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**The effect of a school-based iron intervention on the haemoglobin concentration of school children in north-west Pakistan**

Emily K Rousham[1], Badar Uzaman[2], Daniel Abbott[3], Seunghee F. Lee[3], Shahzad Mithani[2] Natalie Roschnik[3], Andrew Hall[4]

[1] Centre for Global Health and Human Development, Loughborough University, Loughborough, UK.

[2] Save the Children, Pakistan Country Office, House 1, Street 15 A, Sector F-7/2, Khyaban-i-Iqbal, Islamabad, Pakistan.

[3] Save the Children US, 2000 L Street, NW; Suite 500; Washington, DC 20036, USA.

[4] Centre for Public Health Nutrition, School of Life Sciences, University of Westminster, London, UK.

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**Address for correspondence**:

Emily K Rousham, Centre for Global Health and Human Development, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire, LE11 3TU.

Email: e.k.rousham@lboro.ac.uk

Tel: 01509 228812

Fax: 01509 223940

Daniel Abbott: dabbott@savechildren.org

Andrew Hall: ah2108@gmail.com

Seunghee F. Lee: slee@savechildren.org

Shahzad Mithani: Shahzad.Mithani@savechildren.org

Natalie Roschnik: nroschnik@savechildren.org

Badar Uzaman: Badar.Uzaman@savechildren.org

**Abstract**

Objective: To assess the effectiveness of iron supplements administered to school children through a longitudinal school health intervention in terms of child haemoglobin concentration and anaemia prevalence.

Subjects and Methods: Children and adolescents aged 5-17 years were selected from 30 schools in north-west Pakistan for a longitudinal iron supplement intervention. Children received once-weekly iron supplements (200mg ferrous sulphate containing 63mg of elemental iron) for 24 weeks (n=352); or the same supplements twice-weekly for 12 weeks (n=298) or received no tablets (n=298). Haemoglobin concentration was estimated in finger-prick blood samples at baseline, 12 and 24 weeks. Follow-up samples were taken at 36 weeks.

Results: A non-significant increase in haemoglobin concentration was observed in children receiving iron supplements after 12 weeks (mean 1.4 g/l SD 15.0 g/l in once-weekly vs 2.5 g/l SD 14.5 g/l in twice-weekly) compared with the group receiving no iron supplements. There was no significant reduction in the prevalence of anaemia in the once-weekly or twice-weekly group compared with the unsupplemented group. The prevalence of anaemia increased in all three groups during the follow-up period (24 to 36 weeks).

Conclusion: Once-weekly and twice-weekly iron supplements were not associated with significant increases in haemoglobin concentration compared with unsupplemented children. In all groups, baseline haemoglobin concentration was the strongest predictor of haemoglobin increase. The lack of improvement may stem from the moderate baseline prevalence of anaemia (33%); other micronutrient deficiencies; variable compliance, or the worsening of haemoglobin status due to seasonal changes in dietary iron and other nutrients.

## Introduction

Iron supplements are recommended both to treat and prevent iron deficiency anaemia in populations at risk, particularly pregnant women and young children in communities where diets are inadequate [(1-2)]. Most iron supplement trials have targeted pre-school children since they undergo rapid growth and often consume a diet containing little iron[(2)]. Fewer studies have examined the effect of iron supplements on the haemoglobin concentration of older children and adolescents. This represents an important gap in research since the prevalence of anaemia in school children can be high [(3)] and has been associated with impaired educational attainment and cognitive function [(4)].

Previous iron supplement interventions have varied in duration and frequency of treatment and have had varying results [(1, 5-6)]. Some studies comparing once-weekly and twice-weekly or daily supplementation have reported no differences in the change in haemoglobin concentrations with different treatment regimens [(7-9)]. Other studies have reported greater increases in haemoglobin when iron supplements are given more frequently [(10)]. Some trials have reported no significant improvements in haemoglobin concentration in children who have received iron supplements even for 12 months [(11-12)]; hence, there are still uncertainties about the efficacy of iron supplementation programmes delivered to whole populations and the relative benefits of once weekly versus twice weekly administration.

