

Original Article

The effect of daily consumption of the small fish *Amblypharyngodon mola* or added vitamin A on iron status: a randomised controlled trial among Bangladeshi children with marginal vitamin A status

Anna B Andersen MSc¹, Lise KH Schmidt MSc¹, Daniel Faurholt-Jepsen PhD¹, Nanna Roos PhD¹, Henrik Friis PhD¹, Katja Kongsbak PhD¹, Mohammed A Wahed MSc², Shakuntala Haraksingh Thilsted PhD³

¹Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Denmark

²International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Mohakhali, Dhaka, Bangladesh

³WorldFish, Banani, Dhaka, Bangladesh

Background and Objectives: Mola (*Amblypharyngodon mola*) is a nutrient-rich, small fish found in ponds and rice fields in Bangladesh. The aim of the present intervention was to assess the effect of mola consumption on iron status in children with marginal vitamin A status. **Methods and Study Design:** Bangladeshi children (n=196), aged 3-7 years, with marginal vitamin A status were randomly allocated to one of three intervention groups served different fish curries: mola curry (experimental group); rui (*Labeo rohita*) curry with added retinyl palmitate (positive control group); or rui curry (negative control group). The intervention meals were served 6 days/week for 9 weeks. The experimental and positive control meals were designed to contain similar amounts of retinol activity equivalents per portion. The mola curry contained four times more iron compared to the rui curries due to different iron content in the two fish species. Haemoglobin, ferritin, serum transferrin receptor and C-reactive protein were measured at screening and endpoint. **Results:** In the experimental group receiving mola, serum transferrin receptor concentration declined 0.73 mg/L (95% CI 0.17, 1.28, $p=0.01$) compared to the positive control group, while there were no differences between groups in ferritin or haemoglobin. **Conclusions:** Consumption of mola instead of rui has potentially an effect on iron status in children with marginal vitamin A status, seen as a decrease in serum transferrin receptor concentration.

Key Words: iron status, vitamin A, serum retinol, small nutrient-rich fish, Bangladesh

INTRODUCTION

Iron deficiency is widespread in low-income countries especially among children and women of reproductive age.¹ Iron deficiency can cause impaired cognitive and physical development in children, as well as increased risk of infections.² Several common foods contain iron, but the bioavailability from diets dominated by plant foods is often poor due to inhibitors (e.g. phytate and polyphenols) of non-heme iron. Animal-source foods are the best sources of the more absorbable heme iron; however, these foods are not readily accessible to the majority of the population in low-income countries.^{3,4}

Small indigenous freshwater fish species is a common and irreplaceable animal-source food for many rural families in Bangladesh. Mola (*Amblypharyngodon mola*) is a nutrient-rich, small freshwater fish found in ponds and rice fields in Bangladesh and other Asian countries, and consumed by all socio-economic groups.⁵ Mola is often eaten whole including the head, eyes, bones and viscera. Mola contains a large amount of vitamin A of which

about 80% is found as 3, 4-dehydroretinol (vitamin A-2).⁶ Using a conversion factor of 40% from vitamin A-2 to retinol (vitamin A-1),⁷ this amounts to more than 2000 µg retinol activity equivalents (RAE) per 100 g of edible parts. Mola also contains a moderate amount of bioavailable iron, and is a good source of calcium.⁵ Larger fish species such as the indigenous carp, rui (*Labeo rohita*) contain no or very little vitamin A in the edible parts, and less iron than mola.

Previous studies have suggested that vitamin A deficiency impairs iron metabolism, and positive effects of vitamin A-1 supplementation on iron status.⁸⁻¹⁰ Studies in

Corresponding Author: Dr Shakuntala Haraksingh Thilsted, WorldFish, Bangladesh & South Asia Office, House 22 B, Road 7, Block F, Banani, 1213 Dhaka, Bangladesh.

Tel: (+88) 01925 155040; Fax: (+88) 02 8811151

Email: s.thilsted@cgiar.org

Manuscript received 13 July 2014. Initial review completed 30 September 2014. Revision accepted 26 May 2015.

doi: 10.6133/apjcn.092015.11

rats confirmed that vitamin A deficiency specifically impaired erythropoiesis.¹¹ It is unknown whether intake of vitamin A-2 could have the same effect as vitamin A-1. We assessed the effect of mola consumption or added vitamin A on iron status among Bangladeshi children with marginal vitamin A status.

