.001
TfR (mg/L)		<b>8.9 ± 2.7</b>	9 ± 2.6	9.1 ± 2.6	8.5 ± 2.5	8.9 ± 3.1	0.001
% children with high TfR (TfR > 8.3 mg/L)	1882	<b>47.8</b>	43.7	43.5	52.4	51.4	0.003
% children with iron deficiency anemia (anemia with high TfR and/or low ferritin)		<b>7.7</b>	6.5	5.6	9.5	9.0	0.096
total body iron (mg/kg)		<b>5.9±2.3</b>	6±2	6±2.2	5.8±2.4	5.8±2.5	0.061
% children with low iron status (total body iron < 4 mg/kg)		<b>15.1</b>	12.9	13.5	15.9	18.1	NS
zinc (umol/L)	1546	<b>7.6 ± 1.7</b>	7.2 ± 1.6	7.8 ± 1.7	7.5 ± 1.7	7.8 ± 1.7	0.000
% zinc deficient children ***		<b>93.2</b>	98.1	91.2	93.7	91.9	0.000
% severely zinc deficient children ****		<b>53.7</b>	51.1	48.9	55.5	51.1	0.000
iodine (ug/L)		<b>32.8</b>	22.4	25.3	49.0	33.6	0.000
% children < 50 ug/L	1824	<b>17.2</b>	6.4	9.0	35.0	17.4	0.000
% children >200 ug/L		<b>0.3</b>	0.0	0.0	1.3	0.0	0.001
RBP (mmol/L)		<b>1.6 ± 0.4</b>	1.6 ± 0.4	1.7 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	0.000
% vitamin A deficient children (RBP< 0.7 umol/L)	1882	<b>0.9</b>	0.6	0.2	1.2	1.5	NS
% children with marginal vitamin A status (RBP<1.05 umol/L)		<b>9.6</b>	7.3	3.6	14.2	12.9	0.000

\* Hb < 115 g/L for participants <12 y; 120 g/L for teenagers between 12 and 15 y and girls ≥15 y; 130 g/L for boys ≥15 y

\*\* FER corrected for inflammation <15 mg/L

\*\*\* serum zinc <0.65 mg/L for participants <10 y ; for participants ≥10 y, cut-offs are 0.66 mg/L for girls and 0.70 mg/L for boys

\*\*\*\* serum zinc <0.5 mg/L

NS : Non Significant

**TABLE 30 COGNITION OUTCOMES AND EFFECT SIZES AFTER 6 MONTHS OF INTERVENTION FOR ALL CHILDREN**

<b>Block design score</b>						
Effect of variables		Interaction term*				
	P value		mean	SE	$\beta$ coefficient [95% CI]	P-value
group	0.075		Baseline			
age	0.000	UltraRice®original	13.6	0.4	-	-
gender	0.000	UltraRice®new	14.1	0.4	-	-
visit	0.000	NutriRice®	13.6	0.4	-	-
school(group)	0.000	placebo	13.1	0.4	-	-
groupxvisit	0.003		Endine			
		UltraRice®original	22.0	0.5	1.17 [ 0.12 ; 2.22 ]	0.029
		UltraRice®new	20.5	0.5	-0.66 [ -1.71 ; 0.38 ]	0.214
		NutriRice®	20.4	0.5	-0.45 [ -1.50 ; 0.60 ]	0.396
BIC**	25971	placebo	20.5	0.5	-	-
<b>RCPM score</b>						
Effect of variables		Interaction term*				
	P value		mean	SE	$\beta$ coefficient [95% CI]	P-value
group	0.000		Baseline			
age	0.000	UltraRice®original	17.5	0.2	-	-
gender	0.000	UltraRice®new	17.1	0.2	-	-
visit	0.000	NutriRice®	16.4	0.2	-	-
school(group)	0.000	placebo	16.6	0.2	-	-
groupxvisit	0.153		Endine			
		UltraRice®original	21.0	0.2	0.02 [ -0.52 ; 0.57 ]	0.930
		UltraRice®new	20.1	0.2	-0.34 [ -0.87 ; 0.20 ]	0.221
		NutriRice®	19.9	0.2	0.29 [ -0.25 ; 0.82 ]	0.300
BIC**	20736	placebo	20.1	0.2	-	-
<b>Picture completion score</b>						
Effect of variables		Interaction term*				
	P value		mean	SE	$\beta$ coefficient [95% CI]	P-value
group	0.076		Baseline			
age	0.000	UltraRice®original	7.9	1.9	-	-
gender	0.078	UltraRice®new	7.5	1.8	-	-
visit	0.000	NutriRice®	7.8	1.8	-	-
school(group)	0.000	placebo	7.6	1.9	-	-
groupxvisit	0.094		Endine			
		UltraRice®original	11.0	1.9	0.09 [ -0.47 ; 0.64 ]	0.760
		UltraRice®new	10.9	1.9	0.34 [ -0.21 ; 0.89 ]	0.230
		NutriRice®	10.4	1.9	-0.36 [ -0.91 ; 0.19 ]	0.204
BIC**	20640	placebo	10.7	1.9	-	-

Results from generalized mixed models including interventions groups, visit (baseline, endline), intervention groups x visit, age, gender and clustering (school within group) as fixed factors

\* interaction term groupxvisit(=endline) \*\* Bayesian Information Criterion

**TABLE 31 EFFECTS OF RISK FACTORS FOR LOW COGNITIVE SCORES ON THE 6 MONTHS INTERVENTION**

		Overall effect of interaction with the intervention *	UltraRice®original			UltraRice®new			NutriRice®		
		P-value	β coeff*	difference in scores increase **	P- value ***	β coeff*	difference in scores increase **	P- value ***	β coeff*	difference in scores increase **	P- value ***
Stunting	Block design	0.006	-1.411	0.42	0.234	-1.805	0.93	0.144	-2.687	-0.69	0.05
	Picture completion	0.000	-1.146	-0.51	0.015	-0.434	0.68	0.374	-0.243	0.91	0.654
	RCPM	0.352	-0.774	0.14	0.147	-1.131	-0.29	0.042	-0.566	-0.21	0.358
Parasite infection	Block design	0.197	3.152	-1.18	0.009	-0.352	-0.18	0.785	0.935	-0.31	0.524
	Picture completion	0.129	-0.389	-0.29	0.411	0.407	-0.82	0.428	-0.072	-0.32	0.902
	RCPM	0.045	-1.389	-0.08	0.01	-0.121	-0.66	0.835	-0.532	0.03	0.421
Inflammation	Block design	0.141	-1.130	-0.80	0.314	0.471	-0.67	0.706	-0.136	2.08	0.919
	Picture completion	0.873	0.275	0.35	0.534	-0.436	0.00	0.379	-0.01	0.18	0.985
	RCPM	0.058	0.388	0.38	0.442	-0.283	0.04	0.615	0.09	1.30	0.881
Low body iron	Block design	0.157	1.079	0.06	0.453	2.328	-2.91	0.103	-1.582	0.67	0.308
	Picture completion	0.001	-1.011	0.50	0.076	-1.853	-0.51	0.001	0.607	-0.63	0.323
	RCPM	0.211	-1.144	-0.44	0.077	-0.971	-0.66	0.13	-0.102	-0.29	0.884

Results from generalized mixed models including age, gender, clustering (school within group), interventions groups, visit (baseline, endline), intervention groups x visit, and each of risk factor (stunting, parasite infection, inflammation or low body iron) x intervention groups x visit, as fixed factors

\* β coefficient of interaction term group x visit x risk factor (stunting, parasite infection, inflammation or low body iron)

\*\* Difference of improvement of scores between baseline and endline in stunted children compared to non-stunted children (idem for children with parasites compared to children without parasites, children with inflammation compared to children without inflammation, children with low body iron compared to children with normal body iron)

\*\*\* Significance of the difference of improvement of scores

## Discussion

To our knowledge, this is the first paper reporting modest but significant effects of a micronutrient fortified rice provided in a school meal program on cognitive function in primary school children. Beneficial effects of UltraRice®Original on micronutrient status and anemia were reported in small populations ( $n=210$ ) of Mexican women <sup>360</sup>, Indian school-aged children <sup>175</sup> and Thai school-aged children <sup>177</sup>. No effect of NutriRice® was found on Indian children's cognitive function, but the sample size was considerably smaller than in the present study <sup>130</sup>. Duration of these studies was comparable to the FORISCA study, with meals containing UltraRice®Original or NutriRice® being served 5-6 days for 5 to 8 months.

Perhaps surprisingly, the present study found only an effect of UltraRice®Original on cognitive performance, and not of the two other types of fortified rice. UltraRice®Original contained the highest concentration of iron, which is known to be important in brain development, also during pre-adolescence and adolescence. Iron plays a role in neurotransmission, especially in the dopamine pathway, which serves in memory, learning, attention, motor control, and emotional affect modulation <sup>36,60</sup>. Increasing iron intake in iron deficient and/or anemic children using fortification or supplementation has been shown to improve schooling or cognitive outcomes like learning, memory, concentration or school achievement <sup>53,54,382,402-405</sup>. Zinc is also involved in neurotransmission pathways <sup>406</sup>. Moreover, zinc as a component of zinc-dependent enzymes, plays an important role in neuronal genesis and migration, making adolescents particularly sensitive to zinc deficiency due to rapid brain growth, similar to early childhood <sup>378,406,407</sup>. Even though data are lacking about long-term effects of zinc treatment on cognitive performance, there is some evidence that zinc supplementation may improve neuropsychological functions <sup>378</sup> especially reasoning <sup>408</sup>. For example, zinc supplementation had beneficial effects on cognitive scores of Indian adolescent girls, by reducing reaction time, improving memory and RCPM scores <sup>82</sup>. However, a recent meta-analysis found no effect of zinc on cognitive functioning, but reported that there is a lack of high quality trials <sup>409</sup>.

As reported elsewhere, the intervention had a modest but significant and consistent impact on the micronutrient status of children participating in the FORISCA study <sup>280,400</sup>: A significant increase of FER, indicating improvement of iron stores, between baseline and endline was observed only in children receiving UltraRice®New and NutriRice® ( $P<0.001$ ). However, at the same time TFR significantly increased in children receiving UltraRice®New and NutriRice® ( $P<0.001$ ), indicating decreasing iron tissue stores. In contrast, TFR tended to decrease in the UltraRice®Original group ( $P=0.088$ ), indicating improved iron tissue stores. Hence, although it did not improve iron stores UltraRice®Original was the only fortified rice which improved iron tissue status. It is tempting to link the improved iron tissue status in the UltraRice®Original to the improvements of cognitive performance found in this group. And the results also suggest that TFR would be a better indicator for functional iron deficiency than FER, raising questions on which indicator should be used for assessing iron status.

In an overview of randomized controlled trials of multiple micronutrient supplementation in school-aged children, Eilander et al. noticed a potential beneficial effect on fluid intelligence and school performance, but not on crystallized intelligence <sup>383</sup> which is consistent with fortified rice having an impact on block design scores but not on picture completion scores in children participating in the FORISCA study. The block design test measures perceptual reasoning and executive functions, both of which are associated with frontal lobe functions <sup>395,410</sup>. The development of the full range of executive functions is suggested to occur in late childhood and adolescence as myelination of the frontal lobes proceeds <sup>37</sup> with peaks at 7, 9 and 12 years of

age<sup>36,38</sup>. There is some evidence that iron is a key nutrient in the development of these executive functions<sup>36</sup>.

Although UltraRice®Original contained the highest iron content, the difference with the 2 other types of fortified rice was relatively small, and cannot completely explain the observed difference in impact on cognition. Therefore, it is likely that interactions with other micronutrients present in the UltraRice®New and NutriRice®, but not in the UltraRice®Original, also play a role in modifying the effect on cognitive outcomes. UltraRice®New had the lowest concentrations of zinc and iron but the highest of vitamins (A, B6, B12, B1, B9, and B3). Even though vitamin B12, B6, and B9 are known to play a role in cognitive performance by being involved in methylation in the central nervous system<sup>411,412</sup>, UltraRice®New and NutriRice®, fortified with these vitamins, did not improve cognition performance in the present study while, UltraRice®Original, not fortified with these vitamins, did improve cognition performance. Therefore, improvement in cognition performance in the present study is more likely to be related to the additional iron and zinc provided through UltraRice®Original, and not to additional B-vitamins.

Although the impact of UltraRice®Original on RCPM scores did not reach significance, it was significantly lower in children with intestinal parasite infection than in those without parasites. Hence, while being a risk factor in itself for poor cognitive scores, parasite infestation also limited the impact of fortified rice on RCPM scores. Earlier we reported that fortified rice also increased parasite infestation in children participating in the FORISCA study<sup>413</sup>, highlighting the complex interaction between nutrition and infection.

Albeit no significant impact of fortified rice on RCPM and picture completion scores was observed on the general sample, low body iron diminished the increase of these scores over the intervention period regardless of intervention type, indicating that children participating in the FORISCA study with low iron status benefited less from fortified rice with respect to cognition scores, in comparison to children with adequate iron status. Even if increased iron intake during the intervention had an overall beneficial effect on block design scores, this suggests that the amount of bio-available iron in the fortified rice may not be sufficient to reverse the negative effect of iron deficiency on cognition scores.

Inflammation also had a negative effect on RCPM scores improvement. Interestingly, this is in parallel with the observed effects of fortified rice on iron status of children participating in the FORISCA study, which emerged stronger after removing children with inflammation from the analysis<sup>400</sup>. This suggests that the acute-phase-response may disturb the response to fortified foods and therefore the impact on functional outcomes such as of cognitive performance.

### Strengths and limitations of the study

One of the strengths of this study was its design as an effectiveness trial. We assessed the impact of the introduction of fortified rice within an existing program, with minimal interference in the program's daily logistics. Hence, the results obtained provide a realistic indication of what can be expected from the introduction of multi-micronutrient fortified rice on cognitive performance in Cambodian school-aged children. Another strength of the study is the sample size, with >2000 children being followed over the study period. Also, cognitive tools generally suffer from intercultural variability<sup>298,388</sup> but, in order to avoid bias linked to translation in

Khmer language, tests not involving oral items and with predominantly pictorial content have been chosen in the present study<sup>389</sup>.

One limitation of this study was the intervention's short duration of only 6 months; this amount of time may not have been enough to improve cognitive performance to its full potential. An intervention exceeding 6 months may have shown a larger impact on cognitive outcomes. Also, the present study was an effectiveness study, not an efficacy study. Hence, quantities of rice consumed every day were not controlled, nor was the micronutrient status of children enrolled in the study. The prevalence of low iron stores was very low, and larger impact on cognitive function may have been observed in a population with a higher prevalence. Although the schools were randomly allocated to the different intervention groups, groups still differed with respect to thinness, iron status, iodine status, micronutrient deficiencies and parasite infestation. Groups did not differ with respect to gender, age, and stunting. As the subject size is large, and the statistical modeling robust, no attempts were made to correct or amend for this, instead the validity of each analysis was carefully checked. In addition, the pre-post comparison model of the study design further aids the clear interpretation of the data. Another limitation of the FORISCA study is that levels of iron and zinc used in the present study are below the current recommended levels for fortified rice, which might have reduced the effectiveness<sup>180</sup>.

### *Conclusion*

The SMP is known to be an incentive for school attendance and enrollment, and could enhance learning by reducing hunger and increasing concentration<sup>29</sup>. The current study illustrates that fortification of school meals with multiple micronutrients can improve cognitive performance, and thus promote schooling further. However, the improvements in cognitive performance were modest and impact was modified by several external factors such as stunting, intestinal parasite infestation, low body iron and inflammation. This strengthens the case for combining health interventions, such as deworming combined with provision of micronutrients. However, micronutrients used in rice fortification should be carefully selected and appropriately dosed, taking for example the initial nutritional and infection status of the target population also into account.





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*Chapter 5.    **Evaluation of currently  
used indicators for malnutrition**  
using data from Cambodia and Senegal*

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## 5.1. Current MUAC cut-offs to screen for acute malnutrition need to be adapted to gender and age: the example of Cambodia (Published paper)

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### *Introduction*

Wasting or acute malnutrition is a major contributor to the global disease burden and to child mortality. In 2011, >50 million or 8% of all children <5y (years) were affected <sup>3,18</sup>. Acute malnutrition can be divided into severe acute malnutrition, being defined as a weight-for-height Z-score (WHZ) <-3 or a mid-upper arm circumference of <11.5 cm, or moderate acute malnutrition, with a WHZ between -2 and -3 Z-scores or a MUAC (mid-upper-arm circumference) between 11.5 and 12.5 cm. Severe acute malnutrition affected 19 million children in 2011, causing an estimated 500,000 deaths, which represents ~7.5% of all<5y mortality <sup>3</sup>.

For early identification of children with acute malnutrition, an easy, accurate, and low-cost indicator is needed. The golden standard to identify acute malnutrition is weight-for-height z-scores <sup>414</sup>. Unfortunately, scales and/or height board are not always available for screening at community level in many developing countries. Therefore, as subcutaneous fat and muscle mass decrease in undernourished children, MUAC has been used as a proxy indicator to screen for acute malnutrition. Moreover, MUAC was shown to predict child mortality at least as well as WHZ <sup>415-417</sup>. In the revised (2013) guidelines for the management of severe acute malnutrition, the World Health Organization (WHO) recommends using 11.5cm and 12.5cm as cut-offs for admission and discharge criteria for severe and moderate acute malnutrition in children under 5y respectively <sup>418</sup>. However, recently we showed that MUAC and WHZ identify different groups of children with malnutrition, and that a cut-off of 13.3 cm, rather than 11.5 cm would increase sensitivity of MUAC to identify children with a WHZ<-2 or <-3 <sup>419</sup>.

However, it is questionable whether using only 1 cut-off for all children between 6 and 59 months of age is valid, as MUAC was shown to be age and gender dependent in several studies <sup>420,421</sup>. Recent research has highlighted the problems with using one, unique cut-off for MUAC for identifying malnutrition, as it respectively over- and underestimates malnutrition in children under and above 2 years old <sup>421,422</sup>, and identifies more girls than boys with malnutrition. Onis et al (1997) developed a MUAC-for-age z-scores reference based on US data <sup>422</sup>. However, this reference requires an accuracy of the child's age of 1 month. At community level, it is often very difficult to estimate age precisely and a table reference is then needed to verify whether the child is malnourished or not. The latest WHO guidelines for the treatment of severe acute malnutrition recommend refining the cut-offs for MUAC to identify acute malnutrition for different groups in children under 5 y, using perhaps also different cut-offs for stunted and non-stunted children, and establishing cut-offs for children <6 months or >5 y <sup>418</sup>. Malnutrition in the latter age group is important to address also, even though it is less linked to mortality. Acute malnutrition during school years can impair physical and mental development <sup>18</sup>, and acute malnutrition is widely spread also in school-aged children. For example, one third of Southeast Asian school children are affected by thinness <sup>17</sup>.

The present study aimed to answer several research questions posed in the revised WHO guidelines for the treatment of SAM, especially focusing on the discrepancy between MUAC and WHZ, defining new MUAC cut-offs adapted to age and gender in children under 5 y, and MUAC cut-offs for older children. We used pooled data from several surveys carried out in Cambodia between 2011 and 2013. Cambodia is one of the 42 countries with the highest child mortality rates <sup>423</sup>, and prevalence of acute malnutrition is high with 10% of <5y children having a weight-for-height Z-score (WHZ) <-2 <sup>295</sup>.

The main objectives of the study was to find new cut-off values for MUAC in children from 0 to 14 y based on age groups of 0.5 - 2 y, 2 - 5 y, 5 - 8 y, 8 - 11 y and 11 - 14 y, to identify WHZ-scores of <-2 and -3 Z-scores respectively, and to verify whether there were gender differences or differences between stunted and non-stunted children respectively.

### *Methods*

Data on weight, height, MUAC, gender and age were available for a total of 14,157 Cambodian children, from 5 different surveys conducted in Cambodia by several organizations (UNICEF; WHO; WFP; World Vision; International Relief and Development; Institut de Recherche pour le Développement) between 2011 and 2013. All parents of participants gave their written consent. All studies were approved by the National Ethic Committee for Health Research (NECHR) of the Ministry of Health, Phnom Penh, Cambodia. In 4 studies, the target population was children aged 0-4.9 y whereas in 1 study children aged 5-14 years were included.

