The Interrelations Between Nutrition, Social and Economic Trajectories During Adolescence Among Girls in Ghana

Fusta Azupogo

Propositions

1. Consuming multiple-micronutrient fortified biscuits available in the market does not improve micronutrient status and cognitive performance of adolescent girls.
(this thesis)

2. Adolescent boys deserve as much attention as adolescent girls in interventions designed to improve health and welfare.
(this thesis)

3. Scientists with non-significant rather than significant findings go through more stress to have their results accepted for publication.

4. Corruption and neo-colonialism are the main reasons for poverty and under-development in sub-Saharan Africa.

5. Stress and depression are far from the person who loves to sing.

6. An office setting not only makes people more efficient and productive but also improves overall health.

Propositions belonging to the thesis, entitled

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Fusta Azupogo

Wageningen, 31 January 2022

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This research was conducted under the auspices of the Graduate School VLAG (Advanced Studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences)

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 31 January 2022
at 4 p.m. in the Aula.

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The Interrelations Between Nutrition, Social and Economic Trajectories During Adolescence Among Girls in Ghana, 276 pages

PhD thesis, Wageningen University, Wageningen, the Netherlands (2022)
With references, with summary in English

ISBN 978-94-6447-014-7
DOI 10.18174/556418

In loving memory of my beloved, ***Jessica Suurikyibe Baissana-Azupogo***
and my mentor ***Prof. Abdul-Razak Abizari***

Table of Content

Chapter 1

General Introduction

Background

Adolescence and early adulthood are crucially important periods for the development of healthy adults, and adequate nutrition is key, associated with better lives and potential intergenerational benefits. There are approximately 1.8 billion adolescents globally, with 90% residing in low- and middle-income countries (LMICs) [1]. Adolescents make up the most substantial proportion (23%) of the population in sub-Saharan Africa (SSA), and the sub-region is projected to have more adolescents by 2050 than in any other region [2]. According to the Ghana Statistical Service, about a quarter of the Ghanaian population is adolescents [3], and a little over a fifth of the female Ghanaian population comprises adolescents [3]. Adolescents are the future workforce, leaders, and bearers of the future generation, but unlocking their potential is challenged by the disadvantaged nutrition situation adolescents often face.

The WHO defines adolescence as the life stage of approximately 10-19 years [4], characterised by biological and psychosocial changes and increasing maturity. It represents a time in life when the velocity of growth increases, as approximately 45% of total skeletal mass and 15-25% of adult height are gained during adolescence [5, 6]. For girls, the onset of menstruation (menarche) begins 6-12 months after this height increase occurs [7–9], after which height gain continues but at a slower rate for about 4.7 years [10, 11]. Likewise, girls' pelvic bones' growth, critical for preventing birth and pregnancy complications, is gained just before and for 4.7 years after menarche [10, 11].

As a result of the biological changes, energy and nutrient requirements during adolescence are among the highest in the life cycle, making adolescents vulnerable to malnutrition, such as thinness, anaemia, or iron-deficiency anaemia [6, 12, 13]. Although data on adolescents' anthropometric indices are scanty, Candler *et al.* [14] estimated that 7.6% of adolescent girls aged 12-18 years in LMICs are thin. Caleyachetty and *colleagues* [15] estimated that 6% and 4% of adolescent girls aged 12-15 in the WHO Africa sub-region are stunted and thin, respectively. The prevalence of thinness varied from 12.6% (Egypt) to 31.9% (Djibouti) among 11-17 years-old African adolescents [16].

Globally, about a quarter of adolescents are anaemic; more girls are anaemic compared to boys, and the prevalence is generally of extreme public health concern for adolescent girls in much of SSA [17]. Iron deficiency anaemia (IDA) is reportedly the most common cause of anaemia globally [18]. The burden of anaemia and IDA is higher for girls after menarche [18, 19] and particularly for adolescent girls with heavy menstrual blood losses [12, 20, 21]. There is also increasing evidence of other micronutrient deficiencies such as folate, zinc, and vitamin A among SSA girls [22–24].

Besides undernutrition, overnutrition is increasing among adolescents globally, and the trend is notably higher among adolescent girls [25]. Caleyachetty and *colleagues* [15] estimated that about one-fifth of adolescent girls aged 12-15 years in LMICs are overweight or obese, but the prevalence in the WHO Africa sub-region was estimated at 8%. The prevalence of overweight ranged from 8.7% (Ghana) to 31.4% (Egypt) in an analysis of the Global School-based Student Health Survey data for 11-17 years adolescents [16].

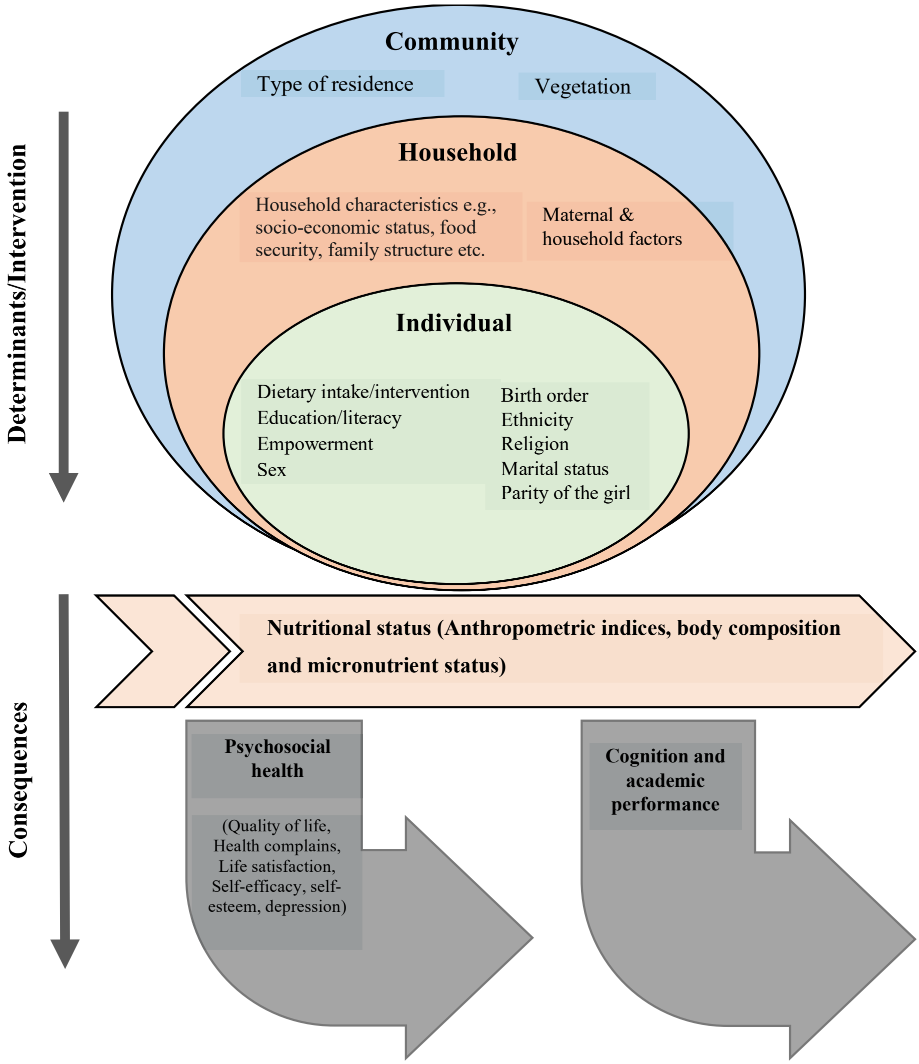


Figure 1: Framework for the socio-cultural and economic determinants of the nutritional status of adolescents *Adapted from* Madjdian *et al.* (2018)[1]

1 Madjdian DS, Azupogo F, Osendarp S, Bras H, Brouwer I. Socio-cultural and economic determinants and consequences of adolescent undernutrition and micronutrient deficiencies in LLMICs: a systematic narrative review. *Ann NY Acad Sci*. 2018; 1416:117–39

Malnutrition, in all its forms, has negative consequences for adolescents, hampering their potential. Undernutrition during adolescence adversely affects attained height [26] and is a high risk for mortality and morbidity; for example, IDA is the third most important cause of lost disability-adjusted life years (DALYs) in adolescents worldwide [18, 27]. SSA has the worst adolescent health profiles with persisting high mortality from maternal and infectious causes [28]. The WHO estimates that 243 deaths per 100000 occur among adolescents in LMICs in African [29]. Undernutrition also has adverse consequences for cognition, psychosocial health, adolescents' socio-economic life trajectories [30–33] and productivity later in life [34]. For adolescent girls, undernutrition has intergenerational effects on their future offspring's nutrition and health [35–37]. In contrast, over-nutrition poses an emerging cardiovascular risk for adolescents; it is a risk factor for obesity in adulthood and diabetes, hypertension, asthma, lower extremity venous oedema, and obstructive sleep apnoea in later life [38, 39].

Attention for adolescent girls' nutrition is vital in improving their health status and breaking intergenerational cycles of malnutrition and deprivation [2, 35–37]. However, interest in adolescents' health is relatively new, and an emphasis on nutrition is even more recent. Nutrition policies in LMICs, including Ghana, have commonly focused on infants and young children (IYC), pregnant and lactating women, neglecting adolescents. Nevertheless, adolescence offers an additional critical window of opportunity besides the first 1000 days of life for interventions to optimise nutrition and health outcomes [35–37]. Understanding the myriad of (context-specific) factors determining adolescent nutrition and gender-specific differences and trends in these and understanding the efficacy and optimal timing (before or after menarche) of adolescent nutrition interventions is crucial for improving girls' nutritional exposure and outcomes in multiple life domains. This thesis contributes to these knowledge gaps by focussing on the nutrition of adolescent girls and boys in Ghana.

Conceptual Framework

The studies described in this thesis are guided by the *socio-ecological framework* depicted in Figure 1, illustrating the socio-cultural and economic (SCE) determinants and consequences (psycho-social health, cognition, and academic performance) of adolescent nutrition status. Generally, nutritional status is influenced by a broader system of SCE contexts, played out at several levels, including environmental/community, household and individual-level factors that interrelate and influence nutrition. **Individual-level factors** such as age, sex, education, occupation, and marital status affect adolescents' nutrition by influencing dietary intake, aside from susceptibility and exposure to infection and access to an adequate diet and health service [13]. At the **household level**, parental education and occupation and household: wealth, size and structure influence the allocation of household resources, including food, health, and sanitation services, through competing needs which often disadvantage girls and influence girls' marital status [1, 13, 40–43]; this contributes to early pregnancies for girls before attainment of adult weight and height [1, 36, 42]. Some household behaviours are determined by cultural and religious norms, prevalent in the community [44] that often discriminate against girls [1], depriving them of essential nutrients, resulting in micronutrient deficiencies [45, 46]. **Community or environmental level** factors such as residence location (rural/urban) and ecological zone are drivers of household factors, including parental education and occupation, poverty, and household food security [47–50]. Community-level factors, individual and household factors are additive factors that influence adolescents' nutrition and not only via the household factors. Overall, besides girls' physiologic disposition to micronutrient deficiencies, SCE deprivations at all levels predispose them to malnutrition, undermining their development into adulthood.

While many studies address the SCE determinants of nutritional status, they mainly focus on IYC and their mothers. Previous efforts at reviewing adolescents' nutrition determinants focussed on overnutrition [51–53] and dietary patterns/behaviour [54]. In the following sections, we summarise what is known about the nutritional status and determinants of Ghanaian adolescent girls and boys, about nutritional and functional outcomes and what is known about nutrition interventions in adolescent girls.

Nutritional Status and Determinants Among Ghanaian Adolescents

Nutritional Status of Ghanaian Adolescents

Nutrition data for adolescents in Ghana is scanty, and until the time of this thesis, no study has examined the trends-over-time or the correlates of adolescents' nutritional status using nationally representative data. The Ghana Demographic and Health Survey (GDHS), conducted since 1988, includes 15-19 years adolescent girls as part of the women of reproductive age group, but only the 2014 GDHS [55] included adolescent males aged 15-19 years as part of a comparative group of men aged 15-49 years. Accordingly, there is presently a gap in national representative data for 10-14 years adolescents in Ghana, but there are a few smaller and location-specific cross-sectional studies.

The above studies indicate there is presently a double burden of malnutrition among adolescents in Ghana. According to the 2014 GDHS [55], thinness and overweight/obesity prevalence for 15-19 years adolescent girls is about 14.4% and 8.7%, respectively. Cross-sectional studies affirm the persistence of undernutrition with an existing and upcoming burden of overnutrition for Ghanaian adolescents [16, 24, 56–58]. The prevalence rates, including stunting (15%-50.3%), thinness (7%-19.4%), and overweight/obesity (6.9%-17%) vary widely between studies, depending on the context in which they were conducted [16, 24, 56–58], related to disparities in socio-economic status and food insecurity [49, 50]. Further, dietary behaviours in Ghana are shifting towards consuming more refined foods, particularly in the urban settings [59], which also partially accounts for the emergence of overnutrition for adolescents.

Between 1990 and 2010, anaemia remained a severe public health problem for females of all ages in Ghana [18]. The 2014 GDHS reports that about 48% of 15-19 years adolescent girls are anaemic [55]. Multiple micronutrient deficiencies, including ID, IDA, vitamin A deficiency (VAD), folate, and vitamin B_{12} co-exist with anaemia among adolescents and non-pregnant Ghanaian women of reproductive age [24, 60]. Although the burden of micronutrient deficiencies is reportedly higher for adolescent girls, micronutrient problems also exist for adolescent boys [24, 61].

Determinants of Nutritional Status of Ghanaian Adolescents

Dietary intake, age, physical activity, and sex have been reported as individual-level factors associated with malnutrition for adolescents in Ghana [24, 61]. Like other LMICs, the diet of Ghanaian adolescents primarily consists of low consumption of fruits and vegetables and high consumption of starch, sweetened foods and beverages and snacks in the form of 'fast-foods' [62–64]. Although quantitative dietary intake data for adolescents in Ghana is scanty, dietary inadequacies of iron, zinc, folate, vitamin A, and vitamin B_{12} have been reported for school-aged children and adolescents [65, 66].

Gosdin *et al.* [61] found that Ghanaian adolescent girls consuming haeme iron-rich foods and iron-fortified foods had a lower prevalence of anaemia and a higher haemoglobin concentration (Hb). Additionally, age was positively associated with anaemia among girls but negatively associated with anaemia among boys [61]. Insufficient physical activity is a known risk factor for higher overweight risk for Ghanaian adolescents [58, 67]. At the household-level, low parental education is associated with undernutrition for adolescents in Ghana [24, 56, 57]. Similarly, low socio-economic status is positively associated with undernutrition but inversely associated with overnutrition for adolescents [24, 57].

Rural residence and residence in the northern savannah ecological zone of Ghana are associated with increased micronutrient deficiencies among adolescents and women of reproductive age [16, 55, 60]. However, geographic scope and sample size limit the generalizability of the existing studies. Moreover, none of the existing studies examined the trends over-time in adolescent nutrition and the factors associated with these trends.

Sex as a Determinant of Nutrition Status of Ghanaian Adolescents

Research on adolescents has focussed mainly on girls, resulting in a scarcity of data for adolescent boys. Further, data from different settings present conflicting results on the association between malnutrition and adolescent sex. Even so, there is growing evidence that adolescent boys, like male IYC, are more vulnerable to stunting and thinness compared to their female peers in Africa, including Ghana [16, 68]. Some studies suggest that adolescent boys are equally susceptible to anaemia [68, 69], partly attributed to their higher predisposition to infections such as helminths [70]. Despite the paucity of data on adolescents' cardiovascular risk, two cross-sectional studies in the Greater Accra and Ashanti regions of Ghana reported a higher prevalence of hypertension and pre-hypertension among adolescent males than their female colleagues [24, 58]. There is a dearth of data on the sex-specific factors associated with the nutritional status, and cardiovascular risk of female adolescents compared to their male peers in SSA.

Timing of Menarche as a Determinant of Nutritional Status of Ghanaian Adolescents

Menarche is the last major event that symbolises sexual maturity in adolescent girls. The onset of menarche varies across countries and even within countries [9, 71]. In Ghana, the average age at menarche (AAM) is approximately 13 years, varying between 12.5 ± 1.3 years in the Greater Accra region to about 13.7 ± 1.9 years in the Northern region [72–74]. AAM is currently declining globally, especially in developed countries and urban areas of some LMIC [9, 75]. Several factors, such as nutrition, genetics, and environmental conditions, including socio-economic status and education level, account for the variation in AAM [9, 72, 75].

Menarche timing (whether later or earlier) is associated with adverse effects on health status; on the other hand, poor nutrition can delay and pronounce the onset of menarche [75]. Well-nourished girls have higher pre-menarche growth velocities and attain menarche earlier than undernourished girls, who grow more slowly for a prolonged period, as menarche is delayed [76]. While growth retardation is related to late menarche [77], a higher body-mass index and fat mass are associated with a lower AAM [75, 78]. Nevertheless, little is known about the interrelations between micronutrient status and menarche. Menstruation necessitates additional dietary iron for girls and may induce a compensatory iron absorption through homeostasis [2, 12]; this is, however, a hypothesis. On the contrary, menstruation is pro-inflammatory [79], which has consequences for micronutrient absorption [80]. As a result of these conflicting processes, it has yet to be established whether a nutrition intervention would yield maximum benefit when implemented pre-or post-menarche.

Nutrition and Functional Outcomes During Adolescence

Undernutrition during adolescence has adverse consequences for cognition, psychosocial health, and the socio-economic life trajectories of adolescents. Like IYC, inadequate micronutrient intake also increases the risk of infections for adolescents, contributing to a cycle of undernutrition, infection, and poor developmental outcomes [13, 81]. Besides physical growth, adolescents experience a substantial structural maturation and development of the brain during which the frontal lobes mature [82, 83]. The frontal lobes are the brain area responsible for higher-order cognitive functions such as logical reasoning, language, attention, inhibiting irrelevant stimulation, planning, problem-solving, and memory [82, 83]. Further, the limbic system, which governs reward processing, appetite, and pleasure-seeking, develops earlier in adolescence than the prefrontal cortex [27].

According to Mesiac *et al*. [12], iron deficiency (ID), besides provoking significant physiological consequences, also adversely affects adolescents' cognitive ability, motor, and mental development. Evidence from the Young Lives Cohort in Ethiopia, India, Peru, and Vietnam shows that linear growth retardation in adolescents is associated with poor cognitive skills and educational performance [30, 31]. A few studies have shown that a better micronutrient status is associated with improved cognitive skills and educational performance [32, 84, 85]. Additionally, improved nutrition during adolescence is associated with enhanced psychosocial health, including health-related quality of life, self-esteem, and self-efficacy [30, 33]. Overall, these findings suggest that the adverse effects of undernutrition on cognition and educational performance are not only limited to childhood but manifest during adolescence as well. However, the evidence has mainly been cross-sectional, making it impossible to establish causality. There is a paucity of data on the effect of micronutrient status other than anaemia on adolescents' cognition and health. Aside from Ethiopia [30, 31, 84] and Uganda [85], data from other SSA countries are commonly lacking. Undernutrition also negatively affects adolescents' life aspirations, through the cognition and psychosocial health pathways, including mental health [30, 86]. For instance, ID leads to fatigue and lethargy and may affect aspirations. Feeling energetic may also increase hopes for the future. However, girls' perspectives on growing up and their future aspirations and the numerous life domains remain unexplored

Additionally, stunted children and adolescents have a higher rate of later arterial hypertension [87]. According to Rotteveel and colleagues [88], young adults born preterm have lower insulin sensitivity and higher blood pressure. Data on the association between nutritional status and cardiovascular health of adolescents in SSA is presently scanty. Nevertheless, a recent scoping review indicates that overweight/obesity during childhood and adolescence in SSA is associated with metabolic syndrome, hypertension, dyslipidaemia, diabetes, and glucose intolerance [39].

Interventions to Improve Nutrition Status of Female Adolescents

In settings with a high burden of anaemia and IDA, the WHO recommends intermittent iron and folic acid supplementation for menstruating women and adolescents [89]. Nonetheless, poor compliance often limits the effectiveness of micronutrient supplementation programmes [90, 91]. Fortification of food without changes in organoleptic properties may be a more practical approach to tackling micronutrient problems in low socio-economic contexts [91]. Biscuits are reported as a convenient food vehicle for fortification as they are handy and easy to manage. More so, biscuits are more likely to be accepted by the adolescent population, who prefer snacks [92]. Many studies have shown that the consumption of multiple micronutrient fortified foods (MMFs) among adolescents effectively improves their haematologic and micronutrient status [93–96].

However, the evidence from LMICs is scanty (Table 1). The few studies from LMICs are primarily from Indian [95,97, 98], Bangladesh [93, 94] and China [96]. Two of the Indian studies [95,98] and that of Adams *et al.* [94] in Bangladesh were, however, limited in their pre-post-test without control design. However, in a randomized controlled trial among Bangladeshi adolescent girls, Hyder *et al.* [93] showed that 6 days/weekly consumption of MMF beverage for 6 months compared to unfortified beverage significantly improves Hb, serum ferritin, and serum retinol. Also, the study of Wang *et al.* [96] in China reported a lower odds of vitamin B_2 and iron deficiencies for those receiving multiple-micronutrient fortified milk compared to unfortified milk in a group of adolescent Chinese girls and boys (72% girls) aged 12-14 years. Overall, in these studies, MMFs were administered 5-7 days/week, for 3-12 months, with an average duration of 6 months [93–98]; the dosage of iron and vitamin A in these efficacy studies ranged from 3.5mg-9.3mg per 100g and 700-1300 IU, respectively. Studies on micronutrient intake and cognition during adolescence are rare, and to the best of our knowledge, the study of Wang *et al.* [96] is the only efficacy trial of MMFs concerning academic performance and some psychosocial competencies of adolescents. In this study, the intervention group were found to have significantly higher scores in several academic subjects ($P < 0.05$), including languages, mathematics, ethics, and physical performance at the end-line; their study also failed to assess fluid cognition. Further, none of the studies mentioned above examined the modifying effect of menarche on the efficacy of MMFs on micronutrient status or adolescent girls' cognition.

Table 1: A review of multiple micronutrient fortified foods in nutrition intervention programmes for adolescents

Study	Study population	The objective of the study	Design of study	Main findings and conclusions
Adams *et al.* (2017) [94]	6-11 yrs. school children in Bangladesh	To explore the acceptability and micronutrient impact of a Bangladesh Government supported school-based micronutrient fortification program for children attending rural primary schools in 10 disadvantaged sub-districts.	Pre-post test without control design with daily intake for a year.	Daily consumption of fortified biscuits by primary school children had a significant positive impact on mean levels of iron, folic acid, vitamin B_{12}, retinol and vitamin D after controlling for sex, baseline deficiency status, CRP, and H. pylori. Levels
Wang *et al.,* (2017) [96]	12-14 yrs. Chinese adolescent boys and girls (n=360)	To test the effects of multi-micronutrient fortified milk in Chinese students.	Using a cluster-randomized trial, participants received either micronutrient-fortified (n = 177) or unfortified (n = 183) milk daily for six months.	Vitamin B_2 deficiency and iron deficiency were less likely in the intervention group. The findings suggest that micronutrient-fortified milk may improve students' academic performance, motivation, and learning strategies.
Thankachan *et al.* (2013) [97]	6-12 yrs. Indian school children	To assess the efficacy of a multiple micronutrient-fortified drink in reducing iron deficiency, IDA, anaemia and improving micronutrient status among school children	A randomized, double-blind, placebo-controlled trial design where children were randomised to receive a multiple-micronutrient fortified drink or placebo 6 days/ week for a period of 8 weeks.	The multi micronutrient-fortified drink was efficacious in reducing the prevalence of ID, IDA, vitamin C and vitamin B_{12} deficiency and improved micronutrient status in schoolchildren.
Goyle and Prakash (2011) [98]	Indian girls aged 10-16 yrs. (n=46) studying in a government school in Jaipur city	To study the effect of micronutrient fortified biscuits on total serum proteins and vitamin A levels of adolescent girls	Pre-test -post-test without control and girls, received biscuits containing 11.4g of protein and fortified with 600 µg vitamin A, 30 mg iron, 100 µg folic acid, 40 mg vitamin C and 150 µg iodine or unfortified biscuits for all working days (5 days) in a 4-month period.	Fortified biscuit consumption improved adequate protein status from 93.5% to 97.8%. Furthermore, normal vitamin A status increased from 56.1% to 73.2% and decreased the percentage of adolescent girls in the low vitamin A status category from 41.5% to 26.8%.

table continues

Study	Study population	The objective of the study	Design of study	Main findings and conclusions
Goyle and Prakash (2010) [95]	Indian girls aged 10-16 yrs. (n=46) studying in a government school in Jaipur city	To study the effect of supplementation of micronutrient fortified biscuits on Hb and serum iron levels	Pre-test -post-test without control and girls, received biscuits containing 11.4g of protein and fortified with 600 µg vitamin A, 30 mg iron, 100 µg folic acid, 40 mg vitamin C and 150 µg iodine or unfortified biscuits for all working days (5 days) in 4 months.	Fortified biscuits consumption increased the percentage of adolescent girls in the ''normal'' category of anaemia from 4.3% to 13.0% and more than the two-fold decrease in the ''moderate'' category of anaemia from 67.4% to 28.3%. Moreover, "normal serum iron levels" increased from 21.7% to 93.5% after intervention by serum iron levels
Hyder *et al*. (2007) [99]	Apparently, healthy adolescent girls aged 9-18 yrs. (n=1125) in rural Bangladesh	The objective of this study was to test the effect of a multiple-micronutrient-fortified beverage on Hb level, micronutrient status, and growth among adolescent girls in rural Bangladesh	12 months, 6 days/weekly: allocated to either a fortified or non-fortified beverage of similar taste and appearance. The beverage was provided at schools. The beverage was fortified with 7mg Fe, 389RE vitamin A, 75µg iodine, 7.5 mg zinc, 120 mg vitamin C, 0.91mg riboflavin, 120 µg folic acid, 1.0 µg B_{12} , 1.0mg B_6 , 10mg vitamin E and 5.0mg niacin	The fortified beverage increased the Hb, serum ferritin, and retinol concentrations at 6 months. Adolescent girls in the non-fortified beverage group were more likely to suffer from anaemia and low serum retinol concentrations. Consuming the beverage for an additional 6 months did not further improve the Hb status, but the serum ferritin level continued to increase.

Rationale and Objectives

There is increasing evidence of all forms of malnutrition among adolescents in Ghana, including stunting, thinness, multiple-micronutrient deficiencies, particularly IDA, and dietary inadequacies, particularly in rural contexts. Adolescent girls in LMICs are a critical target group for nutrition interventions due to their burden of micronutrient deficiencies, the risk of an early pregnancy, and the risk of intergenerational transfer of dis(advantages). Interest in adolescents' health is only recent; until 2016, the focus of nutrition policy in Ghana was primarily on IYC, pregnant and lactating women, and women of reproductive age. However, a weak understanding of the SCE determinants, trend-over-time and contextual correlates of undernutrition and sex-specific analysis delays progress in the design of targeted policies and programmes that can improve adolescents' nutrition holistically. Food fortification could be a useful strategy, but limited knowledge of such interventions' timing, concerning menarche and efficacy, especially in resource-poor settings, delays progress.

This thesis contributes to these knowledge gaps and aims to examine the interrelations between nutritional, social, and economic trajectories when optimising female adolescents' nutrition for better health, education, family formation, and labour participation in Ghana.

The primary objective of the thesis was:

1. To assess (a) the effect of consuming multiple-micronutrient fortified biscuits (MMB) compared to unfortified biscuits (UB) 5 days weekly for 26-weeks on the micronutrient status of adolescent girls in Ghana, (b) the effect of the MMB compared to UB on changes in growth, cognitive and academic performance of the adolescent girls and (c) whether timing (before or after menarche) of the intervention modifies the observed effects.

The secondary objectives were:

1. To identify the socio-cultural and economic determinants and consequences of adolescent undernutrition in low and lower-middle-income countries.
2. To determine the severity of anaemia and contextual factors associated with anaemia and Hb status among adolescents in Ghana.
3. To examine the trend in adolescent girls' nutritional status in Ghana and the factors associated with their nutritional status over time.
4. To compare the risk and correlates of malnutrition and cardiovascular risk among adolescent boys and girls in Ghana.

Outline of the Thesis

Chapter 2 is a systematic literature review to identify the SCE determinants of undernutrition and micronutrient deficiencies among adolescents in lower-middle-income countries. In the review, we identify factors at the individual, household, and broader community level, influencing adolescents' nutrition and health in lower-middle-income countries. We further review the available evidence on the consequences of undernutrition and micronutrient deficiencies during adolescence. **In Chapter 3**, we undertake secondary analysis of data from an impact evaluation of the Ghana School Feeding Programme (GSFP) [100] to determine the severity of anaemia and the contextual factors associated with Hb status and anaemia among rural school-aged children and adolescents. The severity of anaemia from this analysis informs the design of our primary (intervention) study. In **Chapter 4**, we analyse the 2003-2014 GDHS data to map the trends-over-time and the factors associated with malnutrition among adolescent girls in Ghana. The 2014 GDHS, which includes data on anthropometry and blood pressure for both adolescent boys and girls aged 15-19 years, is analysed to compare the prevalence and correlates of malnutrition and pre-hypertension/hypertension among adolescent boys and girls (**Chapter 5**). **Chapter 6** describes the implementation of our primary intervention study, *Ten2Twenty-Ghana* which evaluates the efficacy of MMB compared to UB on micronutrient status, height, cognitive performance of the adolescent girls, and the effect of the intervention timing (before or after menarche). Finally, in **Chapter 7**, we present and discuss the main findings of all the chapters in this thesis. The chapter also includes policy implications based on our findings and recommendations for future research. Figure 2 depicts the focus of each chapter 2-6 in this thesis.

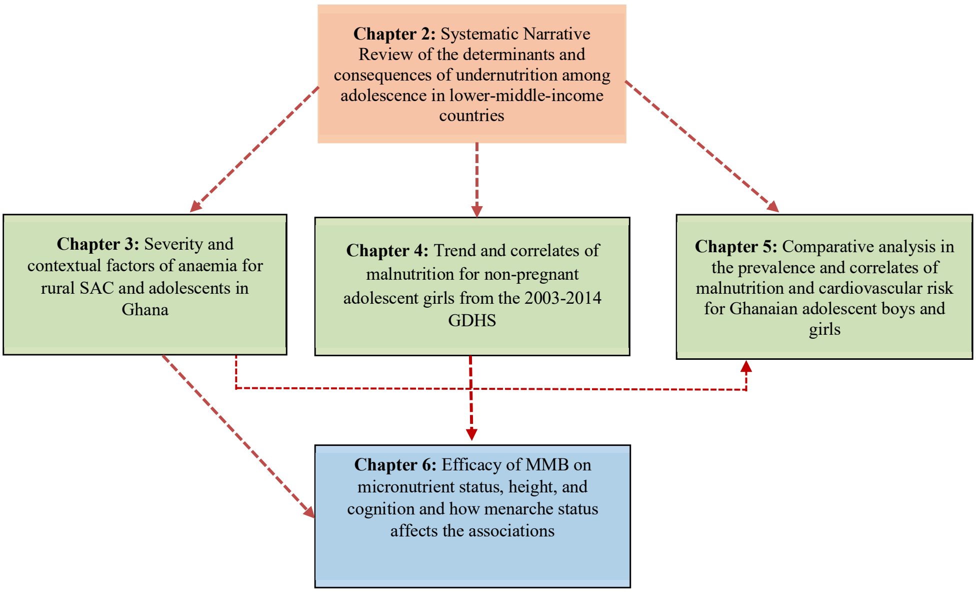
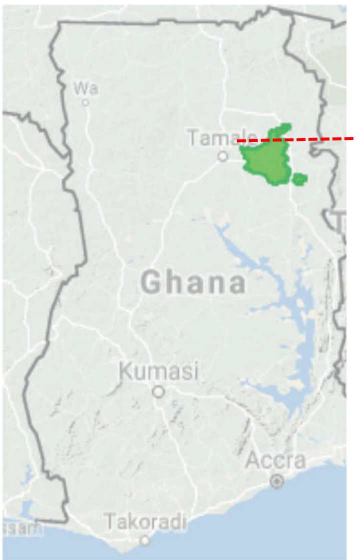


Figure 2: ***An overview of the focus of each chapter in this thesis***; SAC, school-aged children; GDHS, Ghana demographic and health survey; MMB, Multiple-micronutrient fortified foods

Study Setting

In the study evaluating the impact of the GSFP (Chapter 3), adolescent boys and girls were randomly selected from 58 districts in Ghana [100]. The GDHS data, used for Chapter 4 and Chapter 5, is national representative data, available from the DHS MEASURE website *(https://dhsprogram.com/)*. The GDHS included rural and urban areas throughout Ghana, selected using a multistage random sampling approach [55, 101]. The primary study (reported in Chapters 6) was conducted in the Mion District, in North-Eastern Ghana, located in the Northern Region of Ghana between Latitude 90 – 35” North, 00 – 30” West and 00 – 15” East. A total of 19 primary schools in 14 communities across the district were selected for the study. In selecting schools, we obtained data on student enrolment in the district from the Ghana Education Service. The schools were then ranked in descending order (largest to smallest) of girl-child enrolment. As the district is mainly rural, all peri-urban primary schools, also the largest (*n*=4), were first selected and the larger rural primary schools (*n*=15) were then selected for screening until our sample requirement for the survey (n=1040) was met. ***Figure 3a*** is a map of Ghana illustrating the location of Mion district while ***Figure 3b*** illustrates the locations of the communities where the primary study was conducted.



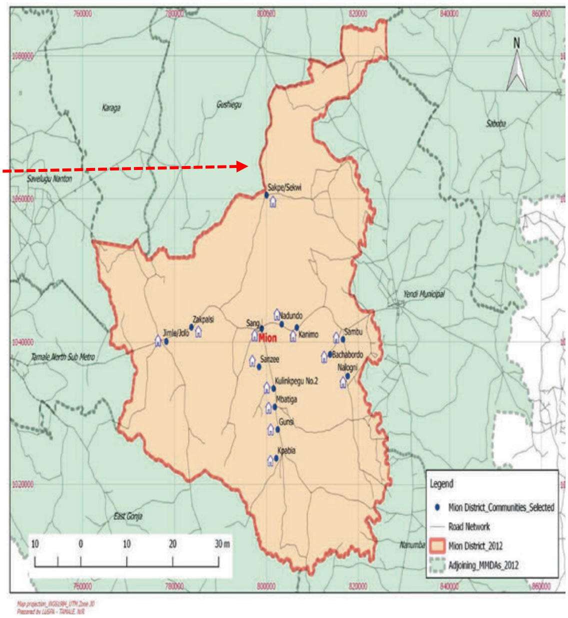


Figure 3a: Map of Ghana with the location of Mion District

Figure 3b: Map of Mion district, Ghana with the communities included in the Ten2Twenty-Ghana study

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Chapter 2

Sociocultural and economic determinants and consequences of adolescent undernutrition and micronutrient deficiencies in LLMICs: a systematic narrative review

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Ann NY Acad Sci. 2018;1416: 117-139.
https://doi.org/10.1111/nyas.13670

Abstract

Adolescent undernutrition is a persisting public health problem in Low and Lower-Middle Income Countries (LLMICs). Nutritional trajectories are complexly interrelated with socio-cultural and economic trajectories. However, a synthesis of the socio-cultural and economic determinants or consequences of undernutrition in adolescents is lacking. We undertook a narrative review of published literature to provide a narrative overview of the socio-cultural and economic determinants and consequences associated with undernutrition among adolescents in LLMICs. We identified 98 articles from PubMed, SCOPUS and CAB-Abstracts on determinants and consequences of undernutrition as defined by stunting, underweight, thinness and micronutrient deficiencies. At the individual level, significant determinants included age, sex, birth order, religion, ethnicity, educational and literacy level, working and marital status. At the household level, parental education and occupation, household size and composition, income, socio-economic status, and resources were associated with undernutrition. Only a few determinants at the community/environmental level, including residence, sanitation, school type, and seasonality were identified. The consequences of adolescent undernutrition were mostly related to education and cognition. This review underscores the importance of the broad range of context-specific socio-cultural and economic factors at several levels that influence adolescent nutritional status and shows that further research on socio-cultural and economic consequences of undernutrition is needed.

Introduction

The world faces the largest cohort of adolescents, aged between 10 and 19 years old, ever [1, 2]. Around 90% of these adolescents live in Low- and Middle-Income Countries (LMICs). As a result of this 'youth bulge,' LMICs are faced with the question of how to harness this demographic dividend, which occurs during a window of opportunity created by a shift to fewer dependent people relative to working-age individuals [3]. Adolescents are the future workforce, leaders, and bearers of the next generation. Improvement of their health and developmental outcomes through nutrition is currently seen as (another) second window of opportunity for "catch-up" growth [4]. Investing in adolescent nutrition not only improves children's health and developmental outcomes, but also those of their offspring, and consequently entire societies [5]. However, development and research programs in LMICs often focus on the first 1000 days, the first five years, or on women in their reproductive age since interventions in these life stages are widely believed to break intergenerational cycles of malnutrition, improving birth and pregnancy outcomes [6, 7].

The life stage of adolescence is characterized by rapid biological growth, in which the social, economic, and cultural context of adolescents is decisive [2, 8, 9]. Many children in LMICs enter adolescence thin, stunted, anaemic and/or micronutrient deficient [10]. Throughout adolescence, nutrition is complexly interrelated with social, cultural, and economic trajectories including education, family formation (e.g., marriage, fertility) and labour participation [11]; disadvantages in these trajectories may influence nutritional status or the other way around. Whilst the attention is shifting towards adolescent nutrition in international development and research [9], evidence concerning socio-cultural and economic characteristics in relation to nutrition throughout adolescence is dispersed, highlighting a research gap in this area.

Additionally, there is a dearth of research on the socio-cultural and economic consequences of undernutrition during adolescence, although the effects of undernutrition during childhood on adult outcomes are well known. For instance, the relations between early childhood nutrition and cognition, learning, or educational achievements [12-15], as well as between early childhood nutrition and economic productivity, wages, marriage, and fertility [16, 17] are well established. But there is a paucity of data on the effects of poor nutrition during adolescents' transitions into adulthood, and how their nutritional status is affected by their everyday life context.

To our knowledge, no reviews exist that summarize the socio-cultural and economic determinants and consequences of undernutrition during adolescence in LMICs. Nonetheless, Viner *et al.* [18] reviewed the social determinants of health in adolescents but did not specifically focus on nutrition or LMICs. Reviews including adolescent nutrition mostly focus on the determinants of overnutrition [19-23] or on the co-occurrence of stunting and overweight [22], which is particularly interesting considering the global nutrition transition [23]. A recent series of reviews on adolescent nutrition consider eating patterns and behavioural patterns during adolescence but do not discuss the "social contexts that directly or indirectly affect adolescent nutrition" in LMICs which may include structural factors at a broader societal level, but also at the level of households and communities [24-27]. Similarly, although some studies focused on the effects of iron deficiencies on cognitive development in children [28, 29], no reviews focus on the socio-cultural and economic determinants or outcomes of adolescents' micronutrient status. The focus of existing reviews on adolescents has mostly been on the effect of micronutrient supplementation [25, 30-32].

Considering the challenge to unlock the potential of adolescents through improved nutrition, a synthesis on what affects, and which effects poor nutrition has throughout adolescence in a particular context is essential to tackle this challenge. Especially in LMICs where adolescents lag in several life domains, such a comprehensive picture could further inform research and context-specific programs that aim to understand and improve the health and developmental outcomes of adolescents. With this review, we aim to fill the research gap by providing a narrative overview of the socio-cultural and economic determinants and consequences associated with protein-energy undernutrition and micronutrient undernutrition/deficiencies among adolescents in Low and Lower-Middle Income Countries (LLMICs). Specific research questions are: 1) what are the SCE determinants of undernutrition and indexes of nutritional status during adolescence in LLMICs; 2) what are the SCE determinants of micronutrient status and deficiencies during adolescence in LLMICs; 3) what are the SCE consequences of undernutrition and micronutrient deficiencies during adolescence in LLMICs? We focus on LLMICs because undernutrition remains the greatest concern and rates are only slowly declining [23]; for instance, more than a quarter of adolescent girls are reported to be underweight in 11 LLMICs and anaemia is a severe public health problem among adolescent girls in 15 out of 21 LLMICs [33]. Such a review may help to understand and improve efforts directed towards optimizing adolescent health and nutrition.

Methods

Undernutrition encompasses both micronutrient deficiencies and macronutrient or protein-energy malnutrition. However, for this review, the term undernutrition refers to stunting, underweight and thinness whilst nutritional status index(es) refers to the Z-scores of height-for-age (HA), weight-for-age (WA), weight-for-height (WH) and BMI-for-age (BA). Micronutrient status and related deficiencies included in this review are vitamin A, vitamin C, vitamin D, vitamin B_{12} iron, haemoglobin status, anaemia, iodine, zinc, folic acid, and calcium; these were selected based on evidence of the common micronutrient deficiencies during adolescence [34].

Search method

A comprehensive search strategy was developed by using a variety of search terms for retrieving relevant literature. Two separate searches were performed between April and May 2017, one focused on undernutrition, the other on micronutrient deficiencies. Search queries were built on five layers with relevant search terms. The first layer referred to 'adolescence', as defined by the WHO (10-19 years) [4]. The second layer included LLMICs in South and East Asia, Latin America, and Sub-Saharan Africa (66 countries) derived from the World Bank list of economies [35]. The third layer included socio-cultural and economic aspects related to trajectories of labour participation, family formation and education (e.g., marriage, cognitive skills, literacy, time use, household structure, and gender). The fourth layer referred to 'associations' (e.g., determinants, factors, outcomes, consequences, interrelations) since we aimed for studies that specifically focused on associations instead of prevalence rates only. The final layer differed for the two searches. In the 'undernutrition' search, terms related to undernutrition (e.g., undernutrition, underweight, weight-for-age, stunting, height-for-age, thinness, weight-for-height and BMI-for-age) were used, whilst for the micronutrient deficiencies search, these terms were replaced by micronutrients and deficiencies including hidden hunger, (iron deficiencies) anaemia, iodine, folate, folic acid, vitamin A, $B_{12,}$ C, $D_{,}$ serum retinol, zinc, and calcium. Search queries were adapted to the requirements of the specific databases: PubMed, Scopus, and CAB Abstracts. Searches were limited to English/Dutch only and as from 1990 onwards. In Scopus, we applied limits on document type, and in PubMed, we used MeSH terms for nutrition and adolescence and limited the search to humans. In total, 2554 papers were found for undernutrition, whiles 685 papers were found for micronutrient deficiencies.

Screening protocol

After duplication removal, a total of 2788 papers were screened based on title and abstract. Quantitative empirical research and working papers were considered for inclusion when they showed associations between the variables of interest. Cohort and longitudinal, cross-sectional and intervention studies were considered for inclusion. Papers were excluded when they focused on diet associations with diseases or other issues (e.g., addictions, helminth infections, anorexia, diabetes, blood pressure), unhealthy adolescents or migrants, biochemical processes, lifestyle/behaviour (e.g., snacking, body image, physical activity), or prevalence only. Studies including a broader age range or just part of the 10-19 years range were excluded when there were no age-specific results (e.g., sample 6-12 should include specific data for 10-12 years). When a paper only reported differences between sexes without explanation or not considering any other variables, we rejected the paper. Qualitative research, methodology papers, review papers, editorials, and intervention studies without baseline information, were excluded. Although we included terms as overweight and obesity in the queries, studies focusing on overnutrition were considered only when they included undernutrition as well. The full-text screening was performed on a total of 248 papers, after which 141 studies were rejected based on the above-mentioned criteria, or when the authors were not able to retrieve the full texts after having requested the papers from authors or research organisations (*n*=18). Afterwards, a manual search was performed in which bibliographies of eligible papers and relevant reviews were screened using the same procedure described above. Furthermore, we asked an external researcher to screen and add to this final list, and we checked our own databases for relevant papers (*n*=20).

