Is iron status associated with markers of non-communicable disease in Indian children?

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ORIGINAL ARTICLE

Is iron status associated with markers of non-communicable disease in Indian children?

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Running title: Iron status and NCD risk

Abstract

Background

High body iron stores have been associated with risk for non-communicable diseases (NCD) like diabetes (high fasting blood sugar, FBS), hypertension (HTN) or dyslipidaemia (high total cholesterol, TC) in adults, but not in adolescent children. This is relevant to iron supplementation and food iron fortification programs that are directed at Indian children.

Methods

The association of NCD with serum ferritin (SF) was examined using logistic additive models, adjusted for confounders such as age, Body Mass Index, C-Reactive Protein, haemoglobin and sex, in adolescent (10-19 years old) participants of the Indian Comprehensive National Nutrition Survey. The interaction of these associations with wealth and co-existing prediabetes was also examined. A scenario analysis was also done to understand the impact of iron fortification of cereals on the prevalence NCD among adolescents.

Results

The odds ratio (OR) of high FBS, HTN and TC were 1.05 (95% CI 1.01-1.08), 1.02 (95% CI: 1.001-1.03) and 1.04 (95% CI: 1.01-1.06) respectively for every 10µg/L increase in SF. The odds for high TC increased with co-existing prediabetes. The scenario analysis showed that providing 10 mg of iron/day by fortification could increase the prevalence of high FBS by 2%-14% across states of India. Similar increments in HTN and TC can also be expected.

Conclusions

High SF is associated with a significant risk for NCD in adolescents, and this is dependent on the wealth class, and on co-existing prediabetes. This should be considered when evaluating the benefits and harms of enhancing iron intake in anaemia prevention programs.

Introduction

The link between body iron status and diabetes has classically been known in relation to hereditary hemochromatosis [1], but more knowledge is now available about the risk of diabetes or other NCD, linked to increased iron intake in otherwise normal individuals [2]. For example, earlier analyses of National Health and Nutrition Examination Survey (NHANES) data showed that the risk of diabetes increased by up to 4-fold when otherwise normal individuals in the highest quintile of serum ferritin concentration were compared to those in the lowest quintile [3]. A meta-analysis of 15 studies showed that greater ferritin levels were associated with a greater risk of type 2 diabetes [4]. In a broader sense, there are also similar linkages of increased iron status as measured by serum ferritin (SF) with other NCD, or their risk factors, like hypertension and dyslipidaemias [5,6]. Many mechanisms for the development of diabetes from increased iron exposure, either as insulin resistance or diminished insulin secretion, have been suggested [2]. Oxidative stress induced by iron could be one pathway to both hypertension and dyslipidaemia [2,16]. It is also worth investigating if the risk for developing one NCD might be magnified by the pre-existence of other NCDs.

In India, iron deficiency anaemia is thought to be rampant [7], and the public health response has been to aggressively supplement dietary iron through iron-folic acid (IFA, 60 mg iron) tablets to adolescent girls and women of reproductive age [8]. Of late, mandatory food fortification with iron is being considered for dietary staple foods like rice, provided in publicly funded safety baskets and feeding programs to women and children [9]. Iron fortification of salt in some states and the voluntary fortification of snacks (also called nutraceuticals), which have also become a common part of India's food landscape even in poor or rural areas [10], further adds to the daily iron intake. The aggressive addition of dietary iron as a solution for anaemia is particularly worrying in India, which has a high adult prevalence of NCD such as diabetes [11], hypertension [12] and dyslipidaemias [13]. This

risk starts early in life, as the recently concluded national survey of Indian children, the CNNS [14], showed that as many as 50% of children, whether thin, stunted or normal, had at least one biomarker of “metabolic obesity” and risk for NCD [15].

Given the enthusiasm for boosting the iron intake of specific populations like women and children through simultaneous iron supplementation [8] and universal fortification of rice [9], particularly in children who already exhibit a high degree of metabolic risk for NCD [15], it becomes important to evaluate the risk that might independently arise from increasing body iron stores. While there are no national adult survey data available, the CNNS [13] measured inflammation corrected serum ferritin (SF), along with NCD biomarkers like blood sugar, plasma lipid concentrations and blood pressure, in a national sample of children. In this report, we evaluated the relationship of SF to the risk of NCD in Indian adolescent children aged 10-19 years in the CNNS, along with the contributory effect of socioeconomic status and pre-existing dysglycaemia. Finally, the effect of increasing SF through iron fortification, and its further effect on NCD, was also modelled.

Subjects and Methods

The data for this analysis were taken from the CNNS [14], a cross-sectional, nationally representative survey of Indian children and adolescents, which was conducted in 30 states of India between 2016-2018, under the aegis of the Ministry of Health and Family Welfare, Government of India, in collaboration with UNICEF, India and the Population Council, Delhi, India. The survey design and sampling methodology are already published elsewhere [14,17,18]. Briefly, a multi-stage, population proportional to size cluster sampling was performed to enrol preschool (1–4 years), school age (5–9 years) children, and adolescents (10–19 years), to adequately represent the national, state, male-female, and urban-rural population. For blood sampling, 50% of all the children who completed

anthropometry were contacted through systematic random sampling. However, due to many reasons described in the CNNS report [14], there were a varying number of sampled children with specific valid biochemical parameters across the age groups for final analyses. Markers of NCD, including fasting blood sugar (FBS), blood pressure (BP; systolic SBP and diastolic DBP) and total cholesterol (TC), along with its fractions such as LDL, HDL and triglycerides, were measured in adolescents (aged 10-19y). FBS, TC and its fractions were measured by quality controlled standard methods [14]. Automated devices were used to measure blood pressure to minimize observer bias or digit preference. The cuff used was appropriate to the size of the adolescent's upper right arm. Three blood pressure readings were taken with a gap of at least two minutes. For SBP and DBP, the average of the second and third reading of systolic and diastolic blood pressures were considered [13]. The FBS cut-off to diagnose prediabetes was 100 mg/dL [19]; the TC cut-off to diagnose hypercholesterolemia was >200mg/dL [20] and hypertension (HTN) was classified as average blood pressure >95th percentile of age, sex and height specific standards [21]. In this analysis, only the data of 10-19 year children were used because not all data were uniformly available in younger children.