Measurements of MUAC (to the nearest 1 mm) were made using a non-stretch tape measure (provided through UNICEF) in all studies. Weight was measured with the child wearing only light clothes, and was recorded to the nearest 0.1 kg by a Salter scale in the 4 studies on children under 5y and by a Seca scale in the study on children above 6 y. Length was measured in children under 2 yrs and height was measured in children above 2 yrs. Length/height was measured to the nearest 0.1 cm with a Holtain infantometer in children under 6 y and with a locally produced stadiometer in children above 6 y. Height boards and scales were daily calibrated. Anthropometric teams were standardized <sup>159</sup>. Age was determined (calculated in the nearest months) by asking of both the child's age and date of birth to mothers of children under 6 y. In the study on children above 6 y, date of birth was collected from school registers. Data were entered into PASW statistics 18 (Chicago, USA) or EpiData Entry (EpiData Association, <http://www.epidata.dk>). Weight for Height z-scores (WHZ), Height for Age z-scores (HAZ), Body Mass Index for Age z-scores (BAZ) were calculated from anthropometric data using WHO AnthroPlus v1.0.4. As recommended by WHO, and using the WHO child growth references <sup>424</sup>, acute malnutrition was defined in children under 5 y by respectively WHZ<-3 z-score (severe acute malnutrition) and WHZ between -3 and -2 z-scores (moderate acute malnutrition), and in children above 5 y by respectively BAZ<-3 z-scores (severe acute malnutrition) and BAZ between -3 and -2 z-scores (moderate acute malnutrition). Stunting was defined as a HAZ<-2 z-scores.

To assess the performance of MUAC cut-offs compared to the golden standard recommended by WHO to define severe and moderate acute malnutrition), receiver operating characteristic curves (ROC curves) were constructed. The sensitivity and false positive rates (1-specificity) of MUAC were determined using wasting (WHZ<-2 z-score in children under 5 y) and thinness (BAZ<-2 z-score in children above 5 y) as gold standards of acute malnutrition. The ROC curve is the plot of sensitivity versus false positive rate of MUAC cut-offs. The area under curve (AUC) is

the area between the curve and the segment (0,0) and (1,1), which corresponds to a random classifier. A larger AUC indicates a more accurate diagnosis of acute malnutrition defined by WHZ cut-offs <sup>425</sup>(17). Data analysis was performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL).

ROC curves were constructed for a large set of MUAC cut-offs, increased by steps of 1 mm, in order to find the new cut-off for severe and moderate acute malnutrition respectively. We generated a dummy variable for each MUAC cut-off, and did this for each MUAC-value in steps of 1 mm. Then, we calculated the AUC for that dummy variable in comparison to the golden standard, using the same methodology as Laillou et al and Fernandez et al <sup>419,426</sup>(8, 18). In order to evaluate the performance of our analysis, the corresponding Youden index, which is the difference between the true positive rate (sensitivity) and the false positive rate, was calculated : 1 indicating a perfect test, and 0 a useless test <sup>427</sup>. These analyses were conducted overall, and by gender and age groups. Several criteria were chosen to select new cut-off presented in this paper. The best correspondence between 2 indicators was first defined as the MUAC cut-off with highest AUC in the ROC curve. However, the sensitivity which one wants to be optimized for screening purposes, in order to miss a minimum of children with acute malnutrition, increased considerably around the MUAC cut-off with the highest AUC, whereas the AUC hardly changed (cf. supporting information figures). Thus, among the highest values of AUC (within 0.02 from highest AUC), the cut-off with the highest sensitivity was selected. As this can lead to many false-positive children to be included after screening, a third criteria was introduced by the authors to define the new cut-off: a false positive rate below 1/3 of non-malnourished children. To summarize, among the highest values of the area under the curve (highest AUC - 0.02), the cut-off with the highest sensitivity and a false positive rate of less than 33% was considered to be the new optimal cut-off. In addition, we calculated the difference of Youden index between the new optimal cut-off and the cut-off obtaining the highest Youden index. These calculations were done for 0.5 - 2 y, 2 - 5 y, 5 - 8 y, 8 - 11 y and 11 - 14 y, for girls, boys and all. Calculations were repeated for stunted and non stunted children under 5 y respectively <sup>418</sup>.

## Results

In total, data was available for 14,157 children (51% male), ranging in age from 0 - 14 y. Characteristics of the children are presented in table 32.

Stunting increased with age with respectively 36% and 41% of children under and above 5 yrs having HAZ <-2 z-scores. The same patterns was seen for acute malnutrition with the prevalence of WHZ<-2 z-scores and WHZ<-3 z-scores being 11% and 1% respectively in children under 5 y, whereas thinness and severe thinness were found in 26% and 5% of the children above 5 respectively. In children under 5 y, prevalence of acute malnutrition and severe acute malnutrition identified using actual WHO MUAC cut-offs of 12.5 cm and 11.5 cm were 3% and 0% respectively (Table 33).

The sensitivity of MUAC ranged from 6.5% to 32.9% in children with acute malnutrition and from 0% to 18.2% in children with severe acute malnutrition with large differences between boys and girls (Table 33).

**TABLE 32 AGE AND GENDER CHARACTERISTICS OF THE PARTICIPANTS AND PROPORTION OF CHILDREN SUFFERING FROM ACUTE MALNUTRITION**

Age		Boys	Girls	All
0-23 months	N (%)	2849 (20%)	2736 (20%)	5585 (39%)
	WHZ <-2 z-scores (%)	13%	10%	12%
24-59 months	N (%)	3147 (22%)	3031 (22%)	6178 (44%)
	WHZ <-2 z-scores (%) (%)malnutrition*	10%	9%	10%
5-7.9 years	N (%)	366 (3%)	379 (3%)	745 (5%)
	BAZ <-2 z-scores (%)	19%	14%	16%
8-10.9 years	N (%)	451 (3%)	519 (3%)	970 (7%)
	BAZ <-2 z-scores (%)	26%	21%	23%
11-13.9 years	N (%)	359 (3%)	320 (3%)	679 (5%)
	BAZ <-2 z-scores (%)	40%	36%	38%
All	N (%)	7172 (51%)	6985 (51%)	14157 (100%)
	WHZ or BAZ <-2 z-scores (%)	14%	12%	13%

**TABLE 33 VALIDITY OF ACTUAL WHO CUT-OFF FOR SEVERE AND ACUTE MALNUTRITION IN CHILDREN <5 Y**

	age (y)	false positive rate	sensitivity	AUC
MUAC cut-off 12.5 cm : acute malnutrition				
boys	0-1.9 y	2.1%	17.8%	0.578
	2-4.9 y	0.2%	6.5%	0.531
girls	0-1.9 y	4.9%	32.9%	0.640
	2-4.9 y	0.3%	10.2%	0.549
all	0-1.9 y	3.5%	24.3%	0.604
	2-4.9 y	0.3%	8.1%	0.539
MUAC cut-off 11.5 cm : severe acute malnutrition				
boys	0-1.9 y	0.5%	3.3%	0.514
	2-4.9 y	0.1%	0.0%	0.500
girls	0-1.9 y	0.9%	18.2%	0.586
	2-4.9 y	0.0%	0.8%	0.540
all	0-1.9 y	0.7%	8.6%	0.540
	2-4.9 y	0.0%	2.8%	0.514

New cut-offs are presented in table 34. When segregated into smaller age groups, sensitivity of MUAC to identify acute malnutrition increased considerably, with sensitivities from 49% to 76% for acute malnutrition, and from 55% to 83% for severe acute malnutrition. Sensitivity of the new cut-offs is higher for acute malnutrition than for severe acute malnutrition.

For new cut-offs, in children above 5y, sensitivity ranged from 58% to 94%. Youden index ranged from 31% to 64% for both acute malnutrition and severe acute malnutrition. The difference between the highest Youden index and the Youden index for the selected cut-off was between 0 and 4%.

The new MUAC cut-offs for acute malnutrition and severe acute malnutrition increase with age (Figure 8) over the whole age range from 0 -14 years. In both children below and above 5 y, cut-offs are generally higher for boys than for girls, but the difference between boys and girls was never more than 0.5cm and both cut-offs followed the same pattern. However, in the age group 8-10.9 y cut-off are higher in girls than in boys. The difference in new MUAC cut-off between identifying acute malnutrition (WHZ<-2) and severe acute malnutrition (WHZ<-3) for each gender and age group was small, and varied from 0.2 cm to 1 cm.

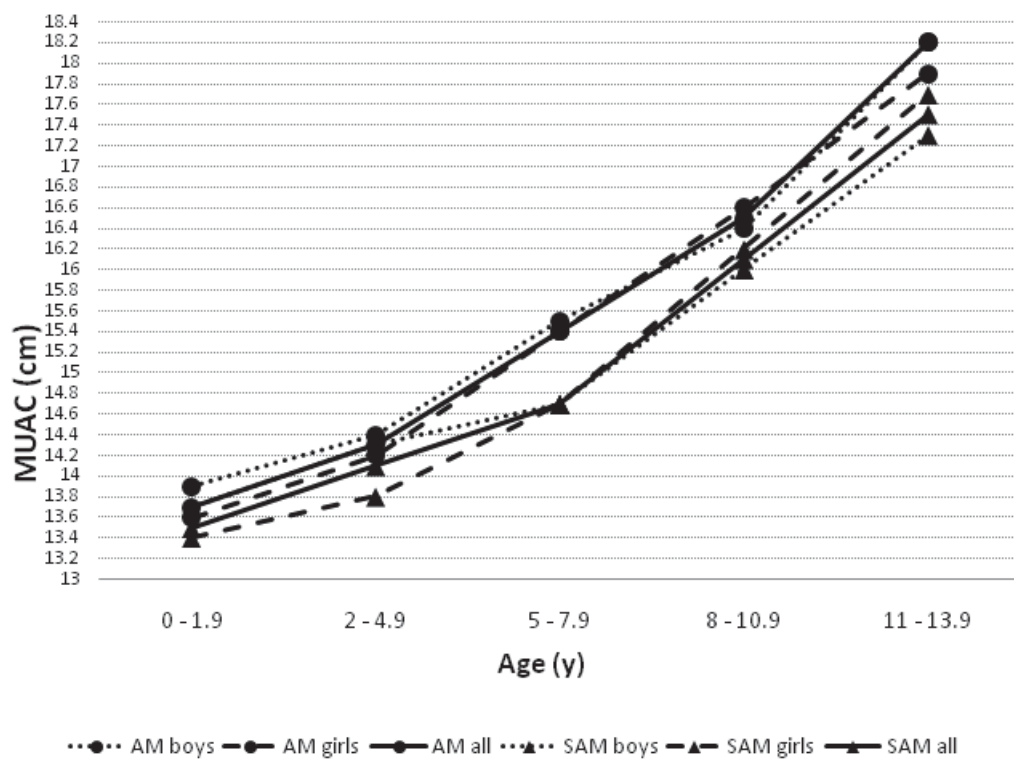
In children below 2 y, MUAC were lower in stunted children than in non-stunted children (table 34). In the children from 2- 5 years, all wasted children were also stunted, so no cut-off for stunted versus non stunted children could be calculated for this age group.



**TABLE 34 NEW CUT-OFFS\* BY AGE GROUP AND GENDER FOR SEVERE AND MODERATE ACUTE MALNUTRITION FOR CHILDREN FROM 0 TO 14Y**

age (y)	MUAC cut-off	false positive rate	specificity	sensitivity	AUC	Youden index	difference with highest Youden index*	MUAC cut-off	false positive rate	specificity	sensitivity	AUC	Youden index	difference with highest Youden index*	MUAC cut-off	false positive rate	specificity	sensitivity	AUC	Youden index	difference with highest Youden index*
Boys							Girls							All							
Acute malnutrition																					
All children																					
0 - 1.9 y	13.9	32%	68%	87%	0.772	54%	0%	13.6	32%	68%	82%	0.746	49%	-2%	13.7	30%	70%	81%	0.753	51%	0%
2 - 4.9 y	14.4	30%	70%	82%	0.762	52%	-3%	14.2	30%	70%	84%	0.770	54%	-1%	14.3	30%	70%	83%	0.762	52%	-1%
5 - 7.9 y	15.5	30%	70%	84%	0.769	54%	-3%	15.4	33%	67%	85%	0.756	51%	-4%	15.4	31%	69%	84%	0.769	54%	0%
8 - 11.9 y	16.4	31%	69%	84%	0.763	53%	-1%	16.6	32%	68%	85%	0.764	53%	0%	16.5	32%	68%	83%	0.758	52%	-1%
11 - 13.9 y	18.2	38%	62%	92%	0.768	54%	0%	17.9	21%	79%	84%	0.813	63%	-1%	18.2	33%	67%	90%	0.784	57%	0%
Stunted children																					
0 - 1.9 y	13.6	31%	69%	85%	0.770	54%	-2%	13.2	31%	69%	83%	0.760	52%	-1%	13.4	31%	69%	80%	0.745	49%	-2%
2 - 4.9 y	14	20%	80%	78%	0.792	58%	0%	14	31%	69%	81%	0.750	50%	0%	14	25%	75%	80%	0.771	31%	0%
Non stunted children																					
0 - 1.9 y	14	29%	71%	85%	0.776	55%	-1%	13.7	32%	68%	83%	0.755	51%	0%	13.9	34%	66%	85%	0.756	51%	0%
2 - 4.9 y	N/A : no wasted non stunted children						0%							0%							0%
Severe acute malnutrition																					
0 - 1.9 y	13.7	29%	71%	82%	0.764	53%	-2%	13.4	19%	81%	73%	0.778	55%	0%	13.5	27%	73%	80%	0.763	53%	-1%
2 - 4.9 y	14.3	31%	69%	83%	0.760	52%	-3%	13.8	20%	80%	84%	0.820	64%	0%	14.1	27%	73%	81%	0.769	54%	-1%
5 - 7.9 y	14.7	11%	89%	58%	0.736	47%	-3%	14.7	17%	83%	73%	0.778	56%	-4%	14.7	14%	86%	63%	0.747	49%	-4%
8 - 11.9 y	16	30%	70%	92%	0.807	62%	-4%	16.2	31%	69%	91%	0.800	60%	-2%	16.1	31%	69%	91%	0.802	60%	-3%
11 - 13.9 y	17.3	25%	75%	82%	0.881	56%	-4%	17.6	33%	67%	89%	0.779	56%	-2%	17.5	31%	69%	89%	0.789	58%	0%

\*cut-off selected according to highest AUC -0.02 AND highest sensitivity AND false positive rate ≤33%



**FIGURE 8 OPTIMAL CUT-OFFS FOR ACUTE MALNUTRITION (AM) AND SEVERE ACUTE MALNUTRITION (SAM) BY AGE GROUP AND GENDER**

## Discussion

In this paper, optimal cut-offs for MUAC to identify acute malnutrition calculated from a pooled dataset of Cambodian children, taking age and gender into account, were higher than the current WHO recommendation. To our knowledge, this is the first paper to report optimal MUAC cut-offs in Asian children, and cut-offs for children >5 y of age. Sensitivity of identifying children with a WHZ <-2 z-score was drastically improved (from <25% to >80%) with the new cut-offs. A consequence of the higher sensitivity is of course a lower specificity, and indeed the number of false-positive cases increased also.

For example, in our dataset, 1045 wasted children (WHZ <-2 z-scores), including 155 children with WHZ <-3 z-score, were not identified as suffering from acute malnutrition by the current WHO MUAC cut-offs of 12.5cm, corresponding to 83% of wasted children below 5 y. Using the new cut-offs for gender and age as recommended in the current paper to screen for acute malnutrition, we missed only 154 wasted children (12.3%). However, using the cut-offs recommended in this paper, 4152 children would have been wrongly identified as having acute malnutrition, while using the current WHO cut-off, only 187 children would have been wrongly identified for acute malnutrition. The difference between new cut-offs for acute and severe acute malnutrition is narrow (table 34) so we suggest to only retain cut-offs for acute malnutrition.

Half a century ago, MUAC was assumed to be almost stable between 0 and 5 years, based on well-nourished Polish children <sup>428</sup> but more recent studies suggested that MUAC is age and gender specific <sup>420,429</sup>. A MUAC for age z-scores reference was built in 1997 but it requires knowledge of the age of the child to the nearest month, something which is often difficult to obtain in the field <sup>295</sup>. Therefore, the present study tried to find a balance between practicality and ideal by dividing children under 5 y in 2 groups using 2 y as a threshold.

The reliability of WHZ as a gold standard to define acute malnutrition is questioned <sup>430</sup>. WHZ is related to body shape and may overestimate the prevalence of acute malnutrition in some populations <sup>431</sup>. Also, it was reported that MUAC performs at least as well or even better than WHZ in predicting mortality among malnourished children <sup>417,432</sup>. However, assessing the immediate death risk is not the only purpose of diagnosing acute malnutrition. Acute malnutrition defined by WHZ<-2 also contributes to increased morbidity, impaired physical and cognitive development, and is associated with micronutrient deficiencies <sup>418</sup>. Therefore acute malnutrition screening should not be reduced to mortality risk screening. Identifying only children at high risk for mortality may prevent to treat children suffering from less advanced acute malnutrition but still at high risk for impaired development. Also, we showed that in the Cambodian context, MUAC and WHZ identified a different sub-set of children as being malnourished. Hence, we have argued that both MUAC and WHZ should be used to identify malnutrition, and one cannot use one or the other <sup>419</sup>.

However, to avoid overburdening the health system with false-positive cases of malnutrition, we suggest distinguishing 2 uses for MUAC cut-off. The first use will be the prevention of death, and the current WHO MUAC cut-off should be used to initiate treatment for severe acute malnutrition in. The newly proposed MUAC cut-offs should be used to screen for children suffering from acute malnutrition. These children may not be at immediate risk for mortality, but early detection of acute malnutrition may prevent them for later risk for mortality and for impaired development.

MUAC and WHZ are associated with different aspects of body composition, and therefore identify different groups of children with malnutrition. Therefore, both indicators should be used to start the treatment <sup>419</sup>. Currently, only 1 cut-off for MUAC is being recommended and used for both screening and initiation of treatment for acute malnutrition. Earlier, we have proposed to use 2 different cut-offs for MUAC: one for screening, and one for initiation of treatment for acute malnutrition <sup>419</sup>. The new cut-offs for acute malnutrition proposed in the present paper are meant for screening at community level for acute malnutrition, with treatment for severe acute malnutrition started using WHZ < -3 z-score as indicator of acute malnutrition or the current WHO cut-offs for MUAC. This way, sensitivity of identifying children with acute malnutrition is increased to >80%, whereas specificity for starting treatment remains the same. Using new MUAC cut-off would therefore only increase the number of children being sent to a primary health center to be re-measured for MUAC, weight and height, therefore the global cost of screening malnutrition would increase, as well as more children being correctly treated for malnutrition <sup>429</sup>. As obtaining a MUAC is a non-invasive procedure without any health risks, this poses not a health but an economical issue, as overtreatment can be a burden to the health system. But in return, parents of those children who are likely to be moderately acute malnourished could receive prevention counseling to avoid their children to become severe as no community management of acute malnutrition is being implemented in Cambodia at scale.

Based on the same sample of children under 5 y, Laillou et al recommend to use 13.3 cm as MUAC cut-off to screen severe acute malnutrition in children from 6 m to 5 y <sup>419</sup>, which is lower than the recommended cut-offs in the current paper. This difference can be explained by the fact that in the current paper, the age group was split into 2 (0 - 1.9 y and 2 - 4.9 y). In addition, in the current paper, steps of 0.1 cm were used to identify the new MUAC cut-off, instead of 0.25 cm as in paper by Laillou et al. Finally, we have added the criteria of highest sensitivity and maximum acceptable false positive rate to define the new MUAC cut-off. However, although the cut-offs differ slightly, it is clear that current practice to identify children with acute malnutrition can be improved considerably by increasing the current WHO recommended cut-offs for MUAC.