Current WHO guidelines recommend the intermittent administration of iron supplements as a public health intervention for school age children in settings where the prevalence of anaemia is 20% or higher [(13)]. Previous iron supplementation programmes delivered through schools have led to significant improvements in the haemoglobin concentration of children [(14-15)]. These programmes have the additional advantage of using an existing infrastructure to deliver treatments as school staff can be trained to give iron tablets.

The aim of the present study was to examine the effect of iron supplements given to school children in a remote area of north-west Pakistan. The study objectives were to evaluate the change in haemoglobin concentration and anaemia prevalence of once-weekly iron supplements for 24 weeks against an unsupplemented group, and twice-weekly supplements for 12 weeks against an unsupplemented group. A further aim was to examine the change in haemoglobin concentration after supplementation had finished for a follow-up period of 12 weeks (in the once-weekly group) and 24 weeks (in the twice-weekly group) since this has not been examined in previous studies.

The hypothesis was that both treatment regimens would lead to an increase in haemoglobin concentration and reduce the prevalence of anaemia compared with a control group.

It was further hypothesised that 1) after 12 weeks, twice-weekly iron supplements would lead to a significantly greater increase in haemoglobin concentration and reduction in anaemia prevalence than once-weekly supplements or no supplements and 2) once-weekly supplements for six months would lead to a sustained increase in haemoglobin concentration and reduction in anaemia during the follow-up period compared with the same iron dose given in three months (twice weekly), or no iron. It was also important to evaluate which regimen was more practical to implement in a school health programme.

## Materials and Methods

The study started in May 2009 in Allai *tehsil* in Khyber Pakhtunkhwa (KPK) province of Pakistan. A severe earthquake in October 2005 caused substantial damage to the schools and housing in this area.

The sample was drawn from 87 schools, 50 of which were participating in the School Health and Nutrition Program of Save the Children. Twenty of the 50 schools were selected for iron supplementation by simple random sampling. Children from 10 schools were selected to receive a single weekly dose for 24 weeks of 200mg ferrous sulphate providing 63mg of elemental iron, and pupils in 10 schools were given the same iron supplement twice per week for 12 weeks. Of the 37 schools that were not taking part in the School Health and Nutrition program, 10 were randomly selected as the control group in which no treatment was given. Because of the training and infrastructure required for teachers to administer iron tablets it was not possible to randomise treatment across schools outside the School Health and Nutrition program.

A total of 1110 pupils aged 5-17 years were selected for the study with a target sample size of 370 in each group. The estimated prevalence of anaemia was 50% based on a previous survey of school aged children in the Allai *tehsil* [16]. The sample size was calculated on the ability to detect a decrease in anaemia prevalence of 15% (from 50% to 35%) at the 95% confidence level with a power of 80% and applying a design effect of two. The number of children selected from each school was proportionate to the total number of pupils in the school population i.e. population proportion to size (PPS). Similarly, within the school, the more pupils there were in a grade, the more children were selected from that grade.. In each grade, each student was given two pieces of paper containing the same number. Students folded and placed one of these pieces of paper into a basket and kept the other. One student was selected to draw the predetermined number of pieces of paper from the basket. The numbers on these pieces of paper drawn from the basket were read out loud and students with the corresponding numbers on the pieces of paper in their hands were chosen as participants.

Ethical approval for the intervention and follow-up was given by the local Health Department. After explaining the aims and nature of the study, parents of participating students signed a consent form prior to collecting the finger prick blood samples. Inclusion criteria for participating in the study were parental consent and attendance at school. Children were made aware that participation was voluntary and were free to withdraw at any stage. Participating students were given a hygiene kit of soap and nailcutters after giving a finger-prick blood sample.

**Iron supplementation**

Teachers were trained by Save the Children's School Health and Nutrition staff to administer supplements of 200mg ferrous sulphate providing 63mg of elemental iron (Nawabsons Laboratories PVT Ltd, Lahore, Pakistan). The once-weekly supplement group received this tablet on the same day each week, for 24 weeks. The twice-weekly group received a tablet on the same two days each week, for 12 weeks, so both groups were offered the same total number of tablets providing 1.51 g of iron. The comparison group did not receive any supplements during the intervention. Malaria was not endemic in the area.