The lower and upper 0.1% of the data for each biochemical parameter were excluded to avoid undue variability from outliers. All essential parameters were summarized by suitable descriptive statistics and visualized by density plots for understanding the location, scale, and shape of the parameters for the choice of suitable regression techniques to examine the primary aim of the study. The tested exposure to iron intake was SF, and its relation to NCD markers was adjusted for other variables including age, sex, wealth index, Body Mass Index (BMI), plasma C-reactive protein concentration (CRP), and haemoglobin (Hb). The poorest and poorer categories of wealth index (WI) were combined due to their relatively small sample size.

The association of serum ferritin (SF) and fasting blood sugar (FBS) was explored by a linear additive regression model, adjusted for confounders such as age, BMI, CRP, Hb and sex, identified from the literature. Cubic penalized splines with degrees of freedom, auto estimated by generalized cross validation, were used to allow confounders to be nonlinear. In addition, an indicator variable of iron deficiency (SF<15µg/L) was also considered in the model. An adjusted exposure response curve (using a smoothing spline) between SF and FBS was also estimated. Further, the association of SF with FBS across different socioeconomic status was estimated by adding an interaction term between SF and WI in the model, considering it only as an effect modifier. A similar analysis, considering a binary outcome with high fasting blood sugar (FBS>100mg/dL), was also carried out by a logistic additive regression model (Generalized Additive Model with logit link).

The association of SF with systolic and diastolic BP was estimated by similar linear additive model adjusted for all confounders specified above. The effects of SF on BP at different FBS concentrations were also explored by adding an interaction term between BP and FBS. The total effect of SF on BP was then a function of FBS as

$$\hat{\beta}_{Total} = \hat{\beta}_{SF} + (FBS) \times \hat{\beta}_{(SF \times FBS)} \quad (Eq\ 1)$$

The standard error of total effects can be derived from the square root of the variance of total effects as

$$var(\hat{\beta}_{Total}) = var(\hat{\beta}_{SF}) + (FBS)^2 var(\hat{\beta}_{(SF \times FBS)}) + 2(FBS)cov(\hat{\beta}_{SF}, \hat{\beta}_{(SF \times FBS)}) \quad (Eq\ 2)$$

The $\hat{\beta}$'s are the estimated slopes for SF, interaction term (SF×FBS) and total. Further, the total effects of SF at each socioeconomic status were estimated by a linear additive model with interaction of WI with SF and (SF×FBS). Finally, the total effects of SF at different WI classifications, which is the change in BP per unit change in SF at different levels of FBS, were compared visually with the aid of error bars. Logistic additive models were considered for HTN as it is a binary variable.

The association of SF with TC was also examined by the linear additive model structure with and without interaction with FBS and WI described above. The TC values were further interpreted to diagnose children as having either borderline-high cholesterol ($170 < \text{TC} \leq 200 \text{ mg/dL}$) or hypercholesterolemia ($\text{TC} > 200 \text{ mg/dL}$) [19]. An adjusted baseline logistic additive regression model with and without interactions of FBS and WI were considered for them, using $\text{TC} \leq 170 \text{ mg/dL}$ as the baseline category. A similar analysis was performed with serum LDL (borderline: 110-130 mg/dL; high: $> 130 \text{ mg/dL}$) [19] and with serum triglycerides (borderline: 75-100 mg/dl; high: $> 100 \text{ mg/dL}$).

A scenario analysis was also conducted to understand the potential relative impact of rice or wheat fortification with iron, mediated through body iron store status (based on SF), on the prevalence of prediabetes, hypercholesterolemia and HTN among adolescents across states of India. We assumed the delivery of an additional 10 mg of iron per day through iron fortification of rice or wheat when 250 gm of either cereal was eaten daily. For cereal intake of adolescents in each Indian state, data were taken from the 68th round of National Sample Survey Office (NSSO) Consumer Expenditure Survey, conducted between 2011 to 2012 [22]. The intake was calculated per consumer unit (23), which were taken to have a value of 1 for adolescents (similar to the adult value). Based on SF data from Indian efficacy trials with fortified rice or wheat (SF change in the intervention group vs change in the control group), we assumed a 11.5 $\mu\text{g/L}$ increase in SF would occur after 7 months intake of 6 mg per day through a single meal of iron fortified wheat in primary school children (using Fe-EDTA which has a high bioavailability [24]), and a 7.5 $\mu\text{g/L}$ increase in SF would occur after 7 months intake of 20 mg per day through all meals with iron fortified rice (using micronized ground ferric pyrophosphate which has a much lower bioavailability [25]). These iron fortificants are presently included in the regulation to fortify cereals (rice or wheat) in India at

a concentration that would provide ~10.5 mg of iron/day if 250 - 350g cereal were eaten daily [26].