Children above 5 y should also be taken into account in the management of acute malnutrition. Indeed at school age, acute malnutrition, often accompanied by micronutrient deficiencies, can delay maturation, impair muscular strength, bone density and work capacity <sup>18</sup>. Thus malnutrition at school age increase risk of morbidity, of school failure and school drop-out <sup>17</sup>. MUAC cut-offs gradually increased from birth to adolescence in an almost linear manner, but changes are influenced by changes in growth velocity, e.g. during puberty. In our study, cut-offs were higher for boys than for girls (+0.1 - +0.3 cm), except from age group 8 to 10.9 years. We assume that the earlier adolescent growth spurt in girls than in boys, with associated changes in lean and fat mass, underlies this phenomena <sup>433</sup>. Schools could be a practical platform to follow school-aged children for acute malnutrition.

Height of the child was another important factor in determining the new MUAC cut-offs, with lower values in stunted children compared to non stunted children (table 34). Several studies show that stunted children tend to accumulate more fat mass and gain less lean body mass than non stunted children <sup>434,435</sup>, hence perhaps stunted children have lower muscle mass, leading to lower MUAC cut-offs.

A limit of the current study is that data used in this study was from Cambodia and therefore that new MUAC cut-offs presented here are only adapted to Cambodian children. Hence, the aim of the present study is not to suggest using the presented optimal cut-offs as international reference. However, the study highlights that a unique MUAC cut-off for children below 5 y results in a large of children not receiving vital treatment. Therefore we recommend a meta-analysis using the same method on a large dataset from different countries including age, gender, MUAC, height and weight to be conducted, in order to provide an international reference of MUAC cut-offs by gender and age groups to screen for acute malnutrition.

To conclude, new cut-offs for MUAC, adapted to age and gender are needed to improve the sensitivity to identify children with WHZ-score < -2 z-scores. The current WHO MUAC cut-off should be used in targeted interventions and to initiate treatment as it is adapted to detect mortality risk among malnourished children. In the present study, new MUAC cut-offs for screening for acute malnutrition, using age- and gender-specific cut-offs are presented from children from 0 – 14 y. Rapid adaption of these new cut-offs will result in identification of >80% of children with acute malnutrition.

## **5.2. Subclinical inflammation increases plasma transferrin receptor and ferritin and decreases plasma RBP but does not affect plasma zinc in school children and women in Cambodia and Senegal (Manuscript, drafted)**

*To be submitted*

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### *Introduction*

Micronutrient deficiencies are a major public health problem, especially in low-income countries. They are widely prevalent in the world with one child over 3 and one pregnant women over 6 being vitamin A deficient, one child or pregnant women over 5 suffering from iron deficiency anemia, and 17% of the world population being at risk for zinc deficiency <sup>3</sup>. Micronutrient deficiencies are responsible for increased morbidity and cognitive functions damage. By impairing growth and school achievement in children and affecting reproductive functions and fetal development in women <sup>3</sup>, they accentuate the intergenerational cycle of malnutrition <sup>436</sup>.

However, the accuracy of micronutrient deficiencies assessment can be hampered by other factors than micronutrient status affecting biomarkers. Inflammation for example is known to affect biomarkers for iron status (plasma ferritin concentrations) and vitamin A status (plasma retinol concentrations) even in apparently healthy individuals <sup>353</sup>. A complex chain reaction involving immunoregulatory cytokines follows injury or infection and contributes to withholding essential nutrients to infectious agents ('nutritional immunity') and starting repairing process <sup>353</sup>. However, these same mechanisms affect the concentrations of the biomarkers while overall micronutrient status remains the same. At the same time, it appears that micronutrient deficiency can affect the acute phase response <sup>437</sup>. Therefore more precision is needed about the biological response of biomarkers for micronutrient status in relation to inflammation. For biomarkers of vitamin A status (plasma retinol or RBP concentrations) <sup>201,339,438</sup> correction factors based on a meta-analysis have been proposed in order to adjust values in subjects during different stages of inflammation <sup>201</sup>. A similar approach has been used for ferritin concentrations <sup>197</sup>. Iron deficiency is indicated by depleted iron stores, corresponding to low plasma ferritin concentrations (FER) and/or tissue iron deficiency, indicated by elevated soluble transferrin receptor (TFR) concentrations <sup>198</sup>. TFR was long considered to be not or less sensitive to inflammation than FER <sup>198,439</sup> but some studies showed a significant impact of acute phase response on TFR values <sup>440</sup>.

The effect of inflammation on plasma zinc concentration remains unclear too <sup>203,441</sup>. Hence, more data on the impact of acute-phase response on zinc and TFR concentrations is needed. The objective of the present study was to investigate the impact of subclinical inflammation on the assessment of micronutrient deficiencies in 3 different populations and settings (Senegalese school children, Cambodian school children, Cambodian women) by investigating the impact of having elevated acute phase proteins (C-reactive protein and  $\alpha$ 1-Acidglycoprotein) on biomarkers for iron, vitamin A and zinc status.

## *Participants and methods*

### *Study area, design, population surveyed and ethics*

Three datasets were used for the current study.

#### *School children from Senegal*

The study was a representative cross-sectional survey conducted in 2010 in children from primary state schools of Dakar, Senegal <sup>225</sup>. A two-stage cluster sampling method was chosen with schools considered as primary sampling units. Within 30 randomly selected schools, and without criteria for age and gender, 20 children were randomized as final sampling units in each school, resulting in a sample of 594 children. The protocol was approved by the ethical committee of the National Health Research of Senegal. The school directors informed parents of the selected children on the purpose and proceedings of the study. Written informed consent was obtained from all parents at the beginning of the study.

#### *School children from Cambodia*

Data were collected as part of a randomized placebo-controlled trial investigating the impact of multi-micronutrient fortified rices on health and development of Cambodian schoolchildren (FORISCA UltraRice+NutriRice study, FOrtified RIce for School meals in CAMbodia). Baseline data collection was conducted in November 2012 in 20 primary schools from Kampong Speu province in Cambodia <sup>399</sup>. The schools were randomly selected from primary schools participating in school meal or take-home ration programs of the UN World Food Program (WFP). Children attending the selected schools were eligible to be part of the study if they were between 6–16 y of age, had written informed consent from parents/caregivers and did not have any mental or severe physical handicap. In each school, 132 children were randomly selected after stratification by sex and grade, hence 2640 children. 169 children were not recruited because they were absent on the day of data collection or refused to participate. Hence, a total of 2471 schoolchildren aged 6–16 y participated into the blood sample collection. The study was approved by the National Ethic Committee for Health Research (NECHR) of the Ministry of Health, Phnom Penh, Cambodia, the Ministry of Education, Youth and Sports, Phnom Penh, Cambodia, and the Research Ethics Committee of PATH, Seattle, USA.

#### *WRA (Women of Reproductive Age) from Cambodia*

The last dataset was provided by a serological survey for antibodies for Tetanus, Rubella and Measles in WRA conducted in Cambodia in 2010 <sup>442</sup>. 611 enumeration areas (EAs) were selected from the 28 764 EAs in the 2008 Cambodia General Population Census by probability proportional to size (PPS). In each EA, 22 households were randomly selected. All eligible women in selected households were invited to participate after providing written information about the survey and obtaining consent from women. 2117 women participated into the blood sample collection. We used residual serum from this survey to determine ferritin, sTfR, RBP and acute phase proteins.



## Blood collection and laboratory analysis

4-5 mL of venous blood was collected by experienced phlebotomists using sterile single-use material: in a vacutainer with heparin (Terumo heparin Venosafe) in Senegal and in Cambodian women, and in a vacutainer with no anticoagulant (Vacuette, Greiner Bio One, AUstria) in Cambodian children, both trace-element free. Blood samples were stored immediately in an icebox containing ice-packs and transported to the laboratory within a maximum of 5 hours after the first sample withdrawal. Plasma/serum samples were separated by centrifugation, aliquoted, and stored at  $-20^{\circ}\text{C}$ . Centrifugation resulted in serum samples in Cambodian children and in plasma samples in Cambodian women and Senegalese children. However, we will call plasma all samples in the following text.

For plasma zinc measurement, samples were sent with dry ice, to the Nutripass laboratory of the Institut de Recherche pour le Développement (IRD, Montpellier, France) for Senegalese school children or to the National Institute of Nutrition (Hanoi, Vietnam) for Cambodian school children. Zinc status was not measured in Cambodian WRA. Plasma zinc was measured by flame atomic absorption spectrophotometry (AAS), using trace-elements free procedures and urinary iodine (UIC) was measured using an ammonium persulfate method.

Samples from the 3 studies were sent with dry ice and to the CBS laboratory (Willstaett, Germany) for determination of retinol-binding protein (RBP), C-reactive protein (CRP), FER, TFR and  $\alpha 1$ -acid-glycoprotein (AGP). RBP, FER, TFR, CRP, AGP were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) technique <sup>195</sup>.

Inflammation was determined by elevated CRP ( $>5$  mg/L) and/or elevated AGP ( $>1$  g/L) allowing differentiation between incubation phase (high CRP and normal AGP), convalescence phase (both high AGP and CRP) and late convalescence phase (high AGP and normal CRP) <sup>197</sup>. Iron tissue deficiency was defined by TFR  $>8.3$  mg/L <sup>195</sup> [ and depleted iron stores were defined by FER  $<15$   $\mu\text{g/L}$  [WHO, 2011, Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. ]. Low FER and high TFR are both considered as indicators of iron deficiency (ID) <sup>198</sup> so ID was defined by iron stores depleted (low FER) and/or iron tissue deficiency (high TFR). Body iron was calculated according to the formula of Cook: body iron (mg/kg) =  $-(\log(\text{TFR}/\text{FER}) - 2.8229)/0.1207$ . “Body iron deficiency” was defined by body iron  $<0$  <sup>199</sup>. TFR/log FER Index was calculated by dividing TFR (mg/L) by log FER ( $\mu\text{g/L}$ ).  $7.06 = 8.3/\log(15)$  was used to define ID as “high TFR/log TFR index” <sup>443</sup>. Vitamin A status was measured by RBP concentration which reflects plasma retinol concentration because RBP occurs in a 1:1:1 complex with retinol and transthyretin <sup>200</sup>, which is not affected by inflammation <sup>444</sup>. Vitamin A deficiency (VAD) was defined by RBP  $<0.7$   $\mu\text{mol/L}$  <sup>200</sup> and marginal VAD was defined for RBP values  $\geq 0.7$   $\mu\text{mol/L}$  and  $<1.05$   $\mu\text{mol/L}$  <sup>202</sup>. Zinc deficiency was defined by plasma zinc  $<9.9$   $\mu\text{mol/L}$ ,  $10.1$   $\mu\text{mol/L}$  or  $10.7$   $\mu\text{mol/L}$  respectively in children  $<10$  y, girls  $>10$  y and boys  $>10$  y <sup>203</sup>. Children were considered as severely zinc deficient when plasma zinc was  $<7.7$   $\mu\text{mol/L}$  <sup>445</sup>.

## Statistical analysis

Data entry, including quality checks and validation by double entry of questionnaires, was performed with EpiData version 3.1 (EpiData, Odense, Denmark). Data management and analyses were performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL). Significance was

defined as  $P < 0.05$ . The distributions of biomarkers concentration were checked for normality using normality plots and Kolmogorov-Smirnoff test. Because distributions of AGP, CRP, TfR, and FER were skewed, they were log transformed. Spearman's rank correlation coefficients were determined to assess relationships among APP (Acute Phase response Proteins) and micronutrient status biomarkers. For those which are correlated to APP concentrations, we calculated the ratio of the geometric mean or mean values of the biomarker for the group with inflammation, to the reference group without inflammation<sup>197,201,368,440</sup>. The correction factor was calculated as 1/ratio. Prevalence of poor micronutrient status were calculated i) without correction ii) only in the subjects with no inflammation iii) using biomarkers concentrations adjusted with CFs calculated in the present study iv) using FER and RBP concentrations adjusted with CFs recommended by Thurnham<sup>197,201</sup> v) using 30  $\mu\text{g/L}$  as low FER cut-off in children with inflammation<sup>198</sup>. Corrected prevalences were compared to uncorrected prevalence using Mc Nemar's chi-square.

## Results

Biochemical characteristics of the participants from 3 different samples are presented in table 35. Although similar considering gender proportion (half of children were female) and age (10 y), populations of school children from Cambodia and Senegal differed in inflammatory and micronutrient status. Prevalence of inflammation was less than 15% in Senegalese children and Cambodian women but was 40% in Cambodian children, mostly of them being in the late convalescence phase (35% had high AGP and normal CRP). Prevalence of iron deficiency (defined as either a high TFR and/or a low FER) was 39% in Senegalese children, 52% in Cambodian children and 14% in Cambodian women. In Cambodian children, ID was mostly related to a high TFR (only 1% had low FER) while in Senegal children, 33% had a high TFR with 20% having a low FER and in Cambodian women, 10% had a high TFR and 8% had low FER. Prevalence of VAD was less than 5% in all samples but more than 40% of Senegalese children had a marginal vitamin A status. ZD affected 25% of the children in Senegal and more than 90% of the Cambodian children. Correlations between APP and biomarkers of iron, vitamin A and zinc status are presented in table 36. CRP and AGP were highly positively correlated in all samples. FER was highly positively correlated with both CRP and AGP in all samples. TFR was positively correlated to AGP in all samples, and to CRP in the Cambodian school children. Contradictory results were found between indicators based on the TFR/FER ratio (body iron and TFR/log FER index) and APP among the different groups: FER/log FER index was negatively correlated to CRP in both Senegalese children and Cambodian women, while FER/log FER index was positively correlated to AGP in Cambodian children. Similarly, correlations between TBI and AGP were positive in Senegal children and Cambodian women, but negative in Cambodian children.

**TABLE 35 DEMOGRAPHIC AND BIOCHEMICAL CHARACTERISTICS OF PARTICIPANTS: SCHOOL CHILDREN FROM SENEGAL, SCHOOL CHILDREN FROM CAMBODIA, AND WRA FROM CAMBODIA**

	Senegalese school children (n=594)		Cambodian school children (n=2471)		Cambodian WRA (n=2117)	
<b>Age (y)</b>	10.2 ±	2.4	9.6 ±	2.4	26.3 ±	7.0
<b>Female (%)</b>	52.5		50.3		100.0	
<b>CRP (mg/L)</b>	1.2 ±	2.6	1.4 ±	5.0	1.7 ±	4.7
High CRP, >5 mg/L (%)	5.7		5.6		6.8	
<b>AGP (g/L)</b>	0.8 ±	0.2	1.0 ±	0.4	0.7 ±	0.2
High AGP, >1 g/L (%)	10.6		39.9		10.3	
Inflammation, high CRP or high AGP (%)	12.1		40.3		14.1	
No inflammation, normal CRP and normal AGP (%)	87.9		59.7		85.9	
Incubation, high CRP and normal AGP (%)	1.5		0.4		3.8	
Early convalescence, high CRP and high AGP (%)	4.2		5.3		3	
Late convalescence, normal CRP and high AGP (%)	6.4		34.6		7.3	
<b>Ferritin (ug/L)</b>	29.7 ±	17.3	88.4 ±	46.4	79.8 ±	58.1
Low ferritin, <12ug/L (%)	20.4		1.3		7.6	
<b>TfR (mg/L)</b>	8.1 ±	3.1	8.8 ±	2.7	6.1 ±	2.6
High TfR, >8.3 mg/L (%)	33.3		51.4		10.1	
<b>ID (low ferritin and/or high TfR)</b>	38.7		51.6		13.5	
<b>Body iron (mg/kg)</b>	2.6 ±	2.9	6.1 ±	2.2	6.8 ±	3.7
ID (Low body iron, <0 mg/kg) (%)	16.7		1.7		5.1	
<b>TFR/log FER index</b>	6.5 ±	6.0	4.8 ±	2.2	3.9 ±	3.3
ID (index >7.06)	23.9		7		7.2	
<b>RBP (μmol/L)</b>	1.1 ±	0.3	1.5 ±	0.4	1.8 ±	0.7
Vitamin A deficiency, RBP <0.7 μmol/L (%)	3.7		1.0		0.5	
Marginal vitamin A deficiency, RBP <1.05 μmol/L (%)	42.1		11.4		6.0	
<b>Zinc (umol/L)</b>	11.4 ±	2.1	7.5 ±	1.9	6.8 ±	3.7
Zinc deficiency * (%)	27.2		92.8		-	
Severe zinc deficiency ** (%)	4.5		54.2		-	

In most subjects, RBP was negatively correlated to CRP and to AGP. In Cambodian school children however, RBP was negatively associated with CRP but positively associated with AGP. No correlation between zinc and CRP or AGP was found in subgroups but in all subjects combined, zinc was negatively associated with CRP and AGP. For biomarkers that were significantly correlated to CRP and/or AGP (FER, TFR, RBP and zinc), correction factors were calculated for groups according to inflammatory status (tables 37, 38, 39, 40). Mean FER was significantly higher in participants in incubation, in early convalescence, and in late convalescence, or with elevated AGP or CRP in all subjects (table 37). Effect of inflammation was stronger on FER than on TFR with all CFs being lower than 0.83 and almost all P-values being <0.05. Although the changes in FER between the different phases of inflammation were of the same magnitude between the 3 study populations, there were some significant differences in CFs among the different populations.

**TABLE 36 SPEARMAN'S CORRELATION COEFFICIENT (P) BETWEEN INFLAMMATORY AND MICRONUTRIENT STATUS VARIABLES, IN SENEGALESE SCHOOL CHILDREN, CAMBODIAN SCHOOL CHILDREN AND CAMBODIAN WRA**

	Overall (n=5182)	Senegalese school children (n=594)	Cambodian school children (n=2471)	Cambodian WRA n(=2117)
<b>CRP</b>				
AGP	0.44 **	0.58 **	0.62 **	0.34 **
FER	0.26 **	0.27 **	0.26 **	0.24
TFR	-0.02	0.02	0.13 **	-0.04
TBI	0.16 **	0.16 **	0.03	0.16 **
Index	-0.12 **	-0.11 *	0.02	-0.13 **
RBP	0.03 *	-0.23 **	-0.15 **	0.14 **
ZN	-0.08 **	0.00	-0.01	-
<b>AGP</b>				
FER	0.30 **	0.26 **	0.35 **	0.20
TFR	0.40 **	0.09 *	0.34 **	0.16 **
TBI	-0.01	0.11 *	-0.05 *	0.08 **
Index	0.20 **	-0.06	0.17 **	0.01
RBP	-0.01	-0.11 **	0.11 **	-0.05 *
ZN	-0.16 **	-0.04	0.01	-

\* P-value <0.05; \*\* P-value <0.001

Elevated CRP had no influence on TFR concentrations (table 38), whereas elevated AGP was associated with higher TFR concentrations. TFR concentrations were significantly lower in all subjects combined during the early incubation phase, and higher in later stages of inflammation. The maximum increase in TFR concentrations in the later stages of inflammation was less than 25% (lowest CF being 0.78). In children, but not in the women, RBP concentrations were significantly lower when CRP was elevated (table 39). During incubation, mean RBP concentrations were lower in the children but higher in women. Concentrations were lower to the same extent in all populations during early convalescence. In all children combined, zinc concentrations tended to be lower in early convalescence and were significantly lower during late convalescence and with elevated AGP (table 40). These effects disappeared in subgroups of Senegalese children and Cambodian children.

Uncorrected prevalence of high TFR was higher than corrected prevalence in all samples but significantly only in Cambodian children and women (table 41). Uncorrected prevalence of low TFR was lower than corrected prevalence, especially in Senegalese children and Cambodian women. Difference between corrected and uncorrected prevalence of ID defined by low FER and/or high TFR was lower than 2% in Senegalese children and Cambodian women, but was around 8% in Cambodian children. Iron deficiency defined by low body iron or high FER/log FER index was less sensitive to inflammation. Prevalence of vitamin A deficiency was below 4% in all samples and difference between adjusted and unadjusted prevalence was below 1%.