MATERIALS AND METHODS

Study population and screening

The study was conducted from March to July 2002 in an urban slum in Mirpur, a suburb of Dhaka, Bangladesh. Healthy children, aged 3-7 years were screened for serum retinol (s-retinol, vitamin A-1) concentrations. Children who met the inclusion criteria and had s-retinol concentration 0.36-0.70 $\mu\text{mol/L}$, were invited to participate in a randomized, controlled intervention trial for 9 weeks. Prior to screening, all children were given anthelmintic treatment (albendazole 400 mg) twice, with two weeks' interval.¹² Children with s-retinol <0.35 $\mu\text{mol/L}$, severely malnourished (< weight-for-height, 70%, and weight-for-age, 60%, of the US National Center for Health Statistics/WHO international reference median), or with a clinical illness were excluded from the study and referred for treatment. Children who had received vitamin A supplementation within the previous 6 months were also excluded from the study.

The intervention

The selected children were randomly allocated to three treatment groups, using a random number table. All children in each group received a daily meal, 6 days a week for 9 weeks. Rice and a vegetable curry, without vitamin A, formed the basis of all meals and were combined with one of the three fish curry intervention meals.

The experimental group received a mola curry, prepared with whole mola, and thus containing a high amount of vitamin A; the positive control group received a fish curry, made from the fillets of rui, containing no vitamin A, but with added retinyl palmitate (vitamin A-1); the negative control group received rui curry, without retinyl palmitate. All fish curry dishes were homogenized before serving, thus having similar consistencies. The mola and rui curry dishes had a slight colour difference.

The servings of fish curry in the experimental and positive control group were designed to contain 600 μg RAE

per meal, using a conversion factor of 40% for vitamin A-2 to vitamin A-1. In mola, about 80% of the vitamin A is present as vitamin A-2. Retinyl palmitate was added to the rui curry in the positive control meal.

Iron and vitamin A composition of the servings of the dishes are presented in Table 1. The values are extracted from Kongsbak et al (2008),¹² where the full composition of the dishes was reported. Rice and vegetables were served *ad libitum*, together with the fish curry, and contributed to the overall dietary intake. There were no differences in the amounts of rice and vegetables consumed between groups. Details on compositions of the meals and the intakes have previously been reported.¹²

The present study on iron status was a secondary outcome from an intervention study. Sample size was calculated for the primary outcome of the study, as reported elsewhere,¹² thus determined to be able to have a 90% chance of detecting a difference in the mean serum retinol concentration of 0.15 $\mu\text{mol/L}$ between the experimental and negative control group, at the 5% level of significance.

Outcome variables

At screening and endpoint, non-fasting venous blood samples were taken and analysed for haemoglobin, markers of iron status (serum ferritin and soluble transferrin receptor (sTfR)), and vitamin A status (s-retinol, vitamin A-1), as well as the acute phase reactant C-reactive protein (CRP). Data on household socioeconomic status and children's health were obtained by interviews with parents/caregivers. Weight and height of children were measured at screening and endpoint, but not at baseline in order to minimize the discomfort of the children, and thereby assure the acceptability of the study.

Statistical analysis

All statistical analyses were done using STATA/IC 11.2 (StataCorp LP, College Station, USA). Continuous variables were checked for normal distribution with normal probability plots and \log_{10} transformed, if not normally distributed. Continuous variables are presented as mean (SD), geometric mean (geometric SD), and median (interquartile range), as appropriate. Furthermore, percentage (number of observations) was used for categorical variables.

Table 1. Analysed vitamin A, iron, energy and protein composition of the dishes served[†]

	Mola curry (50 g serving)	Rui curry with added retinol (50 g serving)	Rui curry (50 g serving)
Energy (kJ)	487	467	461
Protein (g)	5.0	5.9	5.9
Iron			
Heme iron (mg)	0.83	0.11	0.11
Non-heme iron (mg)	0.58	0.21	0.21
Contribution to iron RDI (%) [‡]	14	3	3
Vitamin A			
3,4-dehydroretinol isomers (μg)	1057	-	-
Retinol isomers (μg)	196	601	-
β -carotene (g)	2.3	1	0.5

[†]Values extracted from Kongsbak et al (2008)¹², where the full nutrient composition of the dishes is reported.