The relative elevation in prediabetes prevalence among adolescents due to the additional iron intake based on the calculation above and their per consumer unit intake of cereal, compared to the current prevalence, was derived as

$$\left(\frac{\text{prob}(100 < FBS_1 < 126 | \text{fortification})}{\text{prob}(100 < FBS_0 < 126 | \text{current})} - 1 \right) \times 100\% \quad (\text{Eq } 3)$$

where, FBS_0 was assumed to be normally distributed with mean μ and SD σ and FBS_1 was assumed to normally distributed with mean $\mu + \delta$ and SD σ ; 100 and 126 are the cut-offs for the diagnosis of prediabetes and diabetes respectively. The δ was derived as

$$\delta = \left[R \times \left(\frac{10}{250} \right) \times \left(\frac{7.5}{20} \right) + W \times \left(\frac{10}{250} \right) \times \left(\frac{11.5}{6} \right) \right] \times \hat{\beta}_{SF} \quad (\text{Eq } 4)$$

Where R and W are the ‘per consumer’ intakes of rice and wheat across the Indian states; $\hat{\beta}_{SF}$ is the effect of SF on FBS at different WI; the numerals 10 and 250 are the assumed delivery of iron (mg/day) and rice (g/day) respectively; 7.5 and 11.5 are the increments in SF ($\mu\text{g/L}$), while 20 and 6 are iron intake (mg/day) through fortification. Similarly, the expected elevation in prevalence of hypercholesterolemia and prevalence of HTN due to fortification among adolescents with prediabetes were derived by respective Odds Ratios (ORs), estimated by the models described above. In these cases, the δ values were derived by substituting the corresponding slope ($=\log \text{OR}/\text{per unit SF}$) in equation (4) and followed by $(e^\delta - 1) \times 100\%$.

Results

The analysis was carried out on the CNNS data available for adolescents, in which 51.3% were boys and 48.7% were girls; their mean age was 14.2 ± 2.8 y (**Figure 1**). Within the analytical sample, 12851 children had valid SF measurements, while valid FBS values

were available for 10534 children, TC for 11964 children and BP measurements for 10272 children. Overall, 20.9% of the children were iron deficient ($SF < 15 \mu\text{g/L}$); the median and IQR of SF were 31.4 (16.8, 53.2) $\mu\text{g/L}$ ranging from 1.1 to 408.4 $\mu\text{g/L}$; 30.4% girls and 11.5% boys were iron deficient. Their mean FBS was $90.8 \pm 10.8 \text{ mg/dL}$ with 13.6% of children with prediabetes; mean TC was $140.6 \pm 31.7 \text{ mg/dL}$ with 4.5% of the children with hypercholesterolemia; the mean SBP was $112.7 \pm 11.1 \text{ mm Hg}$ with 32.5% having HTN (**Supplementary Table 1** and **Figure 2**).

The linear additive model (adjusted) of FBS on SF estimates showed a 0.14 mg/dL increase (95% CI: 0.06, 0.21) in FBS for every 10 $\mu\text{g/L}$ elevation of SF. But when stratified by wealth classes, the richest group exhibited the greatest effect (0.38 mg/dL, 95% CI: 0.26-0.50 for every 10 $\mu\text{g/L}$ elevation of SF), while no significant association was observed for the poorest stratum. **Figure 3** depicts the estimated exposure response curve for this association. The logistic additive model of high FBS ($>100 \text{ mg/dL}$) was significantly associated with SF among the richest class, the estimated OR was 1.05 with 95% CI: 1.01- 1.08 for every 10 $\mu\text{g/L}$ increase in SF (**Table 1**), however, those with $SF > 60 \mu\text{g/L}$ exhibited 1.3 times (95% CI: 1.09-1.55) higher risk of high FBS than those with $SF < 60 \mu\text{g/L}$.

The logistic additive model (adjusted) of HTN on SF, estimated an OR of ~ 1.02 (95% CI: 1.001-1.03) for every 10 $\mu\text{g/L}$ increase in SF. The risk of HTN associated with elevated SF was further magnified by the FBS level in the prediabetes and diabetes range. In the prediabetes range, every 10 $\mu\text{g/L}$ increase in SF was associated with further 5 to 10% increase in the risk of HTN for the middle and poor classes. The same association was not significant for higher wealth classes. A similar pattern was observed for SBP with SF in the adjusted linear additive model. Here, every 10 $\mu\text{g/L}$ increase in SF was associated with 0.13 mm Hg increase in SBP (95% CI: 0.05-0.20). There was no association observed with DBP (**Table 1** and **Figure 4**).