**Haemoglobin measurements**

Field staff were trained to take capillary blood samples from a finger prick using a sterile lancet. Field staff were not informed of the treatment regimen of each school. The haemoglobin concentration was estimated using a portable haemoglobinometer (Hemocue

Hb201+, Angelholm, Sweden) before receiving iron tablets (baseline) and then 12, 24 and 36 weeks later.

As the study site was 1,500 m above sea level the haemoglobin concentration of each child was adjusted to give equivalence with the concentration at sea level by subtracting 5 g/l from each value [17-18]. Thresholds for classification of anaemia were: 115 g/l for ages 5-11.9 y; <120 g/l for ages 12.0-14.9 y; <120 g/l in females ≥15.0 y; and <130 g/l for males ≥15.0 y [18]. There were no laboratory facilities or clinically trained staff on site to diagnose haemoglobinopathies. Children with haemoglobin concentrations indicating severe anaemia were referred to the local health facility.

**Statistical analysis**

Statistical analysis was conducted using STATA (Version 11) with the school specified as the cluster to control for intra-cluster correlation. Repeated measures regression analyses using random effects models were used to examine the haemoglobin concentration at baseline, 12 and 24 weeks in the once-weekly iron supplement group versus non-supplemented groups. The same regression was carried out on haemoglobin concentration at baseline and 12 weeks in the twice weekly iron supplement group versus non-supplemented group. Analyses controlled for school (clusters), age, sex and initial haemoglobin concentration. Binary logistic regression (repeated measures) was employed to estimate the risk of anaemia among once and twice-weekly iron supplemented and non-supplemented groups after controlling for school, age, sex and initial haemoglobin concentration at baseline, 12 and 24 weeks. The differences in anaemia prevalence were examined between groups using differences in proportions. The change in anaemia prevalence within each group was tested using chi-square.

**Results**

Fingerprick blood samples were taken from 1,109 children at baseline and from 983 children after 36 weeks, a loss of 126 subjects (12.8%). There was no statistically significant difference in the mean initial haemoglobin concentration of the children who dropped out and those who completed the study (123.8 g/l vs 125.1 g/l respectively, $P>0.05$). There was no difference in the haemoglobin concentration of boys and girls at baseline (120.0 g/l vs 120.4

g/l respectively, $P>0.05$) nor in the prevalence of anaemia (33.8% vs 31.7% respectively, $P>0.05$). Mean age was 113.5 months (SD 25.0 months) and the mean age of boys and girls within each group is shown in Table 1. There was a smaller proportion of girls than boys within schools which led to a smaller proportion of girls in each group (Table 1). At baseline, the mean haemoglobin concentrations were not significantly different between groups after controlling for clustering by school.

**Effect of iron supplements on haemoglobin concentration**

Table 2 and Figure 1 show the mean haemoglobin concentrations at each assessment. In the once-weekly iron supplement group, the mean haemoglobin concentration showed a non-significant increase between baseline and 12 weeks. From 12 to 24 weeks the mean haemoglobin concentration fell to 121.5 g/l, and then to 119.1 g/l at 36 weeks.

In the twice-weekly iron group, the mean haemoglobin concentration increased between baseline and twelve weeks (120.2 g/l to 122.7 g/l, then fell to 121.2 g/l at 24 weeks and 116.6 g/l at 36 weeks. In the group receiving no iron tablets, the mean haemoglobin concentration showed little change from baseline to 24 weeks, but decreased between 24 and 36 weeks.

Repeated measures linear regression analysis showed no differences in haemoglobin concentration at baseline, 12 and 24 weeks between once-weekly supplemented and unsupplemented groups, and between baseline and 12 weeks in the twice-weekly and unsupplemented groups after controlling for age, sex and baseline haemoglobin concentration. Figure 2 shows that the largest increase in haemoglobin at 12 weeks was observed in children with the lowest baseline haemoglobin concentration, in all groups.