**TABLE 37 CFs AND RATIOS OF FERRITIN BY INFLAMMATORY STATUS IN SENEGALESE SCHOOL CHILDREN, CAMBODIAN SCHOOL CHILDREN AND CAMBODIAN WRA**

	n (%)	FER (µg/L) <sup>1</sup>	P-value <sup>2</sup>	Ratio (95%) <sup>3</sup>			CF <sup>4</sup>
<i>All subjects combined</i>							
No inflammation	3817 ( 74% )	53.7 ± 2.2	-	-			-
Incubation	98 ( 2% )	64.6 ± 2.3	0.023	1.20 (	1.19 - 1.21 )		0.83
Early convalescence	219 ( 4% )	112.2 ± 1.8	0.000	2.09 (	2.08 - 2.09 )		0.48
Late convalescence	1048 ( 20% )	83.2 ± 1.9	0.000	1.55 (	1.55 - 1.55 )		0.65
<i>Senegalese school children</i>							
No inflammation	522 ( 88% )	23.5 ± 1.9	-	-			-
Incubation	9 ( 2% )	28.5 ± 1.7	0.339	1.22 (	1.16 - 1.26 )		0.82
Early convalescence	25 ( 4% )	46.9 ± 1.7	0.000	2.00 (	1.96 - 2.03 )		0.50
Late convalescence	38 ( 6% )	35.1 ± 1.6	0.000	1.50 (	1.47 - 1.52 )		0.67
<i>Cambodian school children</i>							
No inflammation	1476 ( 60% )	68.9 ± 1.7	-	-			-
Incubation	9 ( 0% )	88.9 ± 1.7	0.141	1.29 (	1.27 - 1.31 )		0.77
Early convalescence	130 ( 5% )	125.8 ± 1.6	0.000	1.83 (	1.82 - 1.83 )		0.55
Late convalescence	856 ( 35% )	87.5 ± 1.7	0.000	1.27 (	1.27 - 1.27 )		0.79
<i>Cambodian WRA</i>							
No inflammation	1819 ( 86% )	56.4 ± 2.3	-	-			-
Incubation	80 ( 4% )	67.9 ± 2.4	0.051	1.20 (	1.19 - 1.21 )		0.83
Early convalescence	64 ( 3% )	120.4 ± 1.7	0.000	2.14 (	2.13 - 2.14 )		0.47
Late convalescence	154 ( 7% )	73.8 ± 2.4	0.000	1.31 (	1.30 - 1.32 )		0.76
<i>All subjects combined</i>							
Normal AGP	3915 ( 76% )	54.3 ± 2.2	-	-			-
High AGP	1267 ( 24% )	86.9 ± 1.9	0.000	1.60 (	1.60 - 1.6 )		0.62
<i>Senegalese school children</i>							
Normal AGP	531 ( 89% )	23.5 ± 1.9	-	-			-
High AGP	63 ( 11% )	38.9 ± 1.7	0.000	1.65 (	1.63 - 1.68 )		0.60
<i>Cambodian school children</i>							
Normal AGP	1485 ( 60% )	69.0 ± 1.7	-	-			-
High AGP	986 ( 40% )	91.2 ± 1.7	0.000	1.32 (	1.32 - 1.32 )		0.76
<i>Cambodian WRA</i>							
Normal AGP	1899 ( 90% )	56.8 ± 2.3	-	-			-
High AGP	218 ( 10% )	85.1 ± 2.3	0.000	1.50 (	1.49 - 1.50 )		0.67
<i>All subjects combined</i>							
Normal CRP	4865 ( 94% )	59.2 ± 2.2	-	-			-
High CRP	317 ( 6% )	93.7 ± 2.0	0.000	1.58	1.58	1.59	0.63
<i>Senegalese school children</i>							
Normal CRP	560 ( 94% )	24.1 ± 1.9	-	-			-
High CRP	34 ( 6% )	41.1 ± 1.8	0.000	1.71 (	1.68 - 1.73 )		0.59
<i>Cambodian school children</i>							
Normal CRP	2332 ( 94% )	74.1 ± 1.0	-	-			-
High CRP	139 ( 6% )	123.0 ± 1.6	0.000	1.66 (	1.66 - 1.66 )		0.60
<i>Cambodian WRA</i>							
Normal CRP	1973 ( 93% )	57.6 ± 2.3	-	-			-
High CRP	144 ( 7% )	87.1 ± 2.2	0.000	1.51 (	1.51 - 1.52 )		0.66

<sup>1</sup> Geometric means ± SD

<sup>2</sup> ANOVA on log-transformed FER means of positive group VS control group

<sup>3</sup> Ratio of back-transformed FER concentrations of positive group VS control group

<sup>4</sup> CF=1/Ratio

**TABLE 38 CFs AND RATIOS OF TRANSFERRIN RECEPTOR BY INFLAMMATORY STATUS IN SENEGALESE SCHOOL CHILDREN, CAMBODIAN SCHOOL CHILDREN AND CAMBODIAN WRA**

	n (%)	TFR (mg/L) <sup>1</sup>	P-value <sup>2</sup>	Ratio (95%) <sup>3</sup>	CF <sup>4</sup>
<i>All subjects combined</i>					
No inflammation	3817 ( 74% )	6.8 ± 1.4	-	-	-
Incubation	98 ( 2% )	5.8 ± 1.3	0.000	0.85 ( 0.81 - 0.89 )	1.17
Early convalescence	219 ( 4% )	7.8 ± 1.4	0.000	1.16 ( 1.12 - 1.18 )	0.87
Late convalescence	1048 ( 20% )	8.7 ± 1.3	0.000	1.29 ( 1.27 - 1.29 )	0.78
<i>Senegalese school children</i>					
No inflammation	522 ( 88% )	7.7 ± 1.3	-	-	-
Incubation	9 ( 2% )	7.5 ± 7.7	0.746	0.97 ( 0.32 - 1.63 )	1.03
Early convalescence	25 ( 4% )	8.2 ± 1.3	0.266	1.07 ( 1.00 - 1.13 )	0.94
Late convalescence	38 ( 6% )	7.9 ± 1.2	0.653	1.02 ( 0.97 - 1.08 )	0.98
<i>Cambodian school children</i>					
No inflammation	1476 ( 60% )	8.0 ± 1.3	-	-	-
Incubation	9 ( 0% )	7.2 ± 1.3	0.206	0.90 ( 0.79 - 1.01 )	1.12
Early convalescence	130 ( 5% )	8.8 ± 1.3	0.000	1.10 ( 1.07 - 1.13 )	0.91
Late convalescence	856 ( 35% )	9.3 ± 1.3	0.000	1.15 ( 1.15 - 1.18 )	0.87
<i>Cambodian WRA</i>					
No inflammation	1819 ( 86% )	5.7 ± 1.4	-	-	-
Incubation	80 ( 4% )	5.5 ± 1.3	0.296	0.96 ( 0.91 - 1.02 )	1.04
Early convalescence	64 ( 3% )	6.0 ± 1.4	0.145	1.06 ( 0.99 - 1.11 )	0.94
Late convalescence	154 ( 7% )	6.4 ± 1.4	0.000	1.13 ( 1.08 - 1.16 )	0.89
<i>All subjects combined</i>					
Normal AGP	3915 ( 76% )	6.8 ± 1.4	-	-	-
High AGP	1267 ( 24% )	8.6 ± 1.4	0.000	1.27 ( 1.25 - 1.28 )	0.79
<i>Senegalese school children</i>					
Normal AGP	531 ( 89% )	7.7 ± 1.3	-	-	-
High AGP	63 ( 11% )	8.0 ± 1.3	0.301	1.04 ( 0.99 - 1.08 )	0.96
<i>Cambodian school children</i>					
Normal AGP	1485 ( 60% )	8.0 ± 1.3	-	-	-
High AGP	986 ( 40% )	9.2 ± 1.3	0.000	1.15 ( 1.14 - 1.16 )	0.87
<i>Cambodian WRA</i>					
Normal AGP	1899 ( 90% )	5.7 ± 1.4	-	-	-
High AGP	218 ( 10% )	6.3 ± 1.4	0.000	1.11 ( 1.07 - 1.14 )	0.90
<i>All subjects combined</i>					
Normal CRP	4865 ( 94% )	7.2 ± 1.4	-	-	-
High CRP	317 ( 6% )	7.1 ± 1.4	0.847	1.00 ( 0.96 - 1.01 )	1.00
<i>Senegalese school children</i>					
Normal CRP	560 ( 94% )	7.7 ± 1.3	-	-	-
High CRP	34 ( 6% )	8.0 ± 1.3	0.449	1.04 ( 0.98 - 1.10 )	0.96
<i>Cambodian school children</i>					
Normal CRP	2332 ( 94% )	8.5 ± 1.3	-	-	-
High CRP	139 ( 6% )	8.7 ± 1.3	0.238	1.03 ( 1.00 - 1.05 )	0.97
<i>Cambodian WRA</i>					
Normal CRP	1973 ( 93% )	5.7 ± 1.4	-	-	-
High CRP	144 ( 7% )	5.7 ± 1.4	0.170	1.00 ( 0.96 - 1.04 )	1.00

<sup>1</sup> Geometric means ± SD

<sup>2</sup> ANOVA on log-transformed TFR means of positive group VS control group

<sup>3</sup> Ratio of back-transformed TFR concentrations of positive group VS control group

<sup>4</sup> CF=1/Ratio

**TABLE 39 CFs AND RATIOS OF RBP BY INFLAMMATORY STATUS IN SENEGALESE SCHOOL CHILDREN, CAMBODIAN SCHOOL CHILDREN AND CAMBODIAN WRA**

	n (%)		RBP (μmol/L )		P-value <sup>2</sup>	Ratio (95%) <sup>3</sup>			CF <sup>4</sup>
<i>All subjects combined</i>									
No inflammation	3817	74%	1.58 ±	0.6	-	-			-
Incubation	98	2%	1.80 ±	0.7	0.000	1.14 (	1.05 -	1.23 )	0.8775
Early convalescence	219	4%	1.31 ±	0.4	0.000	0.83 (	0.79 -	0.86 )	1.2097
Late convalescence	1048	20%	1.58 ±	0.5	0.996	1.00 (	0.98 -	1.02 )	1.00
<i>Senegalese school children</i>									
No inflammation	522 (	88% )	1.13 ±	0.3	-	-			-
Incubation	9 (	2% )	0.86 ±	0.2	0.002	0.76 (	0.64 -	0.88 )	1.31
Early convalescence	25 (	4% )	0.97 ±	0.3	0.003	0.86 (	0.75 -	0.97 )	1.16
Late convalescence	38 (	6% )	1.06 ±	0.3	0.092	0.94 (	0.85 -	1.03 )	1.07
<i>Cambodian school children</i>									
No inflammation	1476 (	60% )	1.48 ±	0.4	-	-			-
Incubation	9 (	0% )	1.26 ±	0.5	0.090	0.85 (	0.63 -	1.07 )	1.17
Early convalescence	130 (	5% )	1.26 ±	0.3	0.000	0.85 (	0.82 -	0.89 )	1.17
Late convalescence	856 (	35% )	1.56 ±	0.4	0.000	1.05 (	1.03 -	1.08 )	0.95
<i>Cambodian WRA</i>									
No inflammation	1819 (	86% )	1.79 ±	0.7	-	-			-
Incubation	80 (	4% )	1.96 ±	0.6	0.015	1.09 (	1.02 -	1.17 )	0.91
Early convalescence	64 (	3% )	1.53 ±	0.6	0.002	0.85 (	0.77 -	0.94 )	1.17
Late convalescence	154 (	7% )	1.80 ±	0.7	0.736	1.01 (	0.94 -	1.07 )	0.99
<i>All subjects combined</i>									
Normal AGP	3915 (	76% )	1.58 ±	0.6	-	-			-
High AGP	1267 (	24% )	1.53 ±	0.5	0.003	0.97 (	0.93 -	0.95 )	1.03
<i>Senegalese school children</i>									
Normal AGP	531 (	89% )	1.13 ±	0.3	-	-			-
High AGP	63 (	11% )	1.03 ±	0.3	0.003	0.91 (	0.84 -	0.98 )	1.10
<i>Cambodian school children</i>									
Normal AGP	1485 (	60% )	1.48 ±	0.4	-	-			-
High AGP	986 (	40% )	1.52 ±	0.4	0.014	1.03 (	1.01 -	1.05 )	0.97
<i>Cambodian WRA</i>									
Normal AGP	1899 (	90% )	1.79 ±	0.7	-	-			-
High AGP	218 (	10% )	1.72 ±	0.6	0.136	0.96 (	0.91 -	1.01 )	1.04
<i>All subjects combined</i>									
Normal CRP	4865 (	94% )	1.58 ±	0.5	-	-			-
High CRP	317 (	6% )	1.46 ±	0.6	0.000	0.92 (	0.90 -	0.98 )	1.08
<i>Senegalese school children</i>									
Normal CRP	560 (	94% )	1.13 ±	0.3	-	-			-
High CRP	34 (	6% )	0.94 ±	0.3	0.000	0.83 (	0.74 -	0.92 )	1.20
<i>Cambodian school children</i>									
Normal CRP	2332 (	94% )	1.51 ±	0.4	-	-			-
High CRP	139 (	6% )	1.28 ±	0.4	0.000	0.85 (	0.80 -	0.89 )	1.18
<i>Cambodian WRA</i>									
Normal CRP	1973 (	93% )	1.79 ±	0.7	-	-			-
High CRP	144 (	7% )	1.77 ±	0.7	0.804	0.99 (	0.92 -	1.06 )	1.01

<sup>1</sup> Geometric means  $\pm$  SD

<sup>2</sup> ANOVA on log-transformed RBP means of positive group VS control group

<sup>3</sup> Ratio of back-transformed RBP concentrations of positive group VS control group

<sup>4</sup> CF=1/Ratio



**TABLE 40 CFS AND RATIOS OF ZINC BY INFLAMMATORY STATUS IN SENEGALESE SCHOOL CHILDREN, CAMBODIAN SCHOOL CHILDREN AND CAMBODIAN WRA**

	n (%)	Zinc ( $\mu\text{mol/L}$ ) 1	P-value <sup>2</sup>	Ratio (95%) <sup>3</sup>	CF <sup>4</sup>
<i>All children combined</i>					
No inflammation	1716 ( 67% )	8.41 $\pm$ 1.3	-	-	-
Incubation	15 ( 1% )	9.18 $\pm$ 1.4	0.219	1.09 ( 0.98 - 1.11 )	0.92
Early convalescence	120 ( 5% )	8.00 $\pm$ 1.3	0.053	0.95 ( 0.85 - 1.00 )	1.05
Late convalescence	698 ( 27% )	7.70 $\pm$ 1.3	0.000	0.91 ( 0.95 - 0.97 )	1.09
<i>Senegalese school children</i>					
No inflammation	1203 ( 88% )	11.19 $\pm$ 7.4	-	-	-
Incubation	7 ( 2% )	11.72 $\pm$ 1.1	0.512	1.05 ( 0.96 - 1.08 )	0.95
Early convalescence	95 ( 4% )	10.76 $\pm$ 1.3	0.340	0.96 ( 0.95 - 1.02 )	1.04
Late convalescence	662 ( 6% )	11.34 $\pm$ 1.2	0.703	1.01 ( 0.98 - 1.03 )	0.99
<i>Cambodian school children</i>					
No inflammation	513 ( 60% )	7.45 $\pm$ 1.2	-	-	-
Incubation	8 ( 0% )	6.93 $\pm$ 1.2	0.399	0.93 ( 0.88 - 1.05 )	1.07
Early convalescence	25 ( 5% )	7.41 $\pm$ 1.3	0.795	1.00 ( 0.98 - 1.02 )	1.00
Late convalescence	36 ( 35% )	7.53 $\pm$ 1.3	0.278	1.01 ( 1.00 - 1.02 )	0.99
<i>All children combined</i>					
Normal AGP	1731 ( 68% )	8.42 $\pm$ 1.3	-	-	-
High AGP	818 ( 32% )	7.74 $\pm$ 1.3	0.000	0.92 ( 0.91 - 0.93 )	1.09
<i>Senegalese school children</i>					
Normal AGP	521 ( 90% )	11.20 $\pm$ 1.2	-	-	-
High AGP	61 ( 10% )	11.10 $\pm$ 1.2	0.740	0.99 ( 0.98 - 1.02 )	1.01
<i>Cambodian school children</i>					
Normal AGP	1210 ( 62% )	7.45 $\pm$ 1.2	-	-	-
High AGP	757 ( 38% )	7.52 $\pm$ 1.3	0.339	1.01 ( 1.00 - 1.01 )	0.99
<i>All children combined</i>					
Normal CRP	135 ( 5% )	8.2 $\pm$ 1.3	-	-	-
High CRP	2414 ( 95% )	8.1 $\pm$ 1.3	0.712	0.99 0.96 1.02	1.01
<i>Senegalese school children</i>					
Normal CRP	549 ( 94% )	11.2 $\pm$ 1.2	-	-	-
High CRP	33 ( 6% )	11.0 $\pm$ 1.2	0.593	0.98 ( 0.98 - 1.06 )	1.02
<i>Cambodian school children</i>					
Normal CRP	1865 ( 95% )	7.5 $\pm$ 1.2	-	-	-
High CRP	102 ( 5% )	7.4 $\pm$ 1.3	0.514	0.99 ( 0.98 - 1.05 )	1.01

<sup>1</sup> Geometric means  $\pm$  SD

<sup>2</sup> ANOVA on log-transformed zinc means of positive group VS control group

<sup>3</sup> Ratio of back-transformed zinc concentrations of positive group VS control group

<sup>4</sup> CF=1/Ratio

**TABLE 41 EFFECT OF CORRECTING TFR, PF, RBP CONCENTRATIONS ON THE PREVALENCE OF LOW IRON STATUS AND LOW VITAMIN A STATUS, IN SCHOOL CHILDREN FROM CAMBODIA, SCHOOL CHILDREN FROM SENEGAL AND WRA FROM CAMBODIA**

	Senegalese school children (n=594)		Cambodian school children (n=2471)		Cambodian WRA (n=2117)	
	%	P-value*	%	P-value*	%	P-value*
<b>Low Fer<sup>1</sup></b>						
uncorrected	20.4	NA	1.3	NA	7.6	NA
in participants with no inflammation	22.6	NA	1.4	NA	8.0	NA
corrected for 3 phases of inflammation <sup>2</sup>	21.5	0.016	1.4	0.250	7.9	0.008
corrected for 3 phases of inflammation (Thurnham) <sup>3</sup>	22.9	0.000	1.8	0.000	8.0	0.002
FER < 30 ug/L in children with inflammation	23.2	0.000	2.1	0.000	8.9	0.000
<b>High Tfr<sup>4</sup></b>						
uncorrected	33.3	NA	51.4	NA	10.1	NA
in participants with no inflammation	32.6	NA	42.2	NA	9.5	NA
corrected for 3 phases of inflammation <sup>5</sup>	33.2	1.000	42.9	0.000	9.7	0.021
<b>Iron deficiency (high Tfr and/or low Fer)<sup>14</sup></b>						
uncorrected	38.7	NA	51.6	NA	13.5	NA
in participants with no inflammation	38.5	NA	42.5	NA	13.2	NA
corrected for 3 phases of inflammation <sup>25</sup>	37.9	0.180	44.5	0.000	13.4	0.791
corrected for 3 phases of inflammation (Thurnham) <sup>3</sup>	39.1	0.500	51.6	1.000	13.8	0.016
FER < 30 ug/L in children with inflammation	40.2	0.004	51.7	0.500	14.3	0.000
<b>Iron deficiency (low body iron<sup>6</sup>)</b>						
uncorrected	16.7	NA	1.7	NA	5.1	NA
in participants with no inflammation	18.6	NA	1.7	NA	5.3	NA
corrected for 3 phases of inflammation <sup>25</sup>	17.5	0.002	1.8	0.002	5.2	0.002
corrected for 3 phases of inflammation (Thurnham) <sup>3</sup>	17.7	0.000	1.9	0.000	5.3	0.000
<b>Iron deficiency (high TFR/logFER index<sup>7</sup>)</b>						
uncorrected	23.9	NA	7.0	NA	7.2	NA
in participants with no inflammation	25.7	NA	6.3	NA	7.1	NA
corrected for 3 phases of inflammation <sup>25</sup>	24.6	0.125	6.3	0.000	7.2	1.000
corrected for 3 phases of inflammation (Thurnham) <sup>3</sup>	24.6	0.125	7.7	0.000	7.3	0.500
FER < 30 ug/L for children with inflammation <sup>8</sup>	26.9	0.000	13.2	0.000	7.9	0.000