A positive association between SF and TC was also observed at FBS values that were at, and beyond, the prediabetes level. At a FBS of 200 mg/dL, every 10µg/L increase in SF level was associated with an increase of 4.5 mg/dL (95% CI: 0.6-5.7) in TC. If the FBS was 120mg/dL (as in prediabetes), every 10µg/L increase in SF could lead to an increase of 1.1 mg/dL (95% CI: 0.2-1.6) in TC. Among the richest class, in those with diabetes (FBS at 200 mg/dL), every 10µg/L increase in SF was associated with an increase of 13.8 mg/dL (95% CI: 10.5-17.1) in TC (**Figure 5**; derived by *Eq 1* and *2* above, with inputs from **Supplementary Table 2**). Further, every 10µg/L increase in SF was associated with a 4% (95% CI: 1-6%) increase in odds of hypercholesterolemia, which was magnified by an increase in FBS. With prediabetes (FBS: 100-126mg/dL), the OR of hypercholesterolemia varied from 1.06 (95% CI:1.03-1.09) to 1.14 (95% CI: 1.09-1.19). For prediabetes occurring in the richest class, the OR was 1.07-1.19; while for the richer class this was 1.07-1.15, and was highest for the middle class at 1.07-1.22. The odds of hypercholesterolemia for every 10 µg/L increase in SF was ~78% higher (for the middle class) for a child with diabetes (FBS at 200 mg/dL, **Table 1**). Borderline hypercholesterolemia did not show any association with SF in general, however, for the adolescent with prediabetes, the OR of having borderline hypercholesterolemia was estimated at 1.05-1.16 for the richest class, 1.04-1.13 for the richer class and up to 1.15 for the middle class. No such association observed for the poor class (**Table 1**). A similar analysis with the fractions of TC, such as serum LDL and triglycerides, are reported in **Supplementary Table 3**. For every 10µg/L increase in SF, the OR for high LDL ranged from 1.05 (95% CI: 1.02-1.08%) to 1.11 (95% CI: 1.06-1.16%) at a prediabetes stage (FBS: 100-126 mg/dL). The odds for high LDL remained high in the richest to middle classes, with an OR of 1.16 (95% CI:1.06-1.26). Similarly for every 10µg/L increase in SF, the OR for high triglycerides was about 1.07 at a prediabetes stage (FBS: 100-126mg/dL). The OR for high triglycerides ranged from 1.04 (95% CI:1.01-1.07) to 1.08 (95% CI:1.05-

1.11) in the richest to the middle class. There was a smaller risk of borderline levels of LDL and triglycerides for every SF increase of 10µg/L in prediabetes.

The scenario analysis, that evaluated the risk of prediabetes, HTN or hypercholesterolemia that could occur in adolescents from the daily consumption of 250 g of iron fortified rice or wheat over 7 months (providing about 10 mg of iron/day), showed that the prevalence of prediabetes could increase by 3-14% for the richest children; by 2-9% for the richer class and by 2-10 % for middle-class children, relative to current prevalence, across the different states in India. The national prevalence of prediabetes in the study children was 11.2%, 8.8% and 10% for richest, richer and middle class respectively. The prevalence of hypercholesterolemia among children with prediabetes could increase by 7-70% for the richest children, 10-55% for the richer class and 15-95% for middle-class children, across the states of India. The prevalence of hypercholesterolemia among children with prediabetes in this study was 20.5%, 4% and 6% for richest, richer and middle class, respectively. Similarly, the prevalence of HTN could increase by 1-5% overall, and by 2-6% for children with prediabetes across the states. The prevalence of HTN among prediabetes in this study was 28.8%. The stratification by socioeconomic status was avoided for HTN as associations were not significant across all the wealth categories among those with prediabetes (**Table 2**).

Discussion

This study shows, for the first time in children, that the risk of dysglycaemia or dyslipidaemia can appear with an increase in SF, and this risk is also either not linear, being higher with higher SF exposure, or magnified with co-existing prediabetes or diabetes. The co-existence of a high FBS (as prediabetes or diabetes) could increase the risk for HTN by 2 fold or more. These results converge with reports in adults, for the association between SF

and diabetes, HTN or hypercholesterolemia [1-4,]. For type 2 diabetes, a meta-analysis of 15 prospective studies showed that each 100- μ g/L increment in SF increased its relative risk by 22% (RR, 1.22; 95% CI, 1.14 to 1.31) [4]. These meta-analysis risk values are similar to the risk of prediabetes and diabetes in the present study (equivalent OR of ~1.5 for a 100 μ g/L increase in SF). The association between high SF and HTN has also been noted before [6], with similar risk as in the present study. In addition, a prospective cohort showed that the incidence of HTN in a 4-year follow up was higher (OR 1.54, 95% CI, 1.26-1.88) in men in the highest quartile of SF at baseline [27]. A similar relation of TC with SF has also been noted before [7].

Excess iron can add to the risk of NCD by many mechanisms. For diabetes specifically, the body iron status, as measured by SF, could have a causal role in its pathogenesis by either increasing insulin resistance or by reducing insulin secretion [2]. Several studies have shown a relationship between higher SF and the risk of diabetes [2, 28], including the meta-analysis [4]. The causal direction of the relation between high SF and diabetes is difficult to tease out, since ferritin is also an acute phase reactant that signifies inflammation, as can exist with adiposity [29], and cause insulin resistance. High iron intake and increased SF are inversely associated with adiponectin, due to the lower ferroportin expression on adipocytes [30]. This inverse pattern between SF and adiponectin is similar to what is seen with inflammation, and accounts for a greater insulin resistance. However, inverting the question, through reducing body iron stores and SF by phlebotomy, has been shown to improve dysglycaemia in individuals with metabolic syndrome [31], suggesting a causal relation between high iron status and insulin resistance. The causality linked to high iron intake is also likely to extend beyond SF to blood haemoglobin (Hb) concentrations. A recent large study that evaluated association of Hb levels with over 170 anthropometric, metabolic, and inflammatory parameters in large cross-sectional and longitudinal cohort

studies, showed that those with lower Hb levels had lower BMI, better glucose tolerance, lower TC and blood pressure, less adverse metabolite profiles, and a lower inflammatory load [32]. While this might have been due to higher SF, the role of relative tissue hypoxia and the Hypoxia Inducible Factor (HIF) was thought to be central, although other factors, like blood viscosity and changes in plasma volume, along with endothelial cell dysfunction could also be mediators [32]. However, the relationship between Hb and NCD was not explored in this study, because only about one third of anaemia in this population was due to iron deficiency [13],

There could also be a sexual dimorphism for the risk of diabetes, as a greater risk was observed in women [4], although conversely, a lower SF should be seen in pre-menopausal women, along with higher adiponectin values, protecting them from the risk of insulin resistance. Through its effect on the generation of reactive oxygen species, and general oxidant damage, iron can also directly affect the beta cell of the pancreas [reviewed in 2], and reduce insulin secretion. It is difficult to estimate what proportion of the risk of diabetes is due to insulin resistance or insulin secretion. For the relation observed with children in the present study, it is worth noting that excess iron intake during pregnancy can also lead to a higher risk for GDM [33], which in turn to lead to larger babies and the risk of overweight in those babies as they grow up [34]. Iron deficiency is also associated with increased Reactive Oxygen Species [35], such that the risk could also increase with low iron levels or low SF. The same oxidative stress mechanisms have been attributed to the relation between SF and HTN or hypercholesterolemia [6,7,14].

When modelled in the present study, with an underlying assumption of causality, for a daily intake of 250 g fortified rice (delivering 10 mg) for a period of 7 months, changes in the prevalence of prediabetes, hypertension or hypercholesterolemia were not trivial. For the prevalence of prediabetes, this change ranged from 2-10% of the present value (8.8 to 11.2%,

nationally) depending on wealth. These changes magnify when one considers that fortification can continue for years. More worryingly, while the risk for high FBS is clearly present, the presence of high FBS as prediabetes or diabetes can magnify the risk for hypercholesterolemia. This can also happen with increasing wealth, except for the prevalence of HTN, where significant associations were obtained only in the lower wealth categories. The underlying trend for HTN therefore might not be attributed solely to higher SF, but to unobserved confounders related to poverty.

A vicious cycle can therefore exist between risk of NCD, pre-existing disease and wealth, and this is concerning, considering that in the CNNS, up to half the children, whether normal or thin or stunted, had at least one biomarker (high FBS, high serum cholesterol or triglycerides). These biomarker changes are likely to be chronic rather than acute as there was also an effect on glycated haemoglobin in that analysis [15]. The presence of these biomarkers of overfeeding was called “metabolically obese undersized” or MOU in thin or short children, and “metabolically obese normal weight” or MONW in normal weight individuals. Whether these have the same pathways or not is difficult to evaluate. However, this snapshot of MOU or MONW in a single cross-sectional survey is an indicator of a worrying upward trend. This is not hard to imagine, as India is transitioning rapidly, and probably even faster in rebound to the COVID pandemic. These markers suggest that a positive energy balance exists, which imputes a dynamic process of increasing obesity and NCD, even at this young age.

However, another vicious cycle is likely, particularly in poorer sections and MOU, related to existing iron supplementation programs with enthusiastic cereal feeding as referred to above. This interaction cannot be ascertained, but becomes much more relevant when one considers the mandatory fortification of rice which has been brought forward as a policy in India [36], for supply into the food safety baskets such as the Public Distribution System,

school lunch programs, and the Integrated Child Development Scheme, which looks after under-5 year old children. Here, the supply of iron is likely to be very significant, as the rice could supply about 10 mg iron per day for every 250 g of raw rice; it is possible that even salt could be similarly fortified, delivering an additional 10 mg of iron for every 10 g of salt [37].

It is clear now, from robust studies, that a high SF or high Hb, may be linked to many dimensions of a NCD-prone phenotype [2,4-6,32]. The present study extends this observation to adolescent children, although a limitation might be its cross sectional design, where causality is difficult to determine. There could have been more inflammatory markers measured, and important components of energy balance, like physical activity and energy intake were not explored in this study. The potential escalation of NCD risk should be an additional consideration when evaluating the benefits and harms of multiple public health interventions to enhance iron intake for addressing anaemia. A rational approach that improves erythropoiesis in those who need it, through a wide approach of calibrated intake of diverse diets along with physical activity and a clean environment, is likely to benefit populations much more than single nutrient initiatives.

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Author contributions

HSS and AVK conceived the idea, guided the analysis and drafted the manuscript. SG and TT conducted all statistical analyses. All authors were involved during drafting and approved the final manuscript. All authors had access to raw data.

Conflict of Interest

HSS designed the draft protocol of the CNNS with consultancy support from the UNICEF, India. HSS and AVK were members of the Technical Advisory Committee of the CNNS, constituted by the Ministry of Health and Family Welfare of the Government of India, to oversee its conduct and analysis. HSS is a member of the World Health Organization Nutrition Guidance Expert Advisory Subgroup on Diet and Health. HSS and AVK are members of Expert Groups of the Ministry of Health and Family Welfare on Nutrition and Child Health, and the National Technical Board on Nutrition of the Niti Ayog, Government of India.

Ethical Approval

No separate ethical approval was required for this secondary analysis. The CNNS was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Population Council's International Review Board (New York, USA) and ethics committee of Post Graduate Institute of Medical Education and Research (Chandigarh, India).

Figure Legend

Figure 1: Selection of subjects from the survey

Figure 2: Distribution of Serum Ferritin, Fasting Blood Sugar, BP and Total Cholesterol among children aged 10-19y.

Figure 3: Exposure-response association between Fasting Blood Sugar (FBS) and Serum Ferritin (SF) (left panel) and effect of Serum Ferritin on Fasting Blood Sugar across socioeconomic groups of children aged 10-19y

Figure 4: Exposure-response association of Serum Ferritin and Hypertension (HTN) or measured Systolic Blood Pressure (SBP) of children aged 10-19y (top and bottom left panels). Exposure-response association of Serum Ferritin and Hypertension (HTN) or measured Systolic Blood Pressure (SBP) of children aged 10-19y at different levels of Fasting Blood Sugar (50, 100 and 200 mg/dL) (top and bottom left panels).

Figure 5: Change in Total Cholesterol (TC) at every 10 μ g/L increase in Serum Ferritin (SF) over the range of Fasting Blood Sugar (FBS) of children aged 10-19y (left panel); and across different socioeconomic strata (right panel).

References

1. Swaminathan S, Fonseca VA, Alam MG, Shah SV. The role of iron in diabetes and Its complications. *Diabetes Care*. 2007; 30:1926-1933
2. Simcox JA, McClain DA. Iron and diabetes risk. *Cell Metab*. 2013, 17:329-341.
3. Ford, E.S., and Cogswell, M.E. (1999). Diabetes and serum ferritin concentration among U.S. adults. *Diabetes Care*. 1999; 22:1978–1983.
4. Jiang L, Wang K, Lo K, Zhong Y, Yang A, Fang X, Akezhuoli H, Song Z, Chen L, An P, et al. Sex-Specific Association of Circulating Ferritin Level and Risk of Type 2 Diabetes: A Dose-Response Meta-Analysis of Prospective Studies. *J Clin Endocrinol Metab*. 2019;104:4539-4551.
5. Lee DH, Kang SK, Choi WJ, Kwak KM, Kang D, Lee SH, Lee JH. Association between serum ferritin and hypertension according to the working type in Korean men: the fifth Korean National Health and nutrition examination survey 2010-2012. *Ann Occup Environ Med*. 2018;30:40.
6. Kim YE, Kim DH, Roh YK, Ju SY, Yoon YJ, Nam GE, Nam HY, Choi JS, Lee JE, Sang JE, et al. Relationship between serum ferritin levels and dyslipidemia in Korean adolescents. *PLoS One*. 2016; 11(4): e0153167.
7. Chatterjee P. India's child malnutrition story worsens. *Lancet Child Adolesc Health* 2021;5:P319–20.
8. Anemia Mukht Bharat. Intensified National Iron Plus Initiative (INIPI) [Internet]. New Delhi, India: Ministry of Health and Family Welfare, Government of India; 2020 Available from: https://anemiamukhtbharat.info/wp-content/uploads/2019/09/Anemia-Mukht-Bharat-Brochure_English.pdf [accessed 13 September, 2021].
9. Kurpad AV, Ghosh S, Thomas T, Bandyopadhyay S, Goswami R, Gupta A, Gupta P, John AT, Kapil U, Kulkarni B, et al. Perspective: When the cure might become the

- malady: the layering of multiple interventions with mandatory micronutrient fortification of foods in India. *Am J Clin Nutr.* 2021;114(4):1261-1266.
10. Street A. Food as pharma: marketing nutraceuticals to India's rural poor. *Crit Public Health.* 2015;25:361–72.
 11. India State-Level Disease Burden Initiative Diabetes Collaborators. The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990–2016. *Lancet Glob Health.* 2018; 6:e1352-e1362.
 12. Gupta R, Gaur K, Ram CV. Emerging trends in hypertension epidemiology in India. *J Hum Hypertens.* 2019; 33, 575–587.
 13. Gupta R, Rao RS, Misra A, Sharma SK. Recent trends in epidemiology of dyslipidemias in India. *Indian Heart J.* 2017; 69:382-392.
 14. Ministry of Health and Family Welfare (MoHFW), Government of India, UNICEF and Population Council. Comprehensive National Nutrition Survey (CNNS). National Report 2016–2018. New Delhi, India: MoHFW, Government of India, UNICEF and Population Council; 2019 [accessed 13 September, 2021].
 15. Sachdev HS, Porwal A, Sarna A, Acharya R, Ramesh S, Kapil U, Kurpad AV. Intraindividual double-burden of anthropometric undernutrition and "metabolic obesity" in Indian children: a paradox that needs action. *Eur J Clin Nutr.* 2021;75:1205-1217.
 16. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007; 39:44-84.
 17. Pullakhandam R, Peter R, Ghosh S, Reddy GB, Kulkarni B, Thomas T, Kurpad AV, Sachdev HS, Porwal A, Khan N, et al. Prevalence of low serum zinc concentrations in Indian children and adolescents: findings from the Comprehensive National Nutrition Survey 2016-18. *Am J Clin Nutr.* 2021; 114:638-648.

18. Sachdev HS, Porwal A, Acharya R, Ashraf S, Ramesh S, Khan N, Kapil U, Kurpad AV, Sarna M. Haemoglobin thresholds to define anaemia in a national sample of healthy children and adolescents aged 1-19 years in India: a population-based study. *Lancet Glob Health* 2021; 9:e822-e831.
19. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010; 33:S62–69.
20. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics*. 2011;128:S213–S256.
21. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004; 114:555-576.
22. National Sample Survey Office. Nutritional intake in India: 2011–12, NSS 68th round. New Delhi: National Statistical Organization, Government of India; 2014.
23. Thimmayamma BVS, Rau P, Damayanti K. Dietary assessment as part of nutritional status. In: Bamji MS, Krishnaswamy K, Brahman GNV, editors. *Textbook of human nutrition*. 4th ed. New Delhi (India): Oxford & IBH Publishing Pvt Ltd.; 2016:121–51.
24. Muthayya S, Thankachan P, Hirve S, Amalrajan V, Thomas T, Lubree H, Agarwal D, Srinivasan K, Hurrell RF, Yajnik CS et al. Iron fortification of whole wheat flour reduces iron deficiency and iron deficiency anemia and increases body iron stores in Indian school-aged children. *J Nutr*. 2012;142:1997-2003.
25. Moretti D, Zimmermann MB, Muthayya S, Thankachan P, Lee TC, Kurpad AV, Hurrell RF. Extruded rice fortified with micronized ground ferric pyrophosphate reduces iron