[‡]Calculated based on the recommended daily intake (RDI) of iron of 10 mg/day for children 4-8 years.

- Not detected.

Paired *t* test was used to test for differences between screening and endpoint for haemoglobin (mean) and log₁₀ ferritin and sTfR (geometric means). Linear regression adjusted for age and sex was used to compare the outcome variables by treatment groups; the positive control group was set as reference. This facilitated, firstly, comparison between the experimental and positive control group, receiving equal amounts of vitamin A but different amounts of iron, and, secondly, comparison between the negative and positive control group receiving equal amounts of iron, but different amounts of vitamin A.

Ferritin and sTfR levels are affected by infection, and therefore the concentrations were adjusted by dividing the assessed values by a correction factor, based on corresponding levels of serum CRP. The correction factors were derived from linear regression analyses with log₁₀ ferritin and log₁₀ sTfR among all children screened as the dependent variables, and serum CRP as the independent variable. Serum CRP was coded as dummy variables based on the categories ≤2, >2-5, >5-10 and >10 mg/L. These regression coefficients were used as correction factors.

Ethics

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and the protocol was approved by the Research Review Committee and Ethical Review Committee, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b). Before carrying out the intervention, it was presented to and accepted by the community leaders. Written or oral informed consent was obtained from each child's parent or legal guardian. All children and their family members had access free of charge to the health services, including medicines, given

by the physicians at the icddr,b health clinic. At completion of the intervention, all children received an oral dose of vitamin A (retinyl palmitate, 60 mg (200,000 international units)).

RESULTS

Of the 579 children screened, 196 (33.9%) met the inclusion criteria. Of these, 66 children were randomly allocated to the experimental group, 65 to the positive control group, and 65 to the negative control group. During the intervention period, 12 (5, 1 and 6 in the experimental, positive control and negative control groups, respectively) children dropped out of the study.

The baseline characteristics after randomization are shown in Table 2. Only minor differences were seen for the negative control group compared to the other treatment groups. In all 579 screened children, the ferritin geometric mean was 16.1 (2.5) µg/L, with 55% of the children having values <20 µg/L. Median serum CRP was 2.1 mg/L; 31%, 13% and 7% having values >2-5, >5-10 and >10 mg CRP/L, respectively. Based on linear regression analyses, serum CRP values in the intervals >2-5, >5-10 and >10 mg/L were associated with 1.18, 1.28 and 1.62 times higher ferritin; compared to serum CRP ≤2 mg/L. The ferritin_{corrected} geometric mean was 14.4 (2.5) µg/L, and the proportion of children with values <20 µg/L was 63%. For sTfR, serum CRP >10 mg/L was associated with 1.23 times higher values, whereas serum CRP between 2-5 and 5-10 mg/L were not different from those of the reference category of CRP ≤2 mg/L. By using the sTfR_{corrected} the geometric mean and proportion of children with high values declined marginally from 4.93 (1.54) to 4.86 (1.54) mg/L, and from 7.1% to 6.7%, respectively.