**TABLE 41 (CONTINUED) EFFECT OF CORRECTING TFR, FER, RBP CONCENTRATIONS ON THE PREVALENCE OF LOW IRON STATUS AND LOW VITAMIN A STATUS, IN SCHOOL CHILDREN FROM CAMBODIA, SCHOOL CHILDREN FROM SENEGAL AND WRA FROM CAMBODIA**

	Senegalese school children (n=594)		Cambodian school children (n=2471)		Cambodian WRA (n=2117)	
	%	P-value*	%	P-value*	%	P-value*
<b>Vitamin A deficiency</b> <sup>9</sup>						
uncorrected	3.7	NA	1.0	NA	0.5	NA
in participants with no inflammation	2.9	NA	0.9	NA	0.4	NA
corrected for 3 phases of inflammation <sup>10</sup>	3.4	0.500	0.9	1.000	0.4	0.500
corrected for 3 phases of inflammation (Thurnham) <sup>11</sup>	3.0	0.125	0.7	0.031	0.3	0.250
<b>Marginal vitamin A status</b> <sup>12</sup>						
uncorrected	42.1	NA	11.4	NA	6.0	NA
in participants with no inflammation	39.7	NA	11.0	NA	5.7	NA
corrected for 3 phases of inflammation <sup>10</sup>	39.6	0.000	11.7	0.427	5.9	0.687
corrected for 3 phases of inflammation (Thurnham) <sup>11</sup>	38.9	0.000	8.7	0.000	5.6	0.008
<b>Zinc deficiency</b> <sup>13</sup>						
uncorrected	27.2	NA	92.8	NA	NA	NA
in participants with no inflammation	27.5	NA	93.3	NA	NA	NA
corrected for 3 phases of inflammation <sup>5</sup>	27.3	1.000	92.8	0.500	NA	NA

<sup>1</sup> FER < 15 ug/L for children and WRA, except if notified

<sup>2</sup> cf. CFs table 3

<sup>3</sup> CFs Thurnham for incubation, early convalescence, late convalescence : 0.64 0.39 0.65 in children; 0.73 0.58 0.85 in women

<sup>4</sup> TFR > 8.3 mg/L

<sup>5</sup> cf. CFs table 4

<sup>6</sup> body iron < 0 mg/kg

<sup>7</sup> TFR/logFER > 7.05 (corresponding to 8.3/log15)

<sup>8</sup> TFR/logFER > 5.3 (corresponding to 8.3/log30)

<sup>9</sup> RBP < 0.7 µmol/L

<sup>10</sup> cf. CFs table 5

<sup>11</sup> CFs Thurnham for incubation, early convalescence, late convalescence : RBP 0.87 0.76 0.89

<sup>12</sup> RBP < 1.05 µmol/L

NA : Non applicable

\* McNemar's chi-square of proportion to compare uncorrected prevalence and corrected prevalence

## Discussion

Animal models have shown an effect of inflammation on plasma zinc concentrations. The effect of the APR on plasma zinc concentrations is related to the redistribution of zinc from the plasma to the liver via the activation of immunoregulatory cytokines. For example, interleukin 6 stimulates the production of metallothionin and of zinc transport Zip14, which regulates hepatic zinc uptake and plasma zinc concentration <sup>203,441,446,447</sup>. In animal models, the fall of plasma zinc concentrations occurs before clinical symptoms (hence just after infection) and return to normal quickly <sup>441</sup>. In our cohorts in school children in Cambodia and Senegal, late phases of inflammation slightly affected plasma concentrations of zinc but the effect was modest. The prevalence of zinc deficiency after adjustment was similar to the prevalence among children without inflammation (respectively 27.3% and 27.5% in Senegalese children and 92.8% and 92.8% in Cambodian school children). Duncan et al <sup>448</sup> reported zinc concentrations of Scottish hospital patients being significantly negatively correlated to CRP ( $\rho=-0.16$ ). In these hospital patients, the degree of inflammation was much more severe than in our normal populations, and therefore cannot be classified as 'sub-clinical inflammation'. Indeed, in the study of Duncan et al, zinc concentrations were not significantly lower in patients with CRP concentrations <20 g/L, a situation similar to ours. Therefore our findings suggest that for determining zinc status in normal populations that is in population with only a low prevalence of sub-clinical infection, taking the acute phase response into account will not lead to a significant improvement in the estimate of the prevalence of zinc deficiency. This is consistent with the conclusions of an earlier review indicating no effect of inflammation on zinc status in children <sup>441</sup> but in contrast to the recommendations of the IZINCG working group.

Plasma RBP concentrations are often used as biomarker for vitamin A status, and consider equal as plasma retinol concentrations as retinol:RBP ratio is not altered by inflammation <sup>444</sup>. During the APR proinflammatory cytokines inhibit the hepatic synthesis of RBP and secretion of RBP:retinol complex <sup>438</sup> which decrease retinol mobilization. Currently, it is not clear whether the resultings hyporetinemia is a primary goal of the APR or a secondary consequence <sup>449</sup>.

In our cohorts of children from both Senegal and Cambodia RBP was decreased during incubation and early convalescence, which is consistent with other studies <sup>201,339,438</sup>. Indeed, retinol is known to fall rapidly within 48 hours <sup>353</sup>. During late convalescence, RBP was still lower in Senegalese children but higher in Cambodian children compared to participants with no inflammation, as has been reported earlier too, in a small sample of Indonesian school children <sup>450</sup>. A meta-analysis using the same CRP and AGP cut-offs showed that the APR-induced decrease of retinol lasted into late convalescence <sup>201</sup>. But in this meta-analysis populations with a poor vitamin A status were used (almost all median retinol < 1.05  $\mu\text{mol/L}$ ). Retinol (and hence RBP) concentrations are expected to rebound during convalescence <sup>449</sup>. We believe that baseline vitamin A status might explain this difference between Senegalese and Cambodian children. Vitamin A status in the Senegalese children was poor (40% marginal vitamin A status), whereas in Cambodian children, vitamin A status was good. This means that when correcting for inflammation, baseline vitamin A status should be taken into account. More data on populations with a relative good vitamin A status is needed to confirm this. But if found correct, it will affect the way to correct for inflammation for vitamin A status, as shown by the differences in vitamin A prevalence after using different correction factors. In Senegalese children, the correction factors as proposed by Thurnham (and calculated using populations

with poor vitamin A status) resulted in a prevalence of vitamin A deficiency very close to the prevalence in children without inflammation. In Cambodian subjects however, the correction factors proposed by Thurnham lead to an underestimation of the prevalence of vitamin A deficiency, presumably because vitamin A concentrations rebounded earlier in this population.

In all populations, FER was highly positively correlated with APP as reported earlier <sup>368,440</sup>. In addition, TFR was positively correlated to AGP, and also to CRP in Cambodian school children. But TFR concentrations were only increased in groups with elevated AGP, This is consistent with another study suggesting that FER reacts faster to the APR than TFR <sup>440</sup>. Again, cytokines are involved in the modulation of iron metabolism during the acute phase response: TNF, possibly via stimulation by IFN-  $\gamma$  or IFN- $\alpha$  may induce ferritin synthesis <sup>451,452</sup>. No change of TFR concentrations according to inflammatory status was found in Senegalese children, which could be linked to the low prevalence of inflammation. Although TFR has been long considered as more reliable than FER to assess ID when inflammation was prevalent <sup>198</sup>, in Cambodian women and children, inflammation significantly increased TFR concentrations as it was found in other studies <sup>440,453</sup>. Oppositely, and in line with Righetti et al <sup>440</sup>, a significant decrease of TFR concentrations was observed in all subjects combined during incubation.

Not adjusting ferritin concentrations for inflammation leads to underestimate the proportion of low iron stores, especially in populations with endemic inflammation. In our cohorts adjustment for inflammation increased the prevalence of low ferritin by 2% in Cambodian school children (prevalence of inflammation 40%), by 1.1% in Senegalese children, and by just 0.3% in Cambodian women. These small changes in adjusted prevalence are readily explained by either the low prevalence of inflammation in Cambodian women and Senegalese children and by the low prevalence of low iron stores in Cambodian children and women. Overall, using 30  $\mu\text{g/L}$  as a cut-off for children with inflammation performed less efficiently than adjustment for inflammation by use of APP. To our knowledge, no correction factors for TFR concentration for inflammation have been published. In our cohorts, TFR concentrations were higher during inflammation leading to an overestimation of iron deficiency defined by tissue iron deficiency, in line with results from Righetti et al <sup>440</sup>. In the cohort of Cambodian children, who had a high prevalence of inflammation, the prevalence of high TFR was almost 10% higher as compared to children without inflammation (51% vs 42%). Similar adjustment on TFR were used in Ivorian infants with similarly high proportion of inflammation <sup>440</sup>. In Cambodian women and Senegalese children where inflammation was not very prevalent, the difference between adjusted and not adjusted prevalence of tissue iron deficiency did not exceed 1%.

The use of TFR/log FER rather than TFR/FER to predict iron stores deficiency has been recommended <sup>454</sup>, especially in areas with endemic infection <sup>443</sup>. Instead of calculating CFs to apply to FER/log FER index as it was done by Righetti et al <sup>440</sup>, we used corrected values of FER and TFR to calculate FER/log FER index or body iron and prevalence of ID defined by FER/log FER index > 7.06 or by negative body iron. Depending on the iron status of the population, that is, whether iron deficiency was primarily indicated by low FER or by high TFR, inflammation resulted in either over- or underestimation of the prevalence of ID defined by high FER/log FER index or negative body iron. Overall, the difference between uncorrected prevalence and the reference prevalence was low (< 2%), suggesting that indicators based on both TFR and FER are relatively insensitive to inflammation. The adjusted prevalence of iron deficiency using our CFs for both FER and TFR or Thurnham CFs for FER only were very close in Cambodian women and Senegalese children, suggesting that in these cohorts adjusting FER concentrations for

inflammation is more important than adjusting TFR for inflammation to obtain a more precise estimate of ID. However, using Thunham Cfs for FER only in the Cambodian children led to overestimation of ID, even more than without adjusting for inflammation, because in this cohort, FER concentrations were high, making TFR the most important factor in these 2 indicators.

Prevalence of inflammation is considered to be low under 15% and to only have a modest impact on the assessment of micronutrient deficiencies<sup>439,455</sup>. However consensus about cut-offs to use to define elevated APP is needed. Recently, the sensitivity of the now commonly used cut off for CRP <5 mg/L has been questioned<sup>353</sup>. Several studies used different than 5 mg/L for CRP and 1 g/L for AGP or a series of cut-offs to define categories of inflammation<sup>339,456,457</sup>. Some research showed similar patterns of impact of acute phase response on micronutrient biomarkers using CRP cut-off either 5 mg/L or 10 mg/L<sup>458</sup> while other indicated that the best CRP cut-off could vary between 5, 10 or 20 mg/L depending on the measured biomarker<sup>448</sup>. Different cut-offs across class ages may be needed<sup>339</sup>. Hence, an international recommendation about APP cut-offs used to categorize inflammation is needed.

Our study had several limitations. First, the iron status of populations was heterogeneous with Cambodian children having adequate iron stores but a high proportion of high TFR, while ID in Cambodian women and in Senegalese children was both due to low iron stores and iron tissue deficiency.. But it gave us the opportunity to examine the impact of inflammation on micronutrient status in variable population profiles. Second, the prevalence of inflammation in Senegalese children and Cambodian women was low. Third the size of sample of Senegalese children was small compared to Cambodian children and women. Consequently, the impact of inflammation on some biomarkers in some inflammatory subgroups may have been blurred. Fourth, the golden standard of measuring iron deficiency is bone marrow<sup>371</sup>, which is invasive, expensive and not ethical to perform for research purposes only, so that we did not collect it in our studies.

## *Conclusion*

Our findings suggest to use TFR/log FER to assess populations iron status and iron deficiency prevalence if CRP and AGP are not possible to measure. Indeed, among various indicators of iron deficiency (low FER, high TFR, low FER and/or high TFR, negative body iron, high FER/log FER index), FER/log FER index is the less sensitive to inflammation. Considering the sensitivity of FER and TFR examined alone, they should be adjusted for inflammation preferably by both CRP and AGP, especially in areas with a high infection pressure. So far, only few studies, including the present one, calculated correction factors for TFR<sup>440</sup>. The impact of inflammation on TFR concentrations is clear, as well as the need to adjust TFR concentrations for inflammation in order to avoid the overestimation of ID defined by tissue iron deficiency. However, a large meta-analysis or pooled analyses such as carried out by Thurnham et al on retinol and ferritin<sup>197,201</sup> is needed to quantify better the impact of inflammation in different stages on TFR. The impact of inflammation on zinc status has been found modest in the present study, indicating that plasma zinc concentrations are perhaps less affected by inflammation than previously thought. Different responses of retinol to incubation and convalescence phases were observed in the present study, presumably depending on the vitamin A status of the population. More research should be conducted on APP cut-offs to define the different phases of inflammation, and the sensitivity of micronutrient biomarkers.

## General conclusion

Education is crucial to break the cycle of poverty and malnutrition <sup>379,380</sup>. Nutritional deficiencies are particularly harmful for the physical and cognitive development of school-aged children and adolescents who have high nutrient requirements to support their skeletal and brain growth spurt. The associations between micronutrient deficiencies, parasite infection and poor cognitive performance observed in our research in Cambodia highlight the importance of addressing health and nutrition issues in school-aged children and adolescents in an holistic way, in order to support the educational achievement of the future generation. We showed that some of the adverse effects of malnutrition are still reversible at school age, with cognitive performance being improved by one type of multiple-micronutrient fortified rice. Poor dietary habits and high rates of acute malnutrition and micronutrient deficiencies as observed in the Senegalese urban school children also underline the need for school-based interventions. When entering school, children spend more time out-door, away from their family supervision and consume street and processed foods in urban areas when no school feeding is available <sup>145</sup>, which makes the food transition between preschool and school age a sensitive health issue. Therefore nutrition education and school feeding should be extended, especially in disadvantaged urban areas. To convince policy makers on the need to scale-up of school feeding programs, strong scientific evidence of its benefits on health and nutrition is needed. Our research showed promising beneficial effects of fortified rice on micronutrient status and cognitive performance after only 6 months. A larger impact may be expected from long-term daily consumption of fortified rice. In the future, effectiveness studies of fortified food conducted over more than one school year may stimulate the introduction of fortified staple food in existing school feeding programs.

Furthermore, more research on indicators is needed to better characterize malnutrition in school-aged children and adolescents, taking into account intra-populations variability, effects of environmental factors and specific needs of this age group. We established cut-offs for MUAC in Cambodia, which is a cost-effective tool to screen for acute malnutrition among school children. And we suggested correction factors to eliminate the cofounding impact of the acute-phase response on TFR concentrations, a biomarker of iron status, and verified other correction factors of ferritin and vitamin A status.

More research and efforts should be shifted to the nutritional status of adolescent girls. Nutritional deficiencies among still growing adolescents, who are potential young mothers, may be particularly harmful for both mother and infant, reinforcing the intergenerational cycle of malnutrition. Specific nutrient requirements have to be established for pregnant adolescent girls. School-based and community-based interventions should be implemented to prevent precocious marriage and to improve the nutrition of adolescent girls.

Neglecting the issue of malnutrition in school-aged children and adolescents may compromise the benefits of the historical improvement achieved in younger children. Investing in nutrition in this age range when resources are available is an opportunity to optimize the development of next generations in developing countries.



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## Synthesis

### *Background*

Malnutrition remains a crucial public health problem, especially among young children where it is responsible for growth retardation in one third of them and for half of deaths, mostly in developing countries. Nutritional deficiencies during fetal life and early childhood have long-term and possibly irreversible adverse effects on physical and cognitive development. Consequently, most of the efforts have been focused on improving nutrition among women of reproductive age and children under 5 y. However, prevalence of chronic and acute malnutrition and micronutrient deficiencies are estimated to be also high in older children and adolescents, especially in Africa and in South-East Asia.

	Africa	South-East Asia
Stunting	22%	29%
Thinness	35%	35%
Iron deficiency	29%	20%
Vitamin A deficiency	32%	17%
Iodine deficiency	33%,	33%,
Zinc deficiency	54%	29%

### **Estimation of prevalence of nutritional deficiencies in school-aged children (Best, 2007)**

The growth spurt in adolescence is the second most important in life after the one occurring in the first year of life, which dramatically increases energy, protein and micronutrients nutrient requirements. Although growth retardation reflects primarily nutritional deprivation during the first '1000 days' period, improving nutrition during late childhood and adolescence can prevent the continuation of the stunting process, which is known to be associated with impaired work capacity and obstetric complications. Due to the maternal-fetal nutrient competition in pregnant adolescents supporting the triple burden of poor dietary intake, their own growth and their fetus growth, adolescent mothers and their infants are particularly at risk for nutritional deficiencies in developing countries, where early marriage and childbearing are common.

School age and adolescence are also critical periods for neurological development, with frontal lobes achieving development spurts at 7-9 years and in the mid-teenage years. Research shows beneficial effects of iron or iodine interventions on cognitive performance of school children. Improving micronutrient status in school-aged children reduces morbidity and enhances school performance. Therefore, nutrition at school age and adolescence is crucial for educational achievement and future adult life.

However, data about nutritional status and impact of interventions in school-aged children and adolescents are scarce. The objective of the thesis was to evaluate the prevalence of malnutrition, its determinants and consequences on health outcomes among school-aged children and adolescents from two developing countries, as well as and the effectiveness of school-based interventions. The thesis is based on 2 studies conducted among African and South-East-Asian school-aged children and adolescents.

The first study evaluated the nutritional status and nutrient intake of children from primary state schools of Dakar, the capital of Senegal. Indeed, the urban population has been rapidly expanding in the developing world, and tremendous discrepancies within urban have arisen, which was aggravated by the food price crisis and the global crisis in the past decade. Plus, urban lifestyle and transition from infancy to school age expose children to poor dietary and health habits. However, still few data are available about the nutritional status of school-aged children and adolescents living in urban areas.

The second study is an effectiveness study of micronutrient-fortified rice distributed through the School Feeding Program of World Food Program (WFP) in the Kampong Speu province, Cambodia. School feeding has been scaled up in the past decades because of its incentive effect on school enrollment and attendance but only 18% of school children in low-income countries receive school meals and the impact on nutritional status remains small compared to the costs. Rice being massively consumed in many Asian and Sub-Saharan countries, distributing fortified rice through school feeding may be a cost effective way to improve micronutrient status of school-aged children and adolescents.

## Methods

### • Studies design

In Senegal, a cross-sectional survey was conducted in 2010 on a representative sample of **~ 600 children** aged 5-17 y from primary state schools of the Dakar region (Senegal), selected through a two-stage random cluster sample (30 schools × 20 children) and enrolled after written informed parental consent.

In Cambodia, the FORISCA-UltraRice+NutriRice study was a cluster-randomized, double-blinded, placebo-controlled trial conducted in 2012 among ~2400 children aged 6-16 y in the Kampong Speu province in 2012. Sixteen schools participating in the School Feeding Program of WFP were randomly assigned to receive fortified rice (UltraRice®original, UltraRice®new or NutriRice®) or unfortified rice (placebo) 6 days per week for 6 months. Within each school, 132 children were randomly selected with stratification on grade and gender, and enrolled in the study after written informed parental consent resulting in **~2400 children**. Data were collected before the intervention (baseline), after 3 months (midline) and after 6 months at the end of the intervention (endline).

**Micronutrient composition of uncooked rice per 100g of blended rice**

	Iron (mg)	Zinc (mg)	Vitamin A (IU)	Vitamin B6 (mg)	Vitamin B12 (µg)	Vitamin B1 (mg)	Vitamin B9 (mg)	Vitamin B3 (mg)
UltraRice®original	<b>10.7</b>	<b>3.0</b>	0.0	0.0	0.0	<b>1.1</b>	<b>0.2</b>	0.0
UltraRice®new	<b>7.6</b>	<b>2.0</b>	<b>2140.0</b>	<b>0.0</b>	<b>3.8</b>	<b>1.4</b>	<b>0.3</b>	<b>12.6</b>
NutriRice®	<b>7.5</b>	<b>3.7</b>	<b>960.0</b>	<b>0.9</b>	<b>1.3</b>	<b>0.7</b>	<b>0.1</b>	<b>8.0</b>

- Data collection

Once in Senegal, and at baseline, midline and endline in Cambodia, anthropometric data (height, weight, MUAC, triceps skinfold) and blood and urine samples were collected to measure hemoglobin, plasma concentrations of ferritin (FER), soluble transferrin receptors (TFR), retinol binding protein and zinc, and urinary iodine concentration. Correction factors were used for FER and RBP in subjects with inflammation determined with C-reactive protein (CRP) and  $\alpha$ -1-acid-glycoprotein (AGP).