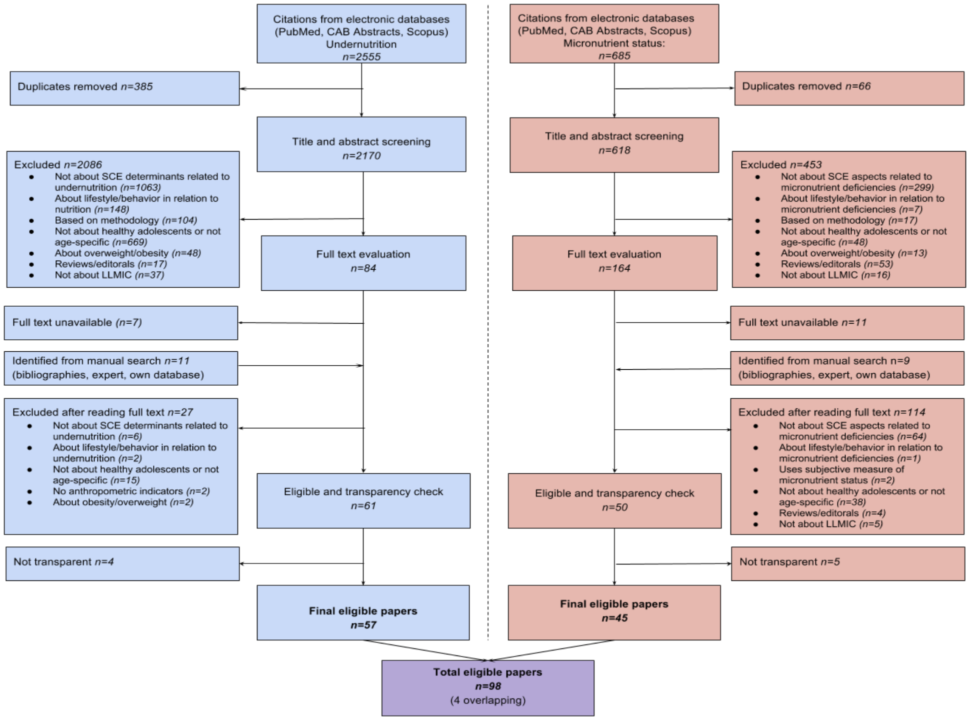

Figure 1. PRISMA flow diagram of the screening process, with undernutrition and micronutrient status combined

Transparency assessment

Finally, 111 papers underwent a transparency check in which they were graded against seven methodological criteria to assess interpretability: research aim or hypothesis, data collection methods, sampling plan and size, analysis method, conclusions and limitations were either available (score 2), partly available (score 1) or missing (score 0). Almost a third of the papers scored at least one zero, but nine papers were excluded because they scored low (1 or 0) on multiple indicators. A total of 57 and 45 papers were included in this review for undernutrition and micronutrient deficiencies respectively. Figure 1 provides an overview of the screening process based on the PRISMA criteria [36].

Data extraction and analysis

Papers were fully read and coded deductively as well as inductively using Atlas Ti for the undernutrition part after which results were transferred to an Excel sheet. For the micronutrient part, data were extracted into an Excel sheet directly. We recorded information on study design, methods, analysis, outcome measures and all associations (significant and non-significant) between undernutrition/micronutrient deficiencies. Then, the two sheets were merged, and findings were cross-checked and discussed by the researchers. Missing data or contradictory data were corrected, and papers were assigned a specific code. Data was entered in four tables, the first including a general overview of characteristics for studies on determinants (Table S1a) and consequences (Table S1b) and focus of the final list of papers; this table also includes all SCE variables studied. Next, two tables were made in which all significant associations (positive/negative) were reported. Table 1 reports on the SCE determinants of undernutrition (categorical) and micronutrient deficiencies (categorical), whilst table 2 includes SCE determinants of nutritional status index (HAZ, BAZ, WAZ and WHZ) and micronutrient status (continuous). Within this categorization, determinants were categorized per level and clustered by domain (education, labour, household composition etc.). Herein, we departed from Bronfenbrenners' human ecological model [37] and Dahlgren and Whitehead's social determinants of health model [38] and acknowledge that an individuals' nutritional status is positioned within and influenced by a broader system of socio-cultural and economic contexts which are played out at several levels. Tables 3a and 3b report on the consequences of undernutrition/nutritional status index and micronutrient deficiencies/status.

Results

Due to the high heterogeneity of outcome measures, the diverse range of study methods, and the lack of transparent methodological descriptions, we could not conduct a meta-analysis. Hence, we focused on the breadth of the studies and synthesized the findings using a narrative approach. Starting with an overview of the papers, we then discuss findings for the two separate searches.

General characteristics

Our sample shows an increase in the number of papers on adolescent nutrition, with a rapid increase after 2008 and again 2013 which might reflect the increasing interest in adolescent undernutrition and micronutrient status, especially after the launches of the 2008 and 2013 Lancet series on maternal and child nutrition (see Figure 2). Most of the published articles in our sample on adolescent undernutrition and micronutrient status focus on both males and females (57.7%). However, research on adolescent females only (38.2%) has been of particular interest in comparison to males (4.1%). Most of the publications on undernutrition and micronutrient status of adolescents originate from India. Most of the publications (*n*=28) from sub-Saharan Africa focussed on undernutrition with less than a half of these publications focussing on adolescents' micronutrient status. We found only two studies originating from LLMICs in Latin America, both of which were on undernutrition. Most of the reviewed studies were cross-sectional in design. The fewer longitudinal studies we found (10.3%) studied mainly associations with adolescent undernutrition and nutrition status index, rather than micronutrient status.

Table 1. Determinants of adolescent undernutrition and micronutrient deficiencies

Association	UNDERNUTRITION						MICRONUTRIENT DEFICIENCIES													
	Stunting		Underweight		Thinness		Vit A		Vit D		Iron def		Anaemia		Iodine		Zinc		Folic	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
INDIVIDUAL LEVEL																				
Determinants																				
Sex																				
Female									m35		m14		m14 m39	m30	m31				m21	
Male	u47 u12 u36 u2 um3 u4 u31 um1 u16 u33		u49 u44 u43 u36 u2 um3 u33		u4 u42 u31 u6 u13 u16 u27 u48 u53								m22							
Age (Female/Male)	u49 m19 u51	um1	u49		u13	u42 u31 u15 u53			m23				m1 m30 m33						m21	
Female	u41	u25 u7			u41								m9-10 m12	m29 m38						
Male	u48												m22	m36						
Birth order	u38		u38		u6															
Ethnicity														m9 m11-12						
Religion (Muslim and Hindu versus Christian)	u36				u4									m9 m11-12, m26						
Marital status (Married vs. unmarried)													m9 m12							

table continues

Association	UNDERNUTRITION Stunting +	 -	 Underweight +	 -	 Thinness +	 -	MICRONUTRIENT DEFICIENCIES Vit A +	 -	 Vit D +	 -	 Iron def +	 -	 Anaemia +	 -	 Iodine +	 -	 Zinc +	 -	 Folic +	 -
Labour																				
Workload						u41														
Working status (Working versus not working)	u44	u4	um3			u4					um3		m19				um3		um3	
Education																				
Attendance		u40 u38																		
Drop-out												um3			m1					
Enrolment		um2 u20		um2	u13									m20						
Literacy level		u41													m26					
Educational level		u4				u7 u4									m9 m19 m25 m34					
No footwear														m38						
HOUSEHOLD LEVEL																				
Parental occupation		u44 u36		u44																
Maternal		u39		u36											um4 m19 m25					
Paternal		u40 um3 u31 um4 u39				um3 u46									m26 m39					
Parental education		u39 u48				u4 u53														
Maternal		u48 u11 u51 u4		u11 u52		u46			m37						m12					

table continues

Association	UNDERNUTRITION						MICRONUTRIENT DEFICIENCIES													
	Stunting		**Underweight**		**Thinness**		**Vit A**		**Vit D**		**Iron def**		**Anaemia**		**Iodine**		**Zinc**		**Folic**	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Paternal		u33 u4		u33		um3 u46									m39					
SES		u16 u48 m19		u2		u7 u16 u25									m9-m10 m12 m21					
Income		um3 u4 um1 u39		u15		u50 u4 u19 u27 um3			um1				m22		m22 m28					
Resources (Land, cattle, latrine, no. of living rooms, rented versus own, housing type, access to piped water)		u44 um3 u33 um4 u49		u44 u33			um3 u53 m30									m26				
Household composition																				
No. of siblings	u36 u39		u36 u38			u38														
No. of servants		u40																		
No. of wives/polygamy	u36 u49																			
No. of sisters/women	u40																			
Living with guardian						u6														
Size	u11 u4 u31 m33 u39		u11 u49			u51 u4									m39					

table continues

Association	UNDERNUTRITION Stunting +	-	Underweight +	-	Thinness +	-	MICRONUTRIENT DEFICIENCIES Vit A +	-	Vit D +	-	Iron def +	-	Anaemia +	-	Iodine +	-	Zinc +	-	Folic +	-
Type of family (joint versus nuclear)	u44					u7										m19				
Food insecurity	u32 u51		u51			u51 u13									um4					
COMMUNITY LEVEL																				
WASH		u51		m30												m19 m30				
Residence																				
Rural (versus urban)	u36 u30 u4 u31 um4		u43 u36 u30		u30 u4 u15								m23		um4	m8-9 m12				
Geographical zone																m9 m20				
School type (Public or poor versus private or rich)	u50 u36 u48 u4		u50 u36		u50										m36					
Scheduled caste (Dalit)	u49																			
Environmental																				
Season (other versus summer)												m23								
Before rain (versus after rains)															m29					
Harvest (versus hunger)									m24					m24	m24	m24				

+, positive association; -, negative association; u, reference for undernutrition; m, reference for micronutrient deficiency; um, both undernutrition and micronutrient deficiency; vit A, vitamin A; vit D, vitamin D

Table 2. Determinants of adolescent nutritional status index (z-score) and micronutrient status

	NUTRITIONAL INDEX						MICRONUTRIENT STATUS															
	H/A		W/A		W/H (BMI/A)		Vit A		Vit C		Vit D		Iron		Hb		Iodine		Calcium		Folic	
Association	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
INDIVIDUAL LEVEL																						
Determinants																						
Sex																						
Female	m17	u28 u35	u52		u52											m17m21						m21
Male	m31	u4 u5 u47		u47		u1 u4		m31			m35											
Age (Female/ Male)	m17m31	u1 u47 um4		u1 u47	u26	u1		m16	m16		m35	m27			m17	m26						
Female		u3 u29 u41	u52	u9 u29	u1 u3 u52	u41	m2								m11							
Male		u26		u26	u1 u26 u4		m4								m4							
Birth order																m7						
Ethnicity	u10 u38																					
Religion (Muslim and Hindu versus Christian)		u26		u26																		
Labour																						
Working status (work versus not working)	u4				u4																	
Time spent in heavy work (carrying heavy goods)	u53																					
Education																						
Attendance	u21 u22 u34 u37		u34u37		u34 u37																	

table continues

	NUTRITIONAL INDEX						MICRONUTRIENT STATUS															
	H/A		W/A		W/H (BMI/A)		Vit A		Vit C		Vit D		Iron		Hb		Iodine		Calcium		Folic	
Association	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Literacy level															m11							
Educational level	u4 u24		u24		u4							m27									m40	
Migration to urban area	u22				u22																	
HOUSEHOLD LEVEL																						
Parental occupation																						
Maternal															m4							
Paternal															m4							
Parental education					u4																	
Maternal	u3 u17		u17		u3 u17																	
Paternal	u3		u3 u33				m4; m5								m34							
Parental literacy																						
Maternal															m11							
Paternal															m11							
SES	u24 u29 u33 u34 m19		u22u24u29u33u34		u34							m32							m32		m40	
Income	u4 u26		u26		u4 u27		m5		m22				m22		m11 m22				m22			
Per capita food expenditure	u17		u17		u17		m4-5															
Resources (Land, cattle, latrine, no. of living rooms, rented versus own, housing type, access to piped water, and electricity)	u33 u35				u35										m3 m11							
Household composition																						

table continues

2

	NUTRITIONAL INDEX						**MICRONUTRIENT STATUS**															
	H/A		**W/A**		**W/H (BMI/A)**		**Vit A**		**Vit C**		**Vit D**		**Iron**		**Hb**		**Iodine**		**Calcium**		**Folic**	
Association	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**
No. of siblings		u33																				
No. of servants																						
No. of wives/polygamy	u29		u29																			
Size		u4				u4		m16		m16				m41		m7						m40
Migration			u9		u9																	
Food insecurity		u8																				
COMMUNITY LEVEL																						
Residence																						
Rural (versus urban)		u4 u29 u30 u43		u30 u43		u4 u30 u34												m24			m24	
Hills (versus lowland)		u52																				
Slum (versus non-slum)		u23				u23																
Geographical zone													m24		m11		m6					
School type (public or poor versus private or rich)		u36; u4		u43; u36																		
Scheduled caste (Dalit)																m11						
Season																						
Season (other versus summer)												m27										
Harvest (versus hunger)								m24								m24		m24				m24

+, positive association; -, negative association; H/A, height-for-age; W/A, weight-for-age; W/H(BMI/A), weight-for-height (body-mass-index-for-age); u, reference for undernutrition; m, reference for micronutrient deficiency; um, both undernutrition and micronutrient status; vit A, vitamin A; vit C, vitamin C; vit D, vitamin D; Hb, Haemoglobin

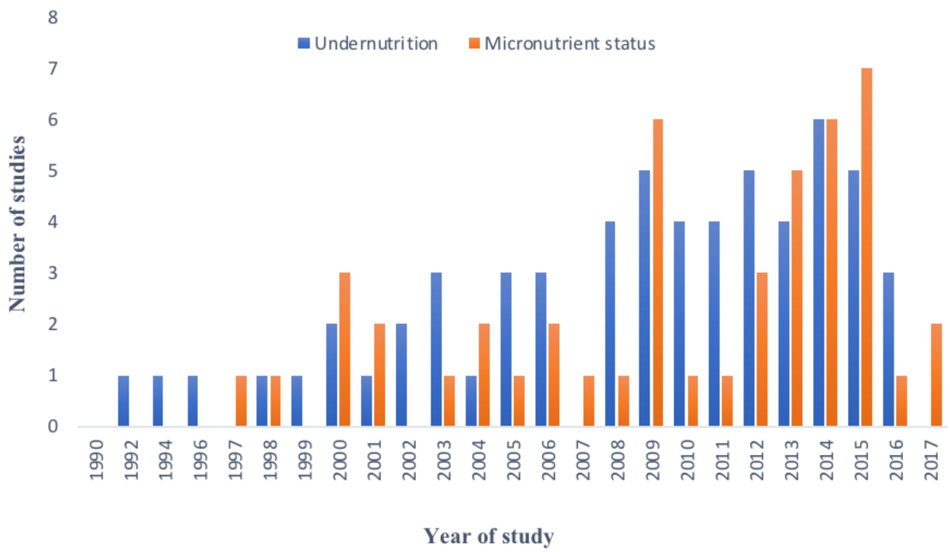

Figure 2. Number of published studies on the SCE determinants of undernutrition and micronutrient status of adolescents in LLMICs between 1990 and 2017

Determinants of undernutrition and nutritional status indexes

In this section, the results on socio-cultural and economic determinants of adolescent undernutrition and nutritional status indexes are summarized per level. Acknowledging the different levels of data analysis, we differentiated between studies focusing on the relation between determinants of undernutrition as indicated by stunting, wasting and thinness (categorical), and between determinants of nutritional status index (continuous) as indicated by height for age (HAZ), weight for age (WAZ), and weight for height (WHZ) and BMI-for-age (BA), in which the latter two were grouped together under WHZ. Although in all studies height and weight measurements were taken, more studies first classified the study population into categories of nutritional status using height and weight indexes and then assessed the associations with SCE aspects, rather than directly analysing growth index in relation to determinants (36 vs. 31 respectively). Stunting and HA were more often used in relation to variables than other indicators or indexes. For the majority of the studies, the WHO/NCHS reference standards were used in which Z-score cut-off points of <-2 SD (standard deviation) were used to classify measurements into undernutrition. Other classifications used were BMI percentiles (WHO), sometimes converted to chronic energy deficiency (CED), or US-CDC reference standards. In general, determinants and consequences significantly associated with undernutrition and nutritional status indexes can be found mostly at the adolescents' individual and household levels (see Table 1).

Individual-level

At the adolescents' individual or micro level, several demographic determinants were identified as risk factors or predictors of stunting, underweight and thinness. Mixed results were found regarding sex, with many studies reporting non-significant differences. Interestingly, studies reporting significant associations showed that boys were often worse in terms of stunting and HAZ [39-49]; underweight and WAZ [39, 41-43, 48, 50-52]; and thinness or WHZ [39, 44, 45, 47, 53-58]. Only three studies in Ghana, Ethiopia and Cambodia found that height or weight for age was lower for girls when compared with boys [59-61]. Age was often reported to influence undernutrition. Four studies found that stunting increased with age in general, and particularly for boys [50, 62-64]. The opposite was found for thinness which decreased with age in four studies [45, 54, 58, 65] compared with only one study [56] which showed an increase. When looking at nutritional status indexes, studies showed similar, but also more varied results. For instance, whilst HA decreased significantly with age during adolescence, for both boys and girls [39, 53, 66-70], here more studies [67, 68, 70] reported that HA and WA in girls decreased more compared to boys. Birth order was only in one study associated with underweight and stunting [71]. Religion was in three studies [41, 44, 69] associated with stunting, thinness and decreased HA and WA, whilst ethnicity was only associated with HAZ in two studies [71, 72]. Migration from a rural to an urban area in Senegal was positively associated with HAZ and WHZ [73]. Finally, two studies reported the negative effects of poor personal hygiene practices on stunting and underweight [63, 74].

Regarding the labour trajectory, generally, working and especially workload, was associated with undernutrition [43, 51]. However, a study in Nepal showed that HA was positively associated with time spent in heavy work [75], and an Ethiopian study found that working was positively associated with HA and WHZ [44].

Education is often mentioned in relation to nutritional status. School attendance and enrolment, educational and literacy levels were in general negatively associated with stunting, underweight and thinness [44, 70, 71, 76-79] and positively with HA, WA and WHZ [44, 73, 77, 80-83]. One Tanzanian study, however, found that school non-enrolment was associated with increased thinness explaining this by the fact that parents often perceived thin adolescents as physically not being ready to attend school [56].

Household-level

At the household level, factors related to parental characteristics, household economic status and resources, household composition and family type were often found to be associated with undernutrition. Generally, parental occupation was associated with lower stunting, underweight and thinness [41, 43, 45, 51, 66, 76, 84], but not with nutritional status indexes. Interestingly, paternal occupation was more often (n=6) associated with stunting and thinness, when compared to maternal occupation which was only in two cases protective against thinness and stunting [41, 84].

Parental education was in general associated with better nutritional status; however, in contrast to parental occupation, here especially maternal education was negatively associated with stunting and underweight [44, 62, 63, 85] and positively with HAZ, WAZ and WHZ [48, 86, 87].

Within the economic domain, household economic status and socio-economic status (SES) were commonly associated with nutritional status. Household and per capita income were negatively associated with stunting, underweight and thinness [43, 44, 46, 57, 65, 84, 88, 89] and to a lesser extent positively with HAZ, WAZ and WHZ [44, 57, 69, 86]. One study showed that per capita food expenditure was positively associated with all nutritional status indexes [86]. Likewise, SES, defined by a wide variety of indicators, was in 15 cases negatively associated with undernutrition or positively with nutritional status indexes [42, 47, 48, 62, 64, 68, 73, 79, 81, 82, 90]. Household resources, including land holdings, possession of cattle, the number of living rooms, rented versus owned home, housing type were negatively associated with undernutrition indicators [43, 48, 51, 58, 66, 74] or, to a lesser extent, positively with HA and WHZ [48, 60]. The lack of latrines (leading to open-air defecation) and having a hand pump (instead of running water) was associated with BAZ [60, 74].

For household composition in relation to adolescent nutrition, several indicators were used. Significant associations were to a greater extent found for indicators of undernutrition than nutritional status indexes. Generally, household size was positively associated with undernutrition [44, 45, 49, 50, 74, 84, 85], but only once with status indexes [44]. The number of siblings was in four studies positively associated with undernutrition [41, 43, 71, 84]. This was more the case for girls, or when there were more girls in a household [76]. Only one study found a similar association with HAZ [48]. Polygamy, or the number of wives in a household, was positively associated with stunting [41, 62], whilst a study in Mali showed how this was negatively associated with HAZ and WAZ [68]. Living with guardians instead of own parents was associated with thinness only in one study [55], and an increasing number of servants in a household was associated with decreased prevalence of stunting [76]. Furthermore, two studies showed that adolescents living in joint families were more likely to be stunted [51] or thin [79]. Similar to migration at the individual level, adolescents living in households that migrated from a rural to an urban area in Senegal had higher WHZ and WAZ than those who did not migrate [91]. Finally, food insecurity at the household level negatively impacted adolescent undernutrition [63, 92]. One study from Ethiopia showed that only in girls decreased HAZ was significantly associated with food insecurity [93].

Community-level

We found only a few determinants that focused on community-level factors. In general, rural residence, living in the hills versus lowlands, or living in slum areas were associated with undernutrition and status indexes [41, 44, 45, 52, 65, 66, 75, 82, 94, 95]. Furthermore, school type was associated with undernutrition, with adolescents attending public, instead of private schools, showing higher rates of undernutrition or poor nutrition [41, 44, 52, 62, 88]. Living in a scheduled caste community was in one Indian study associated with stunting [50].

Determinants of micronutrient status and deficiencies

In this section, the results on SCE determinants of adolescent micronutrient status and deficiencies are outlined. Generally, most of the reviewed studies on micronutrient status examined haemoglobin (Hb) status (*n*=40) and iron status (*n*=13). The determinants of vitamin A status were examined by 10 articles whilst those of vitamin D status were examined by five articles. Few articles (≤ 5) reported on the determinants of folate, zinc, calcium, iodine, vitamin C and vitamin B_{12} status. The statistical analysis procedure was commonly on the determinants of micronutrient deficiencies with logistic regression (*n*=21) or simply bivariate analysis with chi-square (*n*=8). Only two studies used a combination of both categorical (deficiencies) and continuous (status) outcome methods in the statistical analyses.

Individual-level

Like undernutrition, mixed results were found regarding sex, with many studies reporting non-significant differences. Nevertheless, four studies showed that female sex was associated with a higher risk of anaemia [96, 97], iron deficiency anaemia (IDA) [96] and lower Hb levels [64, 98]. Similarly, in India, when compared to adolescent boys, adolescent girls were more likely to be folate deficient [98] and vitamin D deficient [99]. Another study in Cambodia reported female sex as a risk factor for iodine deficiency, but male adolescents were in this study reported to have a lower retinol-binding protein (RBP) concentration and were more likely to have a marginal vitamin A status compared to their female peers [61]. Surprisingly, in a multi-country survey in Lakeside Tanzania, Mozambique, Ghana, Malawi, and Indonesia [100], 12-14 years adolescent boys were more likely to be anaemic than girls, and a study in Ethiopia also reported female sex to be protective of anaemia [74].

Generally, increasing age was found to be a risk factor for anaemia [74, 101], vitamin D deficiency [102], and folate deficiency [98] among male and female adolescents. Likewise, studies in Nigeria [103], India [104], and South Korea [105] found increasing age to be inversely associated with plasma retinol, Hb and serum 25(OH)D respectively for both sexes. Among adolescent girls, four Indian studies reported increasing age as a determinant of anaemia [106-109]. However, increasing age was in Kenya [110] and Ethiopia [111] protective of anaemia for adolescent girls, whilst in Indonesia [112] protective for adolescent boys. Also, serum vitamin C, serum 25 (OH)D and Hb status were in Nigeria [103], India [99], and the Philippines [64] respectively positively associated with increasing age. Among Bangladeshi adolescent girls [113] and boys [114] age was positively associated with serum retinol as well as Hb status. Except in one study on Hb status from Nigeria, birth order was seemingly not an important determinant of poor micronutrient status [115].

Only four studies examined the effect of working status or workload on micronutrient status with two of the studies concluding that working girls had a higher risk of anaemia, iron, and zinc deficiency, compared to their non-working peers [43, 116]. Similarly, only a few (n=5) of the reviewed studies examined the effect of marital status on micronutrient status and this was generally on anaemia. Two studies concluded that being married was related to a higher risk of anaemia for adolescent girls [107, 109].

Late school enrolment [100] and dropping out of school [43] were seemingly risk factors for anaemia and iron deficiency (ID) respectively. However, Ahankari *et al.* found dropping out of school to be protective of anaemia among Indian adolescent girls [106]. Adolescent literacy and a higher educational level were generally protective of anaemia [104, 107, 116-118]. Similarly, literacy [119] and a higher educational [120] level was positively associated with Hb and folate status respectively. Nevertheless, educational level was once found to be inversely associated with serum 25(OH)D among South Korean adolescents [105].

In addition, there were differences in the risk of anaemia by religion and/or caste in India [107, 109, 119]. Personal hygiene was in two studies found to be protective of anaemia in India and Ethiopia [74, 116]. Lastly, one study in Ethiopia found that footwear was protective of anaemia among adolescent girls [111].

Household-level

At the household level, a higher paternal education level was associated with a lower risk of anaemia in Ethiopia [97], higher Hb status in India [118] as well as a higher serum retinol status in Bangladeshi adolescents [114, 121]. Equally, a higher maternal education level was reportedly associated with a lower risk of anaemia [109] and vitamin A deficiency (VAD) [122] in India and Indonesia respectively. Paternal and maternal literacy were also found to positively predict a higher Hb status among Indian female adolescents [119]. Furthermore, a better maternal [66, 116, 117] and paternal [97, 104] occupation status was both protective of anaemia among Indian and Ethiopian adolescents. Likewise, paternal, and maternal occupational status was positively associated with Hb status in Bangladeshi adolescents [114].

Additionally, a higher SES was protective of anaemia [107-109, 116] and positively associated with serum calcium [123] and folate [120] status, yet inversely associated with a higher serum 25[OH]D [123]. Generally, a higher family income was associated with a lower risk of anaemia [124, 125], ID [124] and VAD [46]. Likewise, family income was positively associated with serum retinol [121], serum ferritin [124], and Hb status [119, 124]. Dietary intake of Ca and vitamin C were also reportedly higher with increasing household income level among South Korean adolescent girls [102]. A unit increase in per capita expenditure on food was positively associated with a higher serum retinol among adolescent boys [114] and girls [121] in Bangladesh.

Overall, larger family size was a risk factor for anaemia [97] and inversely associated with serum retinol and vitamin C status [103] besides serum ferritin [126], Hb [115], and folate status [120]. Bangladeshi adolescents living in their parent's houses [127], as well as Indian adolescents living in a household with electricity [119], were found to have a higher Hb status. Moreover, adolescent girls living in households with latrines were at a lower risk of anaemia than those in households without latrines [104]. Remarkably, the prevalence of anaemia was in one study significantly higher among adolescents living in nuclear families compared to their peers in extended or joint families; this was contrary to the association found between family type and stunting/thinness [116]. Finally, food insecurity was in one case reported to be associated with anaemia [66].

Community-level

Surprisingly, residing in a rural community compared to an urban community was protective of anaemia in Uganda and India [107, 109, 128], as well as vitamin D deficiency [102] in South Korea. Only one study found that Ethiopian girls living in rural areas had higher rates of anaemia [66]. Additionally, rural Mozambican adolescent girls had a higher serum folate status when compared to their peers from urban areas; however, rural girls were in this study more at risk of iodine deficiency [129]. Significant variations by geographical location in the prevalence of anaemia, iodine deficiency, serum ferritin, Hb and urinary iodine status were also observed [100, 107, 119, 129, 130].

Among South Korean adolescents, seasons other than summer were associated with a higher risk of vitamin D deficiency [102] or a lower serum 25 [OH]D level [105]. Equally, the risk of anaemia was significantly higher before the rainy season in Kenya [110], while the harvest season in Mozambique was associated with a higher risk of VAD and folate deficiency in all areas (city, coastal and inland) [129]. Lastly, significant variations by season in the prevalence of anaemia and ID were found in Mozambique but these variations were dependent on the residing area [129].

Consequences of undernutrition and poor micronutrient status

We found only 12 papers that reported on the socio-cultural and economic consequences of adolescent undernutrition [53, 61, 80, 82, 83, 111, 116, 131-135]. Most of these studies focused on educational outcomes (Table 3a & b). A study by Dercon and Sanchez [132] showed how non-cognitive skills such as self-efficacy, educational aspirations, and self-esteem, are positively associated with HAZ, using data from the Young Lives multi-country cohort study. Data from the same study [131] and three other studies [61, 80, 135] associated cognitive skills negatively with stunting. School performance (e.g., grade attainment) was worse when adolescents had a low HAZ (stunted) [53, 80, 131, 135], low WAZ (underweight) [53], and low WHZ (thin) [53, 82]. School attendance improved with a higher HAZ [80, 83] and WAZ [83]. At the micronutrient level, two studies found an inverse association between anaemia and grade attainment [111, 116], as well as iron deficiency anaemia and cognitive skills such as Raven's Coloured Progressive Matrices among Cambodian male adolescents [61]. Another study provided significant evidence that memory and scores on Raven's progressive matrices test (intelligence) were positively associated with zinc level, whilst reaction time was negatively associated with zinc levels [134]. Finally, a somewhat older study from Bangladesh associated age at first marriage with weight, showing that greater body weight was associated with earlier age of marriage, even when this effect was adjusted for height, age at menarche and socioeconomic factors. The author suggests that "better-nourished women are more attractive mates owing to their physical appearance and/or better health" [133] (p.94).

Table 3: Consequences of adolescent undernutrition (A) and micronutrient status (B)

A

Outcomes	**Nutritional status index**						**Micronutrient status**	
	HAZ		**WAZ**		**WHZ**		**Zinc**	
	+	**-**	**+**	**-**	**+**	**-**	**+**	**-**
Non-cognitive skills *(Self-efficacy, educational aspirations, self-esteem)*	u18							
Cognitive skills *(Mathematics, language, verbal comprehension, memory, reaction time, intelligence)*	u14; u21						m13	
Educational performance	u1		u1		u1, u34			
School attendance	u37; u21		u37					
Age at marriage				u45				

B

Outcomes	**Undernutrition**						**Micronutrient deficiency**			
	Stunting		**Underweight**		**Thinness**		**Iron deficiency**		**Anaemia**	
	+	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**
Cognitive skills *(Mathematics, language, verbal comprehension, memory, reaction time, picture completion test, intelligence)*		u21, m31 m15						m34		
Educational performance		u1		u1		u1				m19; m38

+, positive association; -, negative association; HAZ, height-for-age; WAA, weight-for-age; WHZ, weight-for-height; u, reference for undernutrition; m, reference for micronutrient deficiency

Discussion

This review is to our knowledge one of the first attempts to capture the wide spectrum of SCE determinants and consequences of adolescent undernutrition and micronutrient deficiencies in LLMICs. We aimed to provide an overview of the SCE determinants of undernutrition and growth (RQ1) as well as micronutrient status and deficiencies during adolescence (RQ2). However, we found most determinants influencing undernutrition and micronutrient deficiencies at the individual and household level, which were mostly comparable for the two indicators of nutritional status. Indeed, such factors are well known to determine health across the life course and cultures [18]. We identified age, sex, birth order, religion, educational and literacy level, working and marital status, and personal hygiene as proximal, individual-level determinants of undernutrition and micronutrient deficiencies in adolescents. Determinants identified at the household level included parental education and occupation, family/household structure and size, household income, food security status, SES and resources or assets within the household. Surprisingly, only a few determinants at the broader community level were identified, which included geographical location, place of residence (urban vs. rural), community and school type, as well as seasonality; however, most of these determinants seem to relate to the physical and economic environment. This denotes the lack of research on the influences of the broader social, cultural, or political context on adolescent nutritional status, and supports the current consensus to address the "major systematic, policy, cultural and environmental barriers in the achievement of improved nutritional health for adolescent girls" but also boys [136]. Likewise, we found a paucity of studies looking at socio-cultural and economic consequences in the domains of education, labour, and family formation (RQ3) of poor nutrition during adolescence in general, highlighting a pressing research gap. Most studies on consequences focused on the associations between adolescent undernutrition or micronutrient status and cognitive skills or educational attainment. Overall, we found evidence from three cohort studies that linear growth retardation or chronic undernutrition in adolescents is associated with poorer cognitive skills and educational performance [116, 131, 132]. These findings suggest that the negative effects of malnutrition on educational performance are not only limited to childhood but manifest during adolescence as well. Similarly, cognitive skills and educational performance were positively associated with micronutrient status, although evidence was mostly cross-sectional which makes it impossible to establish causal relations. Improvements in school attendance were also observed with an increase in height for age, but again, the observed association was cross-sectional. We thus cannot conclude that better-nourished adolescents attend school more regularly, or state that these adolescents have a better nutritional status. In the domain of family formation, we found only one study that showed how nutritional status affected age at marriage, with heavier girls marrying earlier than lighter girls. Possible explanations offered were the correlations between weight and development of secondary sex characteristics or the cultural image that girls with normal weight (vs. underweight) are perceived healthier or more attractive [133].

Figure 3 summarizes the determinants and consequences of adolescent undernutrition and micronutrient deficiency which were derived from the papers. In Figure 3, we hypothesize that the community-level factors exert an influence on the household characteristics which tend to affect the individual-level determinants of nutrition. Under each larger concept are specific determinants that were found to significantly influence the nutrition of adolescents in LLMICs. We could not find determinants at the broader societal level that might influence adolescent nutritional status, indicating a research gap.

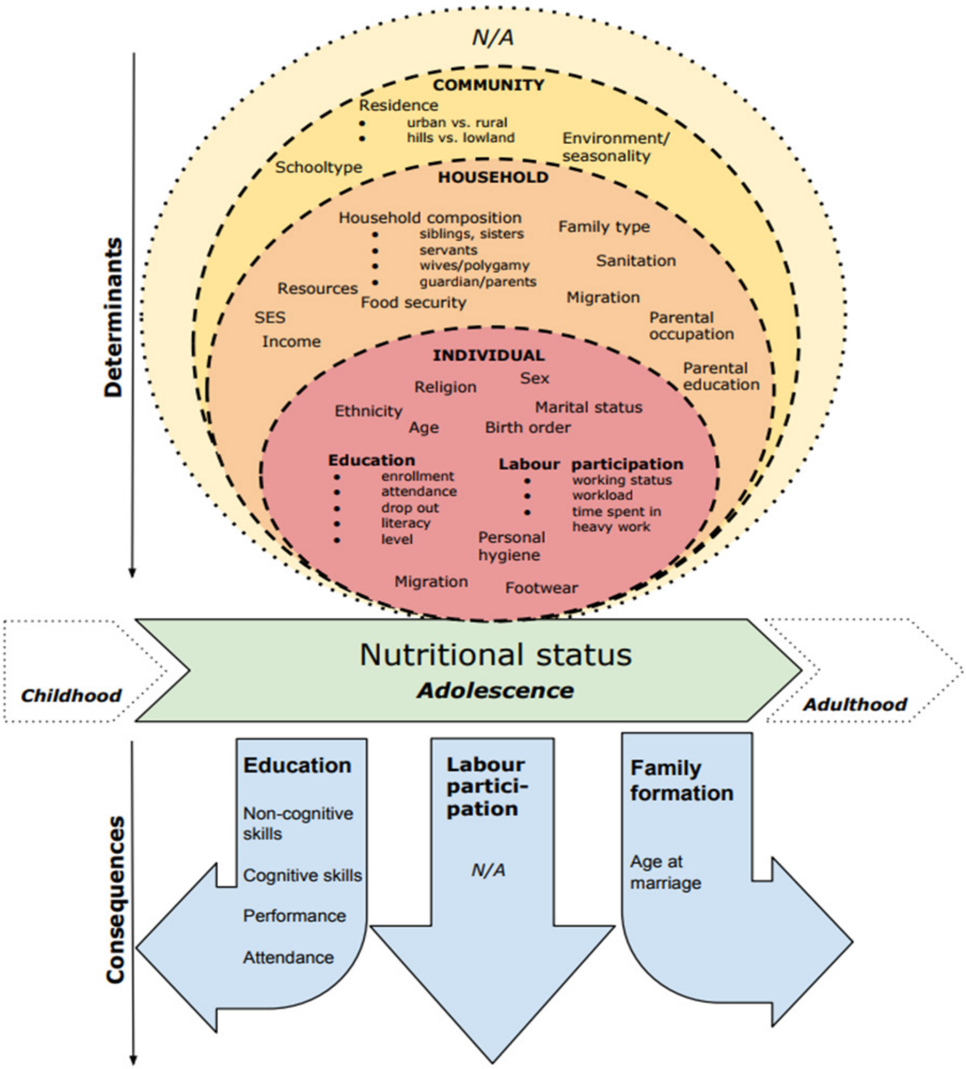

Figure 3. Hypothetical framework summarizing the determinants and consequences of adolescent undernutrition and micronutrient deficiencies in LLMICs

Age and sex

The WHO distinguishes between early (10-14 years) and late (15-19 years) adolescence. We included studies with subjects within this age range but based on the numerous definitions of 'adolescents' we came across, consensus on its definition seems to be lacking with boundaries between being an adolescent or adult somewhat blurred [137]. Particularly in studies targeting women of reproductive age, often, late-adolescent girls are included without referring to adolescence at all.

Unfortunately, from our sample, we could not conclude which determinants were most crucial at what ages (late vs. early adolescence) or for which sex. In general, we found mixed results, significant and non-significant, on the effects of age on nutritional status. Although nutrition differences vary with growth spurt timings [138], a majority of the studies with significant associations between age and micronutrient status or stunting (and HA) in particular, supported increasing age as a risk factor, whilst the prevalence of thinness seemed to decrease with age. This could support evidence that while stunted adolescents (particularly when entering adolescence stunted) might not be able to catch up or compensate for growth sufficiently, especially adolescent girls are better able to improve their body mass (WH) throughout adolescence [138-140]. However, evidence on catching up growth during adolescence is still limited [34, 138].

Similarly, sex differences in undernutrition were inconsistent. However, most studies reporting on sex differences, showed that boys were significantly more likely to be stunted and underweight than girls during adolescence. This is in line with previous studies in Asia and Sub-Saharan Africa [22, 34] that often relate this to boys' later and prolonged growth spurt [40]. In our sample, some authors hypothesize that the finding is related to the work activity hypothesis which refers to the "combined effects of increased energy expenditure and reduced presence at mealtimes" for instance because of work or school (p.359) [39]. In addition, Dapi *et al.* [47] attribute the differences to cultural practices which leads to better nutritional intake for girls, but also the reason that because girls are often involved in cooking and shopping, they might eat in-between meals and during cooking [47]. Studies reporting higher rates of undernutrition in girls often attributed this to gender discrimination and unfavourable intra-household food allocation practices, especially in cases where households had little income or were food insecure. Particularly in South Asia, women are more disadvantaged in accessing food [141]. This is supported by a review of intrahousehold food allocation that shows that inequities are more likely in food insecure or poor households, although this also depends on other factors such as religion, household size, social status, and women's bargaining power [142]. For instance, a study in the far west corner of Nepal showed that adolescent girls ended up second last, or last in the case of daughters-in-law, in the household serving order which could have influenced their nutritional status, especially in food-insecure households [143]. Unequal treatment may thus result when households face extreme circumstances, leading to discrimination against vulnerable women [144]. In terms of micronutrient deficiencies, the opposite effect was found. Here female sex proved to be a risk factor, particularly for low iron status and anaemia. This is in line with other studies

[140] and additionally explained by the increased iron requirements caused by the female growth spurt, menarche, and blood loss during menstruation [7, 145, 146]. Also, when compared to boys, the iron status of girls tends to worsen upon slowing down of growth [138]. Although there were mixed results for the effect of age on micronutrient status, a majority of the studies with significant associations between age and micronutrient status supported increasing age as a risk factor for poor micronutrient status [74, 98, 101-105] for both sexes and notably anaemia among adolescent girls [106-109] which may be related to the increased nutrient requirements with the growth spurt. Another explanation, which was not mentioned by any of these studies and can only be shown by including individual dietary intakes, may relate to pro-male food allocation processes in which girls are allocated less micronutrient-rich foods than boys. Data from the Young Lives cohort shows that such a pro-boy gap and showed how "disparities between mid-adolescent boys and girls are driven by the increased likelihood of boys to consume protein- and vitamin-rich foods" (p.109) [147].

Family and fertility

Although some of the studies excluded adolescent married girls from their sample [106, 117, 130], several Indian studies showed that married adolescent girls were at higher risk of anaemia. In these contexts, marriage during adolescence often leads to early conception, which poses girls at increased risk due to the already increased demands of iron during adolescence [10]. Marrying young also means leaving the natal home and moving in with in-laws, a transition that often leads to a change in social status and access to food which may negatively impact nutritional status [109].

Birth order has been cited as an important determinant of malnutrition among infants and young children showing for instance that earlier-born children (lower birth order) were favoured in terms of intrahousehold food allocation practices, particularly in challenging circumstances [148, 149]. Moreover, some studies show that the poorer nutritional status of later-born children might be due to already depleted maternal stores caused by multiple pregnancies [150]. However, except for three studies [55, 71, 115], we did not find much evidence on the associations between birth order and adolescent nutritional status. It may be that, over time, its effect is diluted. For instance, Horton [148] observed that later-born children are born when per capita resources are smaller as total household income and assets do not increase concomitantly with family size. Thus, the effect of increasing birth order in adolescence may be masked by poor living conditions and its resultant effect of poor dietary intake. Although our sample shows inconsistent findings, results from a Brazilian birth cohort showed that during adolescence, firstborns were heavier and taller than later-born children, due to their greater sensitivity to catch up growth [151].

In contrast, family size, as well as the number of siblings, were often mentioned as risk factors for poor nutritional status. Larger families spend extra resources in meeting their nutrition and health needs thereby putting a strain on already limited resources. The resultant effect may be decreased dietary diversity or intake impacting nutritional status. In such circumstances, vulnerable groups in the household including adolescents may be at a higher risk of malnutrition. The association with the number of siblings was especially found in studies on girls. The authors attribute this to unequal feeding practices and household food distribution [84]. Bird, in her review on the intergenerational transmission of poverty, found that children with more siblings tend to be more malnourished as resources are directed to the youngest or older children, with stronger effects in poor households [152]. Regarding the family type, the prevalence of anaemia was in one study significantly higher among adolescents living in nuclear families compared to their peers in extended or joint families [116] which suggests the relative importance of family support in the prevention of anaemia. Viner *et al.* [18] argued that family connectedness is one of the most important factors that protect against poor health outcomes in adolescence. On the contrary, stunting and thinness were highly prevalent in Indian joint families which could be explained by the effects of family size or lower social status of adolescent girls within these families. Interesting is the link between stunting and polygamy which was found in two Nigerian studies. The authors attribute the higher rates of stunting mainly to poverty and increased household size. The combined effects of polygamy, which occurs more often in low SES groups, and low earning capacity might affect nutritional status [41], however, the authors recommend further research as there might be other underlying mechanisms explaining differences in undernutrition.

Religion and ethnicity

The role of religion and ethnicity in determining nutritional status is quite ambiguous. Within India, the differences in anaemia were context-specific and no particular religion or caste was notably at a higher risk. The differences were mostly attributed to differences in cultural dietary patterns and, or socioeconomic conditions which vary with religion, or caste groups. Likewise, within the same country, variations by geographical location were partly attributed to disparities in diet and prevalence and incidence of infections and diseases. Although an Indian study found that the prevalence of stunting was higher in adolescents who belonged to the Dalit (scheduled caste) community without explaining, Omigbodun *et al.*, who found that Muslim adolescents were worse off in comparison to Christian adolescents in terms of stunting and thinness, argue that religion might act "indirectly in situations where practices within certain social strata would lead to deprivation" (p.670) [41].

Education and occupation

Most studies were conducted in a school setting. This design implies that the prevalence of undernutrition is underestimated if non-enrolled adolescents, who might be more vulnerable and disadvantaged in several life domains, are excluded. Indeed, studies by de Lanerolle-Dias [43] and Hall *et al.* [100] showed that female school dropouts, and adolescents who dropped out in early adolescence or enrolled later in school, were particularly more vulnerable to undernutrition, both in terms of macro and micronutrients and despite the same level of nutritional knowledge. Possible explanations include the additional burden that outside school labour activities place on nutritional status, the relation with SES and household income, and exposure to school nutrition interventions

[44]. On the contrary, Ahankari *et al.* [106] found that school dropouts had a lower risk of anaemia compared to enrolled girls. They argued that non-enrolled girls were generally engaged in agricultural-related employment, with earnings more likely to be spent on nutritional foods which may have improved their Hb. A similar effect was found in other studies where having a job and workload was associated with HAZ and WHZ [44]. Reverse causation, in which undernutrition constrains workload, might be a possible explanation [75]. However, two studies also concluded that working girls had a higher risk of anaemia, iron and zinc deficiency compared to their non-working peers [43, 116]; showing that the additional small income generated by working girls may not always have a positive effect on their nutritional status [153].

The studies underscore the importance of adolescent educational and literacy level as well as parental education and literacy level in reducing the risk of undernutrition, (particularly for stunting) and micronutrient deficiencies. Generally, education and/or literacy may improve healthier behaviour practices and nutritional status via increased awareness and knowledge. Only one study showed how adolescent educational level was inversely associated with serum 25(OH)D [105]. Similarly, another study found SES inversely related to serum 25[OH]D [123], but both associations were attributed to unhealthy lifestyle and sedentary behaviour, a change in practices that is likely to emerge as part of the nutrition transition in LMICs.