Table 2. Background characteristics of the 196 children by treatment group

	Treatment group		
	Experimental group (n=66)	Positive control group (n=65)	Negative control group (n=65)
	Mola curry	Rui curry + vit A-1	Rui curry
	Mean (SD) or % (n) [†]	Mean (SD) or % (n) [†]	Mean (SD) or % (n) [†]
Girls (%)	43.9 (29)	46.2 (30)	38.5 (25)
Age (baseline, months) [‡]	63.6 (15.8)	64.8 (15.8)	63.8 (17.2)
Height (cm) [‡]	101 (10.2)	103 (11.1)	102 (12.1)
Weight (kg) [‡]	14.6 (2.6)	15.1 (3.5)	14.8 (3.2)
Biochemical markers [§]			
Ferritin (µg/L) [¶]	13.6 (2.6)	13.2 (2.7)	13.0 (2.3)
<12 µg/L (%)	40.9 (27)	49.2 (32)	47.7 (31)
Serum transferrin receptor (mg/L) [¶]	5.4 (1.6)	5.4 (1.6)	5.5 (1.7)
>8.5 mg/L (%)	19.7 (13)	16.9 (11)	16.9 (11)
Haemoglobin (g/L)	106 (11.5)	105 (10.9)	105 (12.9)
<110 g/L (%)	60.6 (40)	64.6 (42)	60.0 (39)
Serum retinol (µmol/L) ^{††}	0.62 (0.51; 0.67)	0.63 (0.50; 0.67)	0.63 (0.51; 0.69)
<0.75 µmol/L (%)	100 (66)	100 (65)	100 (65)
C-reactive protein (mg/L) [¶]	3.5 (2.7)	3.7 (3.7)	3.4 (2.4)
≤2 mg/L (%)	36.4 (24)	40.6 (26)	23.1 (15)
>2 - ≤5 mg/L (%)	33.3 (22)	26.6 (17)	50.8 (33)
>5 - ≤10 mg/L (%)	15.2 (10)	14.1 (9)	18.5 (12)
>10 mg/L (%)	15.2 (10)	18.8 (12)	7.7 (5)

[†]Data are mean (SD) or % (n), or otherwise stated.

[‡]Measured at baseline.

[§]Measured at screening.

[¶]Geometric mean (geometric SD).

^{††}Median (interquartile range).

Table 3 shows the absolute changes in iron status from screening to endpoint for all children and by treatment group. Ferritin_{corrected} concentration in all children was 8% (10^B 1.08, 95% CI 0.99, 1.18, $p=0.07$) higher at endpoint, compared to screening; and haemoglobin concentration was 1.7 (95% CI 0.7, 2.8, $p=0.001$) g/L higher, with no indication of difference between groups.

The change in haemoglobin, ferritin_{corrected} and sTfR_{corrected} from screening to endpoint and across intervention groups were assessed in linear regression (Table 4). There was no difference between intervention groups for ferritin_{corrected} and haemoglobin, while the change in sTfR_{corrected} was 0.73 mg/L (95% CI 0.17, 1.28, $p=0.01$) lower in the experimental group compared to the positive control group.

DISCUSSION

The 9 weeks of intervention with fish meals with different contents and sources of vitamin A compounds and iron resulted in an overall increase in haemoglobin and a marginally significant increase in ferritin across all treatment groups. The sTfR improved in the children served mola curry, compared to those served rui curry with added retinyl palmitate, but in general the change across groups was small.

In the present intervention, the iron biomarkers ferritin, sTfR, and haemoglobin were used. The concentration of ferritin reflects the size of the iron stores, with increased concentration of ferritin indicating improved iron storage. sTfR reflects the amount of TfR expressed in the cell membranes, which is responsible for iron uptake into the cells, thus a decrease in sTfR indicates an improved iron status, with increased delivery of iron to the cells.¹³ Haemoglobin is the main pool of iron in the body, and synthesis of haemoglobin is affected by iron intake and absorption, and depleted iron stores can cause iron deficiency anaemia.²

A disadvantage of using ferritin as an indicator of iron status is that external factors, especially inflammation, affect the level of ferritin,^{14,15} and may give an incorrect indication of the iron stores.^{10,14} Also sTfR is affected by inflammation.^{14,16} Hence indicator specific correction factors, based on the association with CRP were used. In this analysis, it was only possible to adjust for elevated serum CRP, and not slower-reacting acute phase reactants, such as α_1 -acid glycoprotein, as has been recommended.¹⁷ As the correlation between CRP and ferritin is non-linear, the correction was made using four CRP cut-off points. Generally, this interaction between biochemical indicators for micronutrient status and acute phase reactions is a challenge in micronutrient interventions, but by making the corrections, the risk of misinterpretations is reduced.

Overall, the children benefited from all the interventions with improved iron status. Compared to the positive control, the experimental group had superior sTfR status at endpoint, but, surprisingly, there was no difference between the experimental and negative groups. The mean daily intake of iron in the mola curry in the experimental group was 1.4 mg compared to 0.32 mg served in the rui curry in the positive and negative control group. Also, the heme iron intake was higher from the mola curry, compared to rui curry.¹² The differences in

iron intake and bioavailability of iron may explain the improvement of sTfR seen in the experimental group, compared to the positive control group, but then we would also expect a difference between experimental and negative control group.

Vitamin A intake could possibly affect the sTfR level,¹⁰ however, no difference was seen between the positive control group receiving rui, with added retinyl palmitate and the negative control group receiving rui, without added retinyl palmitate. It has previously been shown that a combination of vitamin A-1 and iron supplementation can improve sTfR level,¹⁸⁻²⁰ but we did not reproduce this result. The mechanism behind this may be that improving vitamin A-1 status can suppress the synthesis of hepcidin, an iron homeostasis regulating hormone, and thereby improve the absorption of iron from the intestine.¹⁸⁻²⁰ However, it is unknown whether vitamin A₂ has the same effect. The biological value of vitamin A₂ as a vitamin A source was estimated to be 40% of the value of vitamin A-1, based on an early study of the effect of vitamin A compounds on correction of growth faltering in rats.⁷ A more recent study indicates that vitamin A₂ does not convert to vitamin A-1.²¹ Human studies on the biological value of vitamin A-2 compared to vitamin A-1 are lacking. We have previously reported that improved vitamin A status was found in the positive control group but not in the experimental group, compared to the negative control group, assessed only from s-retinol.¹² In this intervention, the vitamin A-2 concentration in serum was not measured.

sTfR was the only iron status indicator that improved relatively more in the children receiving the mola curry, compared to those fed the rui curries. This could be due to the mechanism whereby absorbed iron in iron-depleted individuals is not initially prioritized for storage, but allocated for erythropoiesis in tissues.^{19,22} sTfR is an indirect measure of erythropoiesis, and the higher intake of iron from the mola curry in the experimental group may have resulted in higher supply of iron to the tissues, resulting in the lower sTfR level. The increased iron supply during an intervention of only 9 weeks may be insufficient to detect a difference between treatment groups in ferritin level, as the improvement in iron stores was small. However, participation in the intervention improved iron storage in all children, as indicated by an overall improvement of haemoglobin, and to some degree, ferritin across intervention groups.

A longer intervention period could possibly have resulted in detection of better repletion of iron stores in the experimental group.

The overall improved iron status across the randomised intervention groups could be the result of multiple factors. Prior to the blood sampling at screening, anthelmintic treatment was administered to the children, and during the intervention, the children had free access to medical care. These two factors could have improved the children's general nutritional status by decreasing the susceptibility to infection/infestation.²³ Furthermore, the nutritional status of all children could have been improved by receiving one nutritious meal per day 6 days per week. In addition, the enhancing effect of consumption of fish – the 'meat factor' – on iron uptake from the fish curries could

Table 3. Changes in ferritin, serum transferrin receptor, and haemoglobin from screening to endpoint by treatment group

	Screening			Endpoint			Difference [†]		<i>p</i> value	
	n	Geometric mean	95% CI	n	Geometric mean	95% CI	n	Ratio		95% CI
Ferritin (µg/L) [‡]										
All treatment groups	195	11.2	(9.9; 12.7)	183	12.3	(10.5; 14.4)	182	1.08	(0.99; 1.18)	0.07
Mola curry	66	11.6	(9.2; 14.5)	60	12.0	(9.0; 15.8)	60	1.06	(0.96; 1.24)	0.44
Rui curry + vit A-1	64	11.1	(8.8; 14.0)	64	12.1	(9.3; 15.7)	63	1.08	(0.94; 1.25)	0.27
Rui curry	65	11.0	(8.9; 14.0)	59	12.8	(9.6; 17.2)	59	1.10	(0.94; 1.29)	0.22
Serum transferrin receptor (mg/L) [‡]										
All treatment groups	195	5.29	(4.99; 5.65)	184	5.30	(4.94; 5.68)	183	0.99	(0.96; 1.02)	0.67
Mola curry	66	5.27	(4.70; 5.92)	61	5.12	(4.58; 5.71)	61	0.95	(0.90; 1.01)	0.08
Rui curry + vit A-1	64	5.19	(4.63; 5.82)	64	5.41	(4.80; 6.10)	63	1.04	(0.98; 1.10)	0.16
Rui curry	65	5.41	(4.79; 6.11)	59	5.37	(4.69; 6.15)	59	0.99	(0.95; 1.04)	0.67
	n	Mean	95% CI	n	Mean	95% CI	n	Diff	95 % CI	<i>p</i> value
Haemoglobin (g/L)										
All treatment groups	193	105	(104; 107)	183	107	(105; 109)	183	1.7	(0.7; 2.8)	0.001
Mola curry	65	106	(103; 108)	60	107	(104; 110)	60	1.2	(-0.7; 3.0)	0.21
Rui curry + vit A-1	65	105	(103; 108)	64	107	(105; 110)	64	2.3	(0.4; 4.1)	0.02
Rui curry	63	105	(101; 108)	59	107	(103; 110)	59	1.8	(0.04; 3.5)	0.045

Data are geometric mean (95% CI) (ferritin and serum transferrin receptor) or mean (95% CI) (haemoglobin).

[†]Within group changes from screening to endpoint tested by paired *t* test.

[‡]Ferritin and serum transferrin receptor corrected; a C-reactive protein correction factor.

Table 4. Change in ferritin, serum transferrin receptor and haemoglobin from screening to endpoint between treatment groups with positive control group as reference

	Unadjusted			Adjusted [†]		
	Mean	95% CI	<i>p</i> value	Mean	95% CI	<i>p</i> value
Ferritin (µg/L) [‡]						
Mola curry	0.95	(-2.75; 4.65)	0.61	0.92	(-2.75; 4.60)	0.62
Rui curry + vit A-1 (reference)	-	-	-	-	-	-
Rui curry	3.10	(-0.62; 6.82)	0.10	2.95	(-0.75; 6.65)	0.12
Serum transferrin receptor (mg/L) [‡]						
Mola curry	-0.75	(-1.30; -0.20)	0.008	-0.73	(-1.28; -0.17)	0.01
Rui curry + vit A-1 (reference)	-	-	-	-	-	-
Rui curry	-0.28	(-0.83; 0.28)	0.33	-0.25	(-0.81; 0.31)	0.37
Hemoglobin (g/L)						
Mola curry	-1.10	(-3.61; 1.42)	0.39	-1.12	(-3.65; 1.41)	0.38
Rui curry + vit A-1 (reference)	-	-	-	-	-	-
Rui curry	-0.51	(-3.04; 2.01)	0.69	-0.57	(-3.11; 1.97)	0.69

Data are linear regression with differences between experimental (mola curry), negative control group (rui curry) and positive control group (rui curry + vitamin A-1, reference).

[†]Adjusted for age and sex.

[‡]Ferritin and serum transferrin receptor corrected; a C-reactive protein correction factor.

also have led to the improved iron status across intervention groups.²⁴

The overall increase in haemoglobin and marginal increase in ferritin could also be a consequence of improved vitamin A status. In a cross-sectional study among 164 Bangladeshi children in grades 3-5, a close relation between vitamin A status, measured by s-retinol, and iron status, measured by ferritin and haemoglobin was found. The analysis showed that the higher the s-retinol concentration, the higher were the ferritin and haemoglobin concentrations.²⁵ This association between vitamin A and iron status was shown for vitamin A-1, but it is unknown whether vitamin A-2 has the same effect. In the present intervention, no information is available on whether the vitamin A status increased across groups; however, it is suggested that either improved vitamin A status and/or the iron intake in all three groups improved iron stores.

The contribution of this secondary analysis on impact on iron status derived from a study designed for assessing impact on vitamin A status is to demonstrate how consumption of an iron-rich fish species compared to species with lower iron content can have a positive effect on iron status in a relatively short time. The study was a well-conducted, randomized controlled trial, with a low drop-out rate. The intervention was designed to investigate the impact of vitamin A sources on vitamin A status.¹² However, as mola contains a higher amount of iron than rui, the present intervention is also valid to assess the effect of iron intake from fish on iron status. Selecting children with low iron status as well as low vitamin A status would have optimized the intervention. We conducted the same statistical analyses on the sub-group of children with low iron status at screening, and the results for iron status were similar to the analyses done for all children.

Decrease in the sTfR level indicates that consumption of the small, nutrient-rich indigenous fish, mola can improve iron status of children with marginal vitamin A status. This effect is likely to be due to the moderate higher amount and bioavailability of iron found in mola, compared to the large fish, rui. Synergistic effect with vitamin A is also a possible mechanism. Furthermore, a general positive effect of participation in a 9 week nutrition intervention study was seen, as indicated by an overall haemoglobin increase. The short duration of the intervention period may be a limitation for detecting an effect of the intervention on iron status, thus nutrition trials with longer supplementation and follow-up time are needed.

ACKNOWLEDGMENTS

We thank the children and their families for their participation and interest, as well as the entire field team for their dedication and hard work in successfully conducting the study.

AUTHOR DISCLOSURES

The authors have declared that no competing interests exist. This study was supported by the Danish International Development Assistance (Danida), Ministry of Foreign Affairs, Denmark and Thrasher Research Fund, U.S.A. The funding bodies had no role in the study design, data collection, data analysis, data interpretation or decision to publish the findings.

REFERENCES

1. World Health Organization, World Food Programme, United

- Nations Children's Fund. Preventing and controlling micronutrient deficiencies in populations affected by an emergency. WHO; 2007.
- World Health Organization. Micronutrient deficiencies - iron deficiency anaemia. WHO; 2014 [cited 2014/03]; Available from: <http://www.who.int/nutrition/topics/ida/en/index.html>.
 - Hallberg L, Hulthen L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *Am J Clin Nutr.* 2000;71:1147-60.
 - Hambraeus L. Animal- and plant-food-based diets and iron status: benefits and costs. *Proc Nutr Soc.* 1999;58:235-42.
 - Roos N, Islam MM, Thilsted SH. Small indigenous fish species in Bangladesh: contribution to vitamin A, calcium and iron intakes. *J Nutr.* 2003;133:4021-6.
 - Roos N, Leth T, Jakobsen J, Thilsted SH. High vitamin A content in some small indigenous fish species in Bangladesh: perspectives for food-based strategies to reduce vitamin A deficiency. *Int J Food Sci Nutr.* 2002;53:425-37.
 - Shantz EM, Brinkman JH. Biological activity of pure vitamin A2. *J Biol Chem.* 1950;183:467-71.
 - Hodges RE, Sauberlich HE, Canham JE, Wallace DL, Rucker RB, Mejia LA, Mohanram M. Hematopoietic studies in vitamin A deficiency. *Am J Clin Nutr.* 1978;31:876-85.
 - Mejia LA, Chew F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. *Am J Clin Nutr.* 1988;48:595-600.
 - Zimmermann MB, Biebinger R, Rohner F, Dib A, Zeder C, Hurrell RF, Chaouki N. Vitamin A supplementation in children with poor vitamin A and iron status increases erythropoietin and hemoglobin concentrations without changing total body iron. *Am J Clin Nutr.* 2006;84:580-6.
 - da Cunha MSB, Siqueira EMA, Trindade LS, Arruda SF. Vitamin A deficiency modulates iron metabolism via ineffective erythropoiesis. *J Nutr Biochem.* 2014;25:1035-44. doi: 10.1016/j.jnutbio.2014.05.005
 - Kongsbak K, Thilsted SH, Wahed MA. Effect of consumption of the nutrient-dense, freshwater small fish *Amblypharyngodon mola* on biochemical indicators of vitamin A status in Bangladeshi children: a randomised, controlled study of efficacy. *Br J Nutr.* 2008;99:581-97.
 - Cook JD, Skikne BS, Baynes RD. Serum transferrin receptor. *Annu Rev Med.* 1993;44:63-74.
 - Friis H, Range N, Braendgaard KC, Kaestel P, Changalucha J, Malenganisho W, Krarup H, Magnussen P, Bengaard Andersen A. Acute- phase response and iron status markers among pulmonary tuberculosis patients: a cross-sectional study in Mwanza, Tanzania. *Br J Nutr.* 2009;102:310-7. doi: 10.1017/S0007114508162122
 - Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal. Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. *J Nutr.* 2000;132:3061-6.
 - Kasvosve I, Gomo ZA, Nathoo KJ, Matibe P, Mudenge B, Loyevsky M, Nekhai S, Gordevk VR. Association of serum transferrin receptor concentration with markers of inflammation in Zimbabwean children. *Clin Chim Acta.* 2006;371:130-6.
 - Thurnham DI, McCabe LD, Halder S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. *Am J Clin Nutr.* 2010;92:546-55. doi: 10.3945/ajcn.2010.29284.
 - Mena NP, Esparza AL, Nunez MT. Regulation of transepithelial transport of iron by hepcidin. *Biol Res.* 2006;

- 39:191-3.
19. Viatte L, Lesbordes-Brion JC, Lou DQ, Bennoun M, Nicolas G, Kahn A, Canonne-Hergaux F, Vaulont S. Deregulation of proteins involved in iron metabolism in hepcidin-deficient mice. *Blood*. 2005;105:4861-4.
 20. Dunn LL, Rahmanto YS, Richardson DR. Iron uptake and metabolism in the new millennium. *Trends Cell Biol*. 2007; 17:93-100.
 21. Tanumihardjo SA, Furr HC, Erdman JW Jr, Olson JA. Use of the modified relative dose response (MRDR) assay in rats and its application to humans for the measurement of vitamin A status. *Eur J Clin Nutr*. 1990;44:219-24.
 22. Short MW, Domagalski JE. Iron deficiency anemia: evaluation and management. *Am Fam Physician*. 2013;87: 98-104.
 23. Friis H, Mwaniki D, Omondi B, Muniu E, Thiong'o F, Ouma J, Magnussen P, Geissler PW, Michaelsen KF. Effects on hemoglobin of multi-micronutrient supplementation and multi-helminth chemotherapy: a randomized, controlled trial in Kenyan school children. *Eur J Clin Nutr*. 2003;57:573-9.
 24. Bjorn-Rasmussen E, Hallberg L. Effect of animal proteins on the absorption of food iron in man. *Nutr Metab*. 1979;3: 192-202.
 25. Persson V, Ahmed F, Gebre-Medhin M, Greiner T. Relationships between vitamin A, iron status and helminthiasis in Bangladeshi school children. *Public Health Nutr*. 2000;3:83-9.

Original Article

The effect of daily consumption of the small fish *Amblypharyngodon mola* or added vitamin A on iron status: a randomised controlled trial among Bangladeshi children with marginal vitamin A status

Anna B Andersen MSc¹, Lise KH Schmidt MSc¹, Daniel Faurholt-Jepsen PhD¹, Nanna Roos PhD¹, Henrik Friis PhD¹, Katja Kongsbak PhD², Mohammed A Wahed MSc³, Shakuntala Haraksingh Thilsted PhD⁴

¹Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Denmark

²International Centre for Diarrhoeal Disease Research, Bangladesh (icddr), Mohakhali, Dhaka, Bangladesh

³WorldFish, Banani, Dhaka, Bangladesh

日常小鱼（翻车鱼）摄入或添加维生素 A 对儿童铁状况的影响：一项在孟加拉国临界维生素 A 状态儿童中的随机对照试验

背景和目的：翻车鱼是一种营养丰富在孟加拉国池塘和稻田中发现的小鱼。该干预研究的目的是评估翻车鱼摄入对临界维生素 A 状态儿童铁含量的影响。**方法和研究设计：**196 名年龄在 3-7 岁之间、临界维生素 A 状态的孟加拉国儿童被随机分配到 3 个干预组中的一组，给予不同的鱼咖喱：鱼咖喱（实验组）、瑞（鲛）添加维生素 A 棕榈酸酯咖喱（阳性对照组）；或瑞咖喱（阴性对照组）。干预膳食为每周 6 天共 9 周。实验组和阳性对照组给予相同数量的视黄醇活性当量。翻车鱼铁含量为瑞咖喱铁含量的 4 倍。分别在基线和研究终点测定血红蛋白、铁蛋白、血清转铁蛋白受体和 C-反应蛋白含量。**结果：**与阳性对照组相比，鱼咖喱实验组血清转铁蛋白受体的浓度低至 0.73 mg/L (95% CI 0.17, 1.28, $p=0.01$)，而两组间铁蛋白和血红蛋白含量无统计学意义。**结论：**翻车鱼，而不是瑞咖喱的摄入对临界维生素 A 状态的儿童铁含量有影响，可作为血清转铁蛋白受体水平下降的标志。

关键词：铁、维生素 A、视黄醇、营养丰富的小鱼、孟加拉国