In Senegal, two quantitative 24-hours food recalls were conducted among children and quantitative recipes were collected among mothers in households and in street vendors.

In Cambodia, data on cognitive performance (picture completion and block design from WISC III and Raven's colored progressive matrices) were collected at baseline, midline and endline, as well as feces samples to measure parasite infestation.

***Prevalence of nutritional deficiencies in the studied populations***

	<b>Definition</b>	<b>Senegal</b>	<b>Cambodia</b>
<b>Mean age (y)</b>		9.7	10.2
<b>(Age range)</b>		(5-17)	(6-16)
<b>Stunting (%)</b>	Height-for-Age z-scores (HAZ) <-2	4.9	40.0
<b>Severe stunting (%)</b>	HAZ <-3	0.7	10.9
<b>Thinness (%)</b>	BMI-for-Age z-scores (BAZ) <-2	18.6	25.6
<b>Severe thinness (%)</b>	BAZ <-3	5.6	5.0
<b>Overweight (%)</b>	BAZ > 1	0.3	0.1
<b>Anemia (%)</b>	Hemoglobin (Hb) < 115 g/L (children <12 y); <120 g/L (children 12 - 15 y and girls ≥15 y); <130 g/L for (boys ≥15 y)	14.4	15.7
<b>Severe anemia (%)</b>	Hb <70 g/L	0.1	0.1
<b>Depleted iron stores (%)</b>	FER corrected for inflammation <15 µg/L	21.4	1.5
<b>Tissue iron deficiency (%)</b>	TFR >8.3 mg/L	33.3	51.0
<b>Iron deficiency (ID) (%)</b>	depleted iron stores and/or tissue iron deficiency	39.1	51.2
<b>Iron deficiency Anemia (IDA) (%)</b>	ID and anemia	10.6	10.0
<b>Vitamin A deficiency (%)</b>	RBP corrected for inflammation <0.7 µmol/L	3.0	0.7
<b>Marginal vitamin A status (%)</b>	corrected RBP<1.05 µmol/L	35.9	7.9
<b>Zinc deficiency (%)</b>	zinc <0.65 mg/L (<10 y); <0.70 mg/L (boys≥10 y); <0.66 mg/L (girls ≥10y)	25.9	92.8
<b>Iodine deficiency (%)</b>	urinary iodine <100 µg/L	32.8	17.3
<b>Inflammation (%)</b>	CRP >5 mg/L and/or AGP >1 g/L	12.0	39.5
<b>Parasite infection (%)</b>	≥ 1 egg /g feces	-	18.0

### ***Risk factors for poor nutrition and health outcomes***

Data from Senegal and baseline data from Cambodia were used to evaluate determinants and adverse effects of nutritional deficiencies among school children.

- Nutrient intake and risk factors for micronutrient deficiencies in Senegalese children

Energy and nutrient intakes were adjusted for intra individual variability to obtain estimated usual intakes and prevalence of inadequate intake, using the software PC-Side and according to the intakes recommendations of the Institute of Medicine.

Most of children had insufficient energy intake. 7% had insufficient intake of proteins per kg of body weight. Fiber intake was insufficient in almost 90% of the children. Contribution of proteins and carbohydrates to energy intake was insufficient in respectively 31% and 5% of children. No children had excessive contribution of protein and carbohydrates to energy intake, but respectively 32%, 21% and 14% of children had excessive contribution of lipids, Saturated Fatty Acids (SFAs), and Poly-Unsaturated Fatty Acids (PUFAs) to total energy intake. Half of the children had insufficient iron or vitamin C intake, more than two third had insufficient zinc or vitamin A intake and all of the children had insufficient calcium and folic acid intake. Risks for iron and zinc deficiency were higher when intakes were insufficient in iron, zinc, and protein and excessive in lipid. No micronutrient was consumed above its respective Upper Limits.

These findings indicate a diet poor in dairy products, meat, fruit and vegetables, with a special concern about zinc, vitamin A, folic acid and calcium. Inadequacy of nutrient intake increased with age and is likely to be the main cause of high rates of acute malnutrition and micronutrient deficiencies in the population of primary state schools of Dakar. Reduction of familial care, increase of street food consumption, conjectural food prices crisis, and absence of school meals<sup>145</sup> may lead to a poorer diet at school age compared to infancy, which seem to be aggravated at adolescence in our study. These findings highlight the need of nutritional interventions in Senegalese urban schools such as school feeding program and nutrition education.

- Risk factors for low cognitive scores and impaired growth in Cambodian children

Multivariate analyses were performed to evaluate the associations between cognitive tests scores and variables assessing nutritional status (stunting, anemia and micronutrient status) while taking into account the effect of covariates (age, gender, parasite infection, socio-economic status).

Linear regression analysis was performed with height for age z-scores, plasma zinc and parasite infection with inflammation, age and sex as covariates.

Stunted children had cognitive scores significantly lower than non-stunted children on all tests. In Raven's colored progressive matrices test, boys with iron-deficiency anemia had lower scores than boys with normal iron status (-1.46,  $p < 0.05$ ). In picture completion test, children with normal iron status tended to score higher than iron-deficient children with anemia (-0.81;  $p < 0.1$ ) or without anemia (-0.49;  $p < 0.1$ ). Parasite infection was associated with an increase in risk of scoring below the median value in block design test (OR=1.62;  $p < 0.05$ ), and with lower scores in other tests, for girls only (both  $p < 0.05$ ). Plasma zinc was positively associated with

height for age z-scores ( $aB=-0.033$ ,  $p<0.05$ ), but parasite infection was not. Parasite infection showed a negative association with zinc concentration ( $aB=-0.233$ ,  $p = 0.05$ ).

Poor cognitive performance of Cambodian school-children was multifactorial and significantly associated with long-term (stunting) and recent nutritional status indicators (iron status), as well as parasite infection. Our findings also suggest that factors influencing child growth may depend on prevalence of parasite species and zinc deficiency. Further research is needed to elucidate these relationships and their underlying mechanisms. A life-cycle approach with programs to improve nutrition in early life and at school-age could contribute to optimal cognitive performance.

***Effectiveness of multiple-micronutrient fortified rice on micronutrient status and cognitive performance through the school feeding program of WFP in Cambodia***

Generalized mixed models (linear or binary logistic regression) adjusted for age and gender were used to evaluate the impact of intervention groups on micronutrient status and on the scores of the cognitive tests. Secondary analysis were performed including interactions with low iron status, parasite infection and stunting and the impact of intervention, as these variables were earlier identified as risk factors for low cognitive scores.

After 6 months, children receiving NutrIRice® (NR) and UltraRice®new (URN) respectively had 4 and 5 times less risk of marginal vitamin A status (respectively  $OR=0.24$ ,  $p<0.001$  and  $OR=0.20$ ,  $p<0.001$ ) in comparison to the unfortified rice group. Hemoglobin significantly increased ( $+0.8g/L$ ,  $p<0.05$ ) after 3 months in the URN group in comparison to the unfortified rice group, however, after 6 months, this difference was significant only in children with inflammation ( $+2.1 g/L$   $p<0.01$ ). All cognitive scores improved after 6 months ( $p<0.001$ ). Block design score improvement was significantly higher in children consuming UltraRice®Original ( $p<0.05$ ) compared to the other fortified rice groups and the unfortified rice group. No difference among groups was found on Raven's colored progressive matrices scores or picture completion scores. Stunting, parasite infestation and inflammation negatively affected the impact of the intervention.

Multi-micronutrient fortified rice containing vitamin A effectively improved the vitamin A status of schoolchildren. Impact on hemoglobin and iron status was limited, partly by sub-clinical inflammation. However, fortified rice with the highest concentration of iron improved cognitive performance. Using existing school feeding programs to distribute fortified rice to school children may be a cost-effective way to improve micronutrient status and cognitive performance and thereby improve increase school performance and educational achievements.



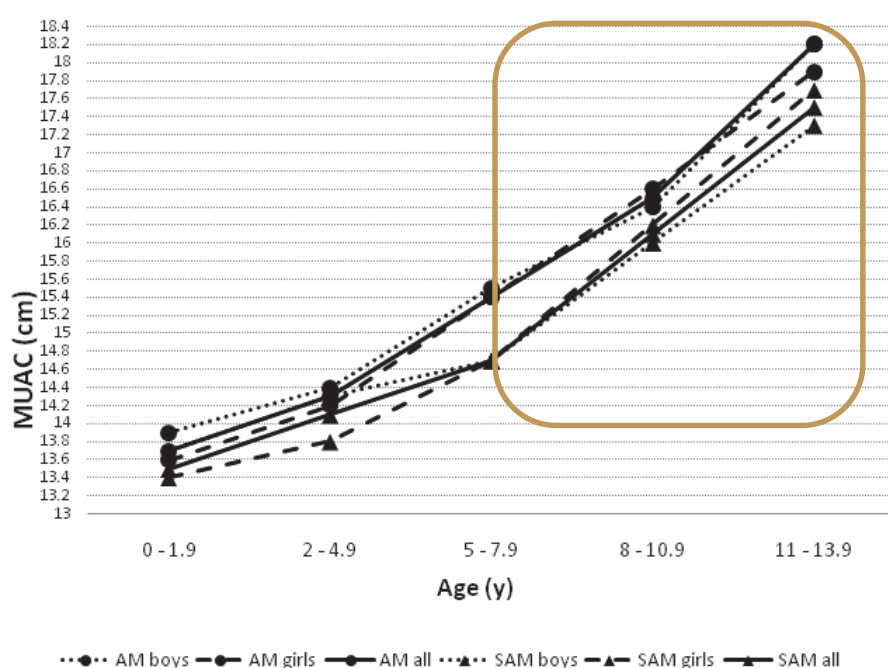
## ***Indicators to evaluate malnutrition among school-aged children and adolescents***

Data from Senegal and baseline data from Cambodia were used to evaluate the relevance of some indicators of malnutrition among school children.

- MUAC cut-offs to screen for acute malnutrition in Cambodian school-aged children and adolescents

As seen in our studies, acute malnutrition is widely spread in school-aged children and adolescents. The latest WHO guidelines for the treatment of severe acute malnutrition recommend establishing MUAC cut-offs for children >5 y. Indeed, even though it is less linked to mortality compared to younger children, acute malnutrition is also important to address during school years as it can impair physical and mental development in this age group.

Anthropometric data from the Cambodian dataset were used. To assess the performance of MUAC cut-offs compared to the golden standard recommended by WHO to define severe and moderate acute malnutrition (BAZ <-2 and BAZ <-3), receiver operating characteristic curves (ROC curves) were constructed. MUAC cut-offs with the highest sensitivity and a false positive rate below 33% were selected among cut-offs with the highest values of area-under-curves (AUC) to be the optimal cut-offs to screen for acute malnutrition.



### **MUAC optimal cut-offs for acute malnutrition (AM) and severe acute malnutrition (SAM) by age group and gender**

MUAC cut-offs gradually increased with age in an almost linear manner, but changes are influenced by changes in growth velocity, e.g. during puberty. Cut-offs were higher for boys than for girls (+0.1 +0.3 cm), except from age group 8 to 10.9 years. We assume that the earlier

adolescent growth spurt in girls than in boys, with associated changes in lean and fat mass, underlies this phenomenon.

Children above 5 y should also be taken into account in the management of acute malnutrition. Indeed at school age, acute malnutrition, often accompanied by micronutrient deficiencies, can delay maturation, impair muscular strength, bone density and work capacity. Thus malnutrition at school age increase risk of morbidity, of school failure and school drop-out. Schools could be a practical platform to follow school-aged children for acute malnutrition.

- Impact of subclinical inflammation on plasma transferrin receptor, ferritin, RBP and zinc in school children in Cambodia and Senegal

Acute-phase response is known to affect plasma ferritin and retinol while micronutrient status remain the same, leading to respectively underestimate and overestimate iron and vitamin A deficiencies. TFR was long considered to be not or less sensitive to inflammation than ferritin but recent research suggest a significant impact of acute phase response on TFR values. The effect of inflammation on plasma zinc concentration remains unclear too. We used data from Cambodia and Senegal to evaluate the impact of subclinical inflammation on the assessment of micronutrient deficiencies among school children.

The ratio of the mean values of the biomarker for the group with inflammation to the reference group without inflammation was calculated. The correction factor was calculated as  $1/\text{ratio}$  and applied to calculate prevalence of micronutrient deficiencies adjusted for inflammation.

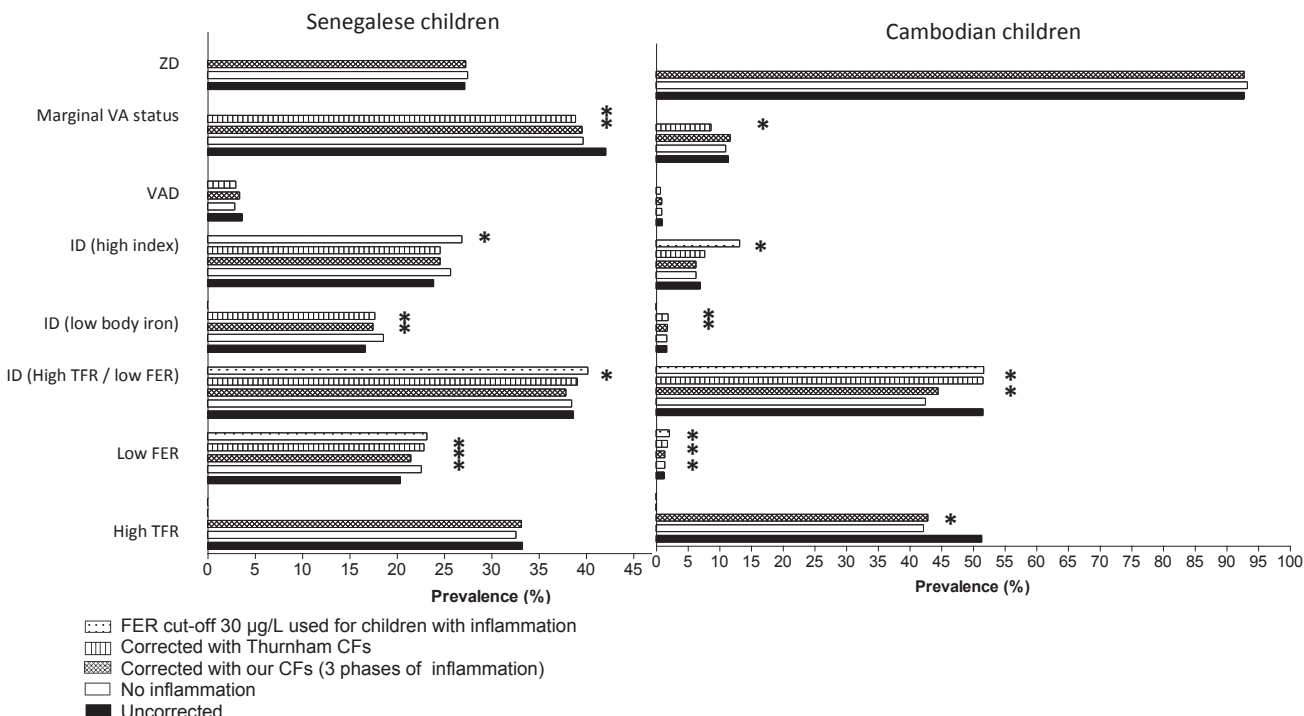
## Ratios and correction factors of ferritin, transferrin receptor, RBP and zinc for inflammation status

	Ferritin			Transferrin receptor		
	Ratio (95%) <sup>1</sup>		CF <sup>2</sup>	Ratio (95%) <sup>1</sup>		CF <sup>2</sup>
<i>Senegalese school children</i>						
Incubation	1.22	[1.16-1.26]	<b>0.82</b>	0.97	[0.32-1.63]	<b>1.03</b>
Early convalescence	2.00	[1.96-2.03]	<b>0.50</b>	1.07	[1.00-1.13]	<b>0.94</b>
Late convalescence	1.50	[1.47-1.52]	<b>0.67</b>	1.02	[0.97-1.08]	<b>0.98</b>
<i>Cambodian school children</i>						
Incubation	1.29	[1.27-1.31]	<b>0.77</b>	0.90	[0.79-1.01]	<b>1.12</b>
Early convalescence	1.83	[1.82-1.83]	<b>0.55</b>	1.10	[1.07-1.13]	<b>0.91</b>
Late convalescence	1.27	[1.27-1.27]	<b>0.79</b>	1.15	[1.15-1.18]	<b>0.87</b>
	RBP			Zinc		
	Ratio (95%) <sup>1</sup>		CF <sup>2</sup>	Ratio (95%) <sup>1</sup>		CF <sup>2</sup>
<i>Senegalese school children</i>						
Incubation	0.76	[0.64-0.88]	<b>1.31</b>	1.05	[0.96-1.08]	<b>0.95</b>
Early convalescence	0.86	[0.75-0.97]	<b>1.16</b>	0.96	[0.95-1.02]	<b>1.04</b>
Late convalescence	0.94	[0.85-1.03]	<b>1.07</b>	1.01	[0.98-1.03]	<b>0.99</b>
<i>Cambodian school children</i>						
Incubation	0.85	[0.63-1.07]	<b>1.17</b>	0.93	[0.88-1.05]	<b>1.07</b>
Early convalescence	0.85	[0.82-0.89]	<b>1.17</b>	1.00	[0.98-1.02]	<b>1.00</b>
Late convalescence	1.05	[1.03-1.08]	<b>0.95</b>	1.01	[1.00-1.02]	<b>0.99</b>

<sup>1</sup> Ratio of back-transformed concentrations of positive group vs. control group

<sup>2</sup> CF=1/Ratio

Secondly, prevalence were adjusted using correction factors for RBP and FER suggested by Thurnham et al.



\* P-value<0.05 Mc Nemar test to compare prevalence with uncorrected prevalence: **ZD**: Zinc deficiency; **VAD** : Vitamin A deficiency; **ID** : Iron deficiency

## Effect of correcting TFR, FER, RBP and zinc for inflammation on the prevalence of micronutrient deficiencies

In previous research, transferrin receptor was thought to be more reliable than ferritin in endemic inflammation areas, but we showed that transferrin receptor is also sensitive to acute-phase response and that it should be adjusted. Our analysis demonstrated that inflammation increases ferritin and transferrin receptor, leading to underestimate low ferritin and overestimate high transferrin receptor so the index transferrin receptor/log ferritin was the less sensitive indicator to inflammation. RBP decreased during inflammation leading to overestimate vitamin A deficiency or marginal vitamin A status in populations with elevated inflammation rates. Our findings suggest that for determining zinc status in normal populations that is in population with only a low prevalence of sub-clinical infection, taking the acute phase response into account will not lead to a significant improvement in the estimate of the prevalence of zinc deficiency. Overall, more research is needed about the indicators of nutritional status among school-aged children and adolescents. The intra-populations variability, the effect of environmental factors and the specific needs of this age range for each gender should be accounted.

## ***Conclusion***

Positive associations between micronutrient deficiencies, parasite infection and poor cognitive performance were observed in Cambodia, underlining the need to address health and nutrition issues among school-aged children and adolescents. Although often omitted by public research, it is known that high nutrient requirements associated to the growth spurt and cerebral development make school-aged children and adolescents particularly vulnerable to poor nutrition, which compromises school achievement, crucial to break the intergenerational cycle of poverty and malnutrition. Vitamin A status and cognitive performance was improved by multiple micronutrient fortified rice in our study in Cambodia, showing that some adverse effects of malnutrition can still be reversed at school age.

Poor dietary intake and elevated rates of acute malnutrition and micronutrient deficiencies in urban school children which do not have access to school feeding as observed in Senegal highlight the need of school-based interventions. Nutrition education and school feeding should be extended, especially in disadvantaged urban areas.

To convince policy makers on the need to scale up school feeding programs, a strong scientific evidence of its beneficial effects on health and nutrition is needed. Promising benefits on micronutrient status and cognitive performance were showed after only 6 months of fortified rice consumption in our research. A larger impact may be expected from long-term daily consumption of fortified rice. In the future, effectiveness studies conducted over more than one school year may stimulate the introduction of fortified staple food in existing school feeding programs.

Moreover, more research on indicators taking into account intra-populations variability, influence of environmental factors and specific needs for school-aged children and adolescents is needed to better identify and determine malnutrition in this age group. We established cut-offs for MUAC in Cambodia, which is a cost-effective tool to screen for acute malnutrition among school children. We suggested correction factors to eliminate the confounding impact of the acute-phase response on TFR plasma concentrations, a biomarker of iron status, and verified correction factors of ferritin and vitamin A status.

Neglecting the issue of malnutrition in school-aged children and adolescents may compromise the benefits of the historical improvement achieved in younger children. Investing in nutrition in these populations is an opportunity to optimize the development of next generations in developing countries.

## Synthèse

### Contexte

La malnutrition est toujours un grave problème de santé publique, notamment parmi les jeunes enfants chez qui elle est responsable du retard de croissance d'un tiers d'entre eux, et de la moitié des décès, majoritairement dans les pays en développement. Les carences nutritionnelles pendant la vie fœtale et la petite enfance causent des dommages à long terme, parfois irréversibles, sur le développement physique et cognitif. Par conséquent, la plupart des efforts ont été concentrés à ce jour sur l'amélioration de la nutrition des femmes en âge de procréer et des enfants âgés de moins de 5 ans. Cependant, on estime que la prévalence de la malnutrition chronique et aigue et les carences en micronutriments est également élevée parmi les enfants plus âgés et les adolescents, notamment en Afrique et en Asie du Sud-Est.

	Afrique	Asie du Sud-Est
Retard de croissance	22%	29%
Maigreur	35%	35%
Carence en fer	29%	20%
Carence en vitamine A	32%	17%
Carence en iode	33%,	33%,
Carence en zinc	54%	29%

**Estimation de la prévalence des carences nutritionnelles chez les enfants d'âge scolaire (Best, 2007)**

Le pic de croissance pendant l'adolescence est le second plus important après celui qui se déroule pendant la première année de vie, ce qui augmente considérablement les besoins en énergie, protéines et micronutriments. Bien que le retard de croissance reflète principalement une privation nutritionnelle pendant la période des premiers « 1000 jours », améliorer la nutrition pendant l'âge scolaire et l'adolescence peut prévenir le prolongement du processus de retard de croissance, dont on sait qu'il est associé à une capacité de travail réduite et à des complications obstétriques. En raison de la compétition nutritionnelle materno-foetal chez les adolescentes enceintes supportant la triple charge d'un régime alimentaire déficient, de leur propre croissance et de la croissance de leur fœtus, les mères adolescentes et leurs nourrissons sont particulièrement à risque de souffrir de carences nutritionnelles dans les pays en développement, où le mariage et la grossesse précoces sont répandus.

L'âge scolaire et l'adolescence sont aussi des périodes critiques pour le développement neurologique, les lobes frontaux connaissant des pics de développement à 7-9 ans et au milieu de l'adolescence. Des recherches mettent en évidence des effets bénéfiques d'interventions en fer ou en iode sur les performances cognitives d'enfants d'âge scolaire. Corriger le statut en micronutriments d'enfants d'âge scolaire réduit la morbidité et améliore les performances

scolaires. Ainsi, une nutrition adéquate à l'âge scolaire et à l'adolescence est cruciale pour la réussite scolaire et la vie future.

Cependant, les données sur l'état nutritionnel et l'impact d'interventions chez les enfants d'âge scolaire et les adolescents sont rares. L'objectif de cette thèse est d'évaluer la prévalence de différentes formes de malnutrition, ses facteurs déterminants et ses conséquences sur la santé chez les enfants d'âge scolaire et les adolescents, ainsi que l'efficacité d'interventions scolaires. Cette thèse est basée sur deux études conduites sur des enfants d'âge scolaire et des adolescents de deux pays en développement (Sénégal et Cambodge).

La première étude évalue le statut nutritionnel et les ingérés en nutriments d'enfants des écoles primaires publiques de Dakar, la capitale du Sénégal. En effet, la population urbaine a connu une expansion rapide dans les pays en développement, et des inégalités massives au sein des zones urbaines sont apparues, ce qui s'est encore aggravé durant la dernière décennie en raison de la crise des prix alimentaires et de la crise économique mondiale. En outre, le mode de vie urbain et la transition de la petite enfance à l'âge scolaire exposent les enfants à de mauvaises habitudes alimentaires et sanitaires. Cependant, très peu de données sont actuellement disponibles sur l'état nutritionnel des enfants d'âge scolaire et les adolescents vivant en zones urbaines.

La deuxième étude est une étude d'efficacité de riz fortifié en micronutriments distribué via le programme d'alimentation scolaire du Programme Alimentaire Mondial (PAM) dans la province de Kampong Speu au Cambodge. L'alimentation scolaire a connu une certaine expansion dans les dernières décennies en raison de son effet incitatif bénéfique sur les inscriptions et la fréquentation scolaires, mais seulement 18% des enfants d'âge scolaire des pays à faible revenu en bénéficient, et l'impact sur l'état nutritionnel demeure modeste par rapport aux coûts engendrés. Le riz étant massivement consommé dans beaucoup de pays asiatiques et subsahariens, distribuer du riz fortifié via les circuits d'alimentation scolaire pourrait être une stratégie à haut rapport coût/efficacité d'améliorer le statut en micronutriments des enfants d'âge scolaire et des adolescents.



## Méthodes

- Plan des études

Au Sénégal, une étude transversale fut conduite en 2010 sur un échantillon représentatif d'environ 600 enfants âgés de 5 à 17 ans des écoles primaires publiques de la région de Dakar (Sénégal), sélectionnés par un échantillonnage aléatoire en grappes (30 écoles x 20 enfants).

Au Cambodge, l'étude FORISCA-UltraRice+NutriRice conduite en 2012 sur environ 2400 enfants âgés de 6 à 16 ans dans la province de Kampong Speu était un essai contrôlé randomisé en grappes en double aveugle. 16 écoles participant au programme d'alimentation scolaire du PAM furent réparties de façon aléatoire pour recevoir du riz fortifié (UltraRice®original, UltraRice®new or NutriRice®) ou non fortifié 6 jours par semaine pendant 6 mois. Au sein de chaque école, 132 enfants furent sélectionnés de façon aléatoire en stratifiant par classe et par sexe et recrutés après signature du consentement éclairé par les parents, résultant en un échantillon d'environ 2400 enfants. Les données furent collectées avant l'intervention (baseline), après 3 mois (midline), et après 6 mois à la fin de l'intervention (endline).

**Composition en micronutriments du riz cru pour 100 grammes de riz mélangé**

	Fer (mg)	Zinc (mg)	Vitamine A (IU)	Vitamine B6 (mg)	Vitamine B12 (µg)	Vitamine B1 (mg)	Vitamine B9 (mg)	Vitamine B3 (mg)
UltraRice®original	<b>10.7</b>	<b>3.0</b>	0.0	0.0	0.0	<b>1.1</b>	<b>0.2</b>	0.0
UltraRice®new	<b>7.6</b>	<b>2.0</b>	<b>2140.0</b>	<b>0.0</b>	<b>3.8</b>	<b>1.4</b>	<b>0.3</b>	<b>12.6</b>
NutriRice®	<b>7.5</b>	<b>3.7</b>	<b>960.0</b>	<b>0.9</b>	<b>1.3</b>	<b>0.7</b>	<b>0.1</b>	<b>8.0</b>

- Collecte des données

Une seule fois au Sénégal, et lors des baseline, midline et endline au Cambodge, des données anthropométriques (taille, poids, périmètre brachial (PB), pli cutané tricipital), ainsi que des échantillons de sang et d'urine furent collectés pour mesurer le taux d'hémoglobine, les concentrations plasmatiques en ferritine (FER), récepteur soluble de la transferrine (TFR), « retinol binding protein » (RBP), et en zinc, et la concentration urinaire en iode. Les concentrations en FER et RBP furent corrigées pour le statut inflammatoire déterminé avec la protéine C-réactive (CRP) et l'  $\alpha$ -1-glycoprotéine acide (AGP).

Au Sénégal, deux rappels alimentaires des 24 heures quantitatifs furent conduits auprès des enfants et les recettes quantitatives furent collectées auprès des mères à domicile et auprès des vendeuses de rues.

Au Cambodge, des données sur les performances cognitives (« picture completion » et « block design » du WISC III et les matrices colorées progressives de Raven) furent collectées lors des baseline, midline and endline, ainsi que des échantillons de fèces pour mesurer l'infestation parasitaire.

## Prévalence des carences nutritionnelles dans les populations étudiées

	Definition	Senegal	Cambodia
Age moyen (ans) (Tranche d'âge)		9.7 (5-17)	10.2 (6-16)
Retard de croissance (%)	z-scores Taille-pour-Age (TAZ) <-2	4.9	40.0
Retard de croissance sévère (%)	TAZ <-3	0.7	10.9
Maigreur (%)	z-scores IMC-for-Age (IAZ) <-2	18.6	25.6
Maigreur sévère (%)	IAZ <-3	5.6	5.0
Surpoids (%)	IAZ > 1	0.3	0.1
Anémie (%)	Hémoglobine (Hb) < 115 g/L enfants <12 ans; <120 g/L enfants 12 - 15 ans et filles ≥15 ans; <130 g/L garçons ≥15 ans	14.4	15.7
Anémie sévère (%)	Hb <70 g/L	0.1	0.1
Stock de fer insuffisant (%)	FER corrigée pour inflammation <15 µg/L	21.4	1.5
Carence en fer des tissus (%)	TFR >8.3 mg/L	33.3	51.0
Carence en fer (%)	carence en fer des tissus et/ou stock de fer insuffisant	39.1	51.2
Anémie ferriprive (%)	carence en fer et anémie	10.6	10.0
Carence en vitamine A (%)	RBP corrigé pour inflammation <0.7 µmol/L	3.0	0.7
Statut marginal en vitamine A (%)	RBP corrigé pour inflammation <1.05 µmol/L	35.9	7.9
Carence en zinc (%)	zinc <0.65 mg/L (enfants <10 ans); <0.70 mg/L (garçons ≥10 ans); <0.66 mg/L (filles ≥10 ans)	25.9	92.8
Carence en iode (%)	iode urinaire <100 µg/L	32.8	17.3
Inflammation (%)	CRP >5 mg/L et/ou AGP >1 g/L	12.0	39.5
Infection parasitaire (%)	≥ 1 oeuf /g fèces	-	18.0

## Facteurs de risque pour de faibles « outcomes » nutrition et santé

Les données du Sénégal et de la baseline au Cambodge furent analysées pour évaluer les facteurs déterminants et les répercussions négatives de la malnutrition chez les enfants d'âge scolaire.

- Apports en nutriments et facteurs de risque de carences en micronutriments

Les apports en énergie et en nutriments furent ajustés en fonction de la variabilité intra-individuelle pour obtenir les « apports usuels estimés » et la prévalence des ingérés inadéquats, en utilisant le logiciel PC-Side et en suivant les recommandations de l'Institut de Médecine.

La plupart des enfants eurent des apports en énergie insuffisants. 7% eurent des apports insuffisants en protéine par kg de poids corporel. Les apports en fibres furent insuffisants chez près de 90% des enfants. La contribution des protéines et des glucides à l'apport énergétique global était insuffisante dans respectivement 31% et 5% des enfants. Aucun enfant n'eut une contribution excessive des protéines et des glucides à l'apport énergétique global, mais respectivement 32%, 21% et 14% des enfants eurent une contribution excessive des lipides, des acides gras saturés (AGS) et des acides polyinsaturés (AGPI) à l'apport énergétique. La moitié des enfants eurent des apports insuffisants en fer et en vitamine C, plus de deux tiers eurent des apports insuffisants en zinc et en vitamine A, et tous les enfants eurent des apports insuffisants en calcium et en acide folique. Les risques de carence en fer et en zinc étaient plus élevés quand les apports étaient insuffisants en fer, zinc, protéines ou excessifs en lipides. Aucun micronutriment ne fut consommé au-dessus de ses limites maximales.

Ces résultats indiquent un régime pauvre en produits laitiers, viande, fruits et légumes, et notamment des apports en zinc, vitamine A, acide folique et calcium particulièrement préoccupants. L'insuffisance des apports augmenta avec l'âge et est probablement la cause principale des taux élevés de malnutrition aigüe et de carences en micronutriments que nous avons observés au sein de la population des écoles primaires publiques de Dakar. La diminution des soins familiaux, l'augmentation de la consommation de nourriture de rue, la crise conjoncturelle des prix alimentaires et l'absence de cantines scolaires peuvent être à l'origine d'un régime alimentaire plus pauvre à l'âge scolaire que pendant la petite enfance, ce qui semble s'aggraver à l'adolescence. Ces résultats mettent en évidence le besoin d'interventions nutritionnelles chez les enfants Sénégalais urbains d'âge scolaire, tels que les cantines scolaires et l'éducation à la nutrition.

- Facteurs de risque pour de faibles scores cognitifs et croissance ralentie chez les enfants cambodgiens

Des analyses multivariées furent effectuées pour évaluer les associations entre les tests cognitifs et les indicateurs de statut nutritionnel (retard de croissance, anémie et statuts en micronutriments) tout en prenant en compte l'effet des covariables (âge, sexe, infection parasitaire, statut socioéconomique).

Des régressions linéaires avec les z-scores taille pour âge, concentration plasmatique de zinc et infection parasitaire furent effectuées, avec âge, sexe et inflammation comme covariables.

Les enfants en retard de croissance eurent des scores cognitifs significativement plus bas que les enfants en croissance normale pour tous les tests. Les garçons souffrant d'anémie ferriprive eurent des scores au test des matrices de Raven inférieurs aux garçons sans carence en fer (-1.46,  $p < 0.05$ ). Les scores « picture completion » des enfants sans carence en fer eurent tendance à être plus élevés que ceux des enfants carencés en fer (-0.81;  $p < 0.1$ ) et souffrant d'anémie ou sans anémie (-0.49;  $p < 0.1$ ). L'infection parasitaire fut associée à une augmentation

du risque d'avoir un score inférieur à la médiane pour le test « block design » (OR=1.62;  $p<0.05$ ), et à des scores plus faibles dans les autres tests, chez les filles ( $p<0.05$  pour les deux tests).

La concentration plasmatique de zinc ( $aB=-0.033$ ,  $p<0.05$ ) mais pas l'infection parasitaire, fut positivement associée avec les z-scores Taille-pour-Age L'infection parasitaire fut négativement associée avec la, concentration plasmatique ( $aB=-0.233$ ,  $p = 0.05$ ).

Les faibles performances cognitives chez les enfants cambodgiens d'âge scolaire furent multifactorielles et significativement associées avec des indicateurs d'historique nutritionnel (retard de croissance) et de statut nutritionnel récent (statut en fer), et avec l'infection parasitaire. Nos résultats suggèrent également que la croissance des enfants pourrait dépendre de l'infection parasitaire et de la carence en zinc. Une recherche plus approfondie est nécessaire pour élucider ces associations et leurs mécanismes sous-jacents. Une approche englobant des programmes d'amélioration de la nutrition pendant la petite enfance et l'âge scolaire pourrait contribuer à optimiser les performances cognitives.

### ***Efficacité du riz fortifié en micronutriments sur les statuts en micronutriments et les performances cognitives via le programme d'alimentation scolaire du PAM au Cambodge***

L'impact de l'intervention sur les statuts en micronutriments et les scores cognitifs fut évalué à l'aide de modèles mixtes généralisés (régressions linéaires ou logistiques binaires). Des analyses secondaires incluant les interactions de l'intervention avec le statut en fer, l'infection parasitaire et le retard de croissance furent effectuées, ces variables ayant été identifiées au préalable comme facteurs de risque pour de faibles scores cognitifs.

Après 6 mois, les enfants recevant NutriRice® (NR) et Ultrarice®New (URN) eurent respectivement 4 et 5 fois moins de risque d'avoir un statut marginal en vitamine A (respectivement OR=0.24,  $p<0.001$  and OR=0.20,  $p<0.001$ ) par rapport à ceux recevant du riz non fortifié. L'hémoglobine diminua significativement (+0.8g/L,  $p<0.05$ ) après 3 mois dans le groupe URN par rapport au groupe « riz non fortifié », cependant, après 6 mois, cette différence était significative seulement chez les enfants sans inflammation (+2.1 g/L  $p<0.01$ ). Tous les scores cognitifs augmentèrent après 6 mois ( $p<0.001$ ). L'augmentation des scores au test "block design" fut significativement plus élevée chez les enfants recevant Ultrarice®Original ( $p<0.05$ ) que chez ceux recevant les autres riz fortifiés et le riz non fortifié. Aucune différence ne fut observée entre les groupes pour les tests des matrices de Raven ou « picture completion ». Le retard de croissance, l'infection parasitaire et l'inflammation eurent un effet négatif sur l'impact de l'intervention.

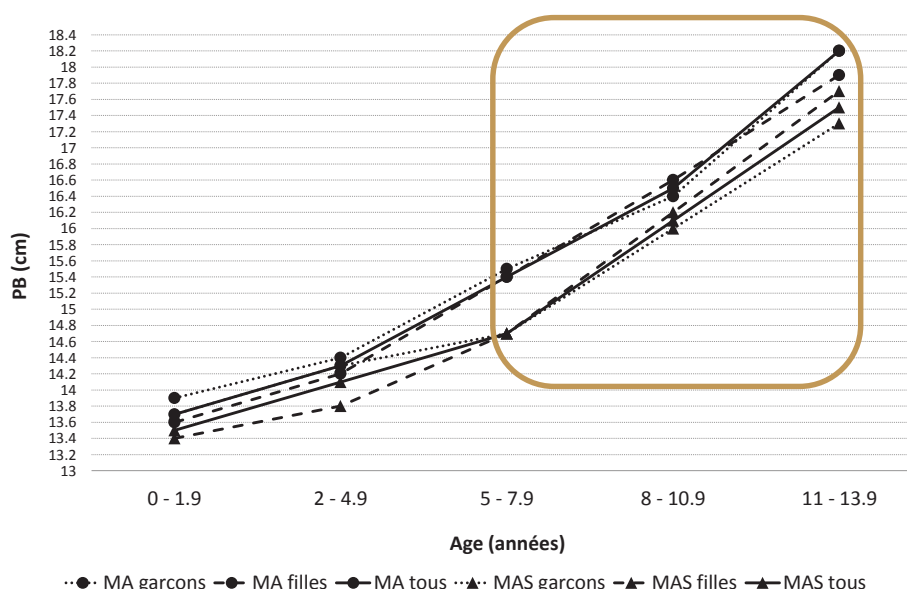
Le riz fortifié en micronutriments contenant de la vitamine A améliora le statut en vitamine A des enfants. L'impact sur l'hémoglobine et le statut en fer fut limité, en partie par l'inflammation sub-clinique. Cependant, le riz fortifié contenant le plus fort taux de fer améliora les performances cognitives. Utiliser les programmes existant d'alimentation scolaire peut être une stratégie rentable d'amélioration des statuts en micronutriments et les performances cognitives et ainsi de favoriser la réussite scolaire.

## Indicateurs d'évaluation de la malnutrition chez les enfants d'âge scolaire et les adolescents

Comme constaté lors de nos études, la malnutrition aigüe est largement répandue parmi les enfants d'âge scolaire et les adolescents. Les dernières directives de l'OMS pour le traitement de la malnutrition aigüe sévère recommandent d'établir des points de coupure pour le périmètre brachial (PB) chez les enfants de plus de 5 ans. En effet, bien que la malnutrition soit moins liée à la mortalité que chez les jeunes enfants, lutter contre la malnutrition à l'âge scolaire est important car elle entrave le développement physique et mental également dans cette tranche d'âge.

- Points de coupure du périmètre brachial (PB) pour détecter la malnutrition aigüe chez les enfants d'âge scolaire et les adolescents cambodgiens

Les données anthropométriques de la base de données du Cambodge furent utilisées. Des courbes ROC (« Receiver operating characteristic ») furent établies pour comparer la performance des points de coupure du PB au « golden standard » recommandé par l'OMS pour définir la malnutrition aigüe sévère et modérée (BAZ <-2 et BAZ <-3). Les points de coupure avec la meilleure sensibilité et un taux de faux positifs inférieur à 33% furent sélectionnés comme les points de coupure de PB optimaux pour dépister la malnutrition aigüe parmi les points de coupure avec les valeurs d'aire sous la courbe (AUC) les plus élevées.



### Points de coupure du PB optimaux pour détecter la malnutrition aigüe et la malnutrition aigüe sévère par tranche d'âge et par sexe

Les points de coupure optimaux augmentèrent graduellement avec l'âge de façon quasi-linéaire, mais cette évolution est influencée par les fluctuations de la vitesse de croissance, notamment pendant la puberté. Les points de coupure furent plus élevés chez les garçons que chez les filles (+0.1 +0.3 cm), sauf dans la tranche d'âge 8-10.9 ans. Nous supposons que le pic de croissance plus précoce chez les filles que chez les garçons et associés à des modifications des masses maigre et grasse explique ce phénomène.

La prise en charge de la malnutrition aigüe devrait prendre en compte les enfants âgés de plus de 5 ans. En effet, à l'âge scolaire, la malnutrition aigüe, qui va souvent de pair avec des carences en micronutriments, peut retarder la maturation sexuelle et altérer la puissance musculaire, la densité osseuse et la capacité de travail. La malnutrition à l'âge scolaire augmente la morbidité, le risque d'échec et d'abandon scolaire. Les écoles peuvent être une plateforme efficace pour le suivi de la malnutrition aigüe chez les enfants d'âge scolaire.

• Influence de l'inflammation subclinique sur les concentrations plasmatiques en récepteurs solubles de la transferrine, ferritine, RBP et zinc chez les enfants d'âge scolaire au Cambodge et au Sénégal

La phase inflammatoire est connue pour altérer les concentrations plasmatiques en ferritine et en rétinol alors que le statut en micronutriment reste inchangé, conduisant à respectivement sous-estimer et surestimer les carences en fer et en vitamine A. La TFR a longtemps été considérée comme non sensible ou moins sensible à l'inflammation que la ferritine mais des recherches récentes ont mis en évidence a impact significatif de la phase inflammatoire sur les concentrations en TFR. L'effet de l'inflammation sur les concentrations plasmatiques en zinc demeure également incertain. Nous avons utilisé les données du Cambodge et du Sénégal pour évaluer l'impact de la phase inflammatoire sur l'évaluation des carences en micronutriments chez les enfants d'âge scolaire.

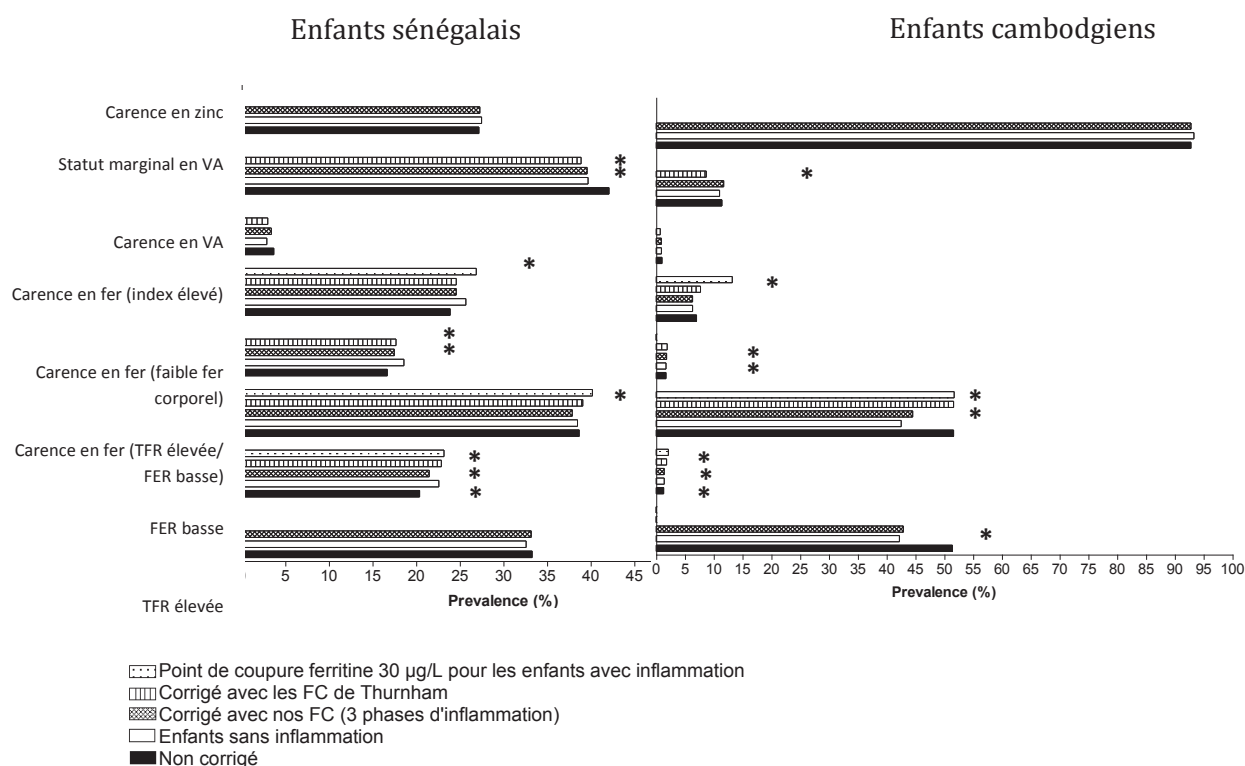
Le ratio des valeurs moyennes du biomarqueur chez les groupes en différentes phases inflammatoires par rapport au groupe sans inflammation fut calculé. Le facteur de correction égal à 1/ ratio fut appliqué aux valeurs du biomarqueurs pour déterminer la prévalence des carences en micronutriments ajustés pour l'inflammation. Les prévalences furent aussi ajustées avec les facteurs de correction pour la ferritine et le RBP proposés par Thurnham et al.

**Ratios et facteurs de correction (FC) en fonction de l'inflammation pour la ferritine, les récepteurs de la transferrine, le RBP et le zinc**

	Ferritine			Recepteur soluble transferrine		
	Ratio (95%) <sup>1</sup>		FC <sup>2</sup>	Ratio (95%) <sup>1</sup>		FC <sup>2</sup>
<i>Enfants sénégalais</i>						
Incubation	1.22	[1.16-1.26]	<b>0.82</b>	0.97	[0.32-1.63]	<b>1.03</b>
Convalescence	2.00	[1.96-2.03]	<b>0.50</b>	1.07	[1.00-1.13]	<b>0.94</b>
Convalescence tardive	1.50	[1.47-1.52]	<b>0.67</b>	1.02	[0.97-1.08]	<b>0.98</b>
<i>Enfants cambodgiens</i>						
Incubation	1.29	[1.27-1.31]	<b>0.77</b>	0.90	[0.79-1.01]	<b>1.12</b>
Convalescence	1.83	[1.82-1.83]	<b>0.55</b>	1.10	[1.07-1.13]	<b>0.91</b>
Convalescence tardive	1.27	[1.27-1.27]	<b>0.79</b>	1.15	[1.15-1.18]	<b>0.87</b>
	RBP			Zinc		
	Ratio (95%) <sup>1</sup>			Ratio (95%) <sup>1</sup>		
<i>Enfants sénégalais</i>						
Incubation	0.76	[0.64-0.88]	<b>1.31</b>	1.05	[0.96-1.08]	<b>0.95</b>
Convalescence	0.86	[0.75-0.97]	<b>1.16</b>	0.96	[0.95-1.02]	<b>1.04</b>
Convalescence tardive	0.94	[0.85-1.03]	<b>1.07</b>	1.01	[0.98-1.03]	<b>0.99</b>
<i>Enfants cambodgiens</i>						
Incubation	0.85	[0.63-1.07]	<b>1.17</b>	0.93	[0.88-1.05]	<b>1.07</b>
Convalescence	0.85	[0.82-0.89]	<b>1.17</b>	1.00	[0.98-1.02]	<b>1.00</b>
Convalescence tardive	1.05	[1.03-1.08]	<b>0.95</b>	1.01	[1.00-1.02]	<b>0.99</b>

<sup>1</sup> Ratio des concentrations dans le groupe en inflammation vs. le groupe sans inflammation

<sup>2</sup> FC = 1/Ratio



\* P < 0.05 Test de Mc Nemar pour comparer prevalence ajustée vs prévalence non ajustée

VA: Vitamine A

### Influence de la correction de TFR, FER, RBP, et zinc en fonction de l'inflammation sur la prévalence des carences en micronutriments

Dans des recherches précédentes, TFR était considéré comme plus fiable que la ferritine dans les zones où l'inflammation est endémique, mais nous avons montré que TFR est également sensible à la phase inflammatoire et qu'elle doit être ajustée en fonction de l'inflammation. Nos analyses ont montré que l'inflammation augmente la ferritine et les récepteurs de la transferrine, conduisant à sous-estimer la prévalence des stocks de fer insuffisants (taux de ferritine bas) et à surestimer la prévalence de la carence des tissus en fer (taux de TFR élevés), faisant de l'index TFR/log FER l'indicateur le moins sensible à l'inflammation. Le RBP diminue pendant l'inflammation ainsi les prévalences de carence en vitamine A et de statut marginal en vitamine A sont surestimées dans les populations avec des taux d'inflammation élevés. Nos résultats suggèrent que pour déterminer le statut en zinc des populations où l'inflammation est sub-clinique, prendre en compte la phase inflammatoire ne modifiera pas significativement l'estimation de la prévalence de la carence en zinc. Globalement, plus de recherche est nécessaire à propos des indicateurs de statut nutritionnel des enfants d'âge scolaire et des adolescents. La variabilité intra-populations, les effets des facteurs environnementaux et les besoins spécifiques de cette tranche d'âge devraient être pris en compte dans la mise au point des indicateurs.



## ***Conclusion***

Des associations positives entre carences en micronutriments, infection parasitaire and faibles performance cognitives furent observées au Cambodge, mettant en évidence la nécessité de traiter les problèmes de nutrition et de santé chez les enfants d'âge scolaire et les adolescents. Même si la recherche cible rarement les enfants d'âge scolaire et les adolescents, on sait que les besoins nutritionnels élevés associés au pic de croissance et de développement cérébral en font une population particulièrement vulnérable aux carences nutritionnelles, ce qui compromet la réussite scolaire, pourtant cruciale pour rompre le cycle intergénérationnel de la malnutrition et de la pauvreté. Le statut en vitamine A et les performances cognitives ont été améliorés grâce au riz fortifié dans notre étude au Cambodge, montrant que certains des effets négatifs de la malnutrition peuvent encore être corrigés à l'âge scolaire.

De faibles apports nutritionnels et des taux élevés de malnutrition aigüe et de carences en micronutriments chez des enfants urbains ne bénéficiant pas d'alimentation scolaire comme nous l'avons observé au Sénégal mettent en évidence le besoin d'interventions à l'école. L'éducation à la nutrition ainsi que l'alimentation scolaire devraient être mis en place dans un nombre croissant d'écoles, notamment dans les zones urbaines défavorisées.

Pour convaincre les décideurs politiques de la nécessité d'étendre ces programmes d'alimentation scolaire, de solides preuves scientifiques de leurs bénéfices sur la santé et la nutrition sont nécessaires. Des effets bénéfiques prometteurs sur les statuts en micronutriments et les performances cognitives furent observés après seulement 6 mois de consommation de riz fortifié lors de notre recherche. Un impact plus important peut être attendu de la consommation quotidienne à plus long terme de riz fortifié. A l'avenir, des études d'efficacité conduites sur plus d'une année scolaire pourrait favoriser l'introduction d'aliments de base fortifiés dans les programmes existants d'alimentation scolaire.

En outre, une recherche plus approfondie prenant en compte la variabilité intra-populations, l'influence des facteurs environnementaux et les besoins spécifiques des enfants d'âge scolaire et des adolescents est nécessaire pour mieux identifier et caractériser la malnutrition dans cette tranche d'âge. Nous avons établi des points de coupure pour le périmètre brachial au Cambodge, un outil peu coûteux pour détecter la malnutrition aigüe chez les enfants scolaires. Nous avons proposé des facteurs de correction pour éliminer l'effet de confusion de la phase inflammatoire sur les concentrations plasmatiques de récepteurs de la transferrine, et vérifié les facteurs de correction des statuts en ferritine et en vitamine A.

Négliger le problème de santé publique que constitue la malnutrition chez les enfants d'âge scolaire et les adolescents risque de compromettre le progrès historiques accompli chez les jeunes enfants. Investir dans la nutrition de ces populations est une opportunité d'optimiser le développement des générations futures dans les pays en développement.

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## Abstract

Undernutrition causes long-term damages on the physical and cognitive development and half of deaths among children under 5 y. Most of the current interventions are concentrated on improving nutrition among young children and mothers. However, malnutrition is also prevalent among older children and adolescents, especially in Africa and in South-East Asia. It has adverse effects on their global development because of the high requirements in energy, protein and micronutrients resulting from growth and brain development spurts occurring these periods of life. Our research evaluated the prevalence of malnutrition and their determinants factors among school-aged children and adolescents in Senegal and Cambodia, as well as the effectiveness of micronutrient fortified food in schools in Cambodia.

A cross-sectional study was conducted on a representative sample of ~ 600 children aged from 5 to 17 years from primary state schools of Dakar area, selected through a two-stage random cluster sample (30 schools × 20 children). Elevated rates of thinness (19%) and micronutrient deficiencies (iron 39%, iodine 33%, zinc 26%) were showed. The food consumption survey revealed insufficient micronutrients and energy intake, as well as contribution to total energy intake insufficient in proteins and excessive in lipids, which reveals poor conditions among school children in Senegal. In Cambodia, a cluster-randomized placebo-controlled double-blind trial was conducted among 2400 children aged 6-16 y to measure the impact of fortified rice consumed during 6 months within the school feeding program of WFP on their micronutrient status and cognitive performance. Before the intervention, poor cognitive performance was associated to stunting, iron deficiency and parasite infestation. The growth was positively associated to zinc status, which was negatively associated to parasite infestation. Rice fortified with vitamin A improved vitamin A status and rice with the highest iron concentration had a positive impact on cognitive performance. Data from Senegal and Cambodia were analyzed to study indicators of malnutrition among school-aged children and adolescents. According to the WHO recommendations, we suggested mid-upper-arm circumference (MUAC) cut-offs to screen for acute malnutrition among children above 5 years in Cambodia (15.5 cm, 16.4 cm and 18.2cm in boys and 15.4 cm, 16.6 cm and 17.9 cm in girls aged 5-7.9y, 8-11.9 y, and 11-13.9 y). We confirmed the need to correct plasma concentrations of retinol-binding-protein and ferritin according to inflammatory status, and showed the interest of a similar correction for transferrin receptor, these corrections improving the estimation of prevalence of vitamin A and iron deficiencies in populations where inflammation is endemic.

Undernutrition remains a public health issue among school-aged children and adolescents in Senegal and Cambodia. Determination of malnutrition in this age range could be improved by research on indicators taking into account intra variability populations, environmental factors and specific needs of these populations. School-based interventions like the one in Cambodia have positive effects on nutrition and development of children, advocating for extension of school feeding, including in disadvantaged urban areas. Distribution fortified food in existing school canteens is a cost-effective strategy to improve nutrition and health. More research and efforts should be shifted to the nutritional status of adolescent girls because of the risk of early childbearing, still commonly practiced in developing countries, and the importance of adequate nutrition during pregnancy. Investing in nutrition in this age range is an opportunity to consolidate the progress achieved in pregnant women and young children through a life-cycle approach and to optimize the development of next generations in developing countries.

**Key-words:** malnutrition, children, school-aged children, adolescents, micronutrients, food fortification, cognition, Africa, Senegal, South-East Asia, Cambodia

## Résumé

### La dénutrition

La dénutrition est responsable de dommages à long terme sur le développement physique et cognitif et de la moitié des décès chez les enfants de moins de 5 ans. La majorité des interventions est concentrée sur la nutrition des jeunes enfants et des mères. Cependant, la malnutrition, également prévalente chez les enfants plus âgés et les adolescents, notamment en Afrique et en Asie du Sud-Est, a des conséquences sur leur développement global, en raison des besoins élevés en énergie, protéines et micronutriments induits par les pics de croissance et de développement cérébral durant ces périodes de la vie. Nos travaux ont évalué la prévalence des malnutritions et leurs facteurs déterminants chez des enfants d'âge scolaire et des adolescents au Sénégal et au Cambodge, ainsi que l'efficacité d'une intervention avec une alimentation enrichie en micronutriments en milieu scolaire au Cambodge.

Une étude transversale fut conduite sur un échantillon représentatif de 600 enfants des écoles primaires publiques de la région de Dakar sélectionnés par un échantillonnage aléatoire en grappes. Des taux élevés de maigreur (19%) et de carences en micronutriments (fer 39%, iode 33%, zinc 26%) ont été démontrés. L'enquête de consommation alimentaire a révélé des apports en micronutriments et en énergie insuffisants, ainsi qu'une contribution à l'apport énergétique déficitaire pour les protéines et en excédent pour les lipides, révélant des conditions précaires de ces scolaires en milieu urbain au Sénégal. Au Cambodge, un essai contrôlé randomisé en grappes en double aveugle conduit sur 2400 enfants a permis de mesurer l'impact de riz enrichis en micronutriments consommés pendant 6 mois, dans le cadre du programme d'alimentation scolaire du PAM, sur leurs statuts en micronutriments et leurs performances cognitives. Avant l'intervention, les faibles performances cognitives étaient associées au retard de croissance, à la carence en fer et aux infections parasitaires. La croissance était positivement associée au statut en zinc, lui-même négativement associé à l'infection parasitaire. Les riz enrichis en vitamine A ont amélioré le statut en vitamine A et le riz avec la concentration en fer la plus élevée a eu un impact positif sur les performances cognitives. Les données du Sénégal et du Cambodge ont été analysées pour étudier les indicateurs de malnutrition chez ces enfants d'âge scolaire et adolescents. Comme recommandé par l'OMS, nous avons déterminé les valeurs seuils du périmètre brachial pour détecter la malnutrition aiguë chez les enfants de plus de 5 ans au Cambodge. Nous avons confirmé la nécessité de corriger les concentrations plasmatiques de retinol-binding-protein et de ferritine en fonction du statut inflammatoire et démontré l'intérêt d'une telle correction pour les récepteurs de la transferrine pour améliorer l'estimation de la prévalence des carences en vitamine A et en fer dans ces populations où l'inflammation est endémique.

La dénutrition reste un problème de santé publique chez les enfants d'âge scolaire et les adolescents au Sénégal et au Cambodge. La détermination des malnutritions dans cette tranche d'âge pourrait être améliorée par des recherches sur les indicateurs prenant en compte la variabilité intra populations, les facteurs environnementaux et les besoins spécifiques de ces populations. Des interventions scolaires comme au Cambodge ont des effets positifs sur la nutrition et le développement, plaidant en faveur d'une extension des programmes d'alimentation scolaire y compris dans les zones urbaines défavorisées. La distribution d'aliments fortifiés dans les cantines scolaires existantes est une stratégie peu coûteuse d'amélioration de la nutrition et de la santé. La nutrition des adolescentes requiert une attention particulière en raison du risque de grossesse précoce particulièrement élevé dans de nombreux pays du Sud. Investir dans la nutrition des enfants d'âge scolaire et des adolescents est une opportunité de consolider le progrès accompli chez les jeunes enfants et d'optimiser le développement des générations futures dans les pays du Sud.

**Mots-clés :** malnutrition, enfants, scolaires, adolescents, micronutriments, fortification des aliments, santé, cognition, Afrique, Sénégal, Asie du sud-est, Cambodge