Parental education was positively associated with nutritional status, particularly stunting seemed to decrease. However, most studies showed an association between maternal education and improved nutrition. This finding is in line with studies on children's nutritional status, showing that maternal education reduces the odds of particularly stunting [153]. However, Vollmer et al. found that maternal and paternal education were equally important in reducing childhood undernutrition [154]. It may be that better-educated parents are more likely to have better-paying jobs. Parental occupation was indeed associated with a better nutritional status. In contrast to education, we found that paternal occupation was more often associated with better nutrition, even though women's increased earning opportunities result in a different allocation of resources in favour of nutrition through improved bargaining power [144]. Additionally, occupation may increase household income and/or SES which were both consistently linked with a lower risk of undernutrition and micronutrient deficiency. Similarly, studies showed that households with more resources lowered the risk of poor nutritional status. Overall, household resources are indicative of SES or income level. Higher SES is generally associated with higher purchasing power and consequently improved household access to diverse foods [155-157]. However, again a complete consensus on the definition of SES is lacking [158]. It is usually measured by determining education, income, occupation, or a composite of these dimensions [159]. Filmer & Pritchett [160] recommended the use of household durable assets index for SES, but in our sample, the concept was interchangeably based on education and/or occupational status, land size, household income, type of school attended (government or private), or (per capita) income. Only seven authors used a more comprehensive description of SES based on these recommendations which make it complex to generalize the effect of SES on adolescent nutritional status. Moreover, as Bradley and Corweyn state, "the relations between particular SES indicators and health factors may be quite complex" (p. 374) with the associations appearing less steep in more egalitarian contexts [158]. Nonetheless, we found that 'SES' was generally positively associated with adolescent nutritional status. This is to be expected in LMICs and supported by previous research on the 'nutrition pathway' which shows that inadequate dietary intake results from low SES, leading to poor nutritional status and delayed growth [158].

Environment and community

At the community level, particularly the place of residence and environmental factors were found significantly associated with malnutrition. Mainly, studies showed that adolescents in rural areas were worse of in terms of stunting, thinness and underweight. However, contrary to the generally held notion that the risks of micronutrient deficiencies are higher in rural than urban communities, several studies showed that residing in a rural community was protective of anaemia, vitamin D deficiency and associated with a higher folate status. Although most studies did not explain the rural-urban variation, this is in line with the literature on the rural-urban divide. In Sub-Saharan Africa for instance, it was found that urban-rural differentials are persistent when controlled for SES, but also that this gap is narrowing in more countries due to the increase of urban malnutrition and widened in a few countries because of the decline of urban malnutrition [161]. Indeed, rapid urbanisation has resulted in an explosion of poor urban settings that house large numbers of adolescents, with increased health risks for young people in such settings [162].

Finally, the observed seasonal variations in micronutrient status were in part attributed to seasonal variation in the availability and access to food, notably, the micronutrient-rich food. Several studies have indeed shown seasonality variations in dietary intake [163-166]. The implication of the finding may be that interventions that aim to improve the nutritional status of adolescents in the context of LMICs need to recognize the role of seasonality on nutritional status to incorporate initiatives to prevent undesirable seasonal declines in nutrient intake and consequently nutritional status.

Limitations

Despite a thorough set-up of this systematic review, certain limitations should be considered when interpreting our findings. First, the set of eligible papers revealed a high heterogeneity in outcome measures, selected socio-cultural and economic variables, data collection methods, levels of data analysis and study setting. This made it infeasible to conduct a meta-analysis within the scope of this review. For instance, although underweight and thinness refer to the same for adolescents and are defined by BAZ < -2SD [167], some of the reviewed authors defined thinness using weight-for-height (WH) while others also defined underweight with weight-for-age (WA), but these were mostly articles published before the recommendations of De Onis and the WHO in 2007.

Also, most of the studies were cross-sectional in design and thus, inferences of possible associations are speculative, and the results are limited to describing co-occurrences. Furthermore, the review is based on primary, quantitative studies only. We acknowledge that SCE determinants and even consequences of undernutrition might be derived from qualitative studies as well. However, we found these studies to be rare, whilst at the same time considering them highly important to consider the adolescents' own perspectives on growing up and nutrition in relation to socio-cultural and economic aspects. Such studies would yield for instance valuable insights into empowerment, decision-making processes, agency, and social status within households which might influence their nutritional status. Although we attempt to consider grey literature as much as possible by conducting extensive electronic and manual searches in three databases, bibliographies, expert advice, and own databases, we cannot be certain we captured all relevant grey literature. Lastly, eligible papers should be assessed for quality to ensure trustworthiness and check whether studies "support an adequate interpretation of their constitutive research practice" (Tamas & Delaney, 2016). However, this requires having access to all available supplementary and process-related information which was not possible due to the heterogeneity and number of papers. Nonetheless, we undertook a transparency check to ensure that the eligible studies were clear in their objective, sampling plan and size, data collection, statistical methods, conclusions, and limitations.

Implications

This review shows that despite increasing interest in adolescent nutrition, few studies consider adolescents' complex everyday life contexts and their entire pathways of transitions into adulthood. Most studies focus on single-factor determinants at the household and individual level, while factors at the community and broader societal level which are the root causes, deserve more attention. The magnitude and direction of associations were found to be context specific. Thus, interdisciplinary, longitudinal research on and *with* adolescents that focuses on the interrelations between context-specific life trajectories is vital to truly understand the transition into adulthood and thereby optimizing health and other developmental outcomes.

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Appendices

Table S1a: General characteristics of reviewed studies

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	**#**	**1st author**	**Country**	**Study design**	**Age**	**Sex**	**(n)**	**Undernutrition**	**Micro-nutrient status**	
[53]	u1	Acham *et al.* (2008)	Uganda	Cross-sectional	9-15	F/M	1003	HAZ, WAZ, BMI-for-age (percentile)		Sex, age
[42]	u2	Adesina *et al.* (2012)	Nigeria	Cross-sectional	10-19	F/M	960	HAZ, BMI /age (percentiles)		Sex, age, parental occupation and education, SES
[67]	u3	Ahmed *et al.* (1998)	Bangladesh	Cross-sectional	10-16	F	384	HAZ, BMI-age (percentiles)		Sex, age, household size, income, food expenditure, paternal education and occupation, SES
[44]	u4	Assefa *et al.* (2015)	Ethiopia	Cohort study	13-16	F/M	2084	HAZ, WHZ (BAZ), WAZ		Sex, age, residence, paternal education, school type, educational level, religion, fertility, occupation, household size, food insecurity
[49]	u5	Ayoola *et al.* (2009)	Nigeria	Cross-sectional	5-20	F/M	623	HAZ, BAZ		Sex, age, ethnicity
[55]	u6	Bamidele *et al.* (2011)	Nigeria	Cross-sectional	15-19	F/M	497	W/H (BMI-A (percentiles)		Sex, age, religion, parental occupation, parental education, birth order, marriage type, living with guardians/parents, number of siblings
[79]	u7	Barman *et al.* (2015)	India	Cross-sectional	15-19	F	300	HAZ, BAZ		Age, SES, family type, religion, literacy level, marital status, educational level, school enrolment
[93]	u8	Belachew *et al.* (2013)	Ethiopia	Cohort study	13-17	F/M	2084	HAZ		Sex, age, household income, food security, educational level, residence
[91]	u9	Benefice *et al.* (1999)	Senegal	longitudinal survey	12-14	F	406	HAZ, WHZ, WAZ		Age, migration
[72]	u10	Benefice *et al.* (2006)	Bolivia	Cross-sectional	0-15	F/M	460	HAZ, WHZ, WAZ		Sex, age, ethnicity

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	#	1st author	Country	Study design	Age	Sex	(n)	Undernutrition	Micro-nutrient status	
[85]	u11	Bhattacharyya *et al*. (2013)	India	Cross-sectional	10-19	F	284	HAZ, W/H (BMI-age) percentiles		Age, literacy level, SES, household size, paternal education
[40]	u12	Bosch *et al.* (2008)	Bangladesh	Cohort study	0-16	F/M	707	HAZ, BAZ and WAZ		Sex, age
[56]	u13	Cordeiro *et al.* (2012)	Tanzania	Cross-sectional	10-19	F/M	542	BMI-age and sex percentiles)		Sex, age, food security, religion, orphanage, school attendance/ enrolment, SES, resources
[131]	u14	Crookston *et al.* (2014)	multi*	Cohort study	8-15	F/M	3375	HAZ		Sex, age, SES, parental education, both parents living in household, birth order, residence, household size
[65]	u15	Dang *et al.* (2010)	Vietnam	Cross-sectional	6-15	F/M	5640	W/H (BMI-age)		Sex, age, SES, residence
[47]	u16	Dapi *et al.* (2009)	Cameroon	Cross-sectional	12-16	F/M	581	W/H (BMI-age) H/A percentiles		Sex, age, SES
[86]	u17	Dasgupta *et al.* (2008)	India	Cross-sectional	7-16	M	2003	HAZ, WHZ (BMI-age) percentiles		Age, parental occupation, parental education, income, birth order, number of siblings, household size, religion, caste, family type
[132]	u18	Dercon & Sanchez (2013)	multi*	Cohort study	7-12	F/M	3300	HAZ		Age, sex
[89]	u19	Ene-Obong *et al.* (2012)	Nigeria	Cross-sectional	10-18	F/M	893	W/H, BAZ		Sex, age, SES, residence, income, literacy level
[78]	u20	Fentiman *et al.* (2001)	Ghana	Cross-sectional	0-18	F/M	4708	HAZ, WHZ, WAZ		Sex, age, parental education, household size, resources, religion, marital status, school enrolment
[80]	u21	Fink & Rockers (2014)	multi*	Cohort study	8-15	F/M	3604	HAZ		Sex, age, school attendance/enrolment attainment, paternal education, household resources,
[73]	u22	Garnier *et al.* (2003)	Senegal	Cohort study	14-16	F	330	HAZ, WAZ, BAZ		Age, living with guardian or parents, birth order, education, migration, residence, SES, hygiene

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	**#**	**1st author**	**Country**	**Study design**	**Age**	**Sex**	**(n)**	**Undernutrition**	**Micro-nutrient status**	
[95]	u23	Izutsu *et al.* (2006)	Bangladesh	Cross-sectional	11-18	F/M	324	BAZ		Sex, age, residence, mental health, religion, ethnicity, educational status, literacy status, occupational status, household income and size
[81]	u24	Joshi *et al.* (2005)	Nepal	Cross-sectional	14-19	F	426	HAZ, BAZ, WAZ		Age, household resources, income, educational level, paternal education and occupation, work status SES
[90]	u25	Joshi *et al.* (2014)	India	Cross-sectional	10-19	F	200	(W/H) BAZ > CED		Age, SES
[69]	u26	Khongsdier *et al.* (2003)	India	Cross-sectional	3-18	M	1351	HAZ, BAZ, WAZ		Age, household income, occupation, resources, SES, religion
[57]	u27	Khongsdier & Mukherjee (2005)	India	Cross-sectional	9-16	F/M	1733	W/H, (BAZ > CED) percentiles		Sex, age, religion, household income, resources
[59]	u28	Lardner *et al.* (2015)	Ghana	Cross-sectional	5-12	F/M	411	WHZ (BMI-for-age (thinness), HAZ		Age, sex, residence
[68]	u29	Leslie & Pawloski (2010)	Mali	Cross-sectional	10-17	F	1157	HAZ, WAZ, BAZ		Age, no. women, number of servants, SES, distance to market, number people in urban area
[94]	u30	Maiti *et al.* (2011)	India	Cross-sectional	10-14	F	2545	HAZ, WAZ, BAZ		Age, residence
[45]	u31	Melaku *et al.* (2015)	Ethiopia	Cross-sectional	10-19	F/M	348	HAZ and BAZ		Sex, age, parental education, residence, household size, sanitation, resources, birth order, number of siblings, religion, paternal occupation
[92]	u32	Miyoshi *et al.* (2005)	Lao PDR	Cross-sectional	3-15	F/M	1075	HAZ, WHZ, WAZ, MUAC-for-height		Sex, age, SES, food security
[48]	u33	Mondal *et al.* (2012)	India	Cross-sectional	6-16	F/M	725	HAZ, WAZ		Sex, age, ethnicity, household income, paternal occupation, resources, no. siblings

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	#	1st author	Country	Study design	Age	Sex	(n)	Undernutrition	Micro-nutrient status	
[82]	u34	Mukudi (2003)	Kenya	Cross-sectional	10-20	F/M	851	HAZ, WHZ, WAZ		Sex, age, residence, school type
[60]	u35	Mulugeta *et al.* (2009)	Ethiopia	Cross-sectional	10-19	F	211	HAZ, BAZ and percentiles		Age, residence, drinking water, latrines household resources, home gardening, food security
[41]	u36	Omigbodun *et al.* (2010)	Nigeria	Cross-sectional	10-19	F/M	1799	HAZ, WAZ, WHZ (BAZ)		Sex, age, school type, maternal occupation, number of siblings
[83]	u37	Omwami *et al.* (2011)	Kenya	RCT	5-15	F/M	554	HAZ, WHZ, WAZ		Sex, age, SES
[71]	u38	Panter-Brick *et al.* (1996)	Nepal	Cross-sectional	6-14	M	307	HAZ, WHZ, WAZ		Age, ethnicity, living with parents, household size, birth order, no. siblings
[84]	u39	Patimah *et al.* (2016)	Indonesia	Cross-sectional	14-18	F	601	HAZ, WAZ		Age, number of siblings, household size, pocket money, SES, paternal occupation, parental education,
[76]	u40	Pawloski *et al.* (2008)	Mali	Cross-sectional	10-17	F	1103	HAZ		Age, SES, household resources, food prices market, no. siblings/sisters, no. women, number of servants, residence, school attendance, paternal occupation
[70]	u41	Rah *et al.* (2009)	Bangladesh	Cohort study	12-19	F	665	HAZ, WHZ/BAZ percentile, WAZ		Age, SES, hygiene
[54]	u42	Rahman & Karim (2014)	Bangladesh	Cross-sectional	10-17	F/M	796	HAZ, BAZ/CED		Sex, age, parental occupation, education, income, household size, birth order, no. siblings, ethnicity
[52]	u43	Raj *et al.* (2009)	India	Cohort study	5-16	F/M	12129	W/H/BMI percentiles		Sex, age, school type, residence
[51]	u44	Rao *et al*. (2006)	India	Cross-sectional	10-17	F	12789	HAZ, WHZ, WAZ, BMI percentiles		Age, religion, family type, household size, occupancy, house type, household resources, occupation, per capita income, age at marriage, residence
[133]	u45	Riley (1994)	Bangladesh	longitudinal survey	10-20	F	1500	HAZ		Age, marital status, fertility

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	#	1st author	Country	Study design	Age	Sex	(n)	Undernutrition	Micro-nutrient status	
[87]	u46	Roba *et al.* (2016)	Ethiopia	Cross-sectional	15-19	F	726	HAZ, BAZ		Age, marital status, ethnicity, religion, parental education, school type, occupation, family size, household income, water and sanitation, income, use of pocket money, time use
[39]	u47	Sellen (2000)	Tanzania	cross-sectional	0-16	F/M	308	HAZ, WHZ, WAZ		Sex, age
[62]	u48	Senbanjo *et al.* (2011)	Nigeria	Cross-sectional	5-19	F/M	570	HAZ		Sex, age, residence, family type, household composition, school type, parental education, SES
[50]	u49	Venkaiah *et al.* (2002)	India	Cross-sectional	14-17	F/M	12124	HAZ, W/H (BMI percentiles)		Sex, age, religion, family type, household size, ethnicity, literacy level, occupation, household resources, occupation, income, marital status
[88]	u50	Wickramasinghe *et al.* (2004)	Sri Lanka	Cross-sectional	8-12	F/M	1266	HAZ, WHZ (BMI percentiles), WAZ		Sex, age, school type
[63]	u51	Wolde *et al.* (2015)	Ethiopia	Cross-sectional	7-14	F/M	450	HAZ, BAZ, WAZ		Sex, age, ethnicity, religion, parental occupation, parental education, household size, household income, HH food security, personal hygiene
[75]	u52	Yamanaka &Ashworth (2002)	Nepal	Cross-sectional	6-17	F/M	237	HAZ, BAZ, WAZ		Sex, age, workload, food intake, time allocation
[58]	u53	Yetubie *et al.* (2010)	Ethiopia	Cross-sectional	10-19	F/M	425	BMI percentiles (W/H)		Sex, age, marital status, household size, parental education, parental occupation, household income, household resources
[106]	m1	Ahankari *et al.* (2017)	India	Cross-sectional	13-17	F	1010		Hb	land holdings, no. of siblings), school dropout, religion, caste

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	#	1st author	Country	Study design	Age	Sex	(n)	Undernutrition	Micro-nutrient status	
[113]	m2	Ahmed *et al.* (1997)	Bangladesh	Cross-sectional	12-19	F	388		Hb, serum retinol and serum total protein	Education level, family size), household income age
[127]	m3	Ahmed *et al.* (2000)	Bangladesh	Cross-sectional	11-16	F	548		Hb, serum ferritin (SF), transferrin saturation (TS), TIBC, serum retinol	Age, parental education, parental occupation, family size, farmland size, household income, housing conditions
[114]	m4	Ahmed *et al.* (2006)	Bangladesh	Cross-sectional	11-16	M	381		Hb, serum retinol	Age, parental education, parental occupation, family size, per capital income, per capital expenditure
[121]	m5	Ahmed *et al.* (2009)	Bangladesh	Cross-sectional	12-15	F	225		Serum retinol, serum, α-tocopherol, plasma vitamin C, serum Zn, serum, Cu, total cholesterol, and serum protein	Age, family size, family income, per capita food expenditure, parental education, and occupation
[130]	m6	Ara *et al.* (2000)	Bangladesh	Cross-sectional	13-19	F	354		Urinary iodine (Spot urine)	Educational level, household asset index; land holding
[115]	m7	Ayogu *et al.* (2015)	Nigeria	Cross-sectional	10-15	F/M	303		Hb	Age, birth order, frequency of illness, family size, type of toilet in house
[128]	m8	Barugahara *et al.* (2013)	Uganda	Cross-sectional	11-14	F	109		Hb	Age, rural vs. urban residence
[107]	m9	Bharati *et al.* (2009)	India	Cross-sectional	10-19	F	177670		Hb	Age, marital status, urban vs. rural residence), ethnicity, religion, caste Standard of Living Index (SLI)

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	**#**	**1st author**	**Country**	**Study design**	**Age**	**Sex**	**(n)**	**Undernutrition**	**Micro-nutrient status**	
[108]	m10	Biradar *et al.* (2012)	India	Cross-sectional	10-19	F	840		Hb	Age and SES
[119]	m11	Bulliyya *et al.* (2007)	India	Cross-sectional	11-19	F	1937		Hb	Age, religion, community, literacy, family type (nuclear/joint), parental literacy, family size, type of house, house electrification, sanitary latrine, household income
[109]	m12	Chellan & Paul (2010)	India	Cross-sectional	10-19	F	311793		Hb	Age, religion, residence, caste, marital status, birth order, maternal age, educational status, maternal education, standard of living
[134]	m13	Chiplonkar & Kawade (2014)	India	Cross-sectional	10-16	F	403		Hb, Plasma Zn (and erythrocyte Zn	Age, family size, parental education, and occupation
[96]	m14	Choe *et al.* (2001)	South-Korea	Cross-sectional comparative	16-17	F/M	660		Hb, SF, TIBC, serum: iron, folic acid, B12 and immunoglobulin G antibody,	Age, parental education and occupation, type of house, Hollingshead index, household size, crowding index, and housing tenure (owned or rented)
[135]	m15	Dissanayake *et al.* (2009)	Sri Lanka	Cross-sectional comparative (iron deficient vs iron sufficient students)	13-15	F/M	188		Hb, SF, serum Zn and T4	Birth order, occupational ambition, educational ambition, enthusiasm towards education, mental health status, problems at home, love affair, hours spent on studies, extra-curriculum activities, hours spent on extra-curriculum activities, distance to school, SES, parental education, parental occupation, availability of electricity

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	#	1st author	Country	Study design	Age	Sex	(n)	Undernutrition	Micro-nutrient status	
[103]	m16	Ene-Obong *et al.* (2003)	Nigeria	Cross-sectional	10-20	F/M	600		Plasma retinol and serum vitamin C	Age, sex, school type nutrition knowledge, household size
[64]	m17	Friedman *et al.* (2005)	Philip-pines	Cross-sectional	7-30	F/M	729	HAZ	Hb	Age, sex, SES
[168]	m18	Geissler *et al.* (1998)	Kenya	Cross-sectional	13-18	F/M	156		Hb, SF, and WBC	Age, sex, parental education, parental occupation, source of income, family size
[116]	m19	Gupta *et al.* (2013)	India	Cross-sectional	10-19	F/M	406		Hb	Age, Sex, family type (nuclear or joint), religion, birth order, caste, hand washing SES, educational status, parental occupation, and education
[100]	m20	Hall *et al.* (2009)	Multi-country**	Cross-sectional	7-19	F/M	1400		Hb	Age, sex, school type, school grade, country, location within country, age-for-grade (index of late school enrolment)
[98]	m21	Jani *et al.* (2015)	India	Cross-sectional	10-17	F/M	244		Hb, red blood cell (RBC) folate,	Age, sex
[124]	m22	Kim *et al.* (2014)	South-Korea	Cross-sectional	10-18	F	1312		Hb, SF, full blood count (RBC, WBC, Hct),	Age, urban vs. rural residence household income
[102]	m23	Kim *et al.* (2014)	South-Korea	Cross-sectional	10-18	F/M	2062		25(OH)D	Age, sex, season, urban vs. rural residence
[129]	m24	Korkalo *et al.* (2015)	Mozambique	Cross-sectional	14-19	F	551		Hb, SF, serum selenium, plasma retinol, serum folate, and urinary iodine	Age, literacy, urban vs. rural residence

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	**#**	**1st author**	**Country**	**Study design**	**Age**	**Sex**	**(n)**	**Undernutrition**	**Micro-nutrient status**	
[117]	m25	Kulkarni *et al.* (2012)	India	Cross-sectional	10-19	F	272		Hb	Age, educational level, family type, SES, maternal occupation
[104]	m26	Laxmaiah *et al.* (2013)	India	Cross-sectional	10-19	F/M	6616		Hb	Age, sex, community (Scheduled Caste (SC) or Scheduled Tribes (ST)), maternal education, occupation household head, household size, sanitary latrine
[105]	m27	Lee *et al.* (2014)	South-Korea	Cross-sectional	12-18	F/M	1510		25(OH)D	Age, sex, season, educational level
[125]	m28	Lee *et al.* (2015)	South-Korea	Cross-sectional	10-20	F/M	1510		25(OH)D, Hb, SF, TIBC	Age, sex, annual household income
[110]	m29	Leenstra *et al.* (2004)	Kenya	Cross-sectional	12-18	F	648		Hb, SF, red cell indices (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC)	Age, residence, bed net use, parental schooling, and SES of caretaker,
[74]	m30	Mahmud *et al.* (2013)	Ethiopia	Cross-sectional	10-15	F/M	431	HAZ, BAZ	Hb	Age, sex, urban vs. rural residence personal hygiene, household size, latrine use, water source
[61]	m31	Perignon *et al.* (2014)	Cambodia	Cross-sectional	6–16	F/M	2443	HAZ	Hb, SF, transferrin receptor, RBP, serum Zn, urinary iodine	Age, sex, household income, educational status caretaker

table continues

General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	#	1st author	Country	Study design	Age	Sex	(n)	Undernutrition	Micro-nutrient status	
[123]	m32	Puri *et al.* (2008)	India	Cross-sectional	6-18	F	3127		25(OH)D, serum Ca, P, alkaline phosphatase	Age, family type, time spent on outdoor activities, occupation of caretaker, SES household head
[101]	m33	Rakesh *et al.* (2015)	India	Cross-sectional	?	F/M	3200		Hb	Age, sex, school type (government or management),
[118]	m34	Rani & Suryaprabha (2013)	India	Cross-sectional	11-19	F	760		Hb	Age, urban vs. rural residence, educational status, marital status, parental occupation and education, religion
[99]	m35	Sahu *et al.* (2009)	India	Cross-sectional	10-20	F/M	121		25(OH)D, serum Ca, serum alkaline phosphatase	Age, season, family size
[112]	m36	Soekarjo *et al.* (2001)	Indonesia	Cross-sectional	12-15	F/M	6486		Hb,	Age, sex, school type (General or Islamic school), parental education
[122]	m37	Soekarjo *et al.* (2004)	Indonesia	RCT	12-15	F/M	3616		Hb, serum retinol	Age, sex, parental education
[111]	m38	Teni *et al.* (2017)	Ethiopia	Cross-sectional	10-19	F	442		Hb	Age, religion, mode of transport to school, marital status parents, sex household head, workload, number of days absent from home, parental education, availability of toilet at home
[97]	m39	Tesfaye *et al.* (2015)	Ethiopia	Cross-sectional	12-19	F/M	408		Hb	Age, sex, parental education and occupation, household size, monthly income
[120]	m40	Thoradeniya *et al.* (2006)	Sri Lanka	Cross-sectional	15-18.9	F	277		Hb, SF, serum folic acid, serum B_{12}, folate intake,	Age, educational status, monthly income, household size, SES

table continues

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General characteristics of the study					Study population			Indicators of undernutrition and micronutrient deficiencies examined		Demographic, socio-cultural, and economic aspects studied
Ref	**#**	**1st author**	**Country**	**Study design**	**Age**	**Sex**	**(n)**	**Undernutrition**	**Micro-nutrient status**	
[126]	m41	Tupe *et al.* (2009)	India	Cross-sectional	15-19	F	173		Hb, SF, nutrient intake	Age, daily workload, occupational status, household size
[46]	um1	Ayogu *et al.* (2016)	Nigeria	Cross-sectional	12-18	F/M	400	HAZ, BAZ	Hb, parked cell volume (PCV), serum retinol	Age, sex, family income, household size, parental occupation
[77]	um2	Beasley *et al.* (2000)	Tanzania	Cross-sectional	7-12	F/M	441	HAZ and WAZ	Hb	Sex, age, housing condition (type, ownership), SES and household possessions
[43]	um3	Lanerolle-Dias *et al.* (2012)	Sri Lanka	Cross-sectional/ RCT	15-19	F	600	BMI	Hb, SF, serum Zn, serum folate and vitamin B_{12}	Age, marital status, working status, household income, age/level of dropping out of school
[66]	um4	Teji *et al*. (2016)	Ethiopia	Cross-sectional	10-19	F	547	HAZ, BAZ	Hb	Age, food security, residence, living with parents, paternal occupation, breadwinner, source of drinking water, parental education

U, undernutrition reference; m, micronutrient reference; F, female; M, male; HAZ, height-for-age z-score; BMI, body-mass index-for-age z-score; WAZ, weight-for-age z-score; BAZ, body-mass index-for-age; WHZ, weight-for-height z-score; SES, socio-economic status

Table S1b: Consequences of undernutrition and micronutrient deficiencies reviewed articles assessed

Ref	#	1st author	Outcome/consequence of undernutrition and micronutrient deficiency
[53]	u1	Acham *et al.* (2008)	Learning achievements (grade in school)
[131]	u14	Crookston *et al.* (2014)	Cognitive skills
[132]	u18	Dercon & Sanchez (2013)	Non-cognitive skills
[80]	u21	Fink & Rockers (2014)	Cognitive skills
[82]	u34	Mukudi (2003)	School performance and attendance
[83]	u37	Omwami *et al.* (2011)	School attendance
[133]	u45	Riley (1994)	Age at marriage
[134]	m13	Chiplonkar & Kawade (2014)	Cognitive performance [(simple-reaction-time (SRT), recognition-reaction-time (RRT), visual-memory, Raven's Progressive Matrices (RPM) test)] and taste acuity [(recognition-thresholds-for-salt (RTS))].
[135]	m15	Dissanayake *et al.* (2009)	Academic performance (marks for mathematics, science, Sinhala language and social science) and intelligence (Raven's Standard progressive matrices)
[116]	m19	Gupta *et al.* (2013)	Academic performance
[61]	m31	Perignon *et al.* (2014)	Cognitive performance (Raven's Coloured Progressive Matrices (RCPM), block design, picture completion, two standardized tests from the Wechsler Intelligence Scale for Children (WISC-III))
[111]	m38	Teni *et al.* (2017)	Academic performance (the average academic score in 2012 - 2013)

Chapter 3

Agro-ecological zone and farm diversity are factors associated with haemoglobin and anaemia among rural school-aged children and adolescents in Ghana

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Matern Child Nutr. 2018; 15:1–11 /
https://doi.org/10.1111/mcn.12643

Abstract

Understanding contextual risk factors for haemoglobin status and anaemia of rural school-age children and adolescents is critical in developing appropriate interventions to prevent anaemia. Secondary data analyses using baseline data of an impact evaluation of the Ghana School Feeding Programme to determine the severity of anaemia and contextual predictors of anaemia and haemoglobin (Hb) status among rural school-age children (6-9 years; *n*=323) and adolescents (10-17 years; *n*=319) in Ghana. We used regression models with variable selection based on backward elimination in our analyses. The mean Hb was 113.8 ± 13.1g/L and the overall prevalence of anaemia was 52.3%, being 55.1% and 49.5% among school-age children and adolescents respectively. We identified child's age (β=2.21, *P*<0.001); farm diversity score (β=0.59, *P*=0.036); and agro-ecological zone (*P*-trend <0.001) as the main predictors of Hb status of school-age children. Household asset index (HAI) (*P*-trend=0.04) and agro-ecological zone (*P*-trend <0.001) were predictors of Hb in adolescents. Agro-ecological zone and age were predictors of anaemia, but the effect of age was only significant for girls and not boys [prevalence odds ratio (POR) 1.35 (95% CI: 1.04, 1.76) vs POR 1.14 (95% CI: 0.88-1.46)]. School-age children in households with maize stock were less likely to be anaemic [POR 0.55 95% CI (0.32, 0.97)]. Household dietary diversity score (β=0.59, *P*=0.033) was associated with Hb status for the full sample only. Anaemia is a severe public health problem among school-age children and adolescents in rural Ghana irrespective of sex. Farm diversity score, availability of maize stock in the household, HAI and agro-ecological zone were the main predictors of Hb and anaemia among the rural school-age children and adolescents.

Introduction

Anaemia is a critical public health condition globally, particularly in low- and middle-income countries where its prevalence tends to be three to four times higher than in advanced economies [1,2]. Besides pregnant women, children (both preschool and school-age) are the most affected group with iron deficiency anaemia because of the rapid growth and the general cognitive development [3]. Studies have shown that adolescents are also at an increased risk of developing anaemia due to increased iron demand during puberty, menstrual losses, limited dietary iron intake and poor dietary habits [4,5]. Adolescent girls are particularly at high risk of developing iron deficiency and/or anaemia, especially amongst those who experience heavy blood losses during menstruation and corresponding decreases in ferritin levels [5,6].

It is estimated that about half of school-age children (SAC) (6-9 years) and pregnant women and close to one-third of non-pregnant women of reproductive age suffer from anaemia globally [2]. In 21 countries assessed by UNICEF, more than one-third of adolescent girls were anaemic [7]. In Ghana, about 73.6% and 44% of rural young children (<5 years) and women are anaemic respectively [8]. About two-thirds of Ghanaian adolescent girls and SAC are reportedly anaemic [7,9]; suggesting anaemia is a severe public health problem in Ghana requiring urgent attention and context-appropriate policies.

The consequences of iron deficiency and anaemia have been well documented; these include impairment of physical and cognitive development of infants and young children [10–12], higher risk of morbidity and mortality for young children and pregnant women [13,14], and increased risk of low birth weight even with moderate preconception anaemia on the part of women in fertile age [15]. The long–term effect of anaemia is reduced cognition in the early years, which is associated with lower productivity later in life [1,16]. The negative consequences of iron deficiency or anaemia on cognitive performance may not be limited only to infants and young children but may continue into adolescence. In a randomized controlled trial with iron supplementation, Bruner *et al.* [17] showed that iron-deficient American adolescent high school girls receiving a 1300mg daily dose of ferrous sulphate for 8 weeks performed better on a test of verbal learning and memory than girls with a similar iron status receiving a placebo.

The primary cause of anaemia is assumed to be iron deficiency; nevertheless, this condition is seldom present in isolation [1]. This is because anaemia is only partly caused by dietary insufficiency of nutrients and it coexists with infectious diseases such as malaria and parasitic infections, haemoglobinopathies as well as socioeconomic and environmental factors such as geographical location, the source of drinking water and sewage system; these determinants are also often context-specific [1,18–20].

Given the context-specificity of the root causes of anaemia, understanding contextual risk factors is critical to guide the implementation of interventions to prevent or reduce anaemia. Nevertheless, there is generally a dearth of data on the major predictors of anaemia among SAC and adolescents as existing studies focus primarily on preschool children (<5 years) or women of reproductive age. Hence, this study aims to fill this critical gap by secondary analyses of data from an impact evaluation to determine the prevalence, severity and contextual determinants of anaemia and haemoglobin (Hb) status among rural Ghanaian SAC and adolescents and provide evidence for policy formulation and programme planning.

Materials and Methods

Study design: We used baseline data from the impact evaluation of the Ghana School Feeding Programme conducted in December 2013. Ethical approval for the impact evaluation was obtained from the Institutional Review Board of the University of Ghana Noguchi Memorial Institute for Medical Research (NMIMR) and Imperial College London Research Ethics Committee. Written and verbal informed consent was obtained from all parents/guardians of the children before the interviews. Details of the impact evaluation study design and the data collected have been reported elsewhere [21].

Study population and sample size: The study population in the impact evaluation was selected through cluster random sampling from the Greater Accra, Ashanti, Brong Ahafo, Central, Eastern, Northern and Upper East Regions of Ghana. In the impact evaluation, a random sub-sample of 717 children aged 4-17 years had measurements of Hb taken. Seventy-five (75) children not meeting the inclusion criteria (age ≥ 6 years and residing in a rural community) were excluded, which led to the final sample of 642 children aged 6-17 years for the analyses.

Measurements

Haemoglobin and anaemia: Hb was measured with HemoCue Hb 201^+ using blood samples from a finger prick test by a trained phlebotomist. Anaemia was defined with the WHO criteria [22]: Hb< 115g/L for 6-11 years children; Hb< 120g/L for 12-14 years children; Hb< 130g/L for males ≥15 years; Hb< 120g/L for non-pregnant females ≥15 years. We also categorized anaemia as severe (Hb < 80g/L), moderate (Hb 80-109g/L) and mild (Hb 110-119g/L) using the WHO criteria [22].

Helminths infestation: Urine and stools samples were collected separately from a sub-sample of the children (N=311) to screen them for ascaris, hookworm, trichuriasis and schistosomiasis. Analyses were carried out by microscopy and/or RDT at the General Laboratory of the Parasitology Department of NMIMR. We defined helminths infestation as the presence of any of the above parasites and a dummy variable was created.

Household dietary diversity: A modified Household Dietary Diversity Score (HDDS) [23] was calculated for each household using data on the consumption of 68 food items grouped into 12 food groups, over the previous 7 days. For each food group, a score of 1 was given if a household consumed at least one food item from the food group in the past week, else 0. The food group scores were summed into the modified HDDS, ranging from 0-12. Food groups included cereals, roots and tubers, vegetables, fruits, meat, eggs, fish and seafood, pulses and nuts, milk and milk products, oils and fats, sugar, and condiments. Furthermore, a household food variety score (HFVS) was constructed similar to the food variety score [24], which consisted of the sum of all the unique food items consumed by a household from the 7-day recall. Similar to the HDDS, the HFVS was a continuous score ranging from 0-68. Lastly, we created a score for household animal food consumption (HAFC) consisting of a sum of all the unique animal foods consumed by the household from the 7-day food recall; the HAFC ranged 0-9. These scores did not consider minimum quantities of intakes.

Farm diversity: A composite crop and livestock count ("farm diversity") captured the number of the different crops cultivated and of the different animals reared by the household in the last farming season. This count indicator has previously been used to assess farm diversity and biodiversity in relation to nutritional outcomes [25,26].

Other covariates: The household questionnaire included modules on household demographics, education, economic activities, and farm income. The data included information on household durables and agriculture assets. We constructed household and agricultural assets indices through principal component analysis [27] and then divided them into terciles. These indices captured household wealth in relation to the ownership of durable and agricultural assets. Furthermore, we included in our statistical models a continuous variable for the proportion of food consumed by the household from its own production in the past month.

We included in the initial models, the following continuous variables: the birth order of the child; the number of days the child bought food from school; the age of the household head; the household's dependency ratio; the number of months the household consumed food from own production; and parental years of schooling. Whilst paternal occupation was coded and analysed as farmer or other (which included off-farm casual paid job and unemployed), maternal occupation was coded and analysed as farmer, trader and other (employed, off-farm casual job and apprentice) based on the data available. Additionally, we analysed as dichotomous variables; availability of maize stock in the house, land ownership, receipt of any form of remittance in the past year, sex of household head and child sickness in the seven days preceding the survey. A categorical variable comprised four agro-ecological zones in Ghana [28,29], namely: northern savannah (Northern and Upper East Regions), coastal savannah (Central Region), transition zone (Brong Ahafo Region) and forest zone (Ashanti and Eastern Regions). We re-coded ethnicity as Akan, Gurma, Mole-Dagbani and others (including Guan, Grusi, Ga-Dangbe, Ewe and other ethnic groups). Children were categorized as school-age (6-9 years) and adolescents (10-17 years) based on their age [30]. Lastly, school grade was categorized as lower primary and upper primary/junior high school.

Statistical analysis: We used SAS 9.3 (SAS Institute Inc., Cary NC.) in all our statistical analyses and a two-tailed *P*-value ≤ 0.05 was considered statistically significant. Population characteristics were presented as means ± SD for continuous variables and percentages for categorical variables. We used One-way analysis of variance (ANOVA) to assess the difference in means between groups for continuous outcome variables whilst Pearson's Chi-square test was employed for categorical variables. Based on the literature, we identified an *a priori* set of potential predictors of Hb and anaemia. These included: child sex and age, helminths infestation, child health status (including malaria), dietary intake, household size, educational status of household head and of the mother, the occupation of the household head and of the mother, household wealth, and ethnicity [20,31–33]. The assumption of normality for linear regression was assessed by visual inspection (Q-Q plots, histograms, and boxplots) and normality violations were corrected by a natural log transformation (proportion of food consumed from own production in the past month) before analysis. The scale of continuous covariates in the logistic regression was assessed with smoothed scatter plots.

We included variables with *P*-values ≤ 0.25 from bivariate analyses along with those thought to be important *a priori* (sex, parental education, and occupation) in backward stepwise multiple regression models using the GLMSELECT PROCEDURE [34] and PROC LOGISTIC PROCEDURE [35] in SAS to respectively determine the regression coefficients (β) and the prevalence odds ratios (POR) of the significant predictors of Hb status and anaemia in the sample. The criteria for variable selection were based on the significance level and the Predicted Residual Sum of Squares Statistic (PRESS). We entered interaction terms to explore potential non-linearities but only the interaction term for age and sex (age*sex) was found to be statistically significant.

The analysis was also stratified for the child's age category (SAC and adolescents). Lastly, as a robustness check, we repeated the analysis with multilevel regression with a random intercept and clustering at the agro-ecological zone using the PROC MIXED PROCEDURE in SAS [36].

Results

Sample characteristics: Table 1 presents the sample characteristics. About 50.3% of the children were of school age and the majority attended lower primary school. About half were females. Children's ethnicity included Akan (38.9%), Gurma (25.9%), Mole-Dagbani (17.6%) and other (17.6%). About 11.5% of the children reported being sick 7 days preceding the survey and none was found to have helminths infestation. Generally, the dependency ratio was high. Furthermore, the mean paternal and maternal years of schooling were 5.6 ± 6.5 years and 3.1 ± 5.6 years respectively.

About 72% of the children belonged to male-headed households. Farming was the main occupation of both parents, but SAC children had more farmer fathers compared to adolescents. A greater proportion of SAC were in lower primary school compared to adolescents. In addition, households of SAC had more land and a higher farm diversity compared to adolescent households. Also, the proportion of food consumed from own production was lower for households of SAC but their households consumed food from their production for a longer duration compared to adolescent households. Lastly, more SAC than adolescents were from the Northern Savannah agro-ecological zone.

The mean Hb concentration of the children in the study was 113.8 ± 13.1g/L and was significantly lower for SAC compared to adolescents [SAC: 111.4 ± 12.8g/L, Adolescents: 116.2 ± 12.9g/L, $P<0.001$] (Table1). The overall prevalence of anaemia was 52.3% and did not differ between SAC and adolescents (SAC: 55.1%; Adolescents: 49.5%, $P=0.16$). However, more SAC were moderately anaemic compared to adolescents and while about 1.2% of SAC were severely anaemic, none of the adolescents was severely anaemic (Table 1).

Table 1: Household and child level characteristics of the rural schoolchildren stratified by age category

Characteristics	Overall n=642	School-age children (6-9 y), n=323	Adolescents (10-17 y), n=319
Child characteristics			
Sex (female), %	48.6	48.3	48.9
Age, y	9.5 ± 2.2	7.7 ± 1.1	11.3 ± 1.3
Haemoglobin concentration (g/L),	113.8 ± 13.1	111.4 ± 12.8	116.2 ± 12.9
Any anaemia, %	52.3	55.1	49.5
Category of anaemia, %			
Mild	15.7	13.3	18.2
Moderate	36.0	40.6	31.4
Severe	0.6	1.2	0
Birth order of child (median)	5	5	5
Number of days child bought food from school	3.6 ± 2.2	3.4 ± 2.3	3.7 ± 2.1
Helminths infestation present (n=311), %	0	0	0
Child sick in the past 7 days (n=589), %	11.5	12.5	10.5
Child's school grade, %			
Lower primary	83.6	97.8	69.3
Upper primary and junior High school	16.4	2.2	30.7
Ethnicity of child, %			
Akan	39.1	37.5	40.8
Gurma	25.9	28.2	23.5
Mole-Dagbani	17.6	17.0	18.2
Other[a]	17.5	17.3	17.6

table continues

Characteristics	Overall n=642	School-age children (6-9 y), n=323	Adolescents (10-17 y), n=319
Household demographic and socio-economic characteristics			
Dependency ratio	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
Sex of household head, %			
Male	72.0	72.1	71.8
Female	28.0	27.9	28.2
Age of household head	45.9 ± 13.1	45.6 ± 12.7	46.2 ±13.4
Paternal years of schooling	5.6 ± 6.5	5.4 ± 6.5	5.7 ± 6.6
Maternal years of schooling	3.1 ± 5.6	2.8 ± 5.4	3.5 ± 5.8
Household receipt of remittance in the past 1 year, %	29.6	26.4	32.9
Occupation of father (n=429), %			
Famer	69.2	77.1	60.7
Other[b]	30.8	22.9	39.3
Occupation of mother (n=596), %			
Farmer	45.1	47.3	43.0
Trader	37.6	35.5	39.7
Other[c]	17.3	17.2	17.3
Household asset index, %			
Lower	34.2	35.9	32.9
Middle	32.9	32.2	33.5
Upper	32.7	31.9	33.5
Household food availability and diet diversity			
HDDS	9.3 ± 1.9	9.3 ± 1.9	9.3 ± 1.9
HFVS	19.2 ± 6.1	19 ± 5.8	19.5 ± 6.3
HAFC	1.9 ± 1.2	1.8 ± 1.0	1.9 ± 1.3
Proportion of food consumed in the past month from own production[d]	1.7 ± 1.7	1.5 ± 1.6	1.9 ± 1.8
Number of months household consumed food from own production	6.9 ± 4.2	7.5 ± 3.9	6.3 ± 4.4
Maize stock available in household, %	23.6	24.4	22.3
Farm diversity	3.5 ± 2.6	3.7 ± 2.6	3.2 ± 2.6
Agriculture asset index, %			
Lower	47.5	45.8	49.2
Middle	34.4	34.4	34.5
Upper	18.1	19.8	16.3
Household owns land, %	78.3	81.7	74.9
Geographical location			
Ecological zone, %			
Northern Savannah	29.6	34.4	24.8
Coastal Savannah	9.0	7.1	11.0
Transitional	22.9	21.1	24.8
Forest	38.5	37.5	39.5

Unless specified, values are mean ± SD; N, sample size; HDDS, household dietary diversity score;

HFVS, household food variety score; HAFC, household animal foods consumption. [a]Other includes Ga-Dangbe, Guan, Grusi, Mande, Ewe and other tribes originating from outside Ghana; [b]Other includes off-farm wage employment, business and unemployed; [c]Other includes off-farm wage employment, apprentice and unemployed; [d]Natural log-transformed variable of the proportion of food consumed in the past month from own production; **P*-value for between-group test (school-aged children vs. adolescents) is statistically significant ($P < 0.05$).