**Effect of iron supplements on anaemia prevalence**

Table 3 shows that among children receiving once-weekly iron tablets for 24 weeks, the prevalence of anaemia decreased slightly from 0-12 weeks, then increased between 12-24 weeks. After an additional 12 weeks without treatment the prevalence of anaemia had risen to 36.1% (36 weeks after baseline). None of the changes in prevalence between each assessment was statistically significant.

The prevalence of anaemia in children receiving twice-weekly iron tablets for 12 weeks decreased from baseline to 12 weeks but was not statistically significant (*P*>0.05). After 12 weeks no more iron supplements were given. From 12 to 24 weeks there was no significant change in anaemia prevalence, but from 24 -36 weeks the prevalence of anaemia increased from 32.7% to 46.2% (*P*<0.001). The prevalence of anaemia in the unsupplemented group did not change between baseline, 12 and 24 weeks (≥40%), but increased significantly from 24 to 36 weeks (41.6% to 53.0%, *P*<0.001) (Table 3).

The prevalence of anaemia in the group receiving no iron tablets was significantly higher at baseline compared with both groups given iron supplements (*P*<0.001, Table 3) and at all subsequent time points. Subsequent analyses therefore controlled for the baseline haemoglobin concentration.

Logistic regression analyses controlling for the baseline haemoglobin concentration, school, age and sex showed no difference in the risk of becoming anaemic among children receiving once or twice-weekly iron tablets compared with controls at 12 or 24 weeks.

As the treatment was randomised by school, rather than by individual, the design effect (DEFF) and intra-cluster correlations (ICC) were calculated for each group at baseline. These values varied considerably for the once weekly, twice weekly and control groups (DEFF of 3.89; 4.82 and 10.62 respectively giving a ICCs of 0.08; 0.12; 0.33), and were particularly high in the control group.

## Discussion

This iron supplementation study was conducted among schoolchildren living in a remote rural area of Pakistan who had an overall anaemia prevalence of 33% at baseline. Children who received ferrous sulphate tablets displayed a small increase in haemoglobin from baseline to 12 weeks of supplementation (1.4 g/l in the once-weekly group; 2.5 g/l in the twice-weekly group), but this was not significantly different to the haemoglobin concentrations in the unsupplemented group. Similarly, there was no reduction in the prevalence of anaemia as a result of iron supplements. The small increase in haemoglobin concentration in the present study is similar to the effect of once-weekly iron supplements given to school children in Mali which led to a mean haemoglobin increase of 1.8 g/l after 10 weeks [14].

Several factors may have contributed to the lack of significant improvement in haemoglobin concentration in the present study. First, anaemia may have been caused by other micronutrient deficiencies such as vitamin A, vitamin B12 or folate. In Tanzania, anaemic children receiving vitamin A alone showed a mean increase in haemoglobin of 13.5 g/l over 12 weeks compared with an increase of 17.5 g/l seen in anaemic children receiving iron alone [19]. In Vietnamese infants, daily multiple micronutrient supplements led to a significant increase in haemoglobin compared with placebo tablets, whereas daily iron supplements did not [20]. However, systematic reviews have concluded that, in the presence of iron deficiency, iron supplements alone are still effective compared with iron administered with other micronutrients [5-6, 13].

Second, the moderate prevalence of anaemia may have limited the potential to increase haemoglobin through low-dose supplements, as seen in previous studies [12, 21] Third, the frequency, duration or dose of treatment may have influenced the findings of this study. The benefits of daily or intermittent iron treatment have been widely debated [22-24]. However, a systematic review of 55 studies concluded overall that iron supplements were effective in increasing haemoglobin concentration in children irrespective of the frequency of treatment [1]. Current recommendations advise once-weekly iron supplements for three months on the basis that this regimen is associated with a low risk of side effects and greater compliance than more frequent doses [6, 13].

Parasitic infections were unlikely to have contributed to the lack of improvement in haemoglobin in this sample since malaria was absent, and a survey of children in the same area found no cases of hookworm and only 30% of children infected with *Trichuris trichiura* [16]. These represent low prevalence rates for soil-transmitted helminths.

This study was designed as part of a school-based nutritional programme with a view to assessing the feasibility and nutritional benefits of iron supplements administered in schools by teachers. As such, there were constraints on the study design which limit the interpretation of findings. Iron supplements were randomised by school, and randomisation was separate for the intervention and control arms which may have led to bias due to potential differences between intervention and control schools. Similarly, the sample was not stratified by age or sex. As the haemoglobin concentration is known to vary with age and sex, these variables were controlled for in the statistical analyses. Finally, compliance with iron supplements was not stringently monitored as it was considered too onerous for teachers to record individual treatments. This is how a programme would be implemented in practice. Large-scale supplement programmes present particular difficulties for monitoring compliance with iron supplements, and data are sparse [6].

Although the statistical analyses controlled for the clustered study design, the intracluster correlations were higher than expected and indicate a higher correlation of haemoglobin concentrations within schools than between schools (clusters). This means that there was a relatively high level of between-cluster variation, particularly in the control group [25]. A *post hoc* power calculation indicates that a prevalence of anaemia of 8% lower could have been detected using the initial prevalence of 33% and applying an ICC of 0.228 derived from data for all 30 schools (DEFF = 8.3), a sample size per cluster of 33, 10 schools per cluster, a power of 80% and a significance of P = 0.05. A larger number of clusters, with fewer participants in each cluster, would have enhanced the power to detect statistically significant changes in haemoglobin concentration [25].

An important finding from this study is the increase in anaemia prevalence during the winter months from 35% to 45% in weeks 24 to 36 (November and February). The children in the study were from predominantly farming households living in a remote, mountainous area. Local fruit and vegetables, including green leafy vegetables, were widely available in the summer months whereas in winter, households were much more reliant upon dried pulses and dried or preserved vegetables. Seasonal changes in micronutrient levels in young children have been previously reported in the Northwest Frontier Province. Among children <2 years, plasma retinol, lutein and alpha-tocopherol increased in the summer months (July-September) corresponding with increased availability of dietary carotenoids [26]. A livelihood survey in the highlands of Northwest Frontier Province found up to 50% of respondents encountered serious problems in providing an adequate food supply in winter (October-February) [27]. In the present study, the increase in the prevalence of anaemia post intervention was smaller in the once-weekly group supplemented for 24 weeks than the twice weekly group supplemented for 12 weeks (7% and 14% increase respectively). Few studies have examined the seasonal prevalence of anaemia in children and this is an important consideration for future research.

In this intervention, once-weekly and twice-weekly iron supplements led to small improvements in haemoglobin concentration which were not statistically significant, but this may in part be due to the imperfect randomisation across the treatment and control arm and the high intra-cluster correlation which was unpredicted. The findings suggest there is potential for iron supplements to buffer children against seasonal iron deficiency which may be worthy of further investigation. This study highlights some of the difficulties of evaluating iron interventions when implemented on a programmatic basis.

**Conflict of Interest**

The authors declare no conflict of interest.

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**Legends to Figures**

Figure 1. Mean haemoglobin concentration (g/l) in the once-weekly and twice-weekly iron supplemented groups and unsupplemented group at baseline and at 12, 24 and 36 weeks follow-up.

Figure 2. Mean change in haemoglobin concentration (g/l) between baseline and 12 weeks against baseline haemoglobin concentration in the once-weekly and twice-weekly iron supplement groups and the unsupplemented group.

Table 1: Characteristics of the sample for the groups at baseline (in month of May) receiving once-weekly iron supplements, twice weekly iron supplements and no iron supplements.

| | Once weekly | Twice weekly | No iron supplements |
|---|---|---|---|
| Number recruited | 370 | 370 | 369 |
| Final sample (n)* | 352 | 333 | 298 |
| Incomplete or lost to follow-up (n) | 18 | 37 | 71 |
| Mean age in months ± SD (n) | | | |
| Boys | 117.44 ±25.67 (303) | 110.47 ±26.37 (251) | 115.84 ± 24.59 (243) |
| Girls | 113.65 ±20.86 (49) | 103.85 ±21.06 (82) | 109.20 ±20.43 (55) |
| % girls | 14 | 25 | 19 |
| Anaemia % within group (n) | | | |
| Not anaemic | 71.9 (253) | 68.8 (229) | 60.7 (181) |
| Anaemic | 28.1 (99) | 31.2 (104) | 39.3 (117) |

Anaemia cut-points for children aged 5-11years: <115g/l. Children aged 12-14 years: <120g/l. Boys over 15 years of age: <130g/l [18].
*Final sample represents cases with a haemoglobin measurement at each of the four assessments.

Table 2: Haemoglobin (Hb) concentrations after adjustment for altitude in the groups receiving once-weekly iron supplements, twice weekly iron supplements and no iron supplements at baseline, 12, 24 and 36 weeks follow-up.

| Group* | | Baseline (May) | 12 weeks (August) | 24 weeks (November) | 36 weeks (February) | 0-12 week change | 0-24 week change | 0-36 week change |
|---|---|---|---|---|---|---|---|---|
| Once-weekly (n=352) | Mean Hb (g/l) | 122.6 | 124.0 | 121.5 | 119.1 | 1.4 | -1.1 | -3.4 |
| | SD | 14.7 | 13.5 | 11.8 | 15.0 | 15.0 | 14.1 | 16.7 |
| Twice-weekly (n=333) | Mean Hb (g/l) | 120.2 | 122.7 | 121.2 | 116.6 | 2.5 | 1.1 | -3.5 |
| | SD | 15.7 | 13.4 | 14.5 | 14.6 | 14.5 | 16.2 | 18.2 |
| No iron (n=298) | Mean Hb (g/l) | 117.1 | 117.2 | 117.8 | 114.2 | 0.1 | 0.7 | -2.8 |
| | SD | 16.0 | 17.6 | 15.3 | 14.3 | 17.0 | 16.2 | 15.6 |
| Between group differences | | P> 0.05 | P>0.05 | P>0.05 | p>0.05 | | | |
| Total (n=983) | Mean Hb (g/l) | 120.1 | 121.5 | 120.3 | 116.8 | 1.4 | 0.2 | -3.3 |
| | SD | 15.6 | 15.1 | 13.9 | 14.8 | 15.5 | 15.5 | 16.9 |

* Once-weekly group received iron supplements for 24 weeks with follow-up blood samples taken at 36 weeks; Twice-weekly group received iron supplements for 12 weeks with follow-up blood samples taken at 24 and 36 weeks.

Table 3: Prevalence of anaemia at baseline, 12, 24 and 36 weeks in the groups receiving once-weekly iron supplements, twice weekly iron supplements and no iron supplements (haemoglobin concentrations were adjusted for altitude before applying anaemia cut-points).

| Group* | | Baseline (May) | 12 weeks (August) | 24 weeks (November) | 36 weeks (February) | Change in prevalence from baseline to 36 weeks (%) |
|---|---|---|---|---|---|---|
| Once-weekly (n=352) | Anaemia % (95% CI) | 29.0 (19.5-38.4) | 28.4 (22.7-34.1) | 31.8 (24.2-39.4) | 36.1[†] (25.6-46.5) | 7.1 |
| Twice-weekly (n=333) | Anaemia % (95% CI) | 31.8 (21.7-42.0) | 28.2 (20.3-36.1) | 32.7[a] (23.9-41.5) | 46.2[a] (35.9-56.6) | 14.4 |
| No iron (n=298) | Anaemia % (95% CI) | 40.3 (23.1-57.4) | 44.0 (26.0-61.9) | 41.6[b] (33.9-50.3) | 53.0[b †] (43.4-62.6) | 12.7 |
| Between group differences | | *P*>0.05 | *P*>0.05 | *P*>0.05 | [†] p<0.05 (once weekly versus control) | |

*Once-weekly group received iron supplements for 24 weeks with follow-up blood samples taken at 36 weeks; twice-weekly group received iron supplements for 12 weeks with follow-up blood samples taken at 24 and 36 weeks.

[a,b] denotes significant change in prevalence between assessments within group (chi-square, p<0.001). Classification of anaemia: <115 g/l children aged 5-11.9 y; <120 g/l children 12.0-14.9 y; <120 g/l in females ≥15.0 y; and <130 g/l for males ≥15.0 y [(18)].