**Josef G. Knoll-Europäischer Wissenschaftspreisträger 2014**

**Josef G. Knoll-European-Science Award Winner 2014**

**Colin Cercamondi "Improving iron nutrition from sorghum and millet based diets in malaria endemic areas", ETH Zurich, 2013**


Summary

**Background:** Iron deficiency (10) without or with anaemia (IDA) is a major global health problem affecting primarily vulnerable population groups such as children <5 years of age and women of reproductive age. In the semi-arid tropics, the aetiology of IDA is multifactorial, but the major factors are low dietary iron intake and bioavailability from monotonous diets based on staple crops, such as sorghum and millets, exacerbated by chronic parasitemia such as malaria infections. Iron fortification of staple foods is considered a promising approach to prevent and correct 10 in certain population groups. 10 in children <5 years of age, not yet consuming significant quantities of staple foods, can be prevented by commercially iron-fortified complementary foods or by complementary food supplements also called *in-home* fortification. A more recent approach to combat 10 is iron biofortification which is the development of iron-enhanced staple crops by traditional plant breeding practices and/or genetic engineering.

Sorghum and millets are important staple crops in areas of the semi-arid tropics where malaria is often endemic. Sorghum and millet foods are low in bioavailable iron and are particularly difficult to (bio)fortify with iron due to high concentrations of phytic acid (PA). In addition to PA, some sorghum and millet varieties contain considerable amounts of polyphenols (PPs) which, like PA, are known to inhibit iron absorption. The application of iron absorption enhancers, such as ascorbic acid (AA), ethylenediaminetetraacetic acid (EDTA) or enzymes degrading PA or PPs in sorghum and millet foods, potentially improves iron bioavailability from sorghum and millet foods. Malaria infections, which are known to interact with human iron homeostasis, must be considered when combaUing 10 in malaria endemic areas.

**Aim:** The overall aim of this thesis was to develop approaches to improve iron nutrition from sorghum and millet based diets in malaria endemic areas. This included an evaluation of the effect of asymptomatic malaria on iron absorption, an investigation into the effect of sorghum PPs on iron absorption and the optimization of iron absorption from a newly developed complementary food fortificant (CFF) added to a thin millet gruel. Moreover, iron bioavailability and total iron absorbed from an iron-biofortified pearl millet paste was compared with that from regular-iron and post-harvest iron-fortified millet.

**Experiments:** The thesis includes four investigations on iron absorption in women or young children using the stable isotope technique. Three investigations were conducted in Benin with women or children and one in Switzerland with women.

*Manuscript 1*: Iron absorption and utilization from an iron-fortified sorghum porridge (3 mg iron) were estimated by using oral and intravenous isotope labels in 23 afebrile Beninese women with a positive malaria smear (asexual P. falciparum parasitemia; >500 parasites/µL blood). The women were studied while infected, treated, and then restudied 10 days after treatment. Iron status, hepcidin, and inflammation indexes were measured before and after treatment.

*Manuscript 2*: Three iron absorption studies were conducted to investigate the inhibiting effects of sorghum PPs on iron absorption and the potential enhancing effects of M, sodium iron EDT A (NaFeEDTA) and polyphenol oxidase treatment using laccase. Studies were conducted in 50 women residing in Switzerland. They consumed dephytinized test meals based on white and brown sorghum varieties with different PP concentrations.

*Manuscript 3*: Optimization of iron bioavailability from a CFF, primarily designed to prevent iron deficiency, was investigated in three absorption studies. Fifty-two young Beninese children consumed test meals consisting of 80 g millet porridge mixed with 30 g CFF and 40 mg AA. Study 1 compared iron absorption from meals fortified with 6 mg iron as ferrous sulphate ($FeSO_4$) with meals fortified with 3 mg iron as $FeSO_4$ and 3 mg iron as NaFeEDTA. Study 2 compared iron absorption from $FeSO_4$-fortifed meals (6 mg iron) without and with 40 mg extra AA (total AA =80 mg). Study 3 compared iron absorption from $FeSO_4$-fortified meals (6 mg iron) with meals containing phytase added prior to consumption once without and once with extra AA.

*Manuscript 4*: A stable isotope study in 20 Beninese women (plasma ferritin <25 µL) using a multiple meal design was carried out to compare iron bioavailability and total iron absorbed from regular-iron, iron-biofortified and post-harvest iron-fortified pearl millet. The three different composite test meals consisted of millet paste either based on regular-iron (1.5 mg iron/serving), iron-biofortified (5.5 mg iron/serving) or post-harvest iron-fortified (5.2 mg iron/serving) pearl millet accompanied by a leafy vegetable sauce or by an okra sauce. Each test meal was consumed 10 times over 5 days (2 portions/day). Test meal servings were labelled with an extrinsic iron isotope tag of 0.4 mg.

**Results:** *Manuscript 1*: Clearance of asymptomatic malaria parasitemia increased dietary iron absorption (from 10.2% to 17.6%; $P< 0.01$) but did not affect systemic iron utilization (85.0% compared with 83.1 %; NS). Malaria treatment reduced low-grade inflammation, as reflected by decreases in serum ferritin, C-reactive protein and interleukin-6, -8. -10 ($P < 0.05$); this was accompanied by a reduction in serum hepcidin of approximately 50%, from 2.7 to 1.4 nmol/L ($P < 0.01$). Treatment decreased serum erythropoietin and growth differentiation factor 15 ($P < 0.05$).

*Manuscript 2*: In study 1, iron absorption from sorghum meals with 17 mg PPs (8.5%) was higher than from sorghum meals with 73 mg PPs (3.2%) and 167 mg PPs (2.7%) ($P< 0.001$). Meals containing 73 and 167 mg PPs showed no difference in absorption ($P= 0.9$). In study 2, iron absorption from meals (167 mg PPs) fortified with NaFeEDTA (4.6%) was higher than from the same meals fortified with $FeSO_4$ (2.7%; $P< 0.001$) but lower compared with $FeSO_4$-fortified meals containing 17 mg PPs (10.7%; $P< 0.001$). In study 3, PP reduction by laccase

(from 167 to 42 mg PPs/meal) did not improve iron absorption compared with meals with 167 mg PPs (4.8% vs. 4.6%; P = 0.4). Adding AA increased iron absorption to 13.6% (P < 0.001).

*Manuscript 3*: In study 1, iron absorption was higher from $FeSO_4$ (8.4%) than from the mixture of NaFeEDTA and $FeSO_4$ (5.9%; P< 0.01). In study 2, adding extra AA to the CFF mixed with millet porridge increased absorption (11.6%) compared with standard AA concentration (7.3%; P< 0.001). In study 3, absorption from test meals containing phytase without or with extra AA (15.8 and 19.9%, respectively) was increased compared with test meals without phytase (8.0%; P< 0.001). The addition of extra AA to meals containing phytase increased absorption when compared with the test meals containing phytase without extra AA (P < 0.05).

*Manuscript 4*: Fractional iron absorption from the test meals based on regular-iron millet did not differ compared with iron-biofortified millet meals (7.5% vs. 7.5%; P = 1.0) resulting in a higher quantity of total iron absorbed from the iron-biofortified millet meals (527 I1g vs. 1125 I1g; P< 0.001). Fractional iron absorption from post-harvest iron-fortified millet (10.4%) meals was higher than from regular-iron and iron-biofortified millet meals (P < 0.01). Total iron absorbed from the test meals based on post-harvest iron-tortitied millet (1500 I1g) was higher than from the regular-iron and iron-biofortified millet meals (P< 0.001 and P< 0.01, respectively).

**Conclusions:** The findings in manuscript 1 show that asymptomatic malaria parasitemia decreases dietary iron absorption but does not influence systemic iron utilization. The negative effect is mediated through low-grade inflammation and it may contribute to ID and IDA or may blunt efficacy of fortification programmes in malaria-endemic areas.

Results of manuscript 2 demonstrate the strong inhibition of PPs from brown sorghum on iron absorption. The results suggest that dephytinization of sorghum-based foods alone would not improve iron absorption if foods contain considerable amounts of PPs from brown sorghum. Furthermore, they show that especially AA and to a lesser extent NaFeEDTA but not laccase are promising enhancers to improve iron bioavailability from foods containing inhibitory PPs from brown sorghum.

As shown in manuscript 3, iron absorption from a lipid-based complementary food fortificant mixed with cereal porridge can be improved by using phytase and AA but not by using a mixture of $FeSO_4$ and NaFeEDTA. Based on the results, the use of a $FeSO_4$/NaFeEDTA mixture as iron fortificant in a lipid-based complementary food cannot be recommended. The addition of phytase and high concentrations of AA is the most promising approach to provide adequate bioavailable iron to young children via a CFF which also delivers energy and protein.

In manuscript 4, the findings show that the total amount of iron absorbed from meals based on iron-biofortified pearl millet is about twice of that from regular-iron millet meals and approximately 2/3 from that of post-harvest iron-fortified millet meals containing the same amount of iron. This indicates that iron biofortification of millet is a promising approach to provide additional bioavailable iron in the diets of millet consuming communities with limited access to conventionally fortified foods and may help to combat ID.

The findings in this thesis demonstrate that improvement of iron nutrition in sorghum and millet consuming communities is challenging because of the negative influence of PA, PPs and malaria. Nevertheless, it can be improved by using iron absorption enhancers in future

fortification programs of sorghum flour (e.g. AA or NaFeEDTA) or in lipid-based iron-fortified supplements (e.g. phytase and AA), and also by iron-biofortified millets providing additional bioavailable iron into the diet.

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