Table 2: Mean (95% C.I) haemoglobin concentration (g/L) and prevalence of anaemia among the rural Ghanaian children stratified by age category and agro-ecological zone

	Mean (95% C.I) of haemoglobin concentration (g/L)				**Prevalence of anaemia**			
Zone	**Overall sample n=642**	**School-age n=323**	**Adolescents n=319**	***P*-value**	**Overall sample n=642**	**School-age n=323**	**Adolescents n=319**	***P*-value**
Forest zone (ref, n=247)	118.6 (117.1, 120.2)	115.9 (113.7, 118.1)	121.2 (119.1, 123.4)	<0.001	36.4	39.7	33.3	0.301
Northern Savannah (n=190)	110.5 (108.9, 112.2)[a]	109.8 (107.6, 111.9)[a, b]	111.6 (109.1, 114.2)[a]	0.277	63.2*	62.2	64.6	0.736
Coastal Savannah (n=58)	109.1 (105.2, 113.0)[a]	102.5 (96.9, 108.1)[a,]	113.4 (108.5, 118.4)[a]	0.005	63.8*	69.6	60.0	0.458
Transitional zone (n=147)	111.7 (109.7, 113.8)[a]	109.2 (106.1, 112.2)[a]	113.9 (111.2, 116.6)[a]	0.023	60.5*	66.2	55.7	0.195
Overall sample (n=642)	113.8 (112.8, 114.8)	111.4 (110.0, 112.8)	116.2 (114.7, 117.6)	<0.001	52.3	55.1	49.5	0.16

n, sample size; ref., reference group; 95% C.I, 95% confidence interval; One-way ANOVA was used for statistical difference in haemoglobin concentration between age groups whilst the chi-square/Fisher's exact test was appropriately used for the prevalence of anaemia.

Note: [a]statistically significantly lower ($P<0.05$) than the forest zone (Tukey-Kramer adjustment); [b] Statistically significantly higher ($P<0.05$) than the coastal savannah zone category (Tukey-Kramer adjustment)). *odds of anaemia were significantly higher with reference to forest zone (*P*-trend <0.001).

Results of the bivariate analyses: We found a significant variation ($P<0.001$) in the mean Hb by agro-ecological zone, ranging from 109.1g/L (95% CI:105.2, 113.0) in the coastal savannah to 118.6g/L (95% CI:117.1, 120.2) in the forest zone (Table 2). Likewise, the mean Hb differed significantly between SAC and adolescents in each agro-ecological zone except for the northern savannah zone but the prevalence of anaemia did not differ significantly between SAC and adolescents in each agro-ecological zone. The overall prevalence rate of anaemia varied from 36.4% in the forest zone to 63.8% in the coastal savannah with the odds of anaemia been significantly higher for all agro-ecological zones compared to the forest zone (Table 2).

Additionally, the results of the bivariate linear regression (Table S1) showed that child's age, school grade, ethnicity (Gurma vs Akan), the number of days child bought food from school, HDDS, HAFC, and agro-ecological zone were significant correlates of the children's Hb status.

Results of the multiple regressions: In the backward multiple linear regression (Table 3), we identified child's age (β=1.21, *P*<0.001), HDDS (β=0.59, *P*=0.033), and agro-ecological zone (*P-trend* <0.001) as significant predictors of the children's Hb. The effects of farm diversity and household asset index (HAI) were not statistically significant for the whole population. When stratifying by age category, the HAI (*P-trend*=0.04) and agro-ecological zone (*P-trend* <0.001) were the only significant predictors of adolescents' Hb, whilst child's age (β=2.21, *P*<0.001), farm diversity (β=0.59, *P*=0.036) and agro-ecological zone (*P-trend* <0.001) significantly predicted Hb in SAC.

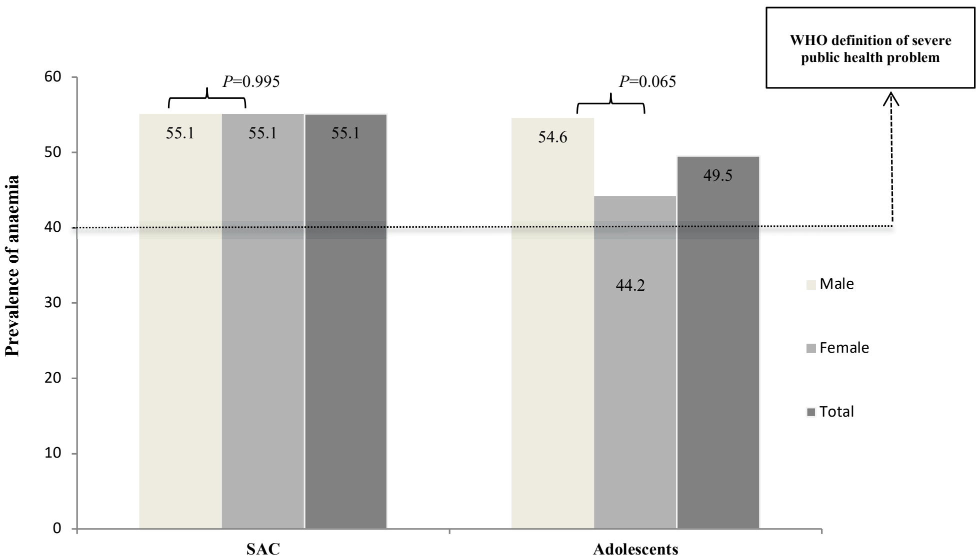

Figure 1: The prevalence of anaemia among the rural Ghanaian school children stratified by age category and sex

Compared to the forest zone, children residing in other agro-ecological zones consistently had a higher odd of anaemia both at the population level and by age category (*P-trend* <0.001 for all models). Furthermore, HDDS [POR 0.91 (95% CI:0.83, 1.00)] and availability of maize stock in the household [POR 0.70 (95% CI:0.47, 1.04)] were associated with lower odds of anaemia for the full sample. Additionally, the availability of maize stock in the household was significantly associated with 45% lower odds of anaemia among SAC. Although a unit increase in HDDS was associated with a lower odd of anaemia among SAC, the effect was not statistically significant. Moreover, the middle tercile of the agriculture asset index was associated with 52% higher odds of anaemia while the upper tercile was associated with 26% lower odds of anaemia among SAC but none was statistically significant. Whilst a unit increase in age was associated with a 23% [POR 0.77 (95% CI: 0.62, 0.96)] *lower* odds in anaemia among SAC, it was associated with 24% higher odds in anaemia among adolescents [POR 1.24 (95% CI:1.03, 1.49)] (Table 4). There was a significant interaction term for age and sex indicating that the effect of age was only significant for female adolescents but not male adolescents [POR 1.35 (95% CI: 1.04, 1.76) vs POR 1.14 (95% CI: 0.88-1.46) for female and male adolescents respectively].

Table 3: Backward multiple linear regression of the predictors of haemoglobin concentration among rural Ghanaian school-age children and adolescents

Predictors	**Overall n=642**			**School age children (6-9 y) n=323**			**Adolescents (10-17 y) n=319**		
	β	**SE (β)**	***P-value***	**β**	**SE (β)**	***P-value***	**β**	**SE (β)**	***P-value***
Age	1.21	0.23	<0.001	2.21	0.62	<0.001	N/S		
Dependency ratio				-5.85	4.03	0.149	N/S		
HDDS	0.59	0.28	0.033		N/S		N/S		
Farm diversity	0.37	0.21	0.059	0.59	0.30	0.036	N/S		
Household asset index			0.099						0.042
Middle vs Lower	1.84	1.20					4.22	1.74	
Upper vs Lower	-0.80	1.29					0.80	1.87	
Ecological zone			<0.001			<0.001			<0.001
Northern Savannah vs Forest	-8.00	1.28		-6.18	1.63		-9.71	1.94	
Coastal Savannah vs Forest	-8.61	1.95		-12.22	2.95		-7.11	2.46	
Transition vs Forest	-7.04	1.32		-5.50	1.92		-7.56	1.83	
Model fit statistics									
F	13.11		<0.001	9.08		<0.001	8.21		<0.001
$R^2_{adjusted}$	0.14			0.14			0.11		
MSE	148.54			142.50			149.78		
AIC	3647.43			1850.36			1792.66		
Press	91386			45077			45496		

n, sample size; β, regression coefficient; SE (β), standard error of regression coefficient; N/S, variable not selected; ref, reference group; MSE, Mean square of residuals; AIC, Akaike criteria

Table 4: Backward multiple logistic regression of the predictors of anaemia among rural Ghanaian school-aged children and adolescents

Predictors	Overall n=642		School age children (6-9 y) n=323		Adolescents (10-17 y) n=319	
	POR (95% C.I)	***P*-value**	**POR (95% C.I)**	***P*-value**	**POR (95% C.I)**	***P*-value**
Age	N/S		0.77 (0.62, 0.96)	0.022	1.24 (1.03, 1.49)*	0.021
Sex	N/S		N/S			0.273
Female vs Male					0.32 (0.04, 2.47)	
Age*sex	N/S		N/S			0.348
Age when sex=female					1.35 (1.04, 1.76)	
Age when sex=male					1.14 (0.88, 1.46)	
HDDS	0.91 (0.83, 1.00)	0.054	0.89 (0.78, 1.02)	0.085	N/S	
Maize stock available in household		0.074		0.038		
Yes vs No	0.70 (0.47, 1.04)		0.55 (0.32, 0.97)			
Agriculture asset index				0.099		
Middle vs Lower	N/S		1.52 (0.87, 2.65)		N/S	
Upper vs Lower	N/S		0.74 (0.39, 1.41)		N/S	
Ecological zone		<0.001		<0.001		<0.001
Northern Savannah vs Forest	3.23 (2.14, 4.89)		2.60 (1.48, 4.57)		3.68 (2.01, 6.71)	
Coastal Savannah vs Forest	2.94 (1.58, 5.48)		3.54 (1.27, 9.86)		3.21 (1.46, 7.05)	
Transition vs Forest	2.78 (1.80, 4.28)		3.10 (1.56, 5.81)		2.46 (1.36, 4.40)	
Model fit statistics						
Log likelihood ratio	50.13	<0.001	38.26	<0.001	32.58	<0.001
Wald test	46.90	<0.001	33.02	<0.001	29.36	<0.001
Nagelkerke's R^2	0.11		0.16		0.13	

n, sample size; POR, prevalence odds ratio; 95% C.I, 95% confidence interval; N/S, variable not selected; ref, reference group

Discussion

To our knowledge, this is the first study to investigate the prevalence of anaemia as well as the predictors of Hb status and anaemia among rural SAC and adolescents in Ghana. About one in two children in our sample were found to be anaemic with most children being moderately anaemic (36.0%), which signals the presence of a serious public health issue [22]. Anaemia prevalence rates ranging between 21.1%-82.6% have been reported for SAC and adolescents in Sub-Saharan Africa [20,33,37,38]. In countries like Ghana, poor dietary intake due to food insecurity and/or consumption of monotonous plant-based diets and infections in rural settings are key drivers of inadequate micronutrient intake and anaemia [39–42].

Compared to our results, a recent study in the transition zone of Ghana reported a lower prevalence rate (30.8%) of anaemia among rural 6–12-year SAC [43]. However, two recent studies in the northern savannah zone of Ghana reported a higher prevalence rate (64%) than our study among 5-12-year-old rural SAC [9,44]. Although the apparent differences with our findings may be related to differences in sample size and socioeconomic conditions among others, the results suggest a variation in the prevalence of anaemia based on geographic/contextual circumstances. Agro-ecological zone consistently stood out as a key significant predictor of both Hb and anaemia in all models. Specifically, residing in any other agro-ecological zone compared to the forest zone was associated with a significant reduction in Hb level and a higher odd of anaemia (*P-trend* <0.001). The within-country variations in anaemia prevalence may partly be attributed to geographical disparities in dietary patterns, prevalence, and incidence of diseases as well as socio-economic factors. In Ghana, people residing in the northern savannah zone are notably more food insecure [45], consume fewer fruits than those in the coastal savannah and forest zones [46] and have a higher prevalence of malaria, which may be all contributing to anaemia [47]. The finding corroborates that of Hall *et al.* [48] who found a significant difference in the mean Hb and prevalence of anaemia between children residing by Lake Victoria and those residing on the coast of Tanzania.

In the present study, older children had higher levels of Hb, which has already been reported in other contexts [32,37,49]. However, in our stratified analysis, the observed trend remained statistically significant only for SAC, for whom a year increase in age was associated with a 2.21g/L increase in Hb and a 23% significant reduction in the odds of anaemia, suggesting young age was a risk factor for SAC. Younger children are generally more vulnerable to poor health as they are often more at risk of dietary inadequacy and infections such as malaria and helminths; for instance, poor dietary diversity and a high prevalence of malaria (81.3%) and sub-clinical inflammation (48.7%) have been reported among rural SAC in Ghana [9].

Age was not significantly associated with the Hb status of adolescents in the multiple regression; however, we found that after peaking at 11 years, the Hb of females declined (Figure S1a) which corroborates the findings of Sabale *et al.* [50] who found that Hb decreases significantly as age increases from 9-15 years among Mumbai school girls aged 9-19 years. The mean age of the adolescent girls (11.2 ± 1.3 years) in the present study corresponded with the mean age of the onset of menarche in Ghana [51,52] and may partly explain our finding. Unfortunately, we could not further test this hypothesis, as the baseline data did not include information on age at menarche.

Furthermore, a unit increase in age was significantly associated with 24% higher odds of anaemia for adolescents in the multiple logistic regression. However, the inclusion of an interaction term for age and sex showed that the odds of anaemia for a unit increase in age was only significantly higher for adolescent girls and not boys. This is not surprising considering the growth spurt in early adolescence and the increased iron requirements for girls at menarche [4]. Indeed, adolescent girls are particularly at high risk of developing iron deficiency and/or anaemia, especially amongst those who experience heavy blood losses during menstruation and corresponding decreases in ferritin levels [5,6]. The finding emphasizes the need for intervention programmes to curtail anaemia in early adolescence to compensate for the additional nutrient requirements for growth and puberty as well as the extra losses due to menstruation.

In contrast to many other studies that have reported a higher prevalence and risk of anaemia among females compared to males [20,31,43], though not significant, our findings pointed to a higher prevalence of anaemia among adolescent males compared to females and a similar prevalence for both school-age males and females (Figure 1). The finding is nevertheless similar to the result of Hall *et al.* [48], who found a higher prevalence of anaemia for 12-14 years boys compared to girls in rural Volta region of Ghana. Although the mean Hb of adolescent males was slightly lower than that of adolescent females in the present study (Boys: 115.3g/L, Girls: 117.0g/L, P=0.98), the WHO criterion classifies adolescent males aged ≥15 years as anaemic at a higher threshold compared to adolescent females with similar age (130 vs 120) g/L. Thus, more adolescent males than females were classified as anaemic and may partly account for the finding. Nonetheless, this finding implies that adolescent boys should also be targeted for anaemia control programmes besides adolescent girls, the usual priority group.

In agreement with evidence that high socioeconomic status is protective of anaemia [49,53] (Abdel-Rasoul *et al.*, 2015; Ngesa & Mwambi, 2014), belonging to the middle or upper terciles of the HAI was associated with a significant increase in Hb compared to those in the lower tercile of the HAI for adolescents. In addition, a unit increase in HDDS was associated with a 0.59 g/L significant increase in the Hb level of the children, but the association did not hold in the stratified analysis by age category, which could be because the sub-samples were underpowered to detect any statistical certainty. Farm diversity seemed to have a weak association with Hb but the association was clearer for SAC for whom a unit increase in farm diversity was significantly associated with a 0.59 g/L increase in Hb. We found no interaction between farm diversity and HDDS in the present study;

nevertheless, studies have shown that farm diversity improves household dietary diversity [25,26]. The HDDS is a measure of household food security rather than dietary quality [54], but it has been shown that household food security influences the quality of individual diets and micronutrient adequacy [55,56]. SAC children in households with maize stock were less likely to have anaemia compared to their peers in households without maize stock. Maize is generally a staple crop in most Ghanaian homes and food secured households are most likely to have maize stock in the household year-round; this suggests that SAC may be more vulnerable to the effects of household food insecurity, compared to adolescents.

Several studies have reported that parental education has a protective effect against anaemia in resource-poor settings [20,37,49]; therefore, it is surprising this association does not hold in our study with either education as a categorical variable or a continuous variable. This may be due to the relative lack of variation in educational status in our sample, with most of the parents having completed only a few years of schooling [mothers: 3.1 ± 5.6 years, fathers: 5.6 ± 6.5 years]. Likewise, we did not find any association between maternal occupation nor paternal occupation with Hb status or anaemia in contrast to several other studies [20,32,37].

Even though helminths infestation is a key significant determinant of anaemia [31,57], remarkably, we did not find any helminths infestation among a random sub-sample of the children (n=311) which was partly attributable to the mass deworming of school children which is being implemented in Ghana by the Ghana Health Service since 2009. Ghana started its national deworming campaign for schistosomiasis and soil-transmitted helminths in that year. Indeed, schools can constitute effective platforms for reaching this population through integrated deworming, water, hygiene, and sanitation programmes [58].

Strengths and limitations of the study

The results were generally robust when modelling with backward regression, multilevel regression and OLS. Nonetheless, some limitations inherent in the present study should be considered in the interpretation of our findings. Firstly, because these are observational, cross-sectional data, these cannot be interpreted as causal findings. We, therefore, limit our interpretation to describing associations. Even so, we thoroughly modelled several potential explanatory variables including subject and household demographics, education, livelihood, wealth, farming and production orientation and quality of household dietary intake, as well as used different methods to test the robustness of these associations.

Furthermore, intakes of vitamin A-rich and iron-rich foods are well-recognized predictors of anaemia [40], yet it was not possible to evaluate the contribution of dietary intake to the improvement of Hb in the present study as we lacked data on intakes of these foods specifically. Malaria is another factor that may contribute to anaemia [57,59] and a high prevalence of malaria among Ghanaian children has previously been reported [9,47]. Although we did not include malaria incidence in our statistical models, child sickness in the last 7 days preceding the survey was captured, which we presume, may have sufficiently accounted for any malaria incidence.

Our study population is rural, which limits the generalization of our findings to all SAC and adolescents in Ghana. Moreover, only SAC and adolescents enrolled in school were studied; but studies have shown that non-enrolled children may be more anaemic than those in school since enrolled children are in a better position to understand health and nutritional risks as this could be taught in school or sensitization programmes may be carried out in their schools. This could have underestimated the true prevalence of anaemia in our study but considering that 36% of children in rural areas never enter school in Ghana [60], the study population may well represent all rural children.

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Appendices

Table S1: Bivariate regression of the factors associated with haemoglobin concentration among rural Ghanaian schoolchildren stratified by age category

Predictors	Overall n=642			School-age children (6-9 y) n=323			Adolescents (10-17 y) n=319		
	β	SE (β)	*P-value*	β	SE (β)	*P-value*	β	SE (β)	*P-value*
Child characteristics									
Sex									
Male vs Female	0.75	1.03	0.468	-0.23	1.42	0.871	1.68	1.45	0.247
Age	1.25	0.23	<0.001	2.22	0.63	0.001	0.30	0.55	0.593
Child sick in the past 7 days									
Yes vs No	-0.50	1.32	0.703	-0.76	1.79	0.670	0.16	1.91	0.934
Child's birth order (continuous)	0.10	0.24	0.678	0.10	0.35	0.648	0.15	0.32	0.772
Child's school grade									
Upper primary/JHS vs Lower Primary	4.35	1.38	0.002	8.75	4.86	0.073	1.55	1.57	0.325
Ethnicity									
Gurma vs Akan	-2.60	1.30	0.046^{-}	-2.44	1.77	0.170	-2.07	1.87	0.269
Mole-Dagbani vs Akan	-2.58	1.48	0.081	-2.42	2.08	0.245	-2.69	2.04	0.188
Other[a] vs Akan	-2.82	1.48	0.057	-1.71	2.06	0.408	-3.75	2.07	0.070
Number of days child bought food from school	0.47	0.24	0.052^{-}	0.34	0.32	0.288	0.45	0.35	0.192
Household demographic and socioeconomic characteristics									
Dependency ratio	-2.74	2.78	0.324	-6.05	4.03	0.135	-0.16	3.73	0.967
Sex of household head									
Female vs Male	-0.05	1.15	0.966	-1.29	1.58	0.417	1.15	1.61	0.475
Age of household head	0.06	0.04	0.161	0.11	0.06	0.042	-0.01	0.05	0.917
Household asset index									
Middle vs Lower	1.89	1.26	0.133	-0.11	1.73	0.950	3.72	1.78	0.160
Upper vs Lower	0.17	1.26	0.891	-2.38	1.73	0.169	2.50	1.78	0.037
School years of father	0.11	0.08	0.175	0.16	0.11	0.154	0.04	0.11	0.701
School years of mother	-0.07	0.09	0.430	-0.24	0.13	0.073	0.02	0.13	0.881
Occupation of father (n=429)									
Other[b] vs Famer	1.74	1.35	0.199	-1.02	2.05	0.618	2.46	1.81	0.176
Occupation of mother (n=596)									
Trader vs Famer	1.14	1.14	0.316	1.39	1.55	0.371	0.42	1.62	0.797
Other[c] vs Famer	-0.41	1.46	0.782	-0.13	1.97	0.947	-0.93	2.10	0.657
Household received some remittance in the past 1 year									
Yes vs No	-0.31	1.23	0.789	-1.44	1.58	0.364	0.34	1.56	0.827
Household food availability and diet diversity									
HDDS	0.55	0.27	0.044	0.54	0.38	0.157	0.50	0.38	0.183
HFVS	0.14	0.08	0.110	0.10	0.12	0.427	0.14	0.11	0.224
HAFC	0.89	0.43	0.036	1.03	0.66	0.119	0.77	0.68	0.254
Proportion of food consumed in the past month from own production[d]	0.19	0.31	0.541	0.88	0.45	0.052	-0.02	0.41	0.967
Number of months household consumed food from own production	0.04	0.12	0.732	0.19	0.18	0.310	0.08	0.16	0.604
Maize stock in household									

Predictors	Overall n=642			School-age children (6-9 y) n=323			Adolescents (10-17 y) n=319		
	β	SE (β)	*P-value*	β	SE (β)	*P-value*	β	SE (β)	*P-value*
Yes vs No	0.89	1.22	0.467	3.16	1.64	0.055	-1.24	1.74	0.479
Farm diversity	0.33	0.20	0.103	0.86	0.27	0.002	-0.03	0.28	0.913
Household agriculture asset index									
Middle vs Lower	0.66	1.15	0.568	-0.82	1.60	0.611	2.28	1.61	0.157
Upper vs Lower	1.62	1.42	0.257	2.20	1.91	0.250	1.47	2.07	0.479
Household owns land									
Yes vs No	0.29	1.25	0.814	1.69	1.84	0.360	0.03	1.67	0.986
Geographical location									
Ecological zone in Ghana									
Northern Savannah vs Forest	-8.08	1.21	<0.001	-6.17	1.60	<0.001	-9.57	1.77	<0.001
Coastal Savannah vs Forest	-9.54	1.83	<0.001	-13.46	2.77	<0.001	-7.78	2.35	0.001
Transitional vs Forest	-6.91	1.30	<0.001	-6.77	1.85	<0.001	-7.31	1.77	<0.001

β,regression coefficient ; SE (β), standard error of regression coefficient; ref, reference group; HDDS, household dietary diversity score; HFVS, household food variety score; HAFC, household animal foods consumption; [a]Other includes Ga-Dangbe, Guan, Grusi, Mande, Ewe and other tribes originating from outside Ghana; [b]Other includes off-farm wage employment, business and unemployed; [c]Other includes off-farm wage employment, apprentice and unemployed; [d]Natural log-transformed variable of proportion of food consumed in the past month from own production;

Table S2: Univariate logistic regression of the factors associated with anaemia among rural Ghanaian schoolchildren stratified by age category

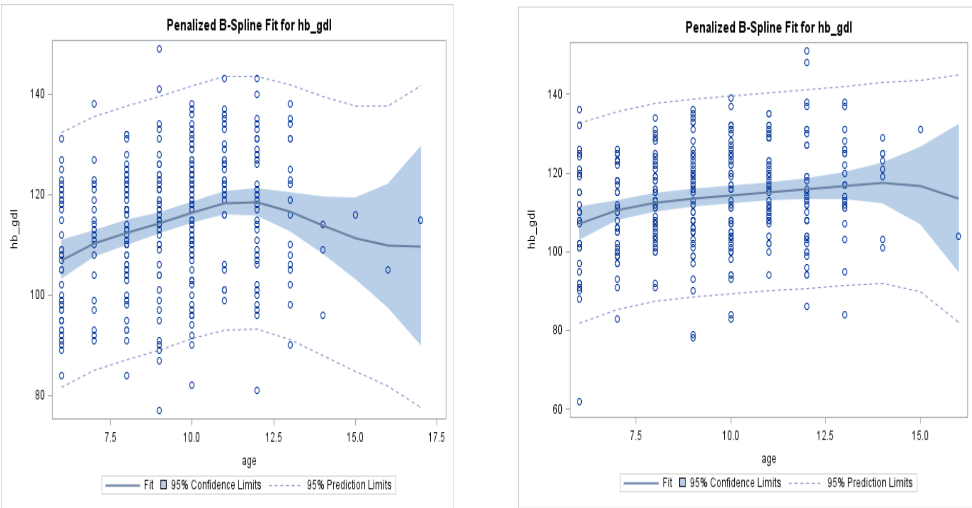
Predictors	Overall n=642		School-age children (6-9 y) n=323		Adolescents (10-17 y) n=319	
	POR (95% C.I)	*P*-value	POR (95% C.I)	*P*-value	POR (95% C.I)	*P*-value
Child characteristics						
Sex						
Male vs Female	1.23 (0.90, 1.68)	0.190	1.00 (0.64, 1.55)	0.995	1.52 (0.98, 2.36)	0.065
Age	0.96 (0.89, 1.03)	0.271	0.77 (0.63, 0.95)	0.012	1.22 (1.03, 1.45)	0.023
Child sick in the past 7 days						
Yes vs No	1.01 (0.68, 1.51)	0.954	1.16 (0.66, 2.01)	0.610	0.86 (0.48, 1.53)	0.610
Child's birth order (continuous)	0.98 (0.92, 1.06)	0.652	1.00 (0.91, 1.11)	0.984	0.96 (0.87, 1.07)	0.472
Child's school grade						
Upper primary and JHS vs Lower Primary	0.84 (0.55, 1.27)	0.398	0.32 (0.06, 1.67)	0.175	1.03 (0.64, 1.65)	0.911
Ethnicity		0.021		0.072		0.125
Gurma vs Akan	1.72 (1.16, 2.55)		2.04 (1.17, 3.56)		1.39 (0.78, 2.45)	
Mole, Dagbani vs Akan	1.38 (0.87, 2.16)		1.74 (0.91, 3.33)		1.12 (0.60, 2.08)	
Other[a] vs Akan	1.75 (1.12, 2.75)		1.44 (0.76, 2.72)		2.13 (1.12, 4.06)	
Number of days child bought food from school	0.94 (0.87, 1.01)	0.074	0.93 (0.84, 1.03)	0.163	0.95 (0.85, 1.05)	0.319

Predictors	Overall n=642		School-age children (6-9 y) n=323		Adolescents (10-17 y) n=319	
	POR (95% C.I)	*P*-value	POR (95% C.I)	*P*-value	POR (95% C.I)	*P*-value
Household demographic and socioeconomic characteristics						
Dependency ratio	1.54 (0.66, 3.55)	0.316	2.65 (0.75, 9.34)	0.132	1.00 (0.32, 3.09)	0.998
Sex of household head						
Female vs Male	0.91 (0.64, 1.28)	0.573	0.96 (0.59, 1.57)	0.881	0.85 (0.52, 1.39)	0.522
Age of household head	0.99 (0.98, 1.00)	0.189	0.98 (0.97, 1.00)	0.062	1.00 (0.98, 1.02)	0.992
Household asset index		0.655		0.791		0.159
Middle vs Lower	0.88 (0.61, 1.29)		1.18 (0.69, 2.01)		0.66 (0.38, 1.13)	
Upper vs Lower	0.84 (0.58, 1.23)		1.16 (0.68, 1.98)		0.61 (0.36, 1.05)	
School years of father	0.98 (0.96, 1.01)	0.157	0.98 (0.95, 1.02)	0.339	0.98 (0.95, 1.02)	0.315
School years of mother	1.00 (0.97, 1.03)	0.996	1.01 (0.97, 1.05)	0.756	1.00 (0.96, 1.04)	0.873
Occupation of father (n=429)						
Other[b] vs Farmer	0.90 (0.60, 1.35)	0.605	1.29 (0.68, 2.43)	0.439	0.73 (0.42, 1.28)	0.267
Occupation of mother (n=596)		0.589		0.622		0.921
Trader vs Farmer	0.83 (0.58, 1.18)		0.78 (0.47, 1.30)		0.99 (0.52, 1.88)	
Other[c] vs Farmer	0.92 (0.58, 1.44)		0.86 (0.45, 1.64)		0.91 (0.55, 1.49)	
Household received some remittance in the past 1 year		0.608		0.881		0.626
Yes vs No	0.92 (0.65, 1.28)		0.96 (0.59, 1.57)		0.89 (0.56, 1.42)	
Household food availability and diet diversity						
HDDS	0.92 (0.85, 1.00)	0.055	0.89 (0.78, 0.99)	0.047	0.96 (0.86, 1.08)	0.485
HFVS	0.98 (0.96, 1.01)	0.187	0.97 (0.94, 1.01)	0.178	0.99 (0.96, 1.03)	0.635
HAFC	0.91 (0.79, 1.05)	0.205	0.91 (0.74, 1.11)	0.351	0.92 (0.75, 1.13)	0.442
Proportion of food consumed in the past month from own production[d]	1.02 (0.93, 1.12)	0.717	0.96 (0.83, 1.10)	0.553	1.05 (0.93, 1.19)	0.443
Number of months household consumed food from own production	1.00 (0.96, 1.04)	0.920	0.99 (0.94, 1.05)	0.800	1.00 (0.95, 1.05)	0.848
Maize stock available in household						
Yes vs No	0.79 (0.55, 1.13)	0.197	0.59 (0.35, 0.98)	0.040	1.06 (0.63, 1.80)	0.822
Agriculture and farm diversity						
Farm diversity	0.98 (0.93, 1.04)	0.581	0.90 (0.82, 0.99)	0.024	1.06 (0.97, 1.15)	0.197
Household agriculture asset index		0.139		0.126		0.318
Middle vs Lower	1.01 (0.71, 1.43)		1.31 (0.79, 2.16)		0.78 (0.48, 1.27)	
Upper vs Lower	0.67 (0.43, 1.02)		0.69 (0.38, 1.24)		0.64 (0.34, 1.20)	

table continues

Predictors	Overall n=642 POR (95% C.I)	*P*-value	School-age children (6-9 y) n=323 POR (95% C.I)	*P*-value	Adolescents (10-17 y) n=319 POR (95% C.I)	*P*-value
Household owns land						
Yes vs No	1.14 (0.79, 1.67)	0.484	1.04 (0.59, 1.83)	0.900	1.19 (0.72, 1.98)	0.499
Geographical location						
Ecological zone in Ghana		<0.001		<0.001		<0.001
Forest	Ref.		Ref.		Ref.	
Northern Savannah	2.99 (2.02, 4.43)		2.50 (1.47, 4.24)		3.64 (2.02, 6.58)	
Coastal Savannah	3.07 (1.70, 5.57)		3.48 (1.33, 9.08)		3.00 (1.39, 6.49)	
Transitional	2.68 (1.76, 4.08)		2.98 (1.60, 5.53)		2.51 (1.41, 4.48)	

n=sample size; POR, prevalence odds ratio ;95% C.I, 95% confidence interval ; ref, reference group; HDDS, household dietary diversity score; HFVS, household food variety score; HAFC, household animal foods consumption; [a]Other includes Ga-Dangbe, Guan, Grusi, Mande, Ewe and other tribes originating from outside Ghana; [b]Other includes off-farm wage employment, business and unemployed; [c]Other includes off-farm wage employment, apprentice and unemployed; [d]Natural log-transformed variable of proportion of food consumed in the past month from own production.



a b

Figure S1: A smoothed scatter plot of the haemoglobin concentration(hb_gdl) of the school age children and adolescents by sex*; females (a) and males (b);* ***interpret*** *with caution as sample size from 14-17 years were small (14 y, n=11; 15 y, n=2; 16 y, n=2 and 17 y, n=1)*

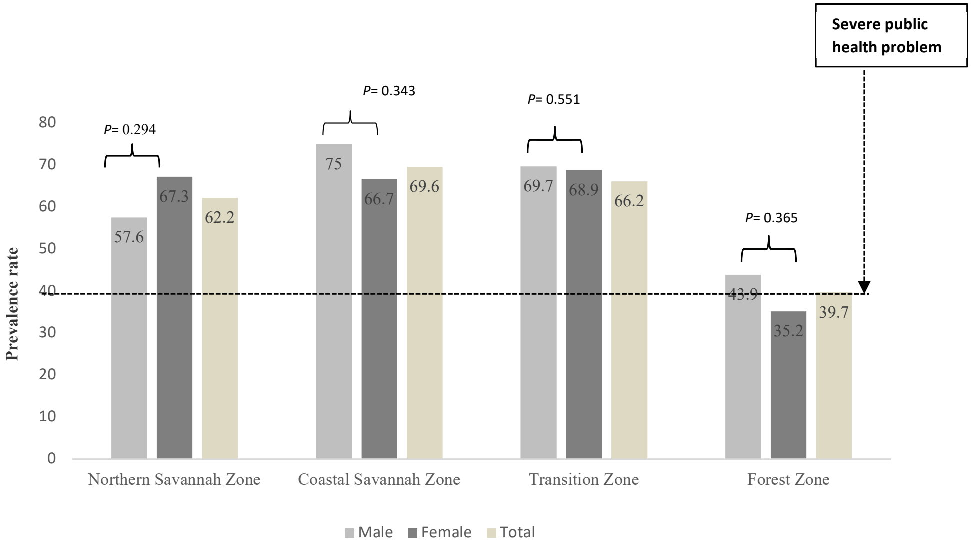


Figure S2: Prevalence of anaemia among school-age children by agro-ecological zone and sex

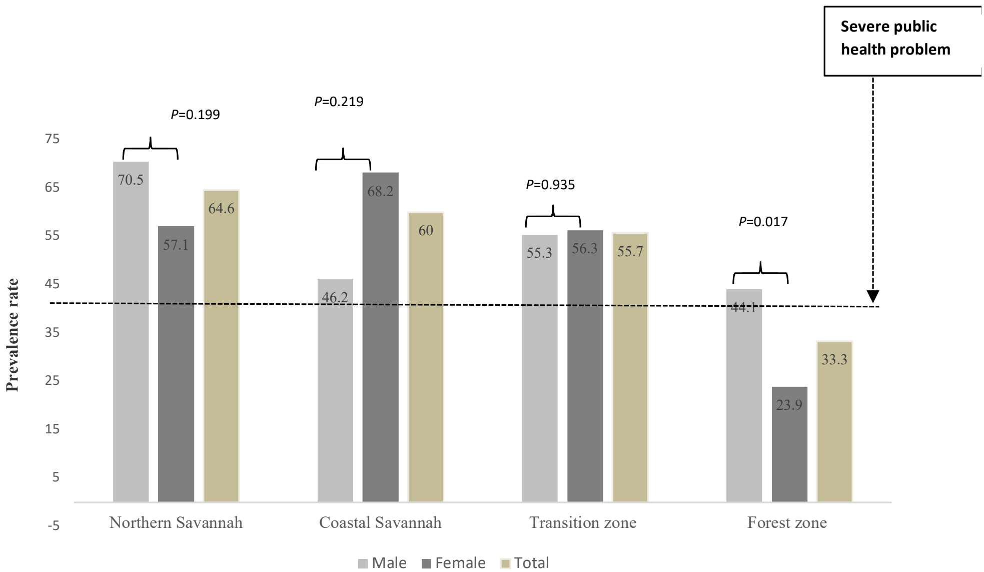


Figure S3: Prevalence of anaemia among adolescents by agro-ecological zone and sex

Chapter 4

Trends and Factors Associated with the Nutritional Status of Adolescent Girls in Ghana: A Secondary Analysis of the 2003-2014 Ghana Demographic and Health Survey (GDHS) Data

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Based upon Public Health Nutr. 2021;:1–41/
https://doi.org/10.1017/s1368980021003827

Abstract

Objective

We examined the trends-over-time and the factors associated with malnutrition among adolescent girls in Ghana.

Design

Cross-sectional analysis from 3 nationwide Ghana Demographic and Health Surveys conducted in 2003 (n=983), 2008 (n=955) and 2014 (n=857). We used Cox proportional hazard models with sample weighting to model the prevalence ratio (PR) of malnutrition.

Setting

Countrywide, covering rural and urban areas in Ghana.

Subjects

Non-pregnant adolescents girls aged 15-19 years.

Results

Compared with 2003 (prevalence 2.0%), thinness declined marginally [prevalence risk (PR) 0.88, 95% CI (0.45, 1.73)] in 2008 and in 2014 [PR 0.71, 95% CI (0.38, 1.56)]. A uniform distribution was found for stunting; the prevalence amounted to 7.9% in 2003 with the PR declining marginally by 19% in 2008 [PR 0.81, 95% CI (0.59, 1.12)], and stayed steady in 2014 [PR 0.81, 95% CI (0.57, 1.17)]. Stunting decreased slightly by only 1.5% points and underweight by less than 1%-point difference in 2014 compared to 2003. We found an increase in overweight/obesity from 10.0% in 2003 with the PR peaking in 2014 [PR 1.39, 95% CI (1.02, 1.88)]; the percentage point difference between 2003 and 2014 increased by 4.3% (95% CI, 0.74, 7.84%) after adjusting for significant predictors in the pooled model. Anaemia remained severe (2003: 44.3%; 2008: 62.1% and 2014: 47.3%) without a clear trend, increasing significantly by 18.1% points in 2008 with a minor increase (2.82%, 95% CI, -1.76, 7.41) in 2014 compared to 2003. A low level of education of the adolescent girl was positively associated with stunting. Increasing age was positively associated with stunting but inversely associated with thinness and anaemia. Girls who had ever given birth were more likely to be anaemic compared to those who never did. A lower level of household wealth and a unit increase in household size were negatively associated with overweight/obesity. Urban dwelling girls were less likely to be stunted.

Conclusion

A steady burden of undernutrition and rising overnutrition emphasise the need for so-called “double-duty actions” to tackle malnutrition in all its forms in Ghanaian adolescent girls.

Introduction

A little over a fifth of the female Ghanaian population is adolescent girls (aged 10-19 years) [1]. In addition to physical growth, adolescence is characterised by profound biological, psychosocial, and cognitive changes [2, 3] related to improved nutrition [4–6]. Besides the first 1000 days of life, adolescence offers an additional (and last) critical window of opportunity for linear growth catch-up [7, 8].

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Nutrient requirements during adolescence are among the highest in the life cycle, making adolescents vulnerable to undernutrition [3, 9] and micronutrient deficiencies, primarily anaemia and iron deficiency anaemia [10, 11]. Some studies also show an increasing overweight prevalence, leading to a double burden of malnutrition among adolescents in low-and middle-income countries (LMICs), particularly for girls [12, 13]. The 2014 Ghana demographic and health survey (GDHS) [14] indicates that 14.0% of 15-19-year-old female adolescents are thin, and 9.0% are overweight. Other studies show that 44.0% of rural Ghanaian adolescent girls aged 10-19 years are anaemic, being higher than 60.0% in the northern and coastal savannah agro-ecological zones [15]. This confirms the presence of the double burden of malnutrition among adolescents in Ghana, which has adverse effects on attained height [16], productivity later in life [17] and cardiovascular risk [18].
Malnutrition is also associated with educational, social, and economic disadvantages that reduce young people's capabilities as they mature, contributing to low social and economic status within the household [19]. About a third of teenage girls in Ghana are married by the age of 18 years [1], and 14.0% of those aged 15-19 years have begun childbearing [14], increasing malnutrition risks for themselves and their children [7, 20]. Girls in Ghana have unhealthier eating habits than boys [21] and are disadvantaged in intra-household food distribution and resource allocation [22]. Ghanaian girls are also more likely to drop out of secondary school than boys [23].

The causes of malnutrition are multi-level and can be explained using a conceptual framework, adapted from a recently proposed socio-ecological framework (Figure 1) for adolescents by our group [24]. The framework recognises the complex hierarchical relationship of determinants of nutrition at the environment/community, household, and individual level. Individual-level characteristics of the girl such as age, sex, disease, birth order, education, occupation, and marital status may affect her nutritional status, mostly through dietary intake, aside from susceptibility and exposure to infection and access to health service [25]. Household-level characteristics influence those at the individual level. Some socio-demographic characteristics of girls, such as marital status, may be influenced by parental education and household wealth [26]. Household characteristics also influence girls' empowerment [27], including education, occupation, and autonomy; empowerment is an essential determinant of nutrition in many developing contexts [27–30]. Place of residence, parental education and occupation, and household wealth influence the household's access to resources, including food, health, and sanitation services [24]. Poor household access to safe water and sanitation facilities leads to an increased risk of infections and diseases, affecting food intake and utilisation [31]. The household's structure such as a large household size may increase the dependency ratio with consequences for dietary intakes [32] due to competing needs for food and health care. Community or environmental level factors are additive factors driving girls' nutrition directly or through household-level determinants. Cultural and religious norms prevalent in the community influence household behaviours [33]. Girls are particularly vulnerable to cultural and gender norms, which often discriminate against them [34].

No national representative data and analysis on determinants of adolescents' nutrition in Ghana are available. Some studies indicate that dietary intake, parental education and occupation, household socio-economic status, type of residence and ecological zone are predictors of adolescents' nutrition in Ghana [11, 35, 36], but the geographic scope and sample size limit the generalizability of these results. Also, no study examined the changes over time in the nutritional status of Ghanaian adolescent girls which may be significant for three (3) reasons. Firstly, there has been a massive improvement in socio-economic conditions in the last three decades in Ghana [37]. Secondly, the country is presently experiencing a nutrition transition, mirrored in more imports in the food environment [38] and the consumption of more processed food [39]. Thirdly, several social protection programmes have been implemented since the turn of the 21st century, including Livelihood Empowerment Against Poverty Programme and Ghana School Feeding Programme, to reduce poverty and undernutrition in marginalised and vulnerable groups.

This study aimed to fill this critical knowledge gap by defining the trends over time in the prevalence of malnutrition, including under- and over-nutrition, and the factors associated with malnutrition among adolescent girls in Ghana using nationally representative data included in the GDHS. Our analyses may provide much-desired evidence for policy formulation and programme planning to optimise interventions that improve nutrition and health for adolescent girls in Ghana.

Methods

Study design: We conducted secondary analyses of the national representative 2003, 2008 and 2014 GDHS data for non-pregnant adolescent girls aged 15-19 years. The GDHS contains data on individual demographic characteristics, household characteristics, fertility, women's empowerment, nutrition, and health of Ghanaian women aged 15-49 years. Although available, we did not use the 1993 and 1998 GDHS data due to the absence of haemoglobin (Hb) data and small sample sizes (see Supplementary Table S1 for population selected for analysis). Details of the sample selection and data collection of the surveys are presented in the DHS Methodology report [40]. The datasets are accessed through the DHS MEASURE website [41]. The Ethical Review Committee of Ghana Health Service, Accra, Ghana, approved the GDHS, and no further ethical approval was required. We obtained permission from DHS MEASURE to download and analyse the data.