- deficiency in Indian schoolchildren: a double-blind randomized controlled trial. *Am J Clin Nutr*. 2006 Oct;84:822-829.
26. The Gazette of India: Extraordinary. Part II—Section 4. Food safety and standards (fortification of foods) regulations 2018. Notification. New Delhi, India: Government of India; 2018.
27. Kim MK, Baek KH, Song K-H, Kang MI, Choi JH, Bae JC, Park CY, Lee WY, Oh KW. Increased serum ferritin predicts the development of hypertension among middle-aged men. *Am J Hypertens*. 2012;25:492–497.
28. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA*. 2004;291:711–717.
29. Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem Soc Trans*. 2005;33:1078–1081.
30. Gabrielsen JS, Gao Y, Simcox JA, Huang J, Thorup D, Jones D, Cooksey RC, Gabrielsen D, Adams TD, Hunt SC, et al.. Adipocyte iron regulates adiponectin and insulin sensitivity. *J Clin Invest*. 2012;122:3529–3540.
31. Houshyar KS, Ludtke R, Dobos GJ, Kalus U, Broecker-Preuss M, Rampp T, Brinkhaus B, Michalsen A. Effects of phlebotomy induced reduction of body iron stores on metabolic syndrome: results from a randomized clinical trial. *BMC Med*. 2012;10: 54.
32. Auvinen J, Tapio J, Karhunen V, Kettunen J, Serpi R, Dimova EY, Gill D, Soininen P, Tammelin T, Mykkänen J, et al. Systematic evaluation of the association between hemoglobin levels and metabolic profile implicates beneficial effects of hypoxia. *Sci Adv*. 2021;7(29):eabi4822.
33. Association between maternal plasma ferritin concentration, iron supplement use, and the risk of gestational diabetes: a prospective cohort study. Zhang X, Wu M, Zhong C,

- Huang L, Zhang Y, Chen R, Zhou X, Xu S, Li Q, Cui W et al. Am J Clin Nutr. 2021; May 21:nqab162. doi: 10.1093/ajcn/nqab162. [accessed 13 September 2021].
34. Sparano S, Ahrens W, De Henauw S, Marild S, Molnar D, Moreno LA, Suling M, Tornaritis M, Veidebaum T, Siani A, Russo P. Being macrosomic at birth is an independent predictor of overweight in children: results from the IDEFICS study. Matern Child Health J. 2013 Oct;17(8):1373-81.
35. Walter PB, Knutson MD, Paler-Martinez A, Lee S, Xu Y, Viteri FE, Ames BN. Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. Proc Natl Acad Sci USA. 2002;99:2264–2269.
36. Department of Food and Public Distribution. Centrally sponsored pilot scheme on “fortification of rice & its distribution under Public Distribution System” [Internet]. New Delhi, India: Department of Food and Public Distribution, Government of India; 2021 [accessed 3 May, 2021]. Available from: https://dfpd.gov.in/Centrally_Sponsored_Pilot_Scheme.htm.
37. The Gazette of India: Extraordinary. Part II—Section 4. Food safety and standards (fortification of foods) regulations 2018. Notification. New Delhi, India: Government of India; 2018.

Table 1: The odds ratio (OR) for different health outcomes with every 10µg/L increase in Serum Ferritin (SF).

Model	OR for every 10µg/L increase in SF				
	Without interaction	With interaction of Fasting Blood Sugar (mg/dL)			
		100	126	150	200
Hypertension					
Overall	1.02(1.01,1.03)	1.04(1.02,1.06)	1.08(1.05,1.12)	1.13(1.07,1.18)	1.22(1.11,1.34)
Richest	1.01(0.99,1.03)	1.02(0.99,1.05)	1.05(0.98,1.12)	1.07(0.97,1.19)	1.13(0.94,1.36)
Richer	1.01(0.99,1.03)	1.02(0.99,1.04)	1.05(0.99,1.12)	1.09(0.98,1.21)	1.17(0.96,1.41)
Middle	1.03(1.01,1.05)	1.05(1.02,1.08)	1.10(1.04,1.17)	1.15(1.04,1.27)	1.26(1.05,1.52)
Poor	1.02(1.00,1.04)	1.04(1.01,1.07)	1.10(1.03,1.16)	1.15(1.05,1.26)	1.28(1.08,1.51)
Border Line Cholesterol (170<Total Cholesterol<200 mg/dL)					
Overall	1.00(0.98,1.02)	1.02(0.99,1.04)	1.11(1.07,1.16)	1.21(1.14,1.29)	1.43(1.28,1.61)
Richest	1.02(0.99,1.05)	1.05(1.02,1.09)	1.16(1.07,1.25)	1.26(1.12,1.43)	1.51(1.21,1.90)
Richer	1.01(0.99,1.04)	1.04(1.00,1.07)	1.13(1.04,1.22)	1.22(1.07,1.38)	1.43(1.13,1.80)
Middle	0.99(0.96,1.02)	1.00(0.97,1.04)	1.14(1.05,1.25)	1.29(1.11,1.50)	1.66(1.26,2.19)
Poor	0.96(0.93,0.99)	0.98(0.94,1.01)	1.06(1.00,1.14)	1.15(1.03,1.28)	1.36(1.11,1.66)
Hypercholesterolemia (Total Cholesterol>200mg/dL)					
Overall	1.04(1.01,1.06)	1.06(1.03,1.09)	1.14(1.09,1.19)	1.22(1.13,1.31)	1.40(1.22,1.61)
Richest	1.04(1.00,1.08)	1.07(1.02,1.12)	1.19(1.08,1.31)	1.31(1.11,1.53)	1.59(1.18,2.15)
Richer	1.05(1.01,1.09)	1.07(1.03,1.12)	1.15(1.05,1.25)	1.22(1.05,1.42)	1.38(1.04,1.83)
Middle	1.05(1.01,1.09)	1.07(1.03,1.12)	1.22(1.12,1.34)	1.38(1.18,1.62)	1.78(1.32,2.40)
Poor	1.01(0.97,1.05)	1.01(0.95,1.06)	0.99(0.88,1.12)	0.98(0.81,1.19)	0.96(0.69,1.34)
High Fasting Blood Sugar (>100mg/dl)					
Overall	1.01(0.99,1.04)				
Richest	1.05(1.01,1.08)				
Richer	0.96(0.92,1.00)				
Middle	1.02(0.99,1.06)				
Poor	1.01(0.98,1.03)				

Table 2: Elevation in potential risk of prediabetes, hypercholesterolemia and Hypertension (HTN) in children aged 10-19y, after consumption of fortified cereal* across states of India.

State	Elevation (%) in Prevalence of prediabetes compared to baseline			Elevation (%) in Prevalence of hypercholesterolemia compared to baseline among those with pre-diabetes			Elevation (%) in Prevalence of HTN	
	Richest	Richer	Middle	Richest	Richer	Middle	Overall	Prediabetes
A & N Island	4.8	2.8	2.8	18.6	13.9	23.5	1.4	3.3
Andhra Pradesh	4.6	2.7	2.7	13.4	11.2	17.4	1.4	3.1
Arunachal Pradesh	5.3	3	3.1	12.8	12.9	22.3	1.6	3.6
Assam	5.3	3	3.1	16	13.4	20.4	1.6	3.6
Bihar	8.5	4.9	4.9	43.8	38.2	62.9	2.5	5.7
Chandigarh	6.5	3.7	3.8	38.1	34.6	54.5	1.9	4.4
Chhattisgarh	5.8	3.3	3.3	17.5	16.2	25	1.7	3.9
D & N Haveli	4.1	2.3	2.4	7.2	12.6	26.2	1.2	2.8
Daman & Diu	4.6	2.6	2.7	22.6	24.2	27.3	1.4	3.1
Delhi	6	3.5	3.5	37.8	31	44.5	1.8	4.1
Goa	4.2	2.4	2.5	15.7	13	20.2	1.3	2.9
Gujarat	5	2.9	2.9	21.4	24	36.5	1.5	3.4
Haryana	8.5	4.9	4.9	61.3	47.1	77.6	2.5	5.7
Himachal Pradesh	8.6	4.9	5	53.7	41.1	68	2.6	5.8
Jammu & Kashmir	6.8	3.9	4	34.1	22.7	37.4	2	4.6
Jharkhand	7	4	4.1	24.9	23.7	43.3	2.1	4.7
Karnataka	3.4	2	2	11.8	10.3	15.3	1	2.3
Kerala	3.9	2.2	2.3	12.3	10.5	16.9	1.2	2.6
Lakshadweep	4.2	2.4	2.4	14.7	12.1	20.1	1.3	2.8
Madhya Pradesh	9.9	5.6	5.7	57.7	52.5	95.4	2.9	6.6
Maharashtra	5.5	3.2	3.2	32.3	27.4	38.5	1.6	3.7
Manipur	5.9	3.4	3.4	16.8	13.3	19.8	1.8	4
Meghalaya	4.2	2.4	2.5	12.6	10	15.6	1.3	2.9
Mizoram	5.3	3	3.1	15.5	11.8	18.1	1.6	3.6
Nagaland	5.2	3	3	15.1	11.5	18.1	1.6	3.5
Orissa	5.9	3.4	3.4	16.4	14.8	24.4	1.8	4
Puducherry	4.2	2.4	2.4	14.1	11.9	16.9	1.3	2.8

Punjab	8.2	4.7	4.7	58.7	45.6	66.4	2.4	5.5
Rajasthan	9.7	5.5	5.6	69.9	55.2	91	2.9	6.5
Sikkim	4.2	2.4	2.5	14.6	11.6	16.3	1.3	2.9
Tamil Nadu	4	2.3	2.3	13	10.6	16.9	1.2	2.7
Tripura	5.7	3.3	3.3	16.4	12.9	20.3	1.7	3.8
Uttar Pradesh	9	5.2	5.2	55.3	43.9	74.2	2.7	6
Uttaranchal	9.1	5.2	5.3	59.8	42.9	69.9	2.7	6.1
West Bengal	5.4	3.1	3.1	20.3	16.5	27	1.6	3.6

Alphabetic order

*: Fortified cereal consumption for seven months, of either 10 mg iron/d iron through daily intake of 250 g fortified rice or 6 mg iron/d iron through a single meal with fortified wheat.

HTN: Hypertension