English summary

Background

Micronutrient deficiencies are estimated to affect about one third of children and women of reproductive age in developing countries. Micronutrient deficiencies, along with poor living conditions, can delay cognitive development, increase morbidity and mortality, keeping millions of children from reaching their full potential in growth and cognitive development. In Cambodia, micronutrient deficiencies among pre-school children are highly prevalent, with 68% and 20% of them being classified as deficient in zinc and folate, respectively. While the prevalence of iron deficiency (serum ferritin concentrations <15 μg/L) is low (<10%), 25% of pre-school children in Cambodia have marginal iron stores (serum ferritin concentrations <50 μg/L). Few studies, though, have investigated the nutritional status of school-age children as well as the reasons of iron deficiency.

Food fortification with multiple micronutrients has the potential to improve micronutrient status, growth and cognitive performance, and reduce morbidity incidence in developing countries. In Cambodia, rice is a staple food, and therefore fortified rice with multiple micronutrients could be a promising strategy to reduce the prevalence of micronutrient deficiencies. However, few studies have investigated the impact of fortified rice on micronutrient status, health and cognitive development in school-age children. Therefore, the aim of this PhD study is to evaluate the impact of fortified rice on nutritional status, health and cognitive performance of Cambodian schoolchildren. This PhD thesis is part of the FORISCA study.

Methods

This PhD thesis includes three papers. In paper I, the stability over time of three main micronutrients: iron, zinc and vitamin A in fortified rice was determined, following different production methods (hot extrusion, cold extrusion, and coating) and storage conditions that resemble real-life conditions in Cambodia (25±5 °C at a humidity of 60% and 40±5 °C at a humidity of 75%) for up to one year. The study was conducted in a laboratory where storage conditions (temperature and humidity) were simulated.

The two other papers are based on data from the FORISCA study, a cluster-randomized intervention study conducted in 2012-2013 in Kampong Speu province, Cambodia. Sixteen
schools in the World Food Program (WFP) school meal program were randomized into four
groups to receive either UltraRice original formula (URO), UltraRice new formula (URN),
NutriRice, or normal unfortified rice (placebo). UltraRice® technology is developed by
PATH (www.path.org) while NutriRice™ technology is developed by DSM/Buhler
(www.dsm.com). Four schools not part of the school meal program served as control group.
Subjects were all children from grades 1 to 6 (ages 6 to 16 years) enrolled in the participating
schools. A sub-sample of approximately 125 children per school was randomly selected for
nutritional assessment of anthropometry, micronutrient status, soil-transmitted helminth
(STH) infection and cognitive performance. Micronutrient status was assessed using standard
biochemical indicators, where body iron was calculated from serum ferritin corrected for
inflammation, and soluble transferring receptor.

Paper II is a cross-sectional analysis of the baseline data from the FORISCA study. The paper
investigates the association between STH, micronutrient status, anaemia and cognitive
performance of schoolchildren. STH infection was identified using the Kato–Katz method.
Cognitive performance was assessed using Raven’s Coloured Progressive Matrices (RCPM),
block design and picture completion. Association between STH infection and cognitive
performance, and various health indicators was analysed using linear and logistic regression
models.

Paper III investigates the impact of the fortified rice intervention on serum zinc concentrations
and serum folate status in the FORISCA study. Serum zinc and folate concentrations were
assessed from morning non-fasting blood samples. Serum zinc were measured at three time-
points: baseline, three and six months of intervention for all intervention arms. Folate
concentrations were measured on a sub-sample serum from baseline and at six months of
intervention in two intervention arms (NutriRice and placebo) only, due to budget constraints.
Generalized mixed models (linear or binary logistic regression) were used to assess the effects
on zinc and folate status, adjusted for age, gender and baseline characteristics.

Results

In Paper I, it is shown that the stability of iron and zinc as micronized iron pyrophosphate and
zinc oxide was high during storage. Retention of vitamin A was significantly affected by the
type of production technique used and storage. Losses for iron and zinc were less than 10%
for any type of fortified rice, while losses for vitamin A ranged from 20% to 93%. This paper
complements another paper from the same research group that reported a high stability of iron and zinc during cooking, but high losses of vitamin A (60%-80%).

In Paper II it is shown that STH infection was prevalent in 18% of the school children participating in the FORISCA study. Hookworm infection was by far the most prevalent STH infection (95% of infected children), while infections with other STH such as *Ascaris* and *Trichuris* was low (<1% of infected children). STH infection (mainly hookworm) was associated with iron status but not vitamin A and zinc status, nor with inflammation or anthropometric status. Body iron was negatively associated with increased intensity of hookworm infection (R = 0.22, P < 0.001). Moreover, STH infection was associated with cognitive performance. After adjusting for age and gender, raw cognitive test scores were significantly lower in hookworm infected children (−0.65; −0.78; −2.03 points for picture completion, RCPM, and block design, respectively; P < 0.05 for all). This effect was most likely mediated through lower body iron. Hence, interventions that are more effective against hookworm infection are needed to contribute to better health and improvement of cognitive performance.

In Paper III we report a very high prevalence of zinc deficiency, assessed as low serum zinc concentrations (90% deficient or 49% severely deficient), based on age and gender specific cut-off values for classifying deficiency (serum zinc < 9.9 – 10.7 μmol/L for deficiency and < 7.6 μmol/L for severe deficiency) in the FORISCA study. After six months of intervention, serum zinc concentrations were significantly increased in all fortified rice groups compared to placebo (+0.98, +0.85 and +1.40 μmol/L for URO, URN and NutriRice respectively). Children receiving fortified rice had a reduced risk of zinc deficiency ranging from 60-70% (OR: 0.35, 0.39, and 0.28 for URO, URN, and NutriRice, respectively), compared to the placebo (p<0.001 for all). The highest increase in serum zinc concentrations was observed in the children receiving the rice with the highest content of zinc (NutriRice). The prevalence of folate deficiency was also reduced in the fortified rice group. The children receiving NutriRice had a significant increase in their serum folate concentrations (2.25 ng/ml) compared to placebo (p=0.007). This paper complements another publication from the FORISCA study on the impacts on iron and vitamin A status.
Conclusion

Iron and zinc were efficiently retained in fortified rice, while vitamin A was lost over months in storage. Therefore, fortified rice could be a suitable vehicle for fortification with zinc and iron but not vitamin A.

Zinc deficiency was highly prevalent while hookworm infection affected 10%-20% of the schoolchildren in the FORISCA study. Hookworm infection was also associated with lower iron status, poor cognitive performance as well as risk for anaemia. Multi-micronutrient fortified rice could help to improve micronutrient status, health and cognitive performance of schoolchildren, but not likely to control anaemia and growth retardation. Introduction of multi-micronutrient fortified rice into school meal programs and for any other target populations is a promising intervention in combination with other health and nutrition-sensitive program such as Water Sanitation and Hygiene (WASH) and effective deworming program.