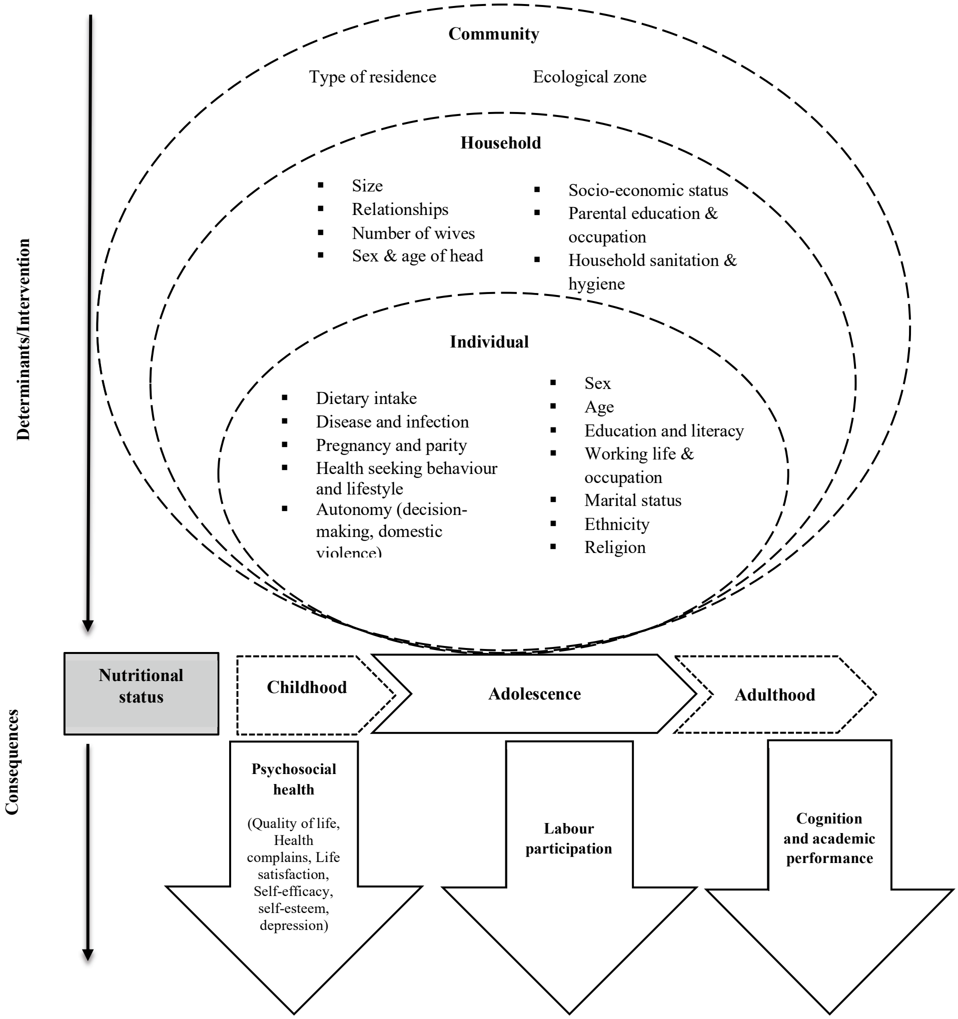

Figure 1. A conceptual framework for the factors associated with the nutritional status of adolescent girls from the 2003-2014 Ghana Demographic and Health Survey

Adapted from Madjdian *et al.* [24]

Dependent variables

Anthropometric indicators: Based on height, weight, age, and sex, height-for-age z-score (HAZ) and body-mass-index for-age z-score (BAZ) were computed with WHO Anthroplus (version1.0.4), using the WHO 2007 growth reference for 10-19 years adolescent girls. We defined stunting (HAZ < -2SD), thinness (BAZ <-2SD), normal weight (-2SD ≤ BAZ ≤ +1 SD), overweight (+1SD < BAZ < +2SD) and obesity (BAZ ≥ +2SD) in conformity to De Onis *et al.* [42].

Anaemia: In all surveys, Hb concentration was measured with the HemoCue 301, using finger prick by trained health technicians from Ghana Health Service. Hb concentration was adjusted for altitude and smoking. Anaemia and severity were defined using the WHO criteria for non-pregnant girls [43], i.e. Hb < 120g/L; severe, moderate, and mild anaemia as Hb < 80g/L, 80 ≤ Hb < 110, and 110 ≤ Hb<120, respectively.

Independent variables

The following explanatory variables for the girls' nutritional status were selected based on the conceptual framework and data availability in the GDHS.

Individual-level variables: Marriage and fertility-related variables included marital status (categorical), having ever given birth (dichotomous), and continuous variables for the age at first birth (if any), and the number of children ever born. The girl's health-seeking behaviour included dichotomous variables of having visited a health facility in the last 12 months, sleeping under a mosquito net, and being covered by the national health insurance scheme (NHIS). Lifestyle factors in the analyses included the frequency of watching TV and of listening to the radio in the past week. The girl's working life included a dichotomous variable for currently working and a categorical variable for occupation. Girls' educational status was assessed as a categorical variable and as the number of completed years of schooling. Other demographic characteristics of girls included age in complete years and categorical variables for religion and ethnicity. Data on dietary intake included the frequency of consuming fruits and vegetables in the past week, only available for the 2008 and 2014 surveys and modelled as continuous variables for the survey specific models. We also included an index of autonomy regarding domestic violence [44] as a proxy of empowerment (see Supplementary Table S2a); the score ranged from 0-5, with a higher score reflecting a greater sense of entitlement and self-esteem and thus higher autonomy [14].

Household-level variables: Data included household size, the number of children aged under five years, and the household head's age as continuous predictors; the sex of the household head (dichotomous), the relationship of the girl to household head (categorical) and the socio-economic status of the household defined by the household wealth index (HWI) quintiles. The HWI is a composite measure of a household's cumulative living standard, calculated using principal components analysis of data on household's ownership of selected assets, materials used for housing construction, types of water access and sanitation facilities, and cooking fuel [40]. In the 2008 and 2014 surveys, dichotomous variables for the household ownership of land and farm animals were also included in the analysis. We constructed a composite index of household water and sanitation facilities (WASH) in conformity to the joint WHO/UNICEF guidelines on improved WASH to prevent oral-faecal contamination [45]; previous studies [46, 47] have used similar indexes (see Supplementary Table S2b). The WASH index ranged from 0-3 based on the available data across surveys.

Community and broader environmental-level variables: These included the type of residence and agro-ecological zone. In the GDHS, the countryside was classified as rural residence, while towns and cities were classified as urban [40]. The previous ten administrative regions of Ghana used for GDHS were classified into 3 agro-ecological zones [48], including the (1) Guinea/Sudan savannah (Northern, Upper East and Upper West Regions), (2) coastal savannah (Central, Greater Accra, and Volta Regions), and (3) forest zone (Brong-Ahafo, Ashanti, Western and Eastern Regions) for the analyses.

Statistical analysis

All statistical analyses were done with SAS 9.4 (SAS Institute Inc., Cary, NC.). Statistical significance was considered as a two-tailed *P*-value ≤ 0.05 at a 95% confidence interval. We presented descriptive statistics as percentages for dichotomous/categorical variables and as means (standard errors) for continuous variables. We used trend graphs to map trends over time in mean HAZ, BAZ and Hb and the prevalence of stunting, thinness, overweight/obesity and anaemia. Cox proportional hazard models were fitted to analyse the prevalence ratios (PR) over time and identify nutritional status determinants over the years with all outcome variables being binary (stunted vs not stunted, thin vs normal weight, overweight/obese vs normal weight, and anaemic vs not anaemic).

Bivariate analyses were first fitted, and all results with *P*-values ≤ 0.25 were further assessed in the multivariable models. In the multivariable models, we explored potential interactions by adding pair-wise interaction terms for the determinants, but none was statistically significant. We first created survey specific models and then pooled the data across all surveys to fit an overall model. In the pooled models, the survey year was included as a categorical variable to examine the trend in the PR with reference to 2003. The log-likelihood ratio test, Akaike information criteria (AIC), Wald test and *P*-value informed the final models. Variables were retained in all final models if they were associated with the outcome variable at a *P*-value of ≤ 0·05. We applied weighting factors in the data and adjusted for strata and cluster effects using the *PROC SURVEY* function in SAS [49], adjusting for differences in the probability of selection and interview due to the intricate survey design. For the pooled analysis, a combined weighting factor was applied. A detailed explanation of the weighting procedure can be found in the DHS Methodology report [40]. In a sensitivity analysis, we repeated all the analyses with linear regression using the "*PROC SURVEYREG*" command in SAS [49] (Supplementary Table S4a-c). We further examined the absolute percentage point decrease/increase in malnutrition prevalence between 2003 and 2014 (Table S5) using SAS "*PROC SURVEYREG*" command [49].

Results

Population characteristics: For all surveys (Table 1), the adolescent girls' mean age was approximately 17 years; about half of the respondents were of Akan ethnicity and more than three-quarters of the adolescents professed Christianity. The majority (≥ 64.5%) were unemployed. Most of the girls had secondary/higher education, and the proportion improved marginally from 65.5% in 2003 to 72.5% in 2014. Less than 5% of the girls were wives of the household head. About half resided in rural areas and Ghana's forest zone. About a quarter of the girls had visited a health facility in the past 12 months. The proportion of girls who slept under a mosquito bed net increased from 4.9% in 2003 to 28.5% in 2014. The proportion of those who were currently married decreased from 9.7% in 2003 to 5.7% in 2014. About a tenth of the girls had ever given birth with a mean number of births of one child across all years. The score for autonomy improved marginally from 3.7 in 2003 to 4.0 in 2014. The frequency of watching TV decreased from 1.6 in 2003 to 1.2 in 2014 but was highest in 2008 (1.7).

Table 1. Population Descriptive Statistics for Adolescent Girls from the 2003-2014 Ghana Demographic and Health Survey Data

Variable	Year of Survey 2003 (n= 983)		2008 (n= 955)		2014 (n= 857)		Pooled data (n= 2795)	
	Mean or Percentage	SE (mean)	Mean or Percentage	SE (mean)	Mean or Percentage	SE (mean)	Mean or Percentage	SE (mean)
Age[1] (years)	16.9	0.1	17.0	0.1	16.8	0.1	16.9	0.0
Health seeking behaviour and lifestyle								
Visited health facility last 12 months	21.0		25.9		24.9		23.9	
Respondent slept under a mosquito bed net	4.9		16.5		28.5		16.6	
Covered by National Health Insurance (NHIS)	-		38.5		56.9		47.7	
Frequency of listening to radio in the past week[1]	2.0	0.0	2.0	0.1	1.3	0.0	1.8	0.0
Frequency of watching television in the past week[1]	1.6	0.1	1.7	0.1	1.2	0.0	1.5	0.0
Dietary intake								
Frequency of fruit intake in the past week[1]	-	-	4.0	0.1	3.0	0.1	3.5	0.1
Frequency of vegetable intake in the past week[1]	-	-	3.8	0.1	3.3	0.1	3.5	0.1
Demographics of the girl								
Religion								
Christian	82.0		79.9		80.6		80.9	
Muslim	15.4		14.9		17.1		15.8	
Other	2.6		5.1		2.3		3.3	
Ethnicity								
Akan	53.0		50.6		49.3		51.0	
Mole-Dagbani	11.3		15.1		16.6		14.3	
Other	35.7		34.3		34.1		34.7	
Occupation of girl								
Unemployed	65.3		67.7		64.5		65.8	

table continues

4

Variable	Year of Survey 2003 (n= 983) Mean or Percentage	SE (mean)	2008 (n= 955) Mean or Percentage	SE (mean)	2014 (n= 857) Mean or Percentage	SE (mean)	Pooled data (n= 2795) Mean or Percentage	SE (mean)
Agriculture/unskilled labour	21.5		21.2		26.9		23.2	
Skilled labour	13.2		11.1		8.6		11.0	
Girl is currently working	32.0		31.8		33.1		32.3	
Years of schooling[1]	2.8	0.1	3.0	0.1	3.0	0.1	2.9	0.0
Highest educational level of girl								
No education	11.3		6.9		3.8		7.3	
Primary school	23.2		20.7		23.7		22.5	
Secondary education /Higher	65.5		72.4		72.5		70.1	
Marriage, fertility, and relations								
Total children ever born[1]	1.1	0.0	1.2	0.0	1.2	0.1	1.1	0.0
Age at first birth[1]	16.9	0.1	16.6	0.2	16.6	0.2	16.7	0.1
Girl has ever given birth	10.2		9.8		10.8		10.3	
Marital status								
Never married	88.5		92.9		93.6		61.6	
Formerly married	1.8		0.9		0.7		6.7	
Currently married	9.7		6.1		5.7		31.7	
Relation of girl to the household head								
Household head	2.1		3.9		1.9		2.6	
Wife	4.6		3.7		3.6		4.0	
Daughter	57.6		59.2		62.4		59.8	
Granddaughter	10.5		9.0		8.0		9.2	
Other family relation	19.2		19.0		15.5		17.9	
Non-family relation	6.0		5.2		8.6		6.5	
Autonomy								
Autonomy index[1]	3.7	0.1	3.9	0.1	4.0	0.1	3.6	0.0
Household characteristics								
Age of household head[1]	36.36	1.8	48.7	0.6	49.2	0.7	48.8	0.4
Household size[1]	6.2	0.1	5.6	0.1	5.7	0.1	5.8	0.1
Number of children < 5years[1]	0.8	0.0	0.7	0.0	0.72	0.0	0.7	0.0
WASH index[1]	2.1	0.0	2.3	0.0	2.3	0.0	2.5	0.0
Sex of household head (male)	57.5		57.7		59.6		58.2	
Household wealth index								
Poorest	14.3		14.5		22.7		17.2	
Poorer	14.2		18.4		22.0		18.2	
Middle	19.1		21.5		18.8		19.8	
Richer	23.7		23.3		17.4		21.5	
Richest	28.7		22.2		19.1		23.3	
Household owns land usable for agriculture	-		49.6		46.1		47.8	
Household owns livestock	-		49.6		48.6		49.1	
Geographical /environmental								
Place of residence								
Rural	55.4		48.8		50.5		51.6	
Urban	44.6		51.2		49.5		48.4	
Agro-ecological zone								
Coastal savannah	35.4		34.6		31.9		34.0	
Forest	51.4		47.7		50.7		49.9	
Guinea/Sudan savannah	13.1		17.7		17.4		16.1	

[1]Values are means with standard errors, all other values are percentage; Autonomy index, a proxy of autonomy regarding domestic violence; WASH, Household water, hygiene, and sanitation; SE (mean), standard error of the mean.

The trend in nutritional status and malnutrition: The mean HAZ and BAZ increased non-significantly from 2003 to 2014 (Figure 2a) and the prevalence of stunting and thinness were comparable across the years (Figure 2b). The prevalence of overweight increased from 10.0% in 2003 to 12.1% in 2008 but virtually flattened off in 2014 (Figure 2b). We observed a V-shaped curve in the adolescent girls' mean Hb status between 2003 and 2014 (Figure 3a) with the mean Hb being higher in 2003 (120.9g/L, SE 0.5) compared to 2008 (113.2g/L, SE 0.6) and 2014 (118.4, SE 0.6) (Table 2b). An inverted V-shape was observed in the prevalence of anaemia between 2003 and 2014, with the 2008 survey recording the highest prevalence of anaemia at 62.1% (Figure 3b). The prevalence of moderate anaemia changed the most for the surveyed years (Figure 3b). Supplementary Table S3 indicates the prevalence rates of the girls' nutritional status by year of the survey.

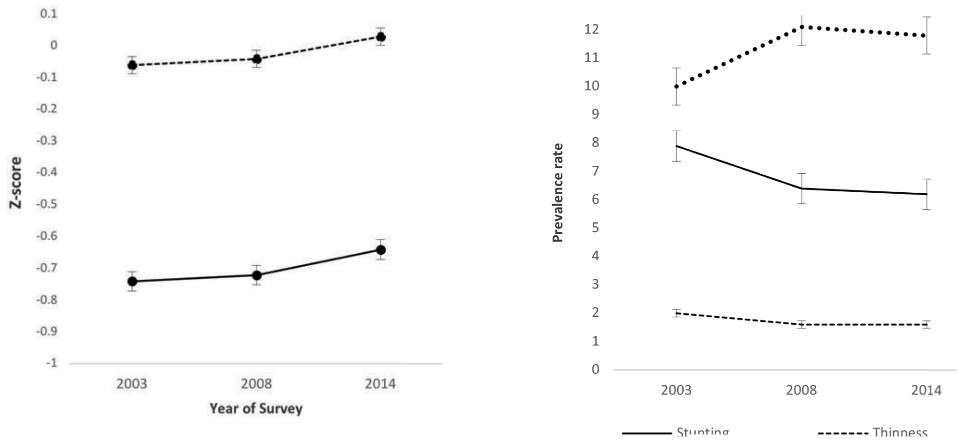

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Figure 2. Trend in the: (a)mean height-for-age z-score (HAZ) and mean body-mass index-for-age z-score (BAZ); (b) prevalence of protein-energy malnutrition among 15-19 years female adolescents from 2003-2014 in Ghana.

Vertical bars are standard errors of the (a) arithmetic means and (b) prevalence rates

Compared to 2003, the PR of stunting decreased non-significantly by 19% (95% CI 0.59, 1.12) in 2008 and 2014 respectively (Figure 4); the PR of thinness declined non-significantly in 2008 compared to 2003, with a further non-significant decrease between 2008 and 2014. Additionally, compared to the 2003 survey, the PR of overweight/obesity increased by 28% in 2008, peaking significantly at 39% (95% CI 1.02, 1.88) in 2014. The PR of anaemia increased significantly by 41% in 2008 compared to 2003, but the trend virtually flattened out in 2014 (Figure 4). Between 2003 and 2014, stunting decreased slightly by only 1.5% points and underweight by less than 1%-point difference but, overweight increased significantly by 4.3% (95% CI, 0.74, 7.84%) points for the adolescent girls after adjusting for significant predictors (Table S4). Anaemia increased significantly by 18.1% points in 2008 with a minor increase (2.82%, 95% CI, -1.76, 7.41) in 2014 compared to 2003.

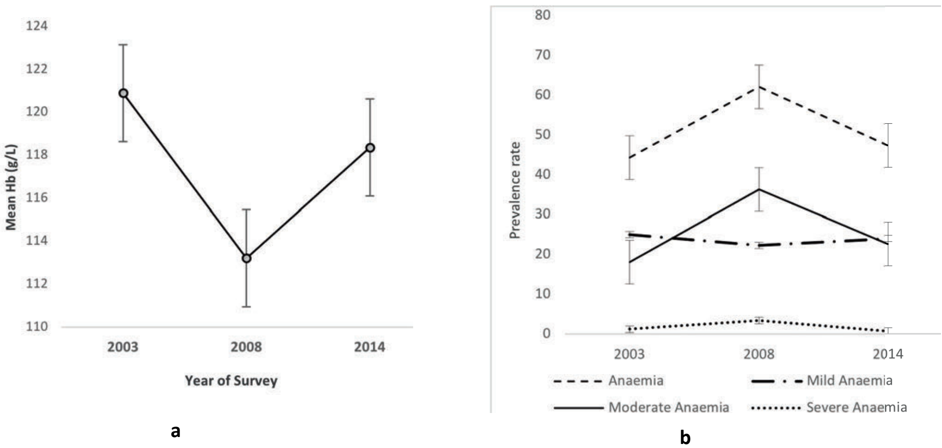

Figure 3. Trend in the: (a) mean haemoglobin (g/L) and (b) anaemia prevalence among female adolescents aged 15-19 years from 2003-2014 in Ghana; *Hb, haemoglobin; anaemia (Hb <120g/dL); mild anaemia (110g/L ≤ Hb ≤ 119g/L); moderate anaemia (80g/L ≤ Hb ≤ 109g/L) and severe anaemia (Hb < 80 g/L). Vertical bars are standard errors of the (a) arithmetic means and (b) prevalence rates.*

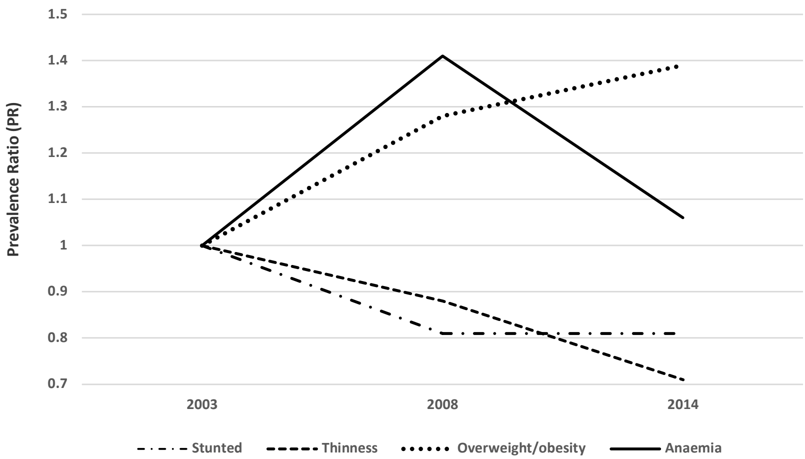

Figure 4. Trend in the adjusted prevalence ratio (PR) of malnutrition among adolescent girls in Ghana from 2003-2014*; all PRs were adjusted for predictors that were significant in the pooled analysis for each outcome variable*

Factors associated with the nutritional status of Ghanaian adolescent girls

Individual-level Factors: Compared to girls with secondary or higher education, those with primary and no education were more likely to be stunted in all but the 2008 survey (Table 2). Girls with no or primary school education were more likely to be thin in 2008 than those with secondary or higher education (Table 3). The PR of anaemia was higher for girls who had ever given birth compared to their peers who had never given birth in both the 2008 and the pooled model (Table 3). In 2014, stunted girls compared to non-stunted girls were more likely to be anaemic (Table 5). Increasing age was positively associated with stunting in 2014 and the pooled model, and with overweight in 2008 (Tables 2 and 4, respectively). Furthermore, increasing age was inversely associated with anaemia in all but 2003 (Table 5). In the 2014 and pooled model, married girls were less likely to be stunted compared to never-married girls (Table 2) and more likely to be overweight/obese compared to never-married girls in 2014 and the pooled model (Table 4). The association between ethnicity and stunting was inconclusive, with conflicting results in 2003 and 2008. However, girls from the Akan ethnicity were significantly less likely to be overweight or obese in both 2008 and the pooled analysis. An increase in the autonomy index was inversely associated with stunting (Table 2) but positively associated with overweight/obesity in 2014 (Table 4). An increase in the frequency of watching TV was inversely associated with thinness in the pooled model (Table 3) and positively associated with overweight/obesity in 2003 (Table 4). For an increase in the frequency of listening to the radio, the PR of thinness increased by 48% in 2003 (Table 3). An increase in fruit consumption frequency significantly reduced the thinness PR in 2014 (Table 3).

Household-level Factors: An increase in the WASH index was positively associated with stunting in 2003 but inversely associated with stunting in 2008 (Table 2). A lower HWI was positively associated with stunting for only the 2003 survey (Table 2). Girls in the first four quintiles of the HWI compared to the fifth quintile were less likely to be overweight or obese for all survey years and the pooled analysis (Table 4). Except for the 2014 survey, an increase in household size was inversely associated with overweight/obesity (Table 4). Household land ownership was significantly associated with anaemia in 2014 (Table 5). Compared to girls that were daughters of the household head, the PR of anaemia was significantly lower for girls who were not related to the household head (Table 5).

Community-level Factors: In our pooled analysis, urban girls were significantly less likely to be stunted than their rural peers (Table 2). Furthermore, compared to girls who resided in the forest zone, those who resided in the coastal savannah zone were significantly more likely to be stunted in 2003 (Table 2) and those residing in the coastal and Guinea/Sudan savannah zones were less likely to be anaemic in 2008 (Table 5). However, girls in the coastal savannah zone were significantly more likely to be anaemic in 2014 (Table 5).

Sensitivity Analysis: Our sensitivity analysis (Table S5a-c) showed a similar trend for HAZ, BAZ, Hb, and the factors associated with each of these outcomes. Lower HWI quintiles were significantly associated with a lower HAZ and BAZ in all the statistical models. Being thin was associated with a lower Hb in all but the 2014 survey model.

Table 2. Multivariate Predictors of Stunting Among Non-Pregnant Adolescent Girls: Analysis of the 2003-2014 Ghana Demographic Health Survey (GDHS) Data

Variables	2003 (n= 983)		2008 (n= 955)		2014 (n= 857)		Pooled (n= 2795)	
	PR	95% CI	PR	95% CI	PR	95% CI	PR	95% CI
Age					1.32	1.11, 1.58	1.11	1.01, 1.23
Ethnicity								
Akan vs Other	1.73	1.06, 2.83	0.58	0.32, 1.06				
Mole-Dagbani vs Other	0.94	0.39, 2.24	0.47	0.23, 0.96				
Highest educational level of girl								
No education vs Secondary education	1.77	0.87, 3.60			3.30	1.42, 7.65	1.96	1.29, 2.99
Primary vs Secondary education	1.99	1.26, 3.15			1.90	0.95, 3.78	1.93	1.37, 2.72
Autonomy index					0.76	0.63, 0.93		
WASH index	1.28	1.03, 1.60	0.67	0.51, 0.88				
Household wealth index								
Poorest vs Richest	4.31	1.93, 9.62						
Poorer vs Richest	6.80	3.09, 14.95						
Middle vs Richest	4.54	2.19, 9.39						
Richer vs Richest	4.05	2.07, 7.93						
Place of residence								
Urban vs Rural							0.63	0.46, 0.87
Agro-ecological zone								
Guinea/Sudan savannah vs Forest	0.60	0.29, 1.23						
Coastal savannah vs Forest	0.45	0.28, 0.70						
Survey Year								
2008 vs 2003							0.81	0.59, 1.12
2014 vs 2003							0.81	0.57, 1.17
Model fit statistics								
Wald test		4.55^{***}		6.81^{***}		8.78^{***}		7.25^{***}
-2 Log-likelihood ratio		950.57		780.34		742.42		2924.71
AIC		972.57		786.34		750.42		2936.71

PR, Prevalence Ratio; 95% CI, 95% confidence interval; $^{***}P \leq 0.001$; AIC; Akaike criteria; WASH, Household water, hygiene, and sanitation

Table 3. Multivariate Predictors of Thinness Among Non-Pregnant Adolescent Girls: Analysis of the 2003-2014 Ghana Demographic Health Survey (GDHS) Data

Variables	**2003 (n= 983)**		**2008 (n= 955)**		**2014 (n= 857)**		**Pooled (n= 2795)**	
	PR	**95% CI**	**PR**	**95% CI**	**PR**	**95% CI**	**PR**	**95% CI**
Age							0.75	0.56, 0.99
Frequency of listening to the radio in the past week	1.46	1.09, 1.96						
Frequency of watching television in the past week	0.61	0.38, 0.98					0.68	0.51, 0.91
Frequency of fruit intake in the past week					0.76	0.58, 0.99		
Highest educational level of girl								
No vs Secondary education	1.79	0.52, 6.11	1.25	0.15, 10.57				
Primary vs Secondary education	2.70	1.02, 7.11	3.53	1.16, 10.73				
Survey Year								
2008 vs 2003							0.88	0.45, 1.73
2014 vs 2003							0.71	0.38, 1.56
Model fit statistics								
Wald test	4.81***			3.59*		3.74*		2.90*
-2 Log-likelihood ratio	228.02			185.55		190.66		711.36
AIC	236.02			189.55		192.66		719.36

PR, Prevalence Ratio; 95% CI, 95% confidence interval; **P*≤0.05; **P≤0.01; ****P*≤0.001; AIC; Akaike criteria

Table 4. Multivariate Predictors of Overweight/Obesity Among Non-Pregnant Adolescent Girls: Analysis of the 2003-2014 Ghana Demographic Health Survey (GDHS) Data

Variables	**2003 (n= 983)**		**2008 (n= 955)**		**2014 (n= 857)**		**Pooled (n= 2795)**	
	PR	**95% CI**	**PR**	**95% CI**	**PR**	**95% CI**	**PR**	**95% CI**
Age			1.15	1.01, 1.30				
Frequency of watching television in the past week	1.29	1.09, 1.53						
Autonomy index					1.27	1.00, 1.62		
Ethnicity								
Akan vs Other			0.62	0.41, 0.93			0.71	0.56, 0.90
Mole-Dagbani vs Other			0.82	0.49, 1.40			0.82	0.56, 1.20
Marital status								
Currently vs Never married					3.52	1.84, 6.75	1.03	0.65, 1.63
Formerly vs Never married					0.67	0.23, 1.91	2.54	1.03, 6.26

table continues

Variables	2003 (n= 983)		2008 (n= 955)		2014 (n= 857)		Pooled (n= 2795)	
	PR	95% CI	PR	95% CI	PR	95% CI	PR	95% CI
Household size	0.91	0.86, 0.97	0.88	0.81, 0.95			0.93	0.89, 0.98
Household wealth index								
Poorest vs Richest	0.15	0.04, 0.64	0.55	0.29, 1.05	0.21	0.10, 0.41	0.24	0.15, 0.39
Poorer vs Richest	0.18	0.08, 0.40	0.53	0.31, 0.92	0.15	0.07, 0.35	0.23	0.15, 0.35
Middle vs Richest	0.24	0.11, 0.55	0.33	0.17, 0.63	0.51	0.28, 0.93	0.34	0.23, 0.49
Richer vs Richest	0.70	0.46, 1.05	0.86	0.56, 1.31	0.79	0.48, 1.29	0.76	0.59, 0.98
Year of Survey								
2008 vs 2003							1.28	0.98, 1.67
2014 vs 2003							1.39	1.02, 1.88
Model fit statistics								
Wald test		11.74***		6.01***		9.10***		11.33***
-2 Log-likelihood ratio		1178.16		1472.42		1400.53		4769.52
AIC		1190.16		1488.42		1414.53		4791.52

PR, Prevalence Ratio; 95% CI, 95% confidence interval; **P*≤0.05; ***P*≤0.01; *P*≤0.001; AIC; Akaike criteria

Table 5. Multivariate Predictors of Anaemia Among Non-Pregnant Adolescent Girls: Analysis of the 2003-2014 Ghana Demographic Health Survey (GDHS) Data

Variables	2003 (n= 983)		2008 (n= 955)		2014 (n= 857)		Pooled (n= 2795)	
	PR	95% CI	PR	95% CI	PR	95% CI	PR	95% CI
Age			0.94	0.91, 0.98	0.88	0.83, 0.94	0.94	0.91, 0.97
Girl has ever given birth								
Yes vs No	1.25	1.03, 1.51	1.21	1.00, 1.45			1.22	1.07, 1.40
Stunting status								
Yes vs No					1.48	1.12, 1.98		
Relation of girl to the household head								
Household head vs Daughter			0.87	0.61, 1.23				
Wife vs daughter			1.07	0.81, 1.40				
Granddaughter vs Daughter			1.01	0.85, 1.21				
Other family relation vs Daughter			0.92	0.79, 1.07				
Non-family relation vs Daughter			0.59	0.39, 0.89				
Household owns land								
Yes vs No					1.27	1.07, 1.50		
Agro-ecological zone								
Guinea/Sudan savannah vs Forest			0.81	0.71, 0.93	0.96	0.80, 1.16		
Coastal savannah vs Forest			0.81	0.71, 0.93	1.23	1.01, 1.50		
Year of Survey								
2008 vs 2003							1.41	1.28, 1.56
2014 vs 2003							1.06	0.95, 1.19

table continues

Variables	2003 (n= 983)		2008 (n= 955)		2014 (n= 857)		Pooled (n= 2795)	
	PR	95% CI	PR	95% CI	PR	95% CI	PR	95% CI
Model fit statistics								
Wald test	5.27*			3.99***		6.77***		19.30***
-2 Log-likelihood ratio	5187.97			7629.05		5787.79		21614.57
AIC	5189.97			7647.05		5797.79		21622.57

PR, Prevalence Ratio; 95% CI, 95% confidence interval; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; AIC; Akaike criteria

Discussion

This study examined the trends-over-time and the factors associated with malnutrition among adolescent girls aged 15-19 years in Ghana using nationally representative data from the 2003, 2008, and 2014 GDHS. Anaemia remained severe (2003: 44.3%; 2008: 62.1% and 2014: 47.3%) without a clear trend, increasing significantly by 18.1% points in 2008 with a minor increase (2.82%, 95% CI, -1.76, 7.41) in 2014 compared to 2003. Between 2003 and 2014, stunting decreased slightly by only 1.5% points and underweight by less than 1%-point difference but, overweight increased significantly by 4.3% (95% CI, 0.74, 7.84%) points for the adolescent girls after adjusting for significant predictors in the pooled model.

According to the WHO criterion, the prevalence of anaemia in all the surveys was of severe public health significance [43] confirming previous studies [15, 50]. The severity of anaemia over the years suggests a high burden of micronutrient deficiencies among adolescent girls in Ghana; inadequate dietary intake, evolving from food insecurity and the consumption of monotonous plant-based diets with little or no animal source foods, has been cited as a common underlying cause [9, 51]. The 2008 survey coincided with the global financial crisis during which macro-economic growth in Ghana was marginal compared to the previous years, with spikes in the prices of fuel and food [52]. Household food and non-food expenditure are associated with household dietary diversity [53]. Hence, the spike in fuel and food prices plausibly influenced household food security and diversity negatively, especially among the middle-class and poor without adequate safety nets. The preceding may partly account for the peak in anaemia in 2008. Besides the effects of the global financial crisis, the finding may also relate to the prevalence and type of disease vectors during the survey. Our analysis showed that the peak of anaemia in 2008 was highest in the forest zone (*not shown*), which was contrary to other studies in Ghana [15, 54]. The forest zone of Ghana has a tropical climate in which malaria exposure is higher [55]; although we could not verify this with the available data, a recent study found that while anaemia in children and reproductive women was associated with iron deficiency in northern Ghana (Guinea savannah zone), it was rather associated with inflammation in the middle and southern belts (forest zone) of the country [56].

Stunting and underweight declined non-significantly between 2003 and 2014 for the adolescent girls, corroborating the finding of Black e*t al.* [57], who report that, globally, stunting is decreasing slowly. Though Ghana attained middle-income status in 2005, inequality has been increasing, and poverty remains prevalent in many areas, with increasing urban poverty resulting from high graduate unemployment [58]. Food security plays a significant role in the prevalence of thinness among adolescents in LMICs [59]. Ghana was among the first African countries to achieve the first MDG of "*eradicating extreme poverty and hunger*" [60]. However, a heavy reliance on rain-fed agriculture, inflation, and high food prices continuously poses a threat to food security even in urban Ghana [61], partly accounting for the steady burden of stunting and minor decrease in thinness. It has been shown that, declines in stunting are only noticeable after a couple of generations of better-nourished mothers [57]; but the 11-year trend in our study sufficed to observe a trend.

Together with the stagnating undernutrition rates, we observed an increasing trend in adolescent overweight/obesity over the years. Although our study is the first to map the trend-over-time in Ghanaian adolescent girls' nutritional status, Ofori-Asenso and colleagues [62] observed an increasing trend in overweight and obesity prevalence for Ghanaian adults in the period 1998–2016 with women more overweight and obese in their study. Increases in overweight and obesity can happen more rapidly than declines in (chronic) undernutrition [12, 13], leading to the co-existence of over-and under-nutrition. Ghana is in the second phase of the nutrition transition [39] with increasing consumption of processed foods, "*fast-foods*" and energy-dense snacks alongside decreasing physical activity levels, which have contributed to overweight and obesity [11, 63, 64]. Buxton [65] found that adolescents in Ghana have unhealthy eating patterns and habits, which are worst among adolescent girls [21] and may partially explain our findings. Also, adolescent girls in Ghana are known to have less physical activity than their male peers [66]. Overall, the co-existence of undernutrition and overnutrition reflects persistent food insecurity and poverty alongside a nutrition transition with an increasingly sedentary lifestyle [64].

Similar to the 2005 WHO report [25], early child-bearing and socio-economic factors (education, household wealth, type of residence) significantly predicted the nutritional status of the adolescent girls in our study. In detail, individual-level characteristics associated with the girls' malnutrition included (1) education, (2) age, and (3) whether the girl had ever given birth. First, educational status is a proxy of socio-economic status and empowerment [67]; accordingly, higher educated girls may be more empowered and less impoverished. Better education may protect against adverse nutrition and health outcomes through the acquisition of positive social, psychological, and economic skills and by influencing lifestyle behaviours such as healthy food choices [68]. Less-educated adolescents are likely to be from households with low socio-economic status [24, 69], associated with a lower HAZ in the present study.

Second, in contrast to our previous study [15], increasing age seemed the most consistent determinant of a reduced anaemia prevalence in our statistical models. Girls in our sample were in fertile age compared to our previous study, where the girls were primarily pre-menarche. Also, other studies found increasing age to be protective of anaemia [70, 71]. One possible reason is that older girls may be less susceptible to chronic infection and inflammation [72]. Many studies have reported younger age as a risk factor for stunting among children and adolescents in LMICs [73, 74], but our study shows that older girls are more stunted. Although this finding conforms with Leslie and Pawloski [75], it was unexpected and does not support the evidence of catch-up growth or compensatory gain among adolescents. Catch-up growth among adolescents may occur only if there is a significant maturational delay of one to two years to allow additional growth [76].

Lastly, girls who had ever given birth before were more likely to be anaemic in our analysis. Pregnancy poses an extra-demand of nutrient requirements for the growing foetus [3, 9, 34]. Adolescent pregnancy negatively affects girls' linear growth, increasing their risk for stunting [20]. Stunted children and adolescents are more susceptible to chronic infections and inflammation [25], this predisposes them to micronutrient deficiencies, including anaemia. Reduced linear growth is also associated with intergenerational consequences of adverse birth outcomes [20]. Our data suggest marriage could influence the association of childbearing and nutrition status as married girls were older and more likely to have given birth. Girls who mature early look older and marry earlier, partly attributed to better secondary sex characteristics for heavier girls [77]. The socio-economic and physiologic deprivations associated with teenage marriage [9, 20, 34, 78] outweigh any possible benefits in girls' nutrition and health. Moreover, any possible benefits of teenage marriage largely depend on the partner's socio-economic status.

Our results suggest that while higher autonomy has benefits for stunting reduction, it is also positively associated with being overweight. Adolescent girls who are more autonomous may have more control over household resources and are better able to make independent decisions regarding their health, including reproductive health [44]; this probably explains the negative association between increasing autonomy index and stunting in 2014. Equally, girls with a higher autonomy may have more purchasing power, which may probably result in more consumption of "*fast-foods*" and energy-dense snacks, explaining the positive trend between the autonomy index and overweight/obesity in 2014.

We observed a positive trend between the frequency of TV watching and overweight/obesity in 2003, with an inverse trend for thinness in our pooled analysis. A combination of the frequency and amount of time spent watching TV or listening to the radio would be a better measure of a sedentary lifestyle [79], but these data were not available. Previous studies showed that a higher frequency of TV watching is significantly associated with overweight for adolescents [69] and women aged 15-49 years [80, 81]. Children who frequently watch TV are also more likely to consume energy-dense snacks and sugar-sweetened beverages [69], which contributes to a higher energy intake, increasing the likelihood of overweight/obesity.

The most consistent household determinant of the adolescent girls' malnutrition was the household size and HWI. Household size was inversely associated with overweight/obesity in our study. A higher dependency ratio may increase household expenditures and competing household needs may lower dietary quantity and quality, with consequences for weight loss, micronutrient deficiencies, infections, and stunting [51]. Girls from households in the first four lower HWI quintiles were consistently less likely to be overweight/obese but were more likely to be stunted in 2003. In our sensitivity analysis with linear regression, lower levels of HWI were negatively associated with HAZ and BAZ, suggesting that increasing household wealth may significantly increase overweight/obesity with a marginal reduction in stunting. One probable reason is that a short-to-medium term exposure to improved household wealth may rapidly improve dietary intake and health; this would improve weight in the short-to-medium term. Also, girls from deprived households may lack the purchasing power to consume "*fast-foods*" and energy-dense snacks, which may reduce overweight/obesity. In contrast, a long-term exposure to improved household wealth would be desirable in reducing stunting [25, 57]. Overall, improving socio-economic conditions is a well-known determinant of a reduced risk of undernutrition but an increased risk of overnutrition [11, 24, 35, 74].

Households with access to agricultural land are more likely to have improved livelihoods [82], especially in rural communities with farm-based livelihoods. Nevertheless, girls from such settings may also be overburdened with farm-related work alongside their gender roles of household chores, compromising their health. The above may explain that girls from households with land were more likely to be anaemic in the 2014 survey. Undeniably, women in Ghana are known to have more substantial burdens in their time than men [83], and adolescent girls may be no exception. The work burden may lead to stress with probable consequences for poor dietary and health-seeking behaviours, impacting health negatively. For instance, the risk of micronutrient deficiencies was reportedly higher among working than non-working girls in Sri Lanka [84]. Also, children who worked longer hours were allegedly more stunted than their peers who worked for shorter hours in Nepal [85].

Community-level determinants of malnutrition for the girls included the agro-ecological zone and the type of residence. However, the type of residence was only a significant determinant of stunting in our pooled analysis, and the association between agro-ecological zone and malnutrition was inconsistent. Overall, poverty and food insecurity are more prevalent in rural parts of the country than in urban settings [58, 61]; this may partly explain that urban-dwelling girls were less stunted. Moreover, many rural communities still have poor access to sanitation services and health care, despite introducing the Community-Based Health Planning and Services compounds in rural Ghana in the early 2000s. Contrary to previous studies [15, 54], girls residing in Ghana's coastal and Guinea/Sudan savannah zones compared to the forest zone were remarkably less likely to be anaemic in the 2008 survey; as earlier mentioned, this partly explains why the prevalence of anaemia peaked in 2008 and somewhat relates to the 2008 global financial crisis and the type and prevalence of disease vectors.

Policy implications: Our findings emphasise the importance of the so-called "double-duty actions" proposed by Hawkes and colleagues [86] to tackle both under- and overnutrition, but evidence of effectiveness for adolescent girls remains unclear. Until recently, nutrition initiatives in Ghana commonly focused on infants, young children, and women, neglecting adolescents. The few interventions targeting adolescents lately have mainly concentrated on improved micronutrient intake for adolescent girls and reduced schistosomiasis and soil-transmitted helminths among school children. The "*Girls, Iron-Folate Tablet Supplementation (GIFTS)"* programme for junior high school girls in Ghana [87] may help reduce anaemia, although compliance-related issues [88, 89] may limit its effectiveness. While the school provides a reliable platform for in-school girls, innovative programmes targeting out-of-school girls are also desirable.

Considering that girls in rural settings were more likely to be stunted, there is a continued need for policies that enhance food security in low-income communities and households and improve girl-child education to mitigate the flattening stunting rate. Likewise, nutrition and public health policies should target girls in high socio-economic settings to overcome the increasing overnutrition trend. Such programmes may include sensitisation and education to improve the consumption of healthier snacks such as fruits and promote a healthy and active lifestyle during adolescence, such as aerobic outdoor games.

The burden of anaemia emphasises a need for a multi-sectoral approach to anaemia prevention. Only about a quarter of the girls ever visited a health facility in the last 12 months; while this may suggest respondents were generally healthy, it also underscores the need to promote health-seeking behaviour among adolescent girls. Our study shows that teenage pregnancy and teenage marriage is still prevalent and efforts to prevent these should be strengthened including improved reproductive health education and care.

Limitations: The present study is the first to map the trend in malnutrition among adolescent girls in Ghana and to assess the factors associated with this malnutrition using national representative data. Nonetheless, our analysis is not without challenges. Firstly, it was impossible to model some potential explanatory variables in the pooled analysis since the data were not available in all datasets. Secondly, dietary intake data was limited to fruits and vegetable consumption, and the data did not include household food security although being an important determinant of adolescent nutritional status [90]. Thirdly, menstruation increases the risk of micronutrient deficiencies, notably, iron deficiency anaemia through iron loss in the blood [9]. However, we could not include menarche status in our analysis. Data on menstruation was related to whether the girl menstruated in the last 6 weeks preceding the survey, but we are not certain whether girls who did not menstruate 6 weeks before the surveys were pre-menarche or simply missed their menstrual period. Fourthly, we selected a subset of women in fertile age (15-49 years.) and were not able to include data for 10-14 years. old adolescents since they are not part of the fertile age group. We were, therefore, limited in examining the trend and correlates of malnutrition for only older adolescents aged 15-19 years. Our findings may, therefore, be extrapolated only to 15-19 years. adolescent girls in Ghana. Finally, the GDHS surveys used a cross-sectional study design, and our findings only depict associations.

Conclusions: Our findings point to a steady burden of undernutrition with an existing and upcoming burden of overnutrition for non-pregnant adolescent girls in Ghana. Nutrition interventions should consider adolescent girls as a major target group besides the usual priority groups, infants, and young children. Our findings emphasise the need for double-duty actions to tackle both under- and overnutrition holistically. Different intervention programmes are needed to meet the nutrition-specific needs of different socio-economic groups of adolescent girls.

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Appendices

Table S1: Flow Chart in the Population for Analysis

	Year of Survey				
	1993	**1998**	**2003**	**2008**	**2014**
Number of women in fertile age (15-49 years)	4562	4843	5691	4916	9396
Number of Adolescents (15-19 years)	140	98	1046	1004	887
Number of those with anthropometry and/or haemoglobin data	140	98	1046	1004	887
Number of those flagged for anthropometry z-score	-	-	19	11	-
Remainder	140	98	1027	993	887
Number of those pregnant	9	-	38	38	30
Population for analysis (non-pregnant girls)	**131**	**98**	**955**	**955**	**857**
Analysed	No	No	Yes	Yes	Yes

Table S2a: Attitude toward wife-beating index from GDHS data for adolescent girls

Variables	**Survey Year**					**Scoring**
	1993	**1998**	**2003**	**2008**	**2013**	**Yes=0 No=1**
Wife beating is justified if:						
Goes out without telling him			√	√	√	
Neglects the children			√	√	√	
Argues with him			√	√	√	
Refuses to have sex with him			√	√	√	
Burns the food			√	√	√	
Maximum attainable score for attitude toward wife beating	**N/A**	**N/A**	**5**	**5**	**5**	

N/A, not applicable

Table S2b: Scoring format for household water and sanitation (WASH) index

No	Variable	Score
1	**Household water index (WI): score range 0-2**	
1.1	Improved source of drinking	
	Piped water supply into the dwelling	1
	Piped water to a yard/plot	
	Public tap/standpipe	
	Tube well/borehole	
	Protected dug well	
	Protected spring	
	Rainwater	
	If the source of drinking water is packaged/sachet-water or bottled and the source of non-drinking water is improved	
	Any other water source	0
1.2	Round-trip time in water haulage between household and water source	
	If the time spent ≤ 30 minutes	1
	If time spent > 30 minutes	0
2	**Household sanitation index (HSI): score range 0-1**	
2.1	Improved household sanitation facility using the type of toilet	
	Flush/pour-flush toilet or latrine that flushes to a sewer, septic tank, or pit.	1
	Ventilated improved pit (VIP) latrine	
	Pit latrines with the pit well covered by a slab	
	Composting toilets are also considered improved	
	Any other toilet	0
	The maximum attainable score for household water and sanitation (WASH)	**3**

Table S3: Prevalence rates and 95% confidence intervals of nutritional status indicators of non-pregnant adolescent girls from the 2003-2010 GDHS

Nutritional status	Prevalence (95% C.I)		
	2003 survey	**2008 survey**	**2014 survey**
Stunting	7.9 (6.4, 9.5)	6.4 (4.7, 8.0)	6.2 (4.4, 8.0)
Thinness	2.0 (1.1, 2.8)	1.6 (0.7, 2.4)	1.6 (0.7, 2.5)
Overweight/obesity	10.0 (7.8, 12.2)	12.2 (10.0, 14.3)	11.8 (9.2, 14.3)
Anaemia	44.3 (40.7, 47.9)	62.1 (58.5, 65.6) [a]	47.3 (43.3, 51.2)

[a] Statistically significantly higher than the 2003 survey with alpha 0.05

Table S4. Prevalence Difference in Stunting, Thinness, Overweight and Anaemia Among Non-Pregnant Adolescent Girls between 2003-2014: Analysis of the Ghana Demographic Health Survey (GDHS) Data

Malnutrition	Crude Estimate			Adjusted Estimate[1]		
	Estimate (%)	95%. C.I	P-value	Estimate (%)	95%. C.I	P-value
Stunting						
2003 survey (Ref.)	0.00					
2008 survey	-1.55	-3.92, 0.81	0.20	-1.44	-3.80, 0.92	0.23
2014 survey	-1.73	-4.24, 0.77	0.17	-1.45	-4.00, 1.10	0.26
Thinness						
2003 survey (Ref.)	0.00					
2008 survey	-0.39	-1.71, 0.93	0.56	-0.20	-1.52, 1.13	0.77
2014 survey	-0.36	-1.73, 1.02	0.61	-0.59	-1.98, 0.80	0.40
Overweight						
2003 survey (Ref.)	0.00					
2008 survey	2.15	-1.04, 5.34	0.19	3.29	0.22, 6.35	0.04
2014 survey	1.77	-1.74, 5.28	0.32	4.29	0.74, 7.84	0.02
Anaemia						
2003 survey (Ref.)	0.00					
2008 survey	17.80	13.49, 22.10	<0.0001	18.11	13.83, 22.39	<0.0001
2014 survey	2.97	-1.66, 7.61	0.21	2.82	-1.76, 7.41	0.23

[1]Estimates were adjusted for predictors which were significantly associated with the outcome in the pooled analysis

Table S5a. Multivariate Predictors of Height-For-Age Z-Score (HAZ) Status Among Non-Pregnant Adolescent Girls: Analysis of the 2003-2014 Ghana Demographic Health Survey (GDHS) Data

Variables	2003 (n= 983)			2008 (n= 955)			2014 (n= 857)			Pooled (n= 2795)		
	Estimate (β)	**SE (β)**	***P-value***	**Estimate (β)**	**SE (β)**	***P-value***	**Estimate (β)**	**SE (β)**	***P-value***	**Estimate (β)**	**SE (β)**	***P-value***
Age							-0.05	0.03	0.04			
Highest educational level of girl			0.02			0.002			0.01			<0.0001
No education vs Secondary/Higher	-0.13	0.11	0.23	-0.35	0.12	0.005	-0.38	0.16	0.02	-0.26	0.07	0.0003
Primary school vs Secondary/Higher	-0.24	0.09	0.01	-0.21	0.08	0.01	-0.23	0.09	0.01	-0.21	0.05	<0.0001
Visited health facility in the last 12 months												
Yes vs No				0.17	0.07	0.02						
Relation of girl to the household head						0.009						
Household head vs Daughter				0.29	0.14	0.04						
Wife vs Daughter				0.12	0.16	0.46						
Granddaughter vs Daughter				0.00	0.11	0.99						
Other family relation vs Daughter				-0.05	0.08	0.48						
Non-family relation vs Daughter				-0.33	0.13	0.01						
Household size	-0.02	0.01	0.03									
Household owns land												
Yes vs No				-0.16	0.06	0.01						
Household wealth index			0.03						0.11			0.001
Poorest vs Richest	-0.09	0.11	0.41				-0.18	0.10	0.12	-0.21	0.07	0.002
Poorer vs Richest	-0.31	0.10	0.002				-0.27	0.11	0.02	-0.24	0.06	<0.0001
Middle vs Richest	-0.18	0.09	0.06				-0.22	0.12	0.06	-0.19	0.06	0.001
Richer vs Richest	-0.24	0.09	0.01				-0.07	0.12	0.60	-0.12	0.06	0.03
Agro-ecological zone						0.093			0.10			0.02
Coastal savannah vs Forest				-0.01	0.07	0.95	0.08	0.08	0.32	-0.02	0.04	0.69
Guinea/Sudan savannah vs Forest				0.17	0.09	0.043	0.21	0.10	0.03	0.15	0.06	0.01
Year of survey												0.05
2008 vs 2003										0.01	0.04	0.85
2014 vs 2003										0.10	0.05	0.03
Model Fit Statistics												
R-Square		0.03			0.05			0.04			0.03	
Root MSE		0.91			0.89			0.86			0.89	
Model (F-Value)		4.93	<0.0001		4.84	<0.0001		2.78	0.004		6.64	<.0001

β: regression coefficient; SE (β): standard error of regression coefficient; ref: reference group; MSE: mean square of residual

Table S5b. Multivariate Predictors of Body-Mass-Index (BMI)-For-Age Z-Score (BAZ) Status Among Non-Pregnant Adolescent Girls: Analysis of the 2003-2014 Ghana Demographic Health Survey (GDHS) Data

Variables	**2003 (n= 983)**			**2008 (n= 955)**			**2014 (n= 857)**			**Pooled (n= 2795)**		
	Estimate (β)	**SE (β)**	***P-value***	**Estimate (β)**	**SE (β)**	***P-value***	**Estimate (β)**	**SE (β)**	***P-value***	**Estimate (β)**	**SE (β)**	***P-value***
Age				0.07	0.03	0.01						
Frequency of listening to the radio in the past week										0.04	0.01	0.01
Frequency of watching television in the past week	0.09	0.03	0.001									
Autonomy index							0.06	0.03	0.04			
WASH index							0.09	0.04	0.01			
Household wealth index			0.02			0.001			0.006			<0.0001
Poorest vs Richest	-0.28	0.10	0.005	-0.36	0.11	0.001	-0.32	0.11	0.004	-0.44	0.06	<0.0001
Poorer vs Richest	-0.26	0.10	0.01	-0.41	0.12	0.001	-0.39	0.11	0.001	-0.43	0.06	<0.0001
Middle vs Richest	-0.29	0.10	0.003	-0.26	0.10	0.01	-0.10	0.11	0.38	-0.26	0.06	<0.0001
Richer vs Richest	0.02	0.09	0.80	-0.09	0.10	0.40	-0.15	0.13	0.26	-0.11	0.06	0.08
Agro-ecological zone			0.004									
Coastal savannah vs Forest	0.02	0.07	0.78									
Guinea/ Sudan savannah vs Forest	-0.27	0.08	0.001									
Year of survey												0.001
2008 vs 2003										0.04	0.05	0.42
2014 vs 2003										0.18	0.05	0.0001
Model Fit Statistics												
R-Square		0.09				0.04		0.07			0.05	
Root MSE		0.85				0.92		0.85			0.88	
Model (F-Value)		12.88	<0.0001		5.85	<0.0001		5.85	<0.0001		13.57	<0.0001

β: regression coefficient; SE (β): standard error of regression coefficient; ref: reference group; MSE: mean square of residuals; WASH, Household water and sanitation

Table S5c. Multivariate Predictors of Haemoglobin (Hb) status Among Non-Pregnant Adolescent Girls: Analysis of the 2003-2014 Ghana Demographic Health Survey (GDHS) Data

Variables	2003 (n= 983)			2008 (n= 955)			2014 (n= 857)			Pooled (n= 2795)		
	Estimate (β)	SE (β)	*P-value*	Estimate (β)	SE (β)	*P-value*	Estimate (β)	SE (β)	*P-value*	Estimate (β)	SE (β)	*P-value*
Girl has ever given birth												
Yes vs No	-2.97	1.09	0.01									
Religion			0.001									
Christian vs Other	3.78	1.17	0.001									
Muslim vs Other	1.56	1.26	0.22									
Relation of girl to the household head						0.15						
Household head vs Daughter				3.81	2.60	0.14						
Wife vs Daughter				0.93	2.27	0.68						
Granddaughter vs Daughter				1.70	1.95	0.38						
Other family relation vs Daughter				2.16	1.50	0.15						
Non-family relation vs Daughter				5.35	2.37	0.02						
Nutritional status			0.03			0.05						0.02
Underweight vs Normal weight	-4.92	1.83	0.0	-7.82	3.95	0.049				-6.80	2.73	0.01
Overweight/ Obese vs Normal weight	-0.44	1.33	0.74	2.82	2.11	0.18				1.37	0.97	0.16
Household wealth index									0.01			
Poorest vs Richest							2.72	1.85	0.14			
Poorer vs Richest							-1.67	2.10	0.43			
Middle vs Richest							3.86	1.87	0.04			
Richer vs Richest							4.21	1.84	0.02			
Agro-ecological zone			0.003			0.001						
Coastal savannah vs Forest	--3.08	0.91	0.001	4.03	1.33	0.003						
Guinea/Sudan savannah vs Forest	-0.55	0.71	0.44	4.90	1.48	0.001						
Year of survey												
2008 vs 2003										-7.73	0.71	<0.0001
2014 vs 2003										-2.57	0.71	0.0003
Model Fit Statistics												
R-Square		0.02			0.04			0.03			0.05	
Root MSE		14.81			15.73			13.70			14.90	
F-Value		7.22	<0.0001		3.70	<0.0001		3.61	0.007		32.57	<0.0001

β: regression coefficient; SE (β): standard error of regression coefficient; ref: reference group; MSE: mean square of residuals

Chapter 5

Malnutrition, Hypertension Risk, and Correlates: An Analysis of the 2014 Ghana Demographic and Health Survey Data for 15-19 Years Adolescent Boys and Girls

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Based upon Nutrients, 2020; 12(2737), 1–23.
https://doi.org/doi:10.3390/nu12092737

Abstract

The sex differences in malnutrition and hypertension during adolescence is largely inconclusive. There is also a paucity of data on the sex-specific correlates of malnutrition and hypertension for adolescents. This study aimed to assess the association between malnutrition, pre-hypertension/hypertension (PHH) and sex among adolescents. The study also aimed to determine and contrast the factors associated with malnutrition and PHH among adolescent boys and girls in Ghana. We analysed data of non-pregnant adolescent girls (n = 857) and adolescent boys (n = 870) aged 15–19 years from the 2014 Ghana Demographic and Health Survey (GDHS). We modelled the prevalence ratio (PR) of malnutrition and PHH using Cox proportional hazard models. Compared to adolescent girls, boys were more than twice likely to be stunted [PR 2.58, 95% CI (1.77, 3.76)] and thin [PR 2.67, 95% CI (1.41, 5.09)] but less likely to be overweight/obese [PR 0.85, 95% CI (0.08, 0.29)] in multiple regression models. Boys were also about twice likely to have PHH [PR 1.96, 95% CI (1.47, 2.59)] compared to their female peers. A low level of education was a stronger predictor of stunting and PHH for girls compared to boys. Empowerment index while reducing the PR of stunting for girls [PR 0.82, 95% CI (0.67, 0.99)], also increased the PR of overweight/obesity [PR 1.31, 95% CI (1.02, 1.68)] for them. A higher household wealth index was associated with overweight/obesity for adolescent girls but was also associated with a lower PR of stunting and PHH for adolescent boys. Improvement in household water, hygiene, and sanitation (WASH) reduced the PR of stunting by 15% for adolescent boys. Overall, our findings suggest a double-burden of malnutrition with an upcoming non-communicable disease burden for adolescents in Ghana. Our findings may also be highlighting the need to target adolescent boys alongside girls in nutrition and health intervention programmes.

Introduction

There are more adolescents in the world than ever, particularly in South-East Asia and Sub-Saharan Africa and the life stage of adolescence (approximately from 10–19 years) is receiving growing attention in international development [1]. About a quarter of the Ghanaian population is adolescents (aged 10–19 years) with the proportion of male adolescents (23.2%) slightly higher than female adolescents (21.7%) [2]. Adolescence is a period of rapid growth and maturation from childhood to adulthood, second to the 1000 days of life [3]; this period is characterized by an increase in nutrient requirements [4].

The association between malnutrition and sex is inconclusive, mainly [5]. However, Abarca-Gómez *et al.* [6] in their analysis of the worldwide trend in body-mass index (BMI), showed that 5-19 years girls in many sub-Saharan African countries have consistently had a higher BMI than their male counterparts from 1975 to 2016. Furthermore, Manyanga *et al.* [7] in their analysis of the Global School Health Survey data for Africa, reported a higher prevalence of overweight for adolescent girls compared to boys in all countries except for Egypt and Malawi. On the contrary, adolescent boys had a higher prevalence of thinness than girls in every country in the analysis of Manyanga *et al.* [7]. In the 2014 Ghana Demographic and Health Survey (GDHS) report [8], 15–19 years adolescents boys compared to their female peers were reported to have a higher prevalence of stunting and thinness while the girls had a higher overweight prevalence. Emerging data from Ghana has also shown varied and relatively higher prevalence rates of stunting (15–50.3%), thinness (7–19.4%), and overweight/obesity (6.9–17%) among adolescent males and females depending on the context in which the study was conducted [9–12]. Although these studies are limited by their cross-sectional design and geographical scope, which mainly includes the metropolitan areas of Accra and Kumasi; the general conclusion is that Ghanaian adolescent boys are often worse off regarding stunting and thinness compared to their female peers. In contrast, adolescent girls are said to be more at risk of being overweight in Ghana compared to their male colleagues.

Literature from Ghana [9–11] has identified poor parental education as a factor associated with undernutrition for adolescents. Moreover, poor sanitation, inadequate dietary intake and household food insecurity are well-known correlates of stunting and thinness among adolescents [5]. While rural residence is linked to undernutrition, residing in an urban setting has been linked with a higher likelihood of overnutrition [5–7]. Similarly, poor socioeconomic status is positively associated with undernutrition but inversely associated with overnutrition [5, 6, 10, 11]. The increasing burden of overweight and obesity has also been attributed to the increasing consumption of "fast foods" loaded with fats, increasing consumption of sugar-sweetened carbonated drinks, and sedentary lifestyles [10, 12–14]. In addition to physical activity [12, 15] and dietary habits [14], which are known to be poorer in adolescent girls in Ghana, no difference between males and females in the other correlates of malnutrition above have been documented for adolescents.

Regardless of the sex differences in under and over-nutrition, while the persisting rates of undernutrition undermine the development of Ghanaian adolescents into adulthood, the evolving burden of over-nutrition poses an emerging cardiovascular risk for them [16]. Though there is a scarcity of data on cardiovascular risk among adolescents in Ghana, two cross-sectional studies in the Greater Accra and Ashanti regions reported a higher prevalence of hypertension and pre-hypertension among adolescent males compared to females [10, 12]. Family history of hypertension, low birth weight, and excess weight are known determinants of hypertension [17]. In a study by Afrifa–Anane *et al.* [12]in Ghana, low physical activity and a higher BMI were associated with higher systolic blood pressure.

To the best of our knowledge, no study has examined the sex-specific correlates of the nutritional status, or the cardiovascular risk of female adolescents compared to their male colleagues in Ghana. The current evidence, as presented, is mostly based on a few cross-sectional studies with relatively smaller sample sizes [9–12]. Age and sex-specific data on the health and nutritional status of adolescents at the national level are urgently needed for policies and programmes for improving the health and nutritional status of adolescents [18]. Hence, the primary objective of this study was to assess the association between malnutrition, pre-hypertension/hypertension (PHH) and sex among adolescents. The study also aimed to determine and contrast the associated factors among the adolescents in Ghana using national representative data from the 2014 GDHS. While contributing to the literature on the sex differences in malnutrition and cardiovascular risk for adolescents, our analyses may help explain the factors moderating the differences.

Materials and Methods

Study Design

The 2014 Ghana DHS data were analysed in this study. The Ghana DHS used a multistage random sampling approach, including both rural and urban areas in all the regions of Ghana. In the multistage sampling, clusters were first selected randomly, followed by a random selection of a community in each cluster; all households in the selected community were interviewed. The 2014 Ghana DHS data included anthropometric data (weight and height) as well as data on systolic and diastolic blood pressure of both adolescent females and males aged 15–19 years, as well as data on demographics, fertility, household characteristics, nutrition, and health. These data are in the public domain and available from the DHS MEASURE website, and details about the surveys can be found in the DHS Methodology report [19]. The Ghana Health Service Ethical Review Committee, Accra, Ghana approved the DHS, and the data was collected by the Ghana Statistical Service and the Ghana Health Service. We obtained permission from "DHS MEASURE" to download and analyse the data, and no further ethical approval was required because anonymous identifiers were used in coding the DHS data. The population for analyses included non-pregnant adolescent girls (n = 857) and adolescent boys (n = 870) aged 15–19 years.

Malnutrition and Hypertension

Anthropometric Indicators: We computed height-for-age *z*-score (HAZ) and body-mass-index for-age *z*-score (BAZ) using WHO AnthroPlus (version 1.0.4). We defined stunting (HAZ < −2SD), thinness (BAZ < −2SD), normal weight (−2SD ≤ BAZ ≤ +1 SD), overweight (+1SD < BAZ < +2SD), and obesity (BAZ ≥ +2SD) as recommended [20].

Blood Pressure and Hypertension: Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in triplicates in the GDHS by trained health technicians, using a digital oscillometer (LIFE SOURCE® UA-767 Plus blood pressure monitor) [8]. We computed and used the mean of SBP and as well as DBP in our analysis of blood pressure (BP) and cardiovascular risk. We defined cardiovascular risk as pre-hypertension or hypertension (PHH). In conformity with the US National Heart, Lung, and Blood Institute's recommended cut-off points, we defined hypertension as SBP and/or DBP ≥ 95th percentile for sex, age, and height [21]. Pre-hypertension was also defined as BP ≥ 120/80mmHg and/or SBP/DBP ≥ 90th percentile but < 95th percentile for sex, age, and height [21].

The Conceptual Framework for the Analyses

The theoretical framework for our analysis was based on Madjdian *et al.* [5] and Lassi *et al.* [22]. The framework of Madjdian *et al.* (2018) postulates that border community-level factors such as type of residence and ecological zone exert an influence on the household characteristics which tend to affect some of the individual-level determinants of nutrition and health. The framework of Lassi *et al.* (2017) asserts that women empowerment, water, and sanitation are mediating factors in improving the nutrition of adolescents and young women.

Individual-level Variables

Variables analysed at the individual level included age, religion, ethnicity, education assessed as the number of completed years of schooling as well as categorical, marital status and, the relationship of the adolescent to the household head. We also included dichotomous variables for whether the adolescent ever had a child, and experienced sex. The variables for the working life of the adolescent included a categorical variable for the type of occupation and a dichotomous variable for any paid work in the past year. Lifestyle factors in the analysis included the frequency of watching TV and of listening to the radio in the past week. Furthermore, the analysis included health-seeking behaviour factors, including having visited a health facility in the last 6 months, sleeping under a mosquito net, and being covered by the Ghana National Health Insurance Scheme (NHIS). Data on dietary intake included the frequency (days) of the consumption of fruits and of vegetables in the past week. Covariates for BP assessment in the data included binary variables (yes/no) for any form of physical activity, smoking, coffee intake, and food intake 10 min before the measurement. The data also included the household consumption of salty foods in the last 24-hour including (1) broth cubes added to food, (2) processed canned meat/fish/legume, (3) salted dried fish/koobi/kako, and (4) any other salty ingredients. A summated score comprising the four listed salty food items denoted the household salty food consumption (SAFC), a proxy for the salt intake of our sample. In the SAFC, a score of 1 else 0 was assigned if the household consumed any of the listed salty food items in the last 24-h. The SAFC ranged from 0 to 4, a higher score reflecting a higher salty food consumption by the household.

We created indexes of attitude towards wife-beating to reflect autonomy regarding domestic violence as well as of property ownership of the adolescent like Amugsi and colleagues [23]. In the attitude towards wife-beating index, a score of 1 else 0 was assigned to a "no" answer to the following questions: wife-beating or hitting is justified if she (1) "goes out without telling him", (2) "neglects the children", (3) "argues with him", (4) "refuses to have sex with him", and (5) "burns the food". A higher score for the adolescent girl meant she was more empowered; but for adolescent boys, it implied, he was more caring and tolerable. Similarly, in the index of property ownership, a score of 1 else 0 was assigned if the adolescent was allowed to own (1) land or (2) a house alone or jointly. The attitude toward wife beating index ranged from 0 to 5 while the property ownership index ranged from 0 to 2. Lastly, a summated empowerment index combined the attitude towards wife-beating and property ownership indexes; the index ranged from 0 to 7 with a higher score implying more empowered. Supplementary Table S1 and S2 illustrates the empowerment index.

Household-level Variables

Household related covariates included the household size, number of children under-five years in the house, the number of persons in the household covered by the NHIS, the age and sex of the household head, and the socioeconomic status of the household defined by the wealth index quintile of the household. In brief, the household wealth index (HWI) in the DHS was computed with the household's ownership of selected assets, such as TV and bicycles, materials used for housing construction, and types of water access and sanitation facilities and cooking fuel [19]. Furthermore, dichotomous variables for the household ownership of land and farm animals were included. In the analysis, we also computed and used the mean years of education of household members as a continuous variable.

Lastly, based on the available data, we constructed a composite index of improved household water, hygiene, and sanitation (WASH), which ranged from 0 to 8 by aggregating scores for an improved sanitation index (SI), improved water index (WI), and improved household hygiene index (HHI). The WASH followed the WHO/UNICEF [24] guidelines on improved household water and sanitation in the prevention of oral–faecal contamination. The SI ranged from 0 to 2, whereby access to an improved toilet facility was scored 1 else 0, household not sharing the toilet facility with others was scored 1 else 0. The WI also ranged from 0 to 3 with a score of 1 else 0 assigned if (1) the household had access to an improved source of drinking water, (2) the round-trip water haulage time between the household and water source was ≤30 min, and (3) water was treated before drinking by the household.

Lastly, the HHI ranged from 0 to 3 and included a score of 1 else 0 for the availability of (1) a place for washing hands in the household, (2) water for washing hands in the household, and (3) soap and detergents for washing hands in the household. Similar indexes have been used in previous studies [25, 26]. The details of the WASH index can be found in the online supplementary material (Table S3).

Broader Community and Environmental Variables

The region of residence was recoded into 3 agro-ecological zones [27] for the analyses including (1) Guinea/Sudan savannah (Northern, Upper East, and Upper West Regions), (2) coastal savannah (Central, Greater Accra, and Volta Regions), and (3) Forest zone (Brong Ahafo, Ashanti, Western, and Eastern Regions). The type of residence (rural or urban) was also included in the analysis. The DHS data defined rural areas as countryside while towns and cities were classified as urban [19]. Finally, we included a dichotomous variable for access to a health program for adolescents accessing health services in our analysis.

Statistical Analysis

Descriptive statistics were presented as percentages for dichotomous/categorical variables and as means (standard errors) for continuous variables. Differences between groups in continuous variables were tested using one-way ANOVA (GLM function in SAS) or the Mann–Whitney test where necessary (for skewed data). In contrast, differences between groups for dichotomous/categorical variables were tested with the Rao–Scott Chi-Square Test. Cox proportional hazard models (stunted vs. not stunted, thin vs. normal weight, overweight/obese vs. normal weight, and PHH vs. normotensive) using "PROC SURVEYPHREG" in SAS were subsequently fitted to compare the prevalence risks and to identify the predictors of malnutrition and cardiovascular risk status. The effect measured was the prevalence ratios (PR). Cox and Poisson models have more robust variance and are thus better alternatives than logistic regression in cross-sectional data analysis [28, 29], explaining our choice of PR with Cox proportional hazard models.

In stratified bivariate analyses by sex, we determined the univariate associations of the different independent variables with the outcome variables. Variables from the bivariate analysis with p-values ≤ 0.25 were subsequently included in multivariable models for further assessment. Finally, we conducted a pooled analysis for both males and females. In the pooled analysis, interaction terms of sex and each of the first-order variables were included, and all interaction terms with p-values ≤ 0.1 were included in the multivariate models for further assessment. The final models were selected based on the log-likelihood ratio test, Akaike information criteria (AIC), Wald test, and p-value. The variance inflation factor (<10), as well as the Pearson and Spearman correlation coefficients, were used to assess multicollinearity in linear regression models. As a result of the complex survey design, we applied the weighting factors in the DHS data and adjusted for strata and cluster effects in all the analyses by using the "PROC SURVEY", function in SAS [30]. A detailed explanation of the weighting procedure can also be found in the DHS Methodology report [19]. All statistical analyses were conducted with SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and a two-tailed *p*-value ≤ 0.05 at 95% confidence interval was considered statistically significant. As a sensitivity analysis, we repeated all the analyses with linear regression using the "PROC SURVEYREG" command in SAS. In the sensitivity analyses, HAZ, BAZ, SBP, and DBP were the outcome variables; the results of the sensitivity analyses are presented as online supplementary material (Table S4, 5).

Results

Population Characteristics

The mean age of female adolescents was 16.8 (SE 0.05) years, while that of male adolescents was 17.0 (SE 0.05) years (Table 1). Furthermore, the proportion of adolescent girls who were unemployed was higher compared to adolescent boys (64.5% vs. 51.2%; *P*= 0.0001) (Table 1). Table 1 also shows that the frequency of listening to the radio (1.6 vs. 1.2 days, *P*< 0.0001), watching TV (1.4 vs. 1.2 days; *P*< 0.0001) were higher among boys compared to girls. However, more adolescent girls visited a health facility in the last six months before the survey compared to adolescent boys (19.2% vs. 10.8%; *P*< 0.0001). The results also showed that more adolescent girls were presently married compared to their male peers (5.7% vs. 0.5%; *P* <0.0001) (Table 1). The frequency of fruit consumption was higher for girls, but the frequency of vegetable consumption was somewhat higher for boys. Overall, about a tenth of the adolescents engaged in some exercise ten minutes before the BP assessment with the proportion of boys marginally higher compared to the girls (15.4% vs. 9.7%; *P*= 0.01). Similarly, less than 1% drunk coffee ten minutes before the BP measurement, with only 0.2% of the boys having smoked.

Additionally, about 3.6% of the adolescent girls were spouses of the household heads. Moreover, more adolescent girls had experienced first sex compared to adolescent boys (41.1% vs. 26.7%; *P*< 0.0001) and more adolescent girls ever had a child compared to boys (10.8% vs. 0.7%; *P*< 0.0001). Of the adolescents who had experienced sex, the mean age for boys was lower compared to girls (14.8 vs. 15.3 years; *P*= 0.001) (Table 1). Nevertheless, the mean age for adolescent girls who ever had a child was lower compared to boys (16.59 vs. 18.15 years; *P*= 0.025). With regards to autonomy and empowerment of the adolescent at the household, the mean autonomy and empowerment index score was higher for males compared to females (4.55 vs. 3.23; *P*< 0.0001). There were more adolescent boys than girls in male-headed households (69.4% vs. 59.6%; *P*= 0.001). The average household size of the sample was about 5.7 (SE 0.10), and the mean age of household heads for the sample was 49.3 years (SE 0.55); none of these differed by the adolescent sex (Table 1). Table 1 also shows that the mean number of children under-five in households of adolescent girls was significantly higher than that of adolescent boys (*P*= 0.022). There were no differences in ethnicity, education, HWI, SAFC, mean household years of education, place of residence, agro–ecological zone, and the proportion of those covered by the NHIS between the adolescent boys and girls (Table 1).

Table 1. Population Descriptive Statistics for adolescent boys and girls aged 15–19 years from the 2014 Ghana Demographic Health Survey Data

Variables	Girls (*n*= 857) Mean or Percentage	Girls SE (mean)	Boys (*n*= 870) Mean or Percentage	Boys SE (mean)	*P*-value [1]
Nutritional status and blood pressure					
Age (years)	16.8	0.05	17.0	0.05	0.048
Arm circumference (cm)	26.3	0.18	25.7	0.17	<0.0001
Weight (kg)	53.4	0.32	54.9	0.39	<0.0001
Height (cm)	158.3	0.23	166.2	0.31	<0.0001
BMI (kg/m^2)	21.3	0.11	19.8	0.09	<0.0001
BAZ	0.03	0.03	-0.59	0.04	<0.0001
HAZ	-0.64	0.03	-1.01	0.04	<0.0001
Systolic blood pressure (mmHg)	107.5	0.45	113.5	0.46	<0.0001
Diastolic blood pressure (mmHg)	69.2	0.39	67.8	0.31	<0.0001
Dietary intake and lifestyle					
Frequency of listening to radio in the past week	1.3	0.03	1.6	0.04	<0.001
Frequency of watching television in week the past week	1.2	0.04	1.4	0.05	<0.001
Frequency of fruit intake in the past week	3.0	0.11	2.9	0.12	0.001
Frequency of vegetable intake in the past week	3.3	0.11	3.9	0.14	<0.001
Health seeking behaviour					
Visited a health facility in the last 6 months [2]	19.2		10.8		<0.0001
Slept under mosquito bed net [2]	28.5		-		-
Covered by National Health Insurance Scheme (NHIS) (yes) [2]	56.9		54.4		0.43
Blood pressure related covariates					
Eaten in the past 10 minutes [2]	58.9		46.9		0.0001
Had coffee in the past 10 minutes [2]	1.1		0.4		0.16
Smoked in the past 10 minutes [2]	0		0.2		-
Exercised in the past 10 minutes [2]	9.7		15.4		0.01
SAFC	1.6	0.04	1.5	0.05	0.70
Demographics					
Religion [2]					0.003
Christian	80.6		76.8		
Muslim	17.1		16.6		
Other	2.3		6.6		
Ethnicity [2]					0.21
Akan	49.3		51.9		
Mole-Dagbani	16.6		13.3		
Other	34.1		34.8		
Highest year of education	3.01	0.08	2.95	0.08	0.012
The highest educational level of adolescent [2]					0.11
No education	3.8		2.5		
Primary school	23.7		20.5		
Secondary education/Higher	72.5		77.0		
Occupation of adolescent [2]					0.0001
Unemployed	64.5		51.2		
Agriculture/unskilled labour	26.9		35.9		
Skilled labour	8.6		12.9		
The adolescent is currently in paid work [2]	33.1		44.7		0.001
Decision making and empowerment indices					
Summated empowerment index	3.23	0.06	4.55	0.05	

table continues

Variables	Girls (*n*= 857)		Boys (*n*= 870)		*P*-value [1]
	Mean or Percentage	SE (mean)	Mean or Percentage	SE (mean)	
Fertility, Marriage and Family Relations					
The adolescent has experienced sex already [2]	41.1		26.7		<0.0001
Age at first sex	15.3	0.11	14.8	0.22	<0.001
The adolescent has a child [2]	10.8		0.7		<0.0001
Age with first child	16.6	0.15	18.2	0.25	0.025
Total children adolescent has	1.2	0.06	1.00	0.00	<0.001
Relation of adolescent to household head [2]					0.99
Household head	1.9		4.5		
Spouse	3.6		N/A		
Child (son/daughter)	62.4		70.2		
Grandchild	8.0		8.0		
Other family relation	15.5		12.6		
Non-family relation	8.6		4.6		
Marital status [2]					<0.0001
Never married	93.6		99.5		
Currently married	5.7		0.5		
Formerly married	0.7		0		
Household characteristics of the adolescent					
WASH index	3.6	0.08	3.6	0.09	0.96
Age of household head (years)	49.2	0.67	49.5	0.83	0.59
Household size	5.7	0.11	5.8	0.13	0.57
Number of children < 5 years	0.7	0.04	0.6	0.04	0.022
Number of household members covered by health insurance	3.6	0.13	3.7	0.22	0.33
Sex of household head (male) [2]	59.6		69.4		0.001
Household wealth index [2]					0.27
Poorest	22.7		22.9		
Poorer	22.0		19.8		
Middle	18.8		19.6		
Richer	17.4		22.0		
Richest	19.1		15.7		
Household owns land usable for agriculture [2]	46.1		52.2		0.04
Household owns livestock [2]	48.6		52.4		0.26
Mean household years of education	5.3	0.16	5.6	0.13	0.22
Geographical/environmental					
Access to a health program for adolescents accessing health services [2]	30.1		34.2		0.25
Place of residence [2]					0.54
Rural	50.5		51.4		
Urban	49.5		48.6		
Agro–ecological zone [2]					0.25
Forest	50.7		48.3		
Guinea/Sudan savannah	17.4		15.3		
Coastal savannah	31.9		36.4		

Between-group test for boys and girls; [2] Values are percentages; all other values are means and standard errors; BMI, Body mass index, BAZ, Body mass index for-age *z*-score; HAZ, height-for-age *z*-score; SAFC, household salty food consumption score; WASH, Water, Sanitation and Hygiene; N/A, not applicable as none of the adolescent boys was a spouse to the household head.

Malnutrition and Cardiovascular Risk

Adolescent girls had a higher mean mid-upper arm circumference (MUAC) (26.3 vs. 25.6 cm; $P < 0.0001$), BAZ (0.03 vs. -0.59; $P < 0.0001$), and HAZ (-0.64 vs. -1.01; $P < 0.0001$) compared to their male colleagues (Table 1). Adolescent boys were more stunted (14.2% vs. 6.2%; $P < 0.0001$) and thin (5.4% vs. 1.6%; $P = 0.0004$) but less overweight/obese (2.4% vs. 11.8%; $P < 0.0001$) compared to their female peers (Figure 1).

Moreover, adolescent boys had a higher SBP (113.5 vs. 107.5 mmHg; $P < 0.0001$) but a lower DBP (67.8 vs. 69.2 mmHg; $P < 0.0001$) compared to their female peers (Table 1). Figure 1 also shows that about a fifth of the adolescents were either pre-hypertensive or hypertensive. The proportion pre-hypertensive (26.4% vs. 14.4%) and hypertensive (0.4% vs. 0.1%) was higher for male compared to female adolescents.

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The multiple (Cox) regression models (Table 2) showed that compared to adolescent girls, adolescent boys were more than twice likely to be stunted [PR 2.58, 95% CI (1.77, 3.76)] and thin [PR 2.67, 95% CI (1.41, 5.09)]. However, adolescent boys were 85% less likely to be overweight/obese compared to their female peers (Table 3). Moreover, compared to adolescent girls, adolescent boys were about twice more likely to have PHH [PR 1.96, 95% CI (1.47, 2.59)] (Table 3). The observed associations were similar at both the univariate (*not shown*) and multiple variable levels.

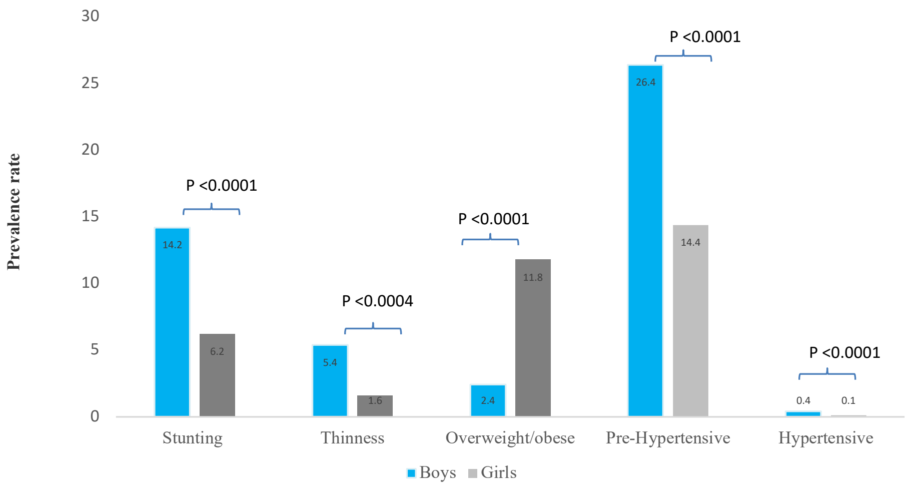

Figure 1. Comparison of the prevalence rates of chronic-energy malnutrition, pre-hypertension, and hypertension between 15–19 years adolescent boys and girls in Ghana

Table 2. Multivariate Predictors of Stunting and Thinness: A Comparative Analysis for Adolescent boys and girls from the 2014 Ghana Demographic Health Survey (GDHS) Data

Variables	Stunting Girls (*n*= 857) PR	95% CI	Boys (*n*= 870) PR	95% CI	Pooled (*n* = 1727) PR	95% CI	Thinness Girls (*n*= 857) PR	95% CI	Boys (*n*= 870) PR	95% CI	Pooled (*n*= 1727) PR	95% CI
Sex												
Boy versus Girl					2.58	1.77, 3.76 ***					2.67	1.41, 5.09 **
Age	1.42	1.19, 1.71 ***										
Girl Menstruated in the last 6 weeks												
Yes vs No	0.23	0.11, 0.48 ***					0.18	0.06, 0.54 **				
The adolescent has experienced first sex												
Yes vs No									0.38	0.15, 0.93 **	0.49	0.23, 1.04
Frequency of fruit intake in the past week	0.88	0.76, 1.01					0.75	0.57, 0.98 *				
Frequency of vegetable intake in the past week	1.13	1.01, 1.28 *			1.09	1.01, 1.17 *						
Empowerment index score	0.82	0.67, 0.99 *			0.76	0.62, 0.93						
Empowerment index*Girl					0.76	0.97, 0.59*						
Ethnicity												
Akan vs Other			1.64	1.10, 2.44 *								
Mole-Dagbani vs Other			0.97	0.62, 1.53								
Relation of girl to the household head												
Household head vs Daughter/son									0.91	0.11, 7.17	0.48	0.07, 3.52
Granddaughter/grandson vs Daughter/son									2.12	1.06, 4.24 *	1.58	0.78, 3.21
Other family relation vs Daughter/son									0.63	0.23, 1.74	0.72	0.33, 1.57
Non-family relation vs Daughter/son									0.19	0.02, 1.46	0.11	0.02, 0.86 *
Marital status												
Currently married vs Never	0.24	0.08, 0.79 *										
The highest educational level of girl												
No education vs Secondary/ Higher	4.03	1.89, 8.60 ***	0.65	0.09, 4.57	1.80	0.87, 3.70			0.41	0.05, 3.17	1.14	0.33, 3.90

table continues

Variables	Stunting Girls (*n*= 857) PR	95% CI	Boys (*n*= 870) PR	95% CI	Pooled (*n* = 1727) PR	95% CI	Thinness Girls (*n*= 857) PR	95% CI	Boys (*n*= 870) PR	95% CI	Pooled (*n*= 1727) PR	95% CI
Primary school vs Secondary/Higher	1.81	0.90, 3.64	1.78	1.22, 2.59 **	1.72	1.21, 2.45 **			2.01	1.10, 3.66 *	1.82	1.08, 3.10 *
Household size									1.08	1.01, 1.17 *		
Water Hygiene and Sanitation index			0.85	0.73, 0.99 *								
Household wealth index												
Poorest vs Richest			3.36	1.15, 9.81 *	1.90	0.86, 4.22						
Poorer vs Richest			5.28	1.93, 14.43 ***	3.16	1.39, 7.19 **						
Middle vs Richest			4.17	1.48, 11.77 **	2.21	0.98, 4.95						
Richer vs Richest			2.42	0.85, 6.91	1.45	0.62, 3.38						
Model fit statistics												
Wald test		28.45 ***		5.27 ***		7.37 ***		8.70 ***		3.90 **		212.08 ***
-2 Log-likelihood ratio		213.09		510.81		824.31		54.10		192.83		277.25
AIC		231.09		528.81		842.31		58.10		208.83		295.25

PR, prevalence ratio; 95% CI, 95% confidence interval; Ref, Reference group; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 3. Multivariate Predictors of Overweight and Pre/Hypertension: A Comparative Analysis for Adolescent boys and girls from the 2014 Ghana Demographic Health Survey (GDHS) Data

Variables	Overweight/Obesity						Pre-Hypertension/Hypertension					
	Girls (*n*= 857)		Boys (*n*= 870)		Pooled (*n*= 1727)		Girls (*n*= 857)		Boys (*n*= 870)		Pooled (*n*= 1727)	
	PR	95% CI	PR	95% CI	PR	95% CI	PR	95% C I	PR	95% CI	PR	95% CI
Sex												
Boy vs Girl					0.15	0.08, 0.29 ***					1.95	1.47, 2.59 ***
Age									1.15	1.05, 1.26 **	1.11	1.03, 1.19 **
The adolescent has a child												
Yes vs No							0.26	0.11, 0.61 **			2.10	0.79, 5.61
An interaction term for having a child * female sex											0.10	0.03, 0.38 ***
Frequency of watching television in the past week							0.72	0.57, 0.90 **				
Empowerment index	1.31	1.02, 1.68 *			1.30	1.04, 1.62 *						
Had coffee in the past 10 min												
Yes vs No									3.64	2.56, 5.19 ***		
Relation of girl to the household head												
Household head vs Daughter /son									1.69	1.12, 2.54 **		
Granddaughter vs Daughter /son									0.89	0.50, 1.61		
Other family relation vs Daughter /son									1.32	1.01, 1.74		
Non-family relation vs Daughter /son									0.74	0.31, 1.77		
Marital status												
Currently married vs Never	0.65	0.23, 1.86										
Formerly married vs Never	3.53	1.86, 6.69 ***										
The highest educational level of girl												
No education vs Secondary/ Higher							0.50	0.15, 1.66			0.80	0.38, 1.67
Primary school vs Secondary/Higher							1.59	1.01, 2.48 *			1.04	0.76, 1.43

table continues

Variables	Overweight/Obesity						Pre-Hypertension/Hypertension					
	Girls (*n*= 857)		Boys (*n*= 870)		Pooled (*n*= 1727)		Girls (*n*= 857)		Boys (*n*= 870)		Pooled (*n*= 1727)	
	PR	95% CI	PR	95% CI	PR	95% CI	PR	95% C I	PR	95% CI	PR	95% CI
An interaction term for highest educational level * sex												
Girl *No education											0.76	0.22, 2.12
Girl *Primary school											1.62	1.01, 2.62 *
Household owns land for agriculture												
Yes vs No							0.59	0.39, 0.90 **				
Sex of household head												
Male vs Female									0.75	0.57, 0.99 *		
Household wealth index												
Poorest vs Richest	0.21	0.10, 0.41 ***	0.21	0.03, 1.56	0.19	0.10, 0.36 ***			2.24	1.25, 4.03 **	2.20	1.26, 3.83 **
Poorer vs Richest	0.15	0.07, 0.35 ***	0.07	0.01, 0.57 *	0.13	0.06, 0.29 ***			1.32	0.69, 2.53	1.48	0.79, 2.76
Middle vs Richest	0.51	0.28, 0.93 *	0.15	0.03, 0.88 *	0.43	0.25, 0.74 **			1.10	0.59, 2.06	1.25	0.76, 2.06
Richer vs Richest	0.78	0.48, 1.28	0.59	0.14, 2.44	0.76	0.46, 1.25			1.60	0.87, 2.93	1.46	0.92, 2.32
Richest vs Richest	Ref.		Ref.		Ref.				Ref.		Ref.	
Agro–ecological zone												
Coastal savannah vs Forest			0.34	0.11, 1.00 *					1.00	0.75, 1.33	1.01	0.76, 1.32
Guinea/Sudan savannah vs Forest			0.31	0.05, 2.15					0.55	0.37, 0.81 **	0.61	0.42, 0.88 **
Thinness vs Normal weight							0.86	0.21, 3.53	1.11	0.64, 1.92	1.00	0.61, 1.65
Overweight vs Normal weight							1.69	1.06, 2.70 *	1.72	0.96, 3.07	1.76	1.20, 2.58 **
Model fit statistics												
Wald test	9.37 ***		3.20 **		16.61 ***		4.65 ***		8.03 **		5.00 *	
−2 Log-likelihood ratio	417.67		77.80		540.65		528.98		984.81		1696.00	
AIC	431.67		89.80		552.65		542.98		1014.81		1730.00	

PR, prevalence ratio; 95% CI, 95% confidence interval; Ref, Reference group; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; BAZ, body-mass-index for-age *z*-score

Factors Associated with Malnutrition

Compared to having secondary/higher education, no education and primary education were consistently associated with a higher PR of stunting for both adolescent boys and girls, but the PR was higher for girls than boys (Table 2). Similarly, compared to the fifth quintile of the HWI, adolescent boys in the first three quintiles were more likely to be stunted in all models; in the pooled model, only the second quintile PR remained statistically significant. Table 2 also shows that an increase in the WASH index was associated with a 15% lower PR of stunting for adolescent boys. Adolescent boys from the Akan ethnicity were meaningfully more likely to be stunted compared to those from other ethnicities [PR 1.64, 95% CI (1.10, 2.44)]. Among, adolescent girls, the PR of stunting was markedly lower for currently married girls compared to never-married girls (Table 2). Furthermore, girls who menstruated in the last six weeks preceding the survey compared to those who did not were 77% less likely to be stunted [PR 0.23, 95% CI (0.11, 0.48)]. Moreover, the PR of stunting was significantly higher for adolescent girls with increasing age. While an increase in the frequency of vegetable consumption in the past week increased the PR of stunting for girls, an increase in the frequency of fruit consumption in the last week reduced the PR for them. Lastly, an increase in the empowerment index decreased the PR of stunting among girls in the statistical models for the adolescent girls and remained significant in the pooled model (*P*-interaction <0.05) (Table 2).

Table 2 also shows that fruit consumption [PR 0.75, (95% CI (0.57, 0.98)] and menstruation six weeks before the survey [PR 0.18, (95% CI (0.06, 0.54)] reduced the PR of being thin among adolescent girls. In the pooled model and that for adolescent boys, the PR of thinness was significantly lower when the adolescent had experienced sex compared to those who were yet to experience sex. Compared to being a daughter/son of the household head, the PR of thinness was significantly higher when the adolescent was a grandson [PR 2.12, 95% CI (1.06, 4.24)] but significantly lower when the adolescent was a non-family relation in the pooled model [PR 0.11, (95% CI (0.02, 0.86)] (Table 2). Furthermore, an increase in household size increased the PR of thinness by 8% for adolescent boys. Lastly, adolescent boys with primary school education compared to those with secondary/higher education were about twice likely to be thin.

Overall, the PR of overweight/obesity was consistently lower for adolescents from households in the first three quintiles of the HWI compared to the fifth quintile in all statistical models (Table 3). Table 3 further shows that married adolescent girls were more likely to be overweight/obese compared to never-married adolescent girls [PR 3.53, 95% CI (1.86, 6.69)]. The PR of overweight/obesity increased by about 30% for an increase in the empowerment index in the pooled model, and in the model for adolescent girls (Table 3). Compared to adolescent boys in the forest zone, those in the coastal savannah zone were less likely to be overweight/obese [PR 0.34, 95% CI (0.11, 1.00)].

Factors Associated with Cardiovascular Risk

Table 3 also shows that the PR of PHH was significantly lower among adolescent girls for an increase in the frequency of watching TV in the past week and when the girl ever had a child compared to those who never had. However, adolescent girls who had primary education compared to those who had secondary/higher education were more likely to have PHH [PR 1.59, 95% CI (1.01, 2.48)]. An interaction term of sex and education in the pooled model showed that the effect of education was significant for adolescent girls compared to boys. Ownership of agricultural land by the household decreased the PR of PHH for adolescent girls. Furthermore, overweight adolescents were consistently more likely to have PHH compared to normal-weight adolescents in all statistical models (Table 3). In the pooled model as well as the model for adolescent boys, the PR of PHH was significantly higher for those in the poorest HWI compared to the wealthiest HWI. Further, adolescent boys in the Guinea/Sudan Savannah zone compared to the forest zone were significantly less likely to have PHH; the association remained in the pooled model.

Sensitivity Analysis

The sensitivity analysis using linear regression showed a similar trend in the risk among adolescent boys compared to girls as well as the factors associated with malnutrition and PHH among adolescents (Table S4,5). Notably, the interaction terms for HWI and sex was statistically significant for HAZ and SBP with boys having significantly lower HAZ and a higher SBP for the lower HWI quintiles compared to the highest quintile. Also, girls who ever had a child had a significantly lower HAZ, and a unit increase in the empowerment index increased the HAZ of girls significantly compared to boys (*P*-interaction <0.05). Lastly, while the experience of sex increased the SBP of girls significantly compared to boys (*P*-interaction < 0.01).

Discussion

The present study compared the risk of malnutrition and PHH among adolescent boys and girls and the factors associated with these risks in Ghana. Overall, adolescent boys were more than twice likely to be stunted and thin compared to their female peers. Madjdian and colleagues [5] found that studies with a significant association between nutritional status and sex point to adolescent boys being worse off than girls in terms of stunting and thinness, partly attributed to boys' later and "sudden" growth spurt [31]. In addition, males are also known to have a high risk for infections [32]; a higher infection risk may increase their risk of undernutrition and may partly account for our findings. Intra-household food distribution in Ghana does not favour girls [33] but Dapi *et al.* [34] argue that girls are often involved in cooking and shopping and might eat in between meals and during cooking, resulting in better nutritional intake for girls compared to boys.

In conformity with several other studies [7, 9, 11, 34], adolescent males were less likely to be overweight/obese compared to their female colleagues. Generally, adolescent girls in Ghana are known to have less physical activity [12, 15] and to have unhealthier eating habits than boys [14], partially explaining our finding. According to Moubarac *et al.* [13], the increasing consumption of nutrient-poor, energy-dense snacks, highly processed foods, sugar-sweetened beverages, and convenient roadside foods (fast-foods) are linked with the emergence of overweight and obesity; this may also partially account for our finding. In the present study, we could not substantiate the findings of Moubarac *et al.* [13] as the 2014 DHS data did not include food intake or snacking habits. Nevertheless, a recent study in the Bekwai Municipality of Ghana indicated that snacking before bed increased the odds of overweight/obesity by more than ten times for 5–17 years children and girls were more overweight (19.4% vs. 7.6%, $p < 0.001$) and obese (10.2% vs. 7.3%, $P= 0.177$) compared to boys [35]. The data on physical activity in the present study may not well reflect the usual physical activity behaviour of our sample. Accordingly, it was not surprising that physical activity did not appear as an important factor in any of our models. Even so, more adolescents boys were found to engage in some form of physical activity ten minutes before the BP assessment. In general, the cultural context in Ghana does not promote much aerobic outdoor games for girls.

Overall, Ghana is presently in the nutrition transition, mirrored in the changing dietary behaviours with more consumption of refined foods, especially in the urban areas [36]. This may partly account for the emergence of overnutrition in our sample. Although a higher frequency of the consumption of fruits and vegetables in the last week may partly explain healthy dietary behaviour, none were essential correlates of overweight/obesity in our models. However, an increase in the frequency of fruit consumption was associated with a lower prevalence of thinness and stunting for girls. Remarkably, an inverse association was found for the frequency of vegetable consumption and stunting for girls. In Ghana, fruits are often consumed as snacks and desserts, but vegetables are commonly consumed as soups and sauces alongside the staple foods. Hence, a high frequency of vegetable intake may represent compensation for reduced calorie intake, especially in settings where food insecurity is prevalent. For instance, in a group of eighth-grade children in the USA, Howard [37] found that children with very low household food security consumed a significantly higher number of servings of carrots. Similarly, Stevens and colleagues [38] reported that the peak of household food insecurity corresponded with higher consumption of dark green leafy vegetables and vitamin A-rich fruits/vegetables in a group of Bangladeshi pregnant women. The former may also explain that boys had a higher frequency of vegetable consumption as their calorie requirements are higher. Nonetheless, fruits and vegetables are rich sources of vitamins and minerals and are linked to improved health outcomes [39].

Considering that over a tenth of the adolescent girls in our study were either overweight or obese, there is a need to institute intervention programmes for the prevention of overnutrition for adolescents, particularly girls. Adolescent overweight/obesity is a known risk factor of obesity in adulthood and diabetes, hypertension, asthma, lower extremity venous oedema, and obstructive sleep apnoea in later life [40]. In our study, being overweight/obese increased the PR of PHH for both adolescent boys and girls. The mechanism of hypertension in overweight/obesity seems to be related to increased sodium sensitivity and the increment of sympathetic nervous system activity [17]. The only data in the GDHS on salt intake for our sample related to the household consumption of salty foods in the last 24-hour, which was not an important covariate of BP or PHH in our analysis. The SAFC in our analysis was only a proxy of the salt intake of our sample and did not sufficiently represent individual salt intake. Although the SAFC included some commonly consumed salty foods in the Ghanaian context, it fails to include all salty foods extensively, quantify the amount of salt consumed from these foods and as well capture the habitual intake of these salty foods. Of particular interest are modifiable factors such as high alcohol intake and high levels of substance use especially among adolescent boys [17], which we could not verify in our data. Considering that only a small fraction (0.2%) of the adolescent boys smoked ten minutes before the BP assessment, smoking behaviour may well not be an important covariate for our sample; it was thus neither a correlate of BP nor PHH in our analysis. Even physical activity ten minutes before the BP assessment was not a relevant correlate of BP in our present study, which may yet be that it did not reflect their usual physical activity level. Overall, the positive association between overweight/obesity and PHH in our analysis emphasises the need for public health intervention programmes to curtail overnutrition. Interestingly, despite the 85% lower PR of overweight/obesity for adolescent boys, they were found to have a higher average SBP, with a higher PR of PHH compared to their female peers, confirming the findings Afrifa–Anane *et al.* [12] in Ghana. According to Abeelen *et al.* [41], inadequate maternal dietary intake during pregnancy is associated with higher hypertension risk among men but not women; they maintain that the nutrition of boys in utero is more dependent on the mother's diet in pregnancy. The literature indicates that stunted children and adolescents have higher rates of later arterial hypertension; undernutrition in childhood and adolescence results in constant physiologic and psychologic stress, increasing the production of stress hormones that weaken the body, decreasing the production of thyroid hormones and insulin-like growth factor that regulates growth [42]. In a follow-up study in the Netherlands, Rotteveel and colleagues [43] showed that young adults born preterm have lower insulin sensitivity and higher blood pressure; low-birthweight and being born preterm are both determinants of stunting and thinness [44]. The above may partly account for the higher PR of PHH for adolescent males in our study since they also had a higher PR of stunting and thinness.

Although TV watching is a known risk factor for overweight, it was rather associated with a lower PR of PHH for adolescent girls in the present study. In our analysis, the frequency of TV watching was somewhat lower for girls compared to the boys and may account for the finding. Conversely, it may be that girls who watched TV frequently were better informed about healthy behaviours. Gyamfi *et al.* [35] recently found TV watching associated with a reduced odds of overweight/obesity in southern Ghana. Like Zimmerman and Bell [45], Gyamfi and colleagues [35] argued that the content of TV shows rather than TV watching itself is associated with poor nutrition outcomes since adolescents who watch educative programmes may be more educated. Nonetheless, we could not explore this further with the available data.

Our findings also suggest that girls may be more at risk of the detrimental effects of poor education on stunting and PHH compared to their male peers. Generally, education is a proxy of socioeconomic status and empowerment [46], and more educated girls are more likely to be empowered. Adolescent girls who are more empowered can better take independent decisions, participate in key decision-making processes, and have more control over household resources; significantly impacting their nutrition and health-seeking behaviours [23]. The preceding also explains that more empowered girls had a reduced PR of stunting in our study. Contrarywise, highly empowered girls, may have more purchasing power and may be more likely to consume the energy-dense, sugar-sweetened beverages and "fast-foods" earlier mentioned; this also partly accounts for the higher PR of overweight/obesity for an increase in the empowerment index among adolescent girls in our analysis. While education may suffice as a proxy of empowerment, the index of empowerment in the present study is a proxy for the autonomy dimension of empowerment, precisely measuring a construct of autonomy using the *"perception and acceptance of domestic violence"* and the ownership of property in the GDHS [8]. We found no multicollinearity between the empowerment index and education in our analysis. Santoso *et al.* [47] argue that empowerment in one domain does not imply empowerment in another; for example, a woman may be educated but does not have the autonomy to own property and/or to make decisions regarding sex and her reproductive health.

In our analysis, a unit increase in the mean household years of education was associated with an increase in SBP for boys, comparable to the HWI in our pooled model (results not shown). However, we excluded the mean household years of education from the SBP pooled model as it was correlated with the education of the adolescent and the HWI in particular; affirming that education is reflective of socioeconomic status. Additionally, girls who lived in households with agricultural land were less likely to have PHH, which may also be that such girls have easy access to agricultural land and are accordingly more economically empowered and less stressed in their livelihoods.

In the present study, a poor HWI was associated with an increased PR of stunting and PHH for boys but a reduced PR of overweight/obesity for girls in conformity with several other studies [5–7, 10, 11]. In general adolescents from poorer households are more likely to have inadequate dietary intake and with a low-purchasing power, may also be less likely to consume "fast-foods" and sugar-sweetened drinks and snacks. While persistent inadequate dietary intake may increase their risk of stunting, reduced calorie intake from the "fast-foods" and snacks may also reduce the risk of overweight/obesity. Contrariwise, those from low socioeconomic households may have poor access to health services, and may thus be more susceptible to poor health, including hypertension. The increased PR of stunting and PHH with a poor HWI for adolescent boys may as well be attributed to their susceptibility to poor health in such conditions. Improvements in household sanitation and hygiene are known to be associated with improved health and nutrition outcomes [48, 49]. It was therefore not surprising that a higher WASH index was associated with a lower PR of stunting among the boys. While this finding may be affirming the susceptibility of males for infections [32], it also suggests improving WASH conditions may offer long-term beneficial effects for nutrition outcomes. In our study, the PR of overweight/obesity was higher when a girl was formerly married compared to being never married. Considering the cross-sectional design of our analysis, the finding may be a reverse causality as studies have shown that heavier girls are more likely to be married earlier compared to their lighter peers [5]. Heavier girls may have better development of secondary sex characteristics, signalling readiness for sexual activity and childbearing [5, 50].

Furthermore, the PR of overweight/obesity was lower for adolescent boys in the coastal savannah zone compared to the forest zone, which may slightly be explained by the differences in cultural dietary patterns, poverty, and food security by ecological zones in Ghana [51, 52]. For instance, the Coastal Savannah zone is next to the Guinea/Sudan savannah zone in the prevalence of food insecurity [51] and poverty [52] in Ghana. In their analysis of food consumption patterns in Ghana, Ecker and Fang [36] observed that Ghana's regions are at different stages of the nutrition transition, and this was partly attributed to inequality in household income and food insecurity in the different regions. Lastly, adolescent males who were household heads were more likely to have PHH compared to those who were sons of the household head; this could somewhat be explained by the stress involved in catering for a household, affecting their health negatively.

In addition to our present findings, adolescent girls remain a vulnerable group in Ghana with disadvantages in intra-household food distribution and resource allocation [33], sexual violence and exploitation [53], poor education [54], more substantial burdens in their time use and less income [55]. Our analysis affirms disadvantages in employment, education, early marriage and childbearing among adolescent girls compared to boys. These deprivations girls encounter have intergenerational consequences for the nutrition and health of their future offspring [56], which may explain that the focus of most nutrition and public health intervention programmes for adolescents in Ghana has mostly been on girls. Nevertheless, our findings may be emphasising the need to include adolescent boys alongside girls in nutrition and public health intervention programmes, ensuring a holistic improvement in the nutrition situation and consequently reducing the health care expenditure. Moreover, there is emerging evidence that there may be some paternal intergenerational effects on the nutrition and health of the offspring [57].

Overall, the prevalence rates of malnutrition for our sample suggest a double burden of malnutrition with an upcoming diet-related non-communicable disease burden in Ghana. According to Hawkes *et al.* [58], a focus on the undernutrition burden of malnutrition may raise risks of poor-quality diets, obesity, and diet-related non-communicable diseases in countries undergoing a rapid nutrition transition. They argue that a more holistic approach is to simultaneously tackle both undernutrition and problems of overweight, obesity, and diet-related non-communicable [58]. Considering that Ghana is presently in the nutrition transition, this holistic approach including both under- and over-nutrition programmes is required to make a meaningful impact. Schools may be a viable place for such comprehensive school-based health intervention programmes. Wei *et al.* [59] recently reported the approach effective in reducing stunting and illness among 4–16 years Zambian children.

It was not feasible to map the trend, and determinants of malnutrition and cardiovascular risk among the adolescents in this analysis since only the 2014 survey included anthropometry of male adolescents aged 15–19 years. More so, data on BP was only available in the 2014 survey. Similarly, the present study did not compare anaemia risk as none of the GDHS surveys has data on the haemoglobin status of adolescent boys. Additionally, the 2014 GDHS did not include any data on dietary diversity; hence, we could not model dietary diversity in any of our statistical analyses. Nonetheless, the inclusion of the frequency of the consumption of fruits and vegetables in the past week may partly account for the adolescents' dietary intake and dietary quality. Although the 2014 GDHS included data on final decision-making in the household, we could not include it in our empowerment index as it was limited to only respondents.

Some aspects of the GDHS data, such as the frequency of the consumption of vegetables and fruits, may be subject to recall bias as this information was based on recalls. Although we could not account for this bias, the bias seems random and may be less likely to influence our conclusions. The inference of a possible causality in this analysis is speculative since the GDHS survey employed a cross-sectional study design. Hence, our findings are limited to the description of observed associations. Nevertheless, we thoroughly modelled several potential explanatory variables, including subject and household-related variables through robust statistical models with weighting, while accounting for the complex survey structure in the GDHS data using the PROC SURVEY function in SAS [30]. Lastly, the use of two different statistical models as in Cox proportional hazard models and linear regression ensured that we minimised the possibility of any biased conclusions in our analyses.

Conclusions

The present study has shown that Ghanaian adolescent boys are more than twice likely to be stunted, thin, and pre-hypertensive/hypertensive compared to their female peers. However, adolescent girls were more likely to be overweight/obese compared to their male colleagues. Our findings suggest poor education may have more detrimental effects on stunting and PHH among girls. Empowerment regarding autonomy from domestic violence and the ownership of property was associated with a reduced PR of stunting for girls, but also increased the PR of overweight/obesity for them. Our findings suggest that improving HWI increases the PR of overweight/obesity for adolescent girls but reduces the PR of stunting and PHH for adolescent boys. Improvement in household WASH conditions may be more beneficial to adolescent boys.

Overall, the prevalence rates of malnutrition and PHH for this sample suggest a double burden of malnutrition with an upcoming non-communicable disease burden and a holistic approach including both under- and over-nutrition programmes are required to make a meaningful impact. Notably, the positive association between overweight and PHH emphasises the need for public health intervention programmes to curtail overnutrition; such programmes may include the promotion of physical activity and healthy dietary behaviours. Our findings may also be highlighting the need to target adolescent boys alongside girls in nutrition and health intervention programmes.

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Appendices

Table S1: Decision making score for adolescent males and females in DHS 2014 data

Variables	Sex	
	Female	**Male**
Index of attitude toward wife beating		
Wife beating is justified if:		
Goes out without telling him	√	√
Neglects the children	√	√
Argues with him	√	√
Refuses to have sex with him	√	√
Burns the food	√	√
The maximum attainable score for attitude toward wife beating	**5**	**5**
Index of property ownership:		
Owns a house alone or jointly	√	√
Owns land alone or jointly	√	√
The maximum attainable score for property ownership	**2**	**2**
Summated empowerment index	**7**	**7**

Table S2: Scoring format from responses for decision-making indexes

Index of attitude toward wife beating scoring format	Index of property ownership Scoring format
No=1	Does not own=0
Yes =0	Alone only=1
	Jointly only=1
	Both alone and jointly=1

Table S3: Improved household water, hygiene, and sanitation index (WASH)

No	Variable	Score
1	**Household water index (WI): score range 0-3**	
1.1	Improved source of drinking	
	Piped water supply into the dwelling	1
	Piped water to a yard/plot	
	Public tap/standpipe	
	Tube well/borehole	
	Protected dug well	
	Protected spring	
	Rainwater	
	If the source of drinking water is packaged/sachet-water or bottled and the source of non-drinking water is improved	
	Any other water source	0
1.2	Round-trip time in water haulage between household and water source	
	If the time spent ≤ 30 minutes	1
	If time spent > 30 minutes	0
1.3	Anything done to water to make it safe for drinking by household	
	Any treatment (boiling, bleach/chlorine, filter, strain through cloth, camphor/naphthalene, solar disinfection, purification tablet, stand and settle or other treatment)	1
	No treatment	0

table continues

No	Variable	Score
2	**Household sanitation index (HSI): score range 0-2**	
2.1	Improved household sanitation facility using the type of toilet	
	Flush/pour-flush toilet or latrine that flushes to a sewer, septic tank, or pit.	1
	Ventilated improved pit (VIP) latrine	
	Pit latrines with the pit well covered by a slab	
	Composting toilets are also considered improved	
	Any other toilet	0
2.2	The household does not share a toilet facility with others	1
3	**Household hygiene index (HHI): score range 0-3**	
3.1	If the household has a place for washing hands	1
3.2	Presence of water at a handwashing place in the household	1
3.3	Soap and other detergents available for hand washing in household	1
	Maximum attainable score for household water, hygiene, and sanitation index (WASH)	**8**

Table S4. Multivariate Predictors of Height-For-Age Z-Score (HAZ) and Body-Mass-Index-For-Age Z-Score (BAZ): A Comparative Analysis for Adolescent Boys and Girls from the 2014 Ghana Demographic Health Survey (GDHS) Data

Variables	**HAZ**						**BAZ**					
	Girls (n= 857)		**Boys (n= 870)**		**Pooled (n= 1727)**		**Girls (n= 857)**		**Boys (n= 870)**		**Pooled (n= 1727)**	
	Estimate (β)	**SE (β)**	**Estimate (β)**	**SE (β)**	**Estimate (β)**	**SE (β)**	**Estimate (β)**	**SE (β)**	**Estimate (β)**	**SE (β)**	**Estimate (β)**	**SE (β)**
Sex												
Boy vs Girl					-0.17	0.13					-0.27**	0.14
Age	-0.08***	0.03										
Girl Menstruated in the last 6 weeks												
Yes vs No	0.41***	0.13					0.25**	0.12				
Adolescent has experienced first sex												
Yes vs No	0.22***	0.07			0.14***	0.04	0.18**	0.07	0.34****	0.08	0.23****	0.05
Adolescent has a child												
Yes vs No					-0.36***	0.13			0.51**	0.26		
Interaction term sex* adolescent has a child												
Girl *Has a child					-0.39**	0.16						
Highest educational level of adolescent												
No education vs Secondary/Higher	-0.38**	0.16	0.29	0.22	-0.31****	0.16						
Primary school vs Secondary/Higher	-0.21**	0.09	-0.35****	0.10	-0.18***	0.09						
Highest education*Sex												
Primary school * Girl					0.51*	0.27						
Primary school*Boy					-0.18	0.13						
Ethnicity												
Akan vs Other					-0.14**	0.06						
Mole-Dagbani vs Other					0.05	0.07						
Marital status												
Currently married vs Never							-0.69**	0.30				
Empowerment score					0.06**	0.03					0.07**	0.03

table continues

Variables	HAZ						BAZ					
	Girls (n= 857)		Boys (n= 870)		Pooled (n= 1727)		Girls (n= 857)		Boys (n= 870)		Pooled (n= 1727)	
	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)
An interaction term for sex*empowerment score												
Girl * empowerment score											0.09**	0.03
Water, hygiene, and sanitation score			0.06**	0.02								
Household wealth index												
Poorest vs Richest	-0.21*	0.11	-0.29**	0.12	-0.16	0.10	-0.42****	0.11	-0.21	0.13	-0.33****	0.08
Poorer vs Richest	-0.27***	0.11	-0.43****	0.13	-0.27***	0.10	-0.44****	0.11	-0.37***	0.13	-0.43****	0.09
Middle vs Richest	-0.24**	0.12	-0.30**	0.13	-0.23**	0.11	-0.15	0.12	-0.29**	0.14	-0.22**	0.09
Richer vs Richest	-0.08	0.12	-0.16	0.12	-0.10	0.12	-0.16	0.14	-0.02	0.14	-0.09	0.09
An interaction term for Sex * Household Wealth Quintile												
Boy* Poorest vs Boy*Richest					-0.35**	0.15						
Boy*Poorer vs Boy*Richest					-0.33**	0.16						
Boy*Middle vs Boy*Richest					-0.21	0.18						
Boy*Richer vs Boy*Richest					-0.15	0.17						
Agro-ecological zone												
Coastal savannah vs Forest	-0.09	0.08										
Guinea/Sudan savannah vs Forest	0.19*	0.10										
Model Fit Statistics												
R-Square	0.07		0.09		0.11		0.07		0.07		0.17	
Root MSE	0.85		0.88		0.87		0.85		0.78		0.82	
Model (F-Value)	3.80****		7.49***		10.51****		6.01****		5.63***		29.38***	

Ref, Reference group; *$P \le 0.1$; ** $P \le 0.05$; ***$P \le 0.01$; **** $P \le 0.001$

Table S5. Multivariate Predictors of Blood Pressure: A Comparative Analysis for Adolescent Boys and Girls from the 2014 Ghana Demographic Health Survey (GDHS) Data

Variables	Systolic blood pressure						Diastolic blood pressure					
	Girls (n= 857)		Boys (n= 870)		Pooled (n= 1727)		Girls (n= 857)		Boys (n= 870)		Pooled (n= 1727)	
	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)
Sex												
Boy vs Girl					3.35**	1.49					-1.56***	0.49
Age			1.37****	0.34	0.74****	0.21			0.32*	0.17	0.40**	0.17
Adolescent has experienced first sex												
Yes vs No	-2.34**	1.09	2.89***	1.12	-0.46	0.85						
Interaction term sex* experienced first sex												
Girl*experienced first sex					3.84***	1.26						
Adolescent has a child												
Yes vs No			18.22****	1.61							-2.47**	1.10

table continues

Variables	Systolic blood pressure						Diastolic blood pressure					
	Girls (n= 857)		Boys (n= 870)		Pooled (n= 1727)		Girls (n= 857)		Boys (n= 870)		Pooled (n= 1727)	
	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)	Estimate (β)	SE (β)
Adolescent exercised in the past 10 minutes												
Yes vs No	4.08***	1.47										
Ate in the past 10 minutes												
Yes vs No			2.55****	0.81	1.69***	0.61						
Had coffee in the past 10 minutes												
Yes vs No			6.39****	1.23	-2.49	2.22					-1.01	1.49
An interaction term for sex*had coffee in the past 10 minutes												
Boy * had coffee in the past 10 minutes					8.32***	2.61					7.32****	2.15
Frequency of fruit intake in the past week			0.32*	0.17								
Visited a health facility in the last 6 months												
Yes vs No					-2.25***	0.79						
Marital status												
Currently married vs Never			-21.70****	5.88					-4.44***	1.67		
Household owns land usage for agriculture												
Yes vs No							-1.44**	0.69				
Household wealth index												
Poorest vs Richest			5.84****	1.40	1.06	1.48						
Poorer vs Richest			1.45	1.41	-0.88	1.50						
Middle vs Richest			1.92	1.27	1.00	1.51						
Richer vs Richest			1.63	1.27	0.28	1.48						
An interaction term for Sex * Household Wealth Quintile												
Boy* Poorest vs Boy*Richest					3.91**	1.75						
Boy*Poorer vs Boy*Richest					2.25	1.93						
Boy*Middle vs Boy*Richest					1.14	1.88						
Boy*Richer vs Boy*Richest					1.40	1.88						
Agro-ecological zone												
Coastal savannah vs Forest			1.90**	0.93	1.07	0.69					-0.52	0.55
Guinea/Sudan savannah vs Forest			-3.48**	1.53	-2.68***	0.93					-2.11****	0.63

table continues

Variables	**Systolic blood pressure**						**Diastolic blood pressure**					
	Girls (n= 857)		**Boys (n= 870)**		**Pooled (n= 1727)**		**Girls (n= 857)**		**Boys (n= 870)**		**Pooled (n= 1727)**	
	Estimate (β)	**SE (β)**	**Estimate (β)**	**SE (β)**	**Estimate (β)**	**SE (β)**	**Estimate (β)**	**SE (β)**	**Estimate (β)**	**SE (β)**	**Estimate (β)**	**SE (β)**
BAZ category												
Underweight vs Normal weight	-6.90	5.19	-2.53	1.29	-3.67**	1.88			-2.98**	1.51	-2.68*	1.53
Overweight vs Normal weight	2.98***	1.02	4.71****	1.39	3.14****	0.97			1.98***	0.76	1.35*	0.78
Adolescent is stunted												
Yes vs No			-3.86***	1.25	-3.23***	1.09						
Agro-ecological zone												
Coastal savannah vs Forest											-0.48	0.55
Guinea/Sudan savannah vs Forest											-1.97****	0.61
Model Fit Statistics												
R-Square	0.04		0.15		0.15		0.01		0.02		0.03	
Root MSE	9.99		10.46		10.30		8.01		8.05		8.01	
Model (F-Value)	5.83****		35.99****		16.76****		4.39**		3.58****		5.32****	

Ref, Reference group; *$P\leq$ 0.1; ** $P\leq$0.05; ***$P\leq$0.01; **** $P\leq$0.001; BAZ, Body-mass index-for-age Z-score

Chapter 6

Fortified biscuits seemed to improve vitamin A status but not iron in post-menarcheal girls: evidence from a randomized control trial in Ghana

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Submitted to ASN Journal of Nutrition

Abstract

Background

Adolescent girls are an important target group for micronutrient interventions. Food fortification is potentially a useful strategy for this group but knowledge of the efficacy and timing (regarding menarche) of such interventions, especially in resource-poor settings is limited.

Objective

To evaluate the effect of consuming multiple-micronutrient fortified biscuits (MMB) 5 days/week for 26 weeks compared to unfortified biscuits (UB) on micronutrient status, height, and cognitive performance of female adolescents. We also explored to what extent the intervention effect varied before or after menarche.

Method

A 26-week double-blind, randomized placebo-controlled trial among adolescent girls aged 10-17 yrs. (n=621) in the Mion District, Ghana. Outcomes were concentrations of haemoglobin, ferritin, soluble transferrin receptor and retinol-binding protein, as well as body-iron. Cognitive function was assessed using the US National Institute of Health toolbox cognition battery. We computed subjects' height-for-age z-score and body-mass index-for-age z-score. Intention-to-treat analysis was complemented with per-protocol analysis.

Results

We found no effect of the intervention on plasma ferritin, transferrin receptor and retinol-binding protein. MMB consumption did not affect anaemia, micronutrient deficiencies and anthropometric indices at the population level. After adjusting for the girl's baseline age, height-for-age z-score and baseline micronutrient status, vitamin A deficiency increased by 6.15% (95% CI 0.72, 11.59) for pre-menarche girls in the MMB compared to the UB group, but deficient/low vitamin A status decreased substantially by 9.63% (95% CI -18.94, -0.32) for MMB girls who were post-menarche compared to their UB peers. Only in anaemic subjects did we find evidence that MMB consumption improves mathematics score and working memory of pre-menarche girls.

Conclusion

In our setting, MMB consumption did not improve iron status, but it decreased the prevalence of a deficient/low vitamin A status in post-menarcheal girls.

Trial Registration

The RCT was registered with the Netherlands Clinical Trials Register (NL7487).

Introduction

Adolescent girls are an important target group for micronutrient interventions, especially in Sub-Saharan Africa where micronutrient problems and adolescent pregnancy are prevalent. Globally, West and Central Africa have the highest adolescent birth rates (115 births per 1000 girls) [1]. In Ghana, a third of girls are married by 18 years [2], and 14% of girls aged 15-19 years have ever given birth; the trend is remarkably higher in rural and northern Ghana [3]. Early marriage contributes to a vicious cycle of early pregnancy, low education, poverty, poor diet, and malnutrition for the girl with intergenerational consequences [4–6]. Pre-pregnancy haemoglobin and iron status are important risk factors for anaemia-related morbidity and mortality during pregnancy [7]. Besides the high physiological requirements during adolescence, pregnancy poses an extra-demand of nutrients for the growing foetus [7–9], negatively affecting girls' attained height, increasing the risk of life-threatening anaemia for girls, maternal morbidity and mortality, and adverse birth outcomes [4, 7, 9–11].

6

Prevalence estimates of iron deficiency (ID) and vitamin A deficiency (VAD) for adolescent girls in Sub-Saharan Africa range 7-33% and 4-32%, respectively [12–14], depending on the context. The burden of anaemia and IDA (iron-deficiency anaemia) is notably higher for adolescent girls due to the increased demand for puberty, menstrual losses, and dietary inadequacies, particularly in low socio-economic settings [4, 8]. Menstruation-induced blood loss may *increase* iron absorption through the homeostatic mechanism of up-regulation during a deficiency [15]. However, menstruation-induced inflammation would lead to *decreased* iron absorption [16]. These opposite mechanisms highlight the uncertainty of a nutrition intervention's best timing, either pre-or post-menarche.

School-based interventions offer an opportunity to optimise the nutrition and health of adolescent girls [17], thereby breaking the intergenerational cycles of malnutrition and deprivation. Multiple-micronutrient fortified foods (MMFs) such as multiple-micronutrient fortified biscuits (MMB) may be a more convenient and effective approach to tackling micronutrient problems of adolescent girls in low-socioeconomic contexts [18]. However, there is a paucity of data on the efficacy of MMFs on the micronutrient status of adolescent girls in Sub-Saharan Africa; the available evidence is mainly from high-income countries [19–21]. Improved micronutrient intake, especially iron and iodine, is essential for brain development and maturation [22–24]. Studies on micronutrient intake and cognition during adolescence are rare, and to the best of our knowledge, the study of Wang *et al.* [25] is the only efficacy trial of MMF concerning academic performance and some psychosocial competencies of adolescents. The present study aims to evaluate the effect of consuming multiple-micronutrient fortified biscuits (MMB) 5 days weekly for 26 weeks compared to unfortified biscuits (UB) on micronutrient status, height, and cognitive performance of female adolescents. We also explored to what extent the intervention effect varied before or after menarche.

Methodology

Study area and participants: The study population consisted of pre-and post-menarche adolescent girls aged 10-17 yrs. in 14 communities in the Mion District, the Northern Region of Ghana. According to the Ghana Statistical Service [26], the district is mainly rural (about 91%), and our previous secondary analysis of data [27] suggests a high prevalence (64.6%) of anaemia among adolescent girls in the rural northern savannah agro-ecological zone.

Study Design: The study was a 26-week follow-up double-blind, randomized placebo-controlled trial (RCT). The study design details, including the inclusion and exclusion criteria, have previously been described in detail elsewhere [28]. A non-targeted approach, including both anaemic and non-anaemic girls, was used to randomize girls into two parallel treatment arms receiving either nutrition/health education (5 different occasions) with a 5-day weekly MMB or UB for 26 weeks. The Navrongo Health Research Centre Institutional Review Board approved the study protocol (NHRCIRB323).

Sample size: We estimated a minimum sample of 155 subjects per group to detect between-group differences [29] in haemoglobin (3.83 g/L), serum ferritin (9.4 μg/L) and serum retinol (0.11μmol/L) with 80% power and a one-sided significance of 0.05. A maximum attrition rate of 10% during follow-up was assumed. A total of 620 adolescent girls (310 pre-menarche and 310 post-menarche) were required for the RCT.

Sampling procedure: Of 1057 girls in a survey, conducted in November/December 2018, we invited and enrolled into the RCT 621 girls who did not meet the exclusion criteria (Table S1). Recruitment of subjects into the RCT was in January/February 2019 (in the dry season) while treatment and follow-up ended in September 2019, during the peak of the rainy season. The study population was limited to primary school girls; junior high school girls were excluded due to the **G**irls' **I**ron **F**olate **T**ablet **S**upplementation programme among adolescent girls in junior high schools [30]. Menarche status was based on recall at screening and the age of the girl (> 13 yrs.) using the average age at menarche in Ghana from the literature [31–33]. A 2-stage sampling procedure with probability proportional to the school and menarche group size was used to randomise the girls into the MMB and UB. Firstly, we generated and sorted in ascending order (lowest to highest), random numbers (between 0-1) by menarche group, within schools in Microsoft Excel (MS Excel, version 2016). Girls from the menarche group of each school were then enrolled until the sample required for the school's menarche group was met. A second set of random numbers between 0 and 1 were generated for selected girls in each menarche group and school, and girls with random numbers < 0.5 were assigned to the UB group, whiles those with random numbers ≥ 0.5 were assigned to the MMB group. *Figure 1* describes participants' flow, including reasons for non-enrolment and drop-out in the RCT.

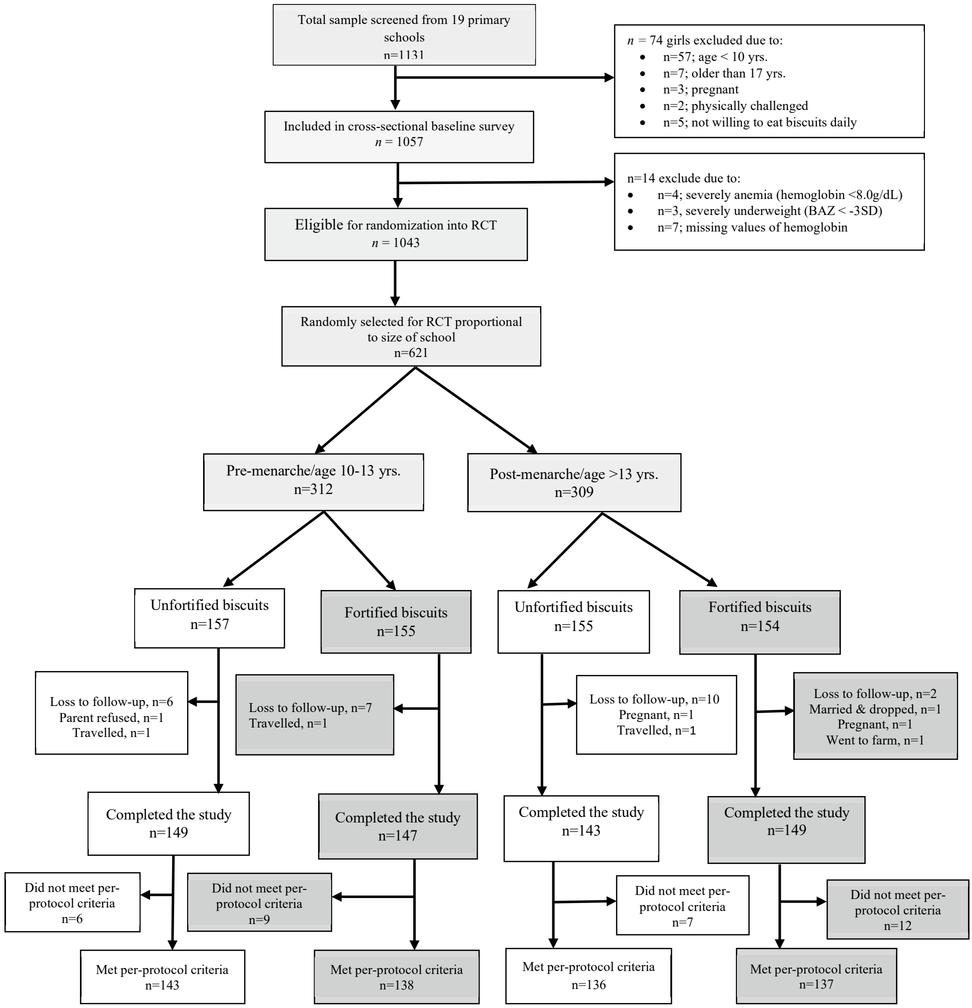

Figure 1. The flow of participants, as per CONSORT guidelines including reasons for non-enrolment and drop-out in the RCT

Treatment and control: Girls in the treatment arm of the RCT received the obaasima MMB, enriched with 11 vitamins (vitamins B_1, B_2, B_6, B_{12}, A, D, K_1, E, niacin, folate and ascorbic acid) and 7 minerals (Zn, Ca, Fe, Cu, I, Se and Mg). Obaasima is a partnership project between the German Development Cooperation (GIZ) and the private sector in Ghana to develop "*Affordable and Nutritious Foods for Women (ANF4W)*". The project presently produces multiple-micronutrient fortified breakfast cereal flour, biscuits and shito (a local spicy sauce), marketed and sold under the brand name "*Obaasima*" in the Ghanaian market (http://obaasimaghana.com/campaign.php). Obaasima products are fortified to meet 15% and 30% of the recommended dietary allowance of the fortified minerals and vitamins respectively, for young women aged 19-30 years. (*Sight and Life*; *personal communication*). Ferrous fumarate (8.2mg/100g) and dry vitamin A palmitate (1.0mg/100g) were the fortificants for iron and vitamin A respectively; Supplementary Table S2 indicates the fortification level of each micronutrient. Placebo recipients consumed biscuits similar in calories and appearance to the MMB. The wheat flour used in producing both biscuits was fortified as by law in Ghana with Fe, Zn, folic acid, vitamins $B_{1,}$ B_2 and B_{12} and niacin; hence, the UB also contained some micronutrients (Table S2). The girls consumed a pack of biscuits (51.3 ± 3.2g) *ad libitum* as a snack on each school day (Monday through to Friday), for 26 weeks, in the teacher and/or field assistant's presence. A pack of the MMB or UB contained between 8-10 pieces of biscuits, with the average weight of a piece being 5.6 ± 0.5g. The whole pack of biscuits had to be consumed at each feeding session but leftovers in pieces (if any) were recorded with a daily case report form, which also captured subjects' attendance at feeding sessions, adverse events, and severe adverse events during the intervention. In our study, blinding of subjects and the research team was achieved by repackaging the biscuits into clear zip-lock bags with yellow and red stickers to distinguish between them [28].

***Plasmodium* infection and deworming:** We screened for current or recent *Plasmodium* infection at baseline, mid-point (thirteenth week) and end-line with malaria rapid diagnostic test (First Response; Premier Medical, Somerset, New Jersey, US). Malaria rapid diagnostic test uses histidine-rich protein-2, which is specific for *P. falciparum* [34], *which* accounts for 75% of *Plasmodium* infections in northern Ghana [35, 36]. Girls who tested positive for *Plasmodium* infection during any time point were given artemether-lumefantrine (80mg/480mg) as treatment, following the Ghana Health Service guidelines [37]. Similarly, girls who reported fever and/or headache during the intervention were also tested and treated for *Plasmodium* infection when positive. Finally, at baseline, all subjects were dewormed with a single dose of mebendazole 400mg chewable tablets, consumed in the presence of the field team with 500mL filtered and packaged sachet water.

Biochemical measurements and analysis: We collected venous blood at baseline and 26 weeks after the start of intervention into Na-Heparin Vacutainers (Becton-Dickinson Diagnostics) for the measurement of plasma concentrations of ferritin (PF), soluble transferrin receptor (TfR), retinol-binding protein (RBP) and inflammation biomarkers: plasma concentrations of C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP). We also assessed haemoglobin, with the HemoCue 301 photometer (Ängelholm, Sweden; 0.1g/dL precision) by finger prick at baseline and using venous blood at the end-line. The VitMin Lab (Willstätt, Germany) measured PF, TfR, RBP, CRP and AGP using a combined sandwich Enzyme-Linked Immunosorbent Assay (ELISA) [38]. All measurements were duplicated and were repeated where the CV (inter-assay) was >10%, and obvious outliers were removed. The CV for the various indicators were SF, 2.3%; TfR, 3.6%; RBP, 3.6%; CRP, 5.8%; and AGP, 8.1%. Certified quality control samples from the CDC/Atlanta and Bio-Rad Liquicheck controls (Bio-Rad) were used to produce calibration curves. We computed body iron stores (BIS) using Cook's formula [39].

Cognitive performance and anthropometry: At baseline and end-line, we assessed the girls' cognitive function with the National Institute of Health toolbox cognition battery using iPads. The cognition battery is a recognized and standardized test tool for measuring cognitive, sensory, motor and emotional function across diverse study designs and settings for ages 3-85 years [40, 41]. We assessed five cognition domains, which, based on literature, are relevant to adolescents' neurological development, including working memory, episodic memory, processing speed, attention and inhibitory control, and executive function [42–44]. The test is computerized on iPads, and the *t*-scores adjusted for the girl's age are estimated automatically at the end of each test.

We also obtained and used the girls' academic grades from their school continuous assessment booklet for 2 academic years, September 2017-July 2018 academic year preceding our study as a benchmark and the September 2018-July 2019 academic year at the end-line as the outcome. The evaluation of academic performance was based on cumulative grades for three core study subjects including English Language, Mathematics and General Science; these subjects form the basic components of academic training in primary schools in Ghana. The overall average score for the 3 subjects for each academic year was computed and used to assess the girls' academic performance. In the extensive survey and after 26 weeks of intervention, we measured height and weight in duplicates to the nearest 0.1 decimal with the Seca stadiometer and digital weighing scale, respectively, following standard procedures [45].

Covariates: We included information on several covariates, at the girl, maternal and household level, collected in face-to-face interviews using a pre-tested questionnaire. The child-level covariates included age, ethnicity, and religion. The data also included the girls' dietary diversity score from a single qualitative 24-hour dietary recall, based on ten food groups [46]. The dietary data also included the frequency of consuming different food groups in the last month, including animal source foods (eggs, fish, meat, dairy products), legumes/nuts/seeds, vitamin A-rich dark green leafy vegetables, and other vitamin A-rich fruits and vegetables. Maternal-level covariates included the age, literacy, education, and work status of the mother. The data also included an index of the mother's household decision-making participation, with the 8-item final decision-making index of the demographic and health survey [47]; the index conformed with Amugsi *et al.* [48]. Household-level covariates in the data included paternal literacy, education, and work status and as well as a household roster, which was used to compute the household size and ratio variables for dependency, literacy, and female to male ratio. Households were classified into categories of food security (food secured, and mild, moderate, and severe food insecurity) based on the Food Insecurity Experience Scale [49]. Lastly, we created a household asset index using principal component analysis and then ranked subjects' households into quintiles of household wealth.

Definitions: Anaemia and the severity of anaemia were defined according to WHO criteria [50]. Inflammation was defined as CRP >5 mg/L and/or AGP >1.0 g/L [51]. After adjusting micronutrient biomarkers for inflammation and *Plasmodium* infection with the BRINDA group approach [52–54], we defined iron deficiency (ID) as (1) PF <15μg/L [51], (2) TfR >8.3 mg/L [38], and (3) PF <15μg/L or TfR >8.3 mg/L. We re-defined ID as PF <15μg/L but PF< 70μg/L for girls with inflammation as recommended by the WHO [51], and lastly as PF <15μg/L but PF< 70μg/L for girls with inflammation or TfR >8.3 mg/L. Iron deficiency anaemia (IDA) was defined as concurrent anaemia and ID (PF <15μg/L or TfR >8.3 mg/L). Vitamin A deficiency (VAD) was defined as RBP <0.7μmol/L, while low/marginal vitamin A status was defined as RBP ≥ 0.7 but <1.05μmol/l [55]. In conformity with de Onis *et al.* [56], we defined being stunted as height-for-age z-score (HAZ) < -2SD and body-mass-index for age z-score (BAZ) was categorized into thinness (BAZ < -2SD), normal weight (-2SD ≤ BAZ ≤ +1 SD), overweight/obesity (BAZ ≥ +1SD). Treatment adherence was defined as the percentage of the total amount (gram) of biscuits each girl consumed, considering the total amount that was scheduled to be consumed for the 26 weeks of intervention.

Statistical analysis

We computed height-for-age z-score (HAZ) and body-mass-index for age z-score (BAZ) using WHO AnthroPlus with the WHO 2007 growth reference for 10-19 years adolescent girls. Data analysis was conducted with SAS 9.4 (SAS Institute Inc., Cary, NC.). We used the Internal Regression Correction (IRC) approach [52–54] of the BRINDA group to adjust PF, TfR and RBP for inflammation biomarkers (CRP and AGP) and *Plasmodium* infection. In the IRC approach, linear regression is used to adjust a biomarker by the concentration of CRP and AGP on a continuous scale and *Plasmodium* infection as a dichotomous variable. We applied the natural log-transformation to linearize the relationship between plasma nutrient markers and inflammatory markers. To avoid bias in regression coefficients for the regression equation, we excluded subjects with CRP below and/or TfR and RBP above the detection limit. Following the BRINDA approach [54], we adjusted TfR for AGP and *Plasmodium* infection but not for CRP. The variance inflation factors were < 2, suggesting no multicollinearity between predictors in the regression models. The lowest CRP decile at baseline and end surveys was the same as the external reference value (0.10mg/L). However, our AGP reference values (baseline=0.36 g/L and end-line=0.40 g/L) were less than the reference values (0.54 g/L and 0.59 g/L) of the BRINDA group [52]. Considering that the majority (81.2%) of our study population was below 15 years, we used the external references values of CRP (0.10mg/L) and AGP (0.59g/L) for the pre-school aged children from the BRINDA group [52].

We presented summary statistics for selected characteristics at baseline by biscuit group to describe the study population. Skewed continuous data were presented as the median and interquartile range. Data normality was visually explored with histograms with normality curves, boxplots, and Q-Q plots. The primary analysis of the treatment effect was intention-to-treat analysis with multiple imputations of missing end-line outcome variables, assuming that the data were missing randomly [57]. The outcome variables for the analyses were haemoglobin, BIS, and the adjusted log-transformed PF, TfR and RBP. We estimated the differences in post-intervention measurements between biscuits groups with adjustment for baseline values of each measurement as in an analysis of covariance (ANCOVA), using a linear mixed model (Proc Mixed). The school (design effect) was included as a random intercept. We did not transform haemoglobin, which was normally distributed, but BIS was analysed as BIS z-score. Similarly, we used the mixed model analysis to examine the effect of the intervention on HAZ, BAZ, cognition, and academic performance.

We estimated the post-intervention prevalence differences [58] in micronutrients deficiencies between MMB and UB groups with the post-estimation command, *adjrr* after running logit models on each micronutrient problem in STATA (StataCorp, v.13.0). Two statistical models were created; model 1 (crude model) was a fixed-effects model and included the biscuits group and the study design effect (menarche status at enrolment). Model 2 adjusted for the girl's baseline micronutrient biomarkers (haemoglobin, PF, TfR and RBP), age, and HAZ. Similar statistical models were used for the secondary outcomes (HAZ, BAZ, cognition, and academic performance). To explore to what extent the magnitude of intervention effects depended on menarche status, we added an interaction term of biscuits and menarche status at enrolment (Biscuits*menarche status) and as well produced stratified estimates by menarche status.

Sensitivity and subgroup analyses

We first conducted a per-protocol analysis, restricted to girls with adherence ≥ 80%. We also conducted a subgroup analysis for subjects who were anaemic at baseline, ensuring that the intervention's effect is plausibly not masked by tissue saturation of nutrient replete girls.

Results

Baseline characteristics: Of 621 girls randomized, 588 (94.7%) completed the study, 94.5% in the MMB group and 94.9% in the UB group. Girls who dropped out of the study (*n*=33) had higher CRP, higher BAZ, and earlier menarche at baseline than the girls who completed the study. Treatment adherence was 90.3% and 88.1% in the MMB and UB respectively. At baseline, the girls' mean age was 12.8 ± 2.0 years (Table 1). The mean HAZ and BAZ were -1.0 ± 1.2 and -0.2 ± 0.9, respectively. Overall, about 17.4% of the girls were stunted, 7.1% were underweight, and less than 2% were overweight/obese at baseline. A little over 40% of the girls had SCI at baseline, mainly the late convalescence phase (high AGP and normal CRP). About 40% of the girls were anaemic; a little over half were iron deficient and close to a quarter had IDA at baseline.

Further, about one-third of the girls were either vitamin A deficient or had a marginal vitamin A status. Compared to the IRC approach, the prevalence rates of ID and IDA were slightly higher when using the exclusion criteria for SCI (Table 1). After 26 weeks of intervention, 37 (6.0%) of the 312 pre-menarche girls attained their menarche.

Table 1. Baseline characteristics of subjects by biscuits group, following intention to treat analysis

Baseline characteristics	Fortified biscuits (n=309)	Unfortified biscuits (n=312)
Vital and personal characteristics		
Age, years	12.8 ± 1.9	12.8 ± 2.0
Height-for-age z-score (HAZ)	-0.9 ± 1.2	-1.0 ± 1.1
Stunted (HAZ < -2SD), %	51 (16.5)	57 (18.3)
Body mass index-for-age z-score (BAZ)	-0.8 ± 0.9	-0.7 ± 0.8
Body mass index z-score category, %		
Underweight (BAZ < -2 SD)	24 (7.8)	20 (6.4)
Overweight/obese (BAZ > + 1SD)	4 (1.3)	5 (1.6)
Positive for *Plasmodium* infection (%)	126 (40.8)	125 (40.1)
Inflammation markers		
C-reactive protein (CRP), mg/L	0.2 (0.1, 0.7)	0.2 (0.1, 0.8)
Alpha-1-acid glycoprotein (AGP), g/L	0.9 (0.6, 1.4)	0.9 (0.6, 1.4)
Inflammation (%)		
CRP >5 mg/L	17 (5.5)	22 (7.1)
AGP > 1 g/L	131 (42.4)	132 (42.3)
Inflammation (CRP > 5 mg/L or AGP> 1 g/L)	133 (43.0)	134 (43.0)
Inflammation category (%)		
Reference	176 (57.0)	178 (57.1)
Incubation	2 (0.7)	2 (0.6)
Early convalescence	15 (4.9)	20 (6.4)
Late convalescence	116 (37.5)	112 (35.9)
Haemoglobin status and Anaemia		
Haemoglobin, g/L	120.0 ± 10.2	120.0 ±10.2
Anemia[1] (haemoglobin < 115/120 g/L) (%)	121 (39.2)	132 (42.3)
Anaemia severity, %		
Mild [2]	73 (23.6)	73 (23.4)
Moderate (80 ≤ haemoglobin ≤109)	48 (15.5)	59 (18.9)
Micronutrient biomarkers and deficiencies after IRC adjustment		
Ferritin (PF), µg/L	47.2 (24.1, 68.0)	44.7 (26.9, 68.1)
Transferrin receptor concentration (TfR), mg/L	8.1 (6.0, 11.6)	8.4 (6.0, 11.3)
Retinol-binding protein (RBP), µmol/L	1.2 (0.9, 1.7)	1.3 (1.0, 1.8)
Body iron stores, mg/kg	3.4 ± 6.4	2.4 ± 8.5
Iron deficiency (PF <15µg), %	38 (12.3)	29 (9.3)
Tissue iron deficiency (TfR >8.3mg/L), %	150 (48.5)	161 (51.6)
Iron deficiency (PF <15µg/L and/or TfR >8.3), %	165 (53.4)	170 (54.5)
Iron deficiency anaemia (anaemia with PF <15µg and/or TfR >8.3mg/L)	64 (21.0)	80 (25.6)
Vitamin A deficiency (RBP < 0.7 µmol/L)	35 (11.3)	23 (7.4)
Low or marginal vitamin A status (0.7 ≤ RBP <1.05µmol/L)	71 (23.0)	71 (22.8)
BIS below zero	41 (13.3)	52 (16.7)
Micronutrient deficiencies excluding children with inflammation (%)		
Iron deficiency (PF <15µg), %	27 (15.3)	18 (10.1)
Tissue iron deficiency (TfR >8.3mg/L), %	93 (52.8)	93 (52.3)
Iron deficiency (PF <15µg/L or TfR >8.3)	107 (60.8)	102 (57.3)
Iron deficiency anaemia (anaemia with PF <15µg and/or TfR >8.3mg/L)	47 (26.70)	40 (22.5)
Vitamin A deficiency (RBP < 0.7 µmol/L)	31 (17.6)	24 (13.5)
Low or marginal vitamin A status (0.7 ≤ RBP <1.05µmol/L)	43 (24.4)	41 (23.0)

Values are means ± SD or median (25th percentile, 75th percentile) unless specified otherwise. HAZ, height-for-age z-score; BAZ, Body-mass index for-age z-score; AGP, Alpha-1-acid glycoprotein; CRP, C-reactive protein; PF, plasma ferritin; TfR, Transferrin receptor concentration; RBP, retinol-binding protein; [1]Anemia, haemoglobin status < 115 g/L for girls aged <12 yrs. and haemoglobin < 120 g/L for girls aged ≥12 yrs.; [2] Mild anaemia: 110 ≤ haemoglobin ≤114 for girls aged 10-11yrs. and 110 ≤ haemoglobin ≤119 for girls aged ≥12yrs.

Table 2 shows that the mean composite fluid cognition and academic performance at baseline were 25.6 ± 9.3 and 54.5 ± 12.8%, respectively. The girls came from relatively low socio-economic households with < 10% of their mothers being literate and only a fifth of the households being food-secure (Table 2). About 90% of the mothers were aged 50 years and above.

Table 2. Baseline cognitive performance, maternal and household-related characteristics

Variable	Fortified biscuits group (n=309)	Unfortified biscuits group (n=312)
Cognition data (t-score)		
Composite fluid cognition	25.4 ± 9.0	25.8 ± 9.6
Executive function	33.7 ± 51.9	33.5 ± 33.5
Attention and inhibitory control	32.8 ± 6.4	32.2 ± 5.8
Working memory test	31.3 ± 13.2	33.7 ± 17.2
Processing Speed	30.3 ± 10.2	31.0 ± 10.0
Episodic Memory Test	43.9 ± 6.3	43.6 ± 6.6
Academic performance		
Overall academic performance	54.2 ± 12.2	54.7 ± 13.5
English Language	53.2 ± 14.8	52.5 ± 14.7
Mathematics	54.7 ±14.3	54.9 ± 14.0
General Science	54.7 ± 13.5	56.7 ± 19.9
Percentage of school attendance	84.4 ± 15.1	83.2 ± 16.4
Maternal characteristics		
Mother is aged 50 years and above (%)	279 (90.3)	285 (91.4)
Mother is literate (%)	31 (10.0)	17 (5.5)
Final decision-making index of mother	5.3 ± 1.3	5.4 ± 1.3
Household characteristics		
Food-secure (%)	64 (20.7)	49 (15.7)
Household wealth index (%)		
Quintile 1	56 (18.1)	68 (21.8)
Quintile 2	76 (24.6)	48 (15.4)
Quintile 3	51 (16.5)	61 (19.6)
Quintile 4	67 (21.7)	54 (17.3)
Quintile 5	59 (19.1)	54(17.3)

Unless otherwise specified, values are means ± SD

Intervention effect on biomarkers of micronutrient status: After 26 weeks of intervention, we found no difference in PF, TfR and RBP in the MMB group compared to the UB group (Table 3). Haemoglobin (-1.20; 95% CI -2.99, 0.60 g/L) and BIS (-0.15; 95% CI -0.27, -0.03 z-score) decreased in the MMB group post-intervention compared to the UB group, unexpectedly (Table 3). We observed a marginal increase in the haemoglobin status of pre-menarche girls (0.43; 95% CI -2.10, 2.97g/L) but a decrease among post-menarche girls (-2.83, 95% CI -5.37, -0.28 g/L). No clear differences were observed in changes in PF, TfR, and RBP between biscuits groups in either pre-or post-menarche girls. Similar results were found when we repeated the analysis using the per-protocol criteria (Table S3) and in the sub-group analysis for girls who were anaemic at baseline (Table S4).

Intervention effect on micronutrient deficiencies: In our study, post-intervention anaemia decreased slightly by 2.30% for pre-menarche girls in the MMB compared to the UB but increased marginally by 9.67% for post-menarche girls in the MMB compared to the UB (Table 4). We found interaction effects between biscuits and menarche for the post-intervention VAD (*P*=0.04) and deficient/low Vitamin A status (*P*=0.03) after adjusting for the girl's baseline age, HAZ, haemoglobin, PF, TfR and RBP. Among pre-menarche girls, MMB increased VAD by 6.15% points more but a minor decrease with MMB was observed for post-menarche girls. In our study, having a deficient/low vitamin A status was 9.63% points lower for post-menarche MMB girls compared to their UB peers; nonetheless, pre-menarche girls in the MMB compared to the UB had a marginal increase (Table 4). Similar results were found when repeating the analysis following per-protocol (Table S5). In the sub-group analysis for anaemic subjects at baseline(*n*=253), the prevalence of anaemia was 14.9% higher in post-menarche on MMB compared to the UB, and the prevalence of IDA was 13.4 % points higher (Table S6).

Table 3. The effect of consuming micronutrient-fortified biscuits compared to unfortified biscuits on micronutrient markers after 26 weeks of intervention in adolescent girls in Ghana

Outcome	Overall sample (n= 621)		Pre-menarche (n= 312)		Post-menarche (n= 309)	
	Estimate (95% C.I)	P-value	Estimate (95% C.I)	P-value	Estimate (95% C.I)	P-value
Ferritin (µg/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	0.31% (-7.20%, 7.82%)	0.94	0.42% (-10.17%, 11.01%)	0.94	0.20% (-10.44%, 10.84%)	0.97
Soluble transferrin (mg/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	2.57% (-3.90%, 9.04%)	0.44	-0.14% (-9.26%, 8.99%)	0.98	5.27% (-3.91%, 14.45%)	0.26
Retinol binding protein (µmol/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	0.60% (-4.33%, 5.53%)	0.81	-2.64% (-9.58%, 4.31%)	0.46	3.83% (-3.16%, 10.82%)	0.28
Body iron store z-score (SD)						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	-0.15 (-0.27, -0.03)	0.01	-0.17 (-0.34, -0.01)	0.04	-0.13 (-0.29, 0.04)	0.13
Haemoglobin concentration (g/L)						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	-1.20 (-2.99, 0.60)	0.19	0.43 (-2.10, 2.97)	0.74	-2.83 (-5.37, -0.28)	0.03

[1]Outcomes variables were log transformed (Ln) and estimates were expressed as percentages increase or decrease.

Table 4. The effect of consuming micronutrient-fortified biscuits compared to unfortified biscuits on post-intervention prevalence difference in micronutrient deficiencies after 26 weeks of intervention in adolescent girls in Ghana

Outcome	Overall sample (n= 621)		Pre-menarche (n= 312)		Post-menarche (n= 309)	
	[1]Prevalence difference (%) (95% CI)	P-value	Prevalence difference (%) (95% CI)	P-value	Prevalence difference (%) (95% CI)	P-value
Anaemia						
Model 1	2.96 (-4.74, 10.67)	0.45	-2.71 (-13.52, 0.08)	0.62	8.69 (-2.29, 19.67)	0.12
Model 2	4.06 (-3.12, 11.24)	0.27	-2.30 (-12.62, 8.03)	0.66	9.67 (-0.21, 19.55)	0.06
Iron deficiency (PF < 15µg/L)						
Model 1	1.71 (-3.20, 6.61)	0.50	0.74 (-5.06, 6.54)	0.80	2.69 (-5.25, 10.62)	0.51
Model 2	1.28 (-2.73, 5.29)	0.53	2.39 (-2.40, 7.18)	0.33	1.09 (-4.97, 7.15)	0.72
Tissue iron deficiency (TfR >8.3)						
Model 1	2.76 (-5.09, 10.61)	0.49	5.16 (-5.91, 16.23)	0.36	0.34 (-10.80, 11.48)	0.95
Model 2	2.26 (-4.94, 9.46)	0.54	3.52 (-6.71, 13.75)	0.50	1.70 (-8.38, 11.77)	0.74
Iron deficiency (PF <15µg/L or TfR >8.3)						
Model 1	1.49 (-6.33, 9.32)	0.71	2.62 (-8.44, 13.67)	0.64	0.36 (-10.72, 11.44)	0.95
Model 2	1.13 (-5.93, 8.19)	0.75	1.06 (-9.05, 11.16)	0.84	1.84 (-7.94, 11.59)	0.71
IDA (anaemia and PF <15µg/L or TfR >8.3)						
Model 1	4.08 (-2.51, 10.68)	0.22	0.92 (-8.29, 10.14)	0.84	7.27 (-2.17, 16.71)	0.13
Model 2	4.49 (-1.30, 10.28)	0.13	1.75 (-6.79, 10.30)	0.69	7.44 (-0.27, 15.15)	0.06
VAD (RBP < 0.7µmol/L)						
Model 1	2.31 (-1.08, 5.70)	0.19	5.86 (0.09, 11.64)	0.05	-1.28 (-4.81, 2.26)	0.48
Model 2	1.96 (-1.34, 5.25)	0.24	6.15 (0.72, 11.59)	0.03	-1.31 (-4.89, 2.28)	0.47
P interaction (biscuits*menarche)		0.04				
Low or VAD (RBP < 1.05 µmol/L)						
Model 1	-1.58 (-9.10, 5.97)	0.68	4.37 (-6.53, 15.28)	0.43	-7.56 (-17.95, 2.84)	0.15
Model 2	-2.35 (-9.33, 4.63)	0.51	5.20 (-5.14, 15.55)	0.32	-9.63 (-18.94, -0.32)	0.04
P interaction (biscuits*menarche)		0.03				

[1]All results are in percentages, reflecting percentage point difference between fortified compared to unfortified biscuits group. Model 1 was a fixed-effects model and included the biscuits group and the study design effect (menarche status at enrolment). Model 2 adjusted for baseline micronutrient biomarkers (haemoglobin, PF, TfR and RBP) and the girl's baseline age, and HAZ

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Intervention effect on cognition, academic performance, and anthropometric indices: No effect of the intervention on HAZ and BAZ was observed (Table 5). Likewise, we found no effect of the intervention on the fluid cognition of the girls. We found a significant interaction effect between treatment and menarche for working memory and episodic memory test. Compared to the UB group, the working memory of pre-menarche subjects in the MMB group increased marginally post-intervention while that of post-menarche girls did not increase (*P*-interaction=0.048). Whereas post-menarche girls in the MMB group compared to the UB group had a marginal increase in their post-intervention episodic memory, that of the pre-menarche girls in the MMB compared to the UB decreased (*P*-interaction=0.014). Adjustments did not substantially affect effect estimates or conclusions. The effect of the MMB on cognition and anthropometric indices remained similar with per-protocol analysis (Table S7). In an additional subgroup analysis of anaemic girls, post-intervention mathematics score was higher in the MMB compared to the UB group (Table S8). Lastly, the post-intervention working memory of pre-menarche subjects who were anaemic at baseline increased distinctly but that of the post-menarche girls decreased (*P*-interaction=0.012).

Table 5. The effect of consuming micronutrient-fortified biscuits compared to unfortified biscuits on selected outcomes after 26 weeks of intervention in adolescent girls in Ghana

Secondary outcomes	Model 1: (Crude model)		Model 2: Adjusted for Child-level factors
	Mean ± SE	β (95% CI)	β (95% CI)
Anthropometric indicators			
Height-for-age z-score			
Unfortified biscuits	-0.87 ± 0.02	Ref.	Ref.
Fortified biscuits	-0.87 ± 0.02	0.00 (-0.05, 0.04)	0.00 (-0.05, 0.04)
Body mass index-for-age z-score			
Unfortified biscuits	-0.58 ± 0.03	Ref.	Ref.
Fortified biscuits	-0.53 ± 0.03	0.04 (-0.01, 0.10)	0.04 (-0.01, 0.10)
Cognition (*t*-score)			
Composite fluid cognition			
Unfortified biscuits	28.86 ± 0.72	Ref.	Ref.
Fortified biscuits	29.35 ± 0.72	0.50 (-0.60, 1.60)	0.44 (-0.66, 1.54)
Executive function			
Unfortified biscuits	34.69 ± 0.59	Ref.	Ref.
Fortified biscuits	35.00 ± 0.60	0.30 (-0.68, 1.29)	0.25 (-0.74, 1.23)
Attention			
Unfortified biscuits	32.39±0.36	Ref.	Ref.
Fortified biscuits	31.79±0.36	-0.60 (-1.44, 0.24)	-0.71 (-1.53, 0.12)
Working memory			
Unfortified biscuits	37.69 ± 1.38	Ref.	Ref.
Fortified biscuits	38.24 ± 1.36	0.55 (-1.81, 2.91)	0.57 (-1.79, 2.92)
Menarche*biscuits*[*]			
Pre-menarche (Ref= unfortified)	38.78 ±1.36	2.86 (-0.43, 6.15)	2.94 (-0.35, 6.23)
Post-menarche (Ref=unfortified)	37.15 ±1.41	-1.76 (-5.13, 1.61)	-1.81 (-5.17, 1.56)
***P*-interaction**		0.051	0.048
Processing speed			
Unfortified biscuits	36.19±0.74	Ref.	Ref.
Fortified biscuits	35.88 ±0.74	-0.24 (-1.68, 1.20)	-0.33 (-1.76, 1.11)
Episodic memory			
Unfortified biscuits	45.04 ± 0.46	Ref.	Ref.
Fortified biscuits	44.98 ± 0.46	-0.06 (-0.91, 0.80)	-0.14 (-0.99, 0.72)

table continues

Secondary outcomes	Model 1: (Crude model)		Model 2: Adjusted for Child-level factors
	Mean ± SE	β (95% CI)	β (95% CI)
Menarche*biscuits [*]			
Pre-menarche (Ref=unfortified)	45.62 ±0.46[a]	-1.16 (-2.37, 0.05)	-1.28 (-2.49, -0.07)
Post-menarche (Ref=unfortified)	44.40 ±0.48	1.04 (-0.17, 2.26)	1.01 (-0.20, 2.22)
***P*-interaction**		0.020	0.014
Academic performance (%)			
Overall performance			
Unfortified biscuits	57.48 ± 1.48	Ref.	Ref.
Fortified biscuits	57.91 ± 1.48	0.43 (-1.07, 1.92)	0.30 (-1.14, 1.74)
	Mean ± SE	**β (95% CI)**	**β (95% CI)**
English language			
Unfortified biscuits	55.95 ± 1.41	Ref.	Ref.
Fortified biscuits	55.52 ± 1.41	-0.43 (-2.18, 1.32)	-0.57 (-2.25, 1.12)
Mathematics			
Unfortified biscuits	57.89 ± 2.04	Ref.	Ref.
Fortified biscuits	58.99 ± 2.04	1.10 (-0.66, 2.87)	0.95 (-0.76, 2.67)
General science			
Unfortified biscuits	58.93 ± 1.45	Ref.	Ref.
Fortified biscuits	59.24 ± 1.45	0.31 (-1.61, 2.23)	0.19 (-1.69, 2.07)

[a]Group mean is significantly higher than the post-menarche group; Model 1 was a fixed-effects model and included the biscuits group and the study design effect (menarche status at enrolment). Model 2 adjusted for baseline micronutrient biomarkers (haemoglobin, PF, TfR and RBP) and the girl's baseline age, and HAZ.

Discussion

In the present study, we hypothesized that MMB compared to UB consumption 5 days weekly for 26 weeks improves adolescent girls' micronutrient status in the short-term and cognition and height in the medium to long-term. We also explored to what extent the intervention effect varied before or after menarche. Overall, our findings do not support our hypothesis; comparing MMB with UB post-intervention, there was only a modest increase in PF and RBP, but not in IDA. However, we observed a significant reduction in deficient/low vitamin A status for post-menarche girls by ~ 10% points.

Our findings are comparable two recent systematic reviews and meta-analyses by Salam *et al* [59] and Eichler *et al.* [60]. Salam *et al* [59] concluded they were uncertain of the effect of multiple-micronutrient fortification on haemoglobin (Mean difference: −0.10 g/dL, 95% CI: −0.88, 0.60). Similarly, Eichler and colleagues [60] also concluded that the consumption of fortified dairy and cereal foods only results in a minor increase in haemoglobin with no differences in anaemia risk for 5-15-year-old children. However, our findings were contrary to other studies that found improvements in post-intervention haemoglobin [29, 61], serum ferritin [20, 29, 61, 62] and retinol concentrations [29, 62]. These differences may be due to differences in food vehicles used, being a fortified beverage [29], a fortified breakfast cereal alongside milk [20]; study length and strength of study design, whether including a control group [61, 62]. The length of our study is relatively short but several other studies [20, 29, 61] reported significant effects of MMFs on the micronutrient status of children and adolescents when consumed for an average of 5 days/week for 6 months.

Although the fortification level per 100g in our study was comparable to several other studies [20, 29, 62], the amount of iron consumed per serving (4.1mg from 51.3 ± 3.2g of biscuits) in our study was lower compared to Powers *et al.* [20] in the UK (6.5mg iron from 50g cereal +150mL milk), Hyder *et al.* [29] in Bangladesh (7mg iron from 200mL beverage) and Adams *et al.* [62] in Bangladesh (7.0 - 9.5mg iron from 75g biscuits). However, the present study assessed the effect of a MMB existing on the market, designed to meet only 15% and 30% of the recommended dietary allowance of iron and vitamin A respectively for women aged 19-35 years, explaining the lower iron level per serving. The bioavailability of fortified iron is lower for children consuming mainly cereal-legume based diets as for our population [63]; hence, although the girls mostly consumed the biscuits about 2 hours before lunch, we cannot rule out the effect of dietary inhibitors of iron absorption, such as phytic acid and/or polyphenols.

We expected a reduction in the TfR of girls in MMB compared to UB; nonetheless, the post-intervention TfR of MMB girls compared to the UB did not reduce, which, according to Zimmermann *et al.* [64], indicates poor iron erythropoiesis. The decreased erythropoiesis suggests continuing ID, which partly explains the non-improvement in haemoglobin for the MMB group in our study. Overall, elevated TfR indicates tissue ID, which may be resulting from the compensation of reduced circulating iron (PF). There is a homeostatic mechanism of up-regulated iron absorption when there is a deficiency [15]; we, therefore, anticipated a larger intervention effect of the MMB in the subgroup of girls with anaemia or ID at baseline. Nevertheless, the post-intervention PF for anaemic subjects in the MMB remained marginal albeit a larger effect size, which yet again suggests poor iron erythropoiesis even in a deficient state. The persistence of anaemia for subjects who had IDA may be due to continuing ID [15]; but the lack of effect of the MMB on anaemia also suggests other nutrients or non-nutritive factors may be involved. Micronutrient deficiencies such as folate, vitamin B_{12} and zinc deficiencies could be additional causes of anaemia [65–67] but the MMB included these nutrients. Non-nutritive factors including infections and diseases (e.g., helminths and malaria) [68], genetic factors (e.g., haemoglobinopathies) including sickle cell traits [69] and socio-economic factors which influence dietary adequacy [70] may be accountable. The decline in BIS for MMB compared to UB in this study is unclear but may relate to the non-nutritive factors mentioned above. Unlike iron, the lack of effect for RBP in the present study is not explained by differences in dosage; studies with significant effects on vitamin A status gave daily doses between 700 and 1300 IU retinol [29, 62], which is like our study (916 IU). The non-nutritive factors cited for iron may also account for the lack of effect of the MMB on RBP. Our findings suggest menarche status probably affects plasma vitamin A status; this was evident in our statistical models for the post-intervention prevalence difference in VAD and deficient/low vitamin A status. Further, stratified analysis by baseline deficient/low vitamin A status (RBP <1.05µmol/L) showed that vitamin A status and menarche status modified the effect of the intervention with post-menarche girls in MMB compared to UB having a substantial increase in post-intervention RBP [12.64%, 95% CI 0.29%, 24.99%]. The demand and use of vitamin A increase during the pre-ovulatory phase to produce reproductive cells and endometrium, and in the post-ovulatory period for the maintenance of the endometrial layer [71]. The increased demand plausibly induces a compensatory absorption of vitamin A for post-menarche girls. However, iron losses, associated with blood loss and the pro-inflammatory [8, 16] nature of menstruation probably has more negative effects on iron status and absorption than any possible benefit from a compensatory absorption during menstrual loss. This possibly explains the modest increase in TfR and substantial reduction in haemoglobin for post-menarche girls in our study.

In contrast, the increase in VAD following MMB consumption in pre-menarche girls suggests vitamin A deficiency may not be the main cause of VAD for pre-menarche girls in this setting. Resistance to infections improves with age among children and adolescents [72], hence, pre-menarche girls were probably more susceptible to the influence of infections on their vitamin A status. Although inflammation did not differ by menarche status, pre-menarche girls were more likely than post-menarche girls to have *Plasmodium* infection at mid-point (22.1% vs. 13.9%, P=0.008).

In our study population, about a third of subjects had either a deficient or marginal vitamin A status at baseline. Interventions that control VAD have been found to improve iron status and control anaemia induced by either ID or infection [64, 73]. However, the improvement in vitamin A status yet decline in haemoglobin for post-menarche girls in our study suggests that improvement in vitamin A may not influence the haemoglobin status of post-menarche girls in some settings. In Western Kenya, Leenstra *et al.* [74], showed that weekly supplementation with vitamin A did not affect ferritin [-1.7µg/L, 95% CI -5.4, 2.7], and haemoglobin [-0.07g/dL, 95% CI -0.38, 0.25] and the effect of weekly iron supplementation was independent of vitamin A among adolescent schoolgirls. Further, the reduction in haemoglobin implies that bone marrow erythropoiesis could not match the pace of blood volume expansion, besides the loss for post-menarche girls. This also possibly explains the modest increase in TfR for post-menarche girls in our study. Overall, this finding implies that menarche status could impact estimates of micronutrient status as well as findings of associations between micronutrient status and health outcomes in girls. Additional research specific to menarche status and micronutrient absorption is needed to inform public health recommendations and improve research methods.

Furthermore, malaria infection alters iron indicators' concentrations (notably TfR) independent of iron status [75, 76]. Wessells and colleagues [75] showed that asymptomatic malaria reduces haemoglobin and RBP while increasing PF and TfR. The increased post-intervention TfR may be a response to haemolysis from malaria [54]. The baseline prevalence of malaria for our sample was about 40%, and the end-line assessment was also during the peak of the rainy season, accompanied by a minor increase in malaria prevalence (~53.5%). About a fifth of the subjects reported an adverse event during the intervention with more than half being a fever/malaria. It is, however, worth mentioning that subjects with malaria at any time point or during the intervention were promptly treated as recommended [37]. Importantly, our mid-point validation of the malaria rapid test kit in a sub-sample (n=68) of the girls showed poor sensitivity (sensitivity=13.3%; specificity=84.9%; *results not shown*), although its reproducibility was 100%. Accordingly, although the internal regression correction approach in our analysis includes malaria as a covariate for the micronutrient biomarkers, the adjustment for malaria was possibly not complete, and we cannot rule out a residual confounding effect from asymptomatic malaria. Even so, the chance of false-negative results was equal in both biscuit groups due to the study's randomized design. Whereas this finding suggests that efficacy trials in malaria-endemic contexts should consider malaria microscopic assessment of malaria status, logistic constraints imply the malaria rapid test kit remains a recommended tool.

Despite the adjustment, inflammation probably influenced our results. A similar efficacy trial with a higher dose (20mg) of iron-fortified biscuits (4 days/week for 6 months) found no effect on hematologic indicators among 6-14 years old Ivorian school children (44% girls), which they partly attributed to a high rate of infections [77]. The baseline prevalence of inflammation in our study was higher than that in the Ivorian study (43% vs 29%) and compared to Hyder *et al.* [29]. Despite deworming all subjects at the baseline, the post-intervention inflammation prevalence remained high for our sample. Our results showed that inflammation was mainly the late convalescence phase, signifying chronic infection and inflammation. The prevalence of inflammation in the present study emphasizes the need to strengthen school children's mass deworming against schistosomiasis and soil-transmitted helminths.

Overall, MMB consumption in our study resulted in only a marginal increase in the anthropometric indices, cognition, and academic performance, collaborating the findings of Eichler and colleagues [60], who concluded in their systematic review and meta-analysis that fortified foods consumption only results in minor improvements in cognition and anthropometric indices of children and adolescents. The modest increase in ferritin for the MMB group may explain these findings. Iron is required for providing oxygen to the brain via erythropoiesis and for myelination of the frontal lobes, which notably takes place during adolescence [22, 23]. Iron is also a cofactor for enzymes involved in neurotransmitter synthesis and plays a role in neurotransmitter metabolism [22]. Iodine also plays an essential role in neurodevelopment, such as neuronal cell maturation and myelination; iodine deficiency can impair adolescents' cognitive function [24]. Although the MMB in our study included iodine, we could not ascertain iodine's role in our sample's cognitive performance, since we did not examine iodine status. The duration of the intervention may also partly explain the lack of effect on cognition. In a randomized trial in Morocco with a higher dose of iron (8mg iron comparing $FeSO_4$, NaFeEDTA or EDTA) using fortified biscuits 6 days weekly for 28 weeks, Bouhouch *et al.* [78] found no effect on cognition of schoolchildren, although the iron status of the children improved in the trial.

The effect of micronutrient interventions on cognition may be more beneficial to micronutrient deficient children. A meta-analysis in children aged 8 years and above [79] showed that iron supplementation positively improved the cognitive function of children, but the effect was stronger for those who were iron-deficient and/or anaemic at baseline. This has been attributed to the homeostatic mechanism of up-regulated iron and iodine absorption during a deficiency [15, 79]. Though modest, the effect of the MMB on hematologic indicators was stronger for anaemic girls, which may explain the substantial increase in the post-intervention mathematics score of anaemic subjects in the MMB and as well the working memory of anaemic pre-menarche girls in the MMB compared to the UB.

To our knowledge, this is the first randomized trial with MMF in a sub-Saharan Africa population of adolescent girls where anaemia and IDA were prevalent. In summary, in this group of rural Ghanaian adolescent girls, MMB consumption did not improve haemoglobin and iron status but reduced the prevalence of deficient/marginal vitamin A status among post-menarche girls. Only in anaemic subjects did we find evidence that MMB consumption improves mathematics score and working memory of anaemic pre-menarche girls. Despite the modest effects observed in our study, food fortification programmes remain relevant, considering the high burden of anaemia, ID, and IDA in the present study. Even though micronutrient supplements may be a better approach, poor compliance still limits its effectiveness [80, 81]. A longer-term consumption of fortified foods alongside regular treatment of infections may prove crucial in improving micronutrient status. An upward review of the iron levels in the obaasima products would be desirable to increase the amount of iron intake per serving. Our findings emphasize the need for malaria and helminths infection programmes besides micronutrient interventions to meaningfully improve adolescent girls' nutrition and health in malaria-endemic and low socio-economic settings. The effect of menarche status on micronutrient status warrants further research.

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Appendix 1

Table S1. The Inclusion and Exclusion Criteria for Ten2Twenty-Ghana Study

No	Inclusion criteria for the survey	Exclusion criteria for RCT
1	Aged 10 to 17 years (verified by birth certificate, health- record, insurance card, school register, or another formal document)	Severely anaemic (Hb < 80 g/L (50))[1]
2	Apparently healthy without any visible sign(s) of poor health	History of medical/surgical events that may significantly affect RCT outcomes
3	Non-pregnant	Sign(s) of chronic infection or metabolic disorder
4	Non-lactating	Clinical sign(s) of vitamin A deficiency and/or iodine deficiency
5	No incompatible mental status	Severely underweight (BAZ[2] < – 3SD)
6	Willing to participate	Taking medical drugs or nutrient supplements at the time of enrolment
7	Informed consent of parent or guardian obtained for the survey	Participating in another food-, supplement-, and/or drug study
8		Not willing to consume biscuits from Monday to Friday for 26 weeks
9		Any known food allergy to biscuits
8		Afraid or not willing to donate approximately 12 mL of blood on 2 different occasions
9		Refusal of parents or guardian
10		[3]Second informed consent from parent or guardian obtained for RCT

[1]Hb: Haemoglobin; those who were severely anaemic were referred to a hospital; [2]BAZ: Body Mass Index- for-age z-scores; [3]Ethical approval requirements demanded that we obtain 2 different informed consents for the extensive survey and the RCT

Table S2. Nutrient Content of biscuits for Ten2Twenty-Ghana RCT

No.	Nutrient	Product name	Nutrient content of fortified biscuits (mg) per serving (51.3g)	[1]Nutrient content (mg) of unfortified biscuits per serving (51.3g)
1	Vitamin A	Dry vitamin A palmitate	0.504	0.10
2	Vitamin D	Dry vitamin D3	0.005	0.00
3	Vitamin E	Dry vitamin E	6.00	0.00
4	Vitamin K	Dry vitamin K1	0.05	0.00
5	Thiamine	Thiamine mononitrate	1.20	0.43
6	Riboflavin	Riboflavin	1.20	0.23
7	Niacin	Niacinamide	14.00	3.03
8	Vitamin B6	Pyridoxine Hydrochloride	1.60	0.00
9	Folic acid	Folic acid	0.311	0.11
10	Vitamin B12	Vitamin B12	0.002	0.001
11	Ascorbic acid	Ascorbic acid	70.00	0.00
12	Calcium	Calcium carbonate	150	0.00
13	Cupper	Cupper Gluconate	0.20	0.00
14	Iodine	Potassium Iodide	0.04	0.00
15	Iron	Ferrous Fumarate	4.05	1.03
16	Magnesium	Magnesium oxide	52.50	0.00
17	Selenium	Sodium Selenite	0.012	0.00
18	Zinc	Zinc Oxide	2.38	1.45

[1]Obtained from the lab division of Mass Industries, Tema-Ghana; the nutrient content reflects the fortification level of wheat flour in Ghana as by law.

Additional information on international regression correction for micronutrient biomarkers

The linear regression equation used to adjust the plasma indicators is shown below.

$LnMB_{adj}$=$LnMB_{unadj}$ - β_1(Ln CRP_{obs}-$LnCRP_{ref}$) - β2($LnAGP_{obs}$-$LnAGP_{ref}$) - β_3***Plasmodium* infection. Where MB, represents the micronutrient biomarker, the subscripts adj and undam represent "*adjusted*" and "*unadjusted*" while the subscripts obs and ref represent "*observed*" and "*reference*".

Table S3. The effect of MMB versus UB on micronutrient status after 26 weeks of intervention in adolescent girls in Ghana: A per-protocol analysis

Outcome	Overall sample (n= 559)		Pre-menarche (n= 282)		Post-menarche (n= 277)	
	Estimate (95% C.I)	P-value	Estimate (95% C.I)	P-value	Estimate (95% C.I)	P-value
Ferritin (µg/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	-0.68% (-8.50%, 7.14%)	0.87	-0.87% (-11.86%, 10.12%)	0.88	-0.48% (-11.60%, 10.63%)	0.93
Soluble transferrin (mg/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	2.30% (-4.48%, 9.07%)	0.51	0.17% (-9.35%, 9.69%)	0.97	4.42% (-5.21%, 14.06%)	0.37
Retinol binding protein (µmol/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	-0.37% (-5.46%, 4.71%)	0.89	-4.53% (-11.68%, 2.61%)	0.21	3.79% (-3.43%, 11.02%)	0.30
Body iron store z-score						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	-0.16 (-0.28, -0.03)	0.015	-0.18 (-0.36, -0.003)	0.047	-0.13 (-0.31, 0.05)	0.15
Haemoglobin status (g/L)						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	-0.82 (-2.71, 1.08)	0.40	0.35 (-2.31, 3.01)	0.80	-1.99 (-4.68, 0.71)	0.15

[1]Outcomes variables were log-transformed (Ln) and estimates were expressed as a percentage increase or decrease.

Table S4. The effect of MMB versus UB on micronutrient status after 26 weeks of intervention in anaemic adolescent girls in Ghana

Outcome	Overall sample (n= 253)		Pre-menarche (n= 132)		Post-menarche (n= 121)	
	Estimate (95% C.I)	P-value	Estimate (95% C.I)	P-value	Estimate (95% C.I)	P-value
Ferritin (µg/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	5.39% (-6.82%, 17.59%)	0.39	9.46% (-8.25%, 2.72%)	0.29	1.31% (-15.48%, 18.11%)	0.88
Soluble transferrin (mg/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	4.93% (-5.95%, 15.81%)	0.37	-0.30% (-16.04%, 15.45%)	0.97	10.16% (-4.82%, 25.13%)	0.18
Retinol binding protein (µmol/L)[1]						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	1.36% (-5.82%, 8.54%)	0.71	-2.17% (-12.56%, 8.22%)	0.68	4.88% (-5.02%, 14.78%)	0.33
Body iron store z-score						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	-0.32 (-0.54, -0.09)	0.01	-0.35 (-0.68, -0.020)	0.04	-0.28 (-0.59, 0.03)	0.08
Haemoglobin status (g/dL)						
Unfortified biscuits	Ref.		Ref.		Ref.	
Fortified biscuits	-2.39 (-5.33, 0.55)	0.11	-0.20 (-4.47,4.07)	0.93	-04.58 (-8.63, -0.52)	0.03

[1]Outcomes variables were log-transformed (Ln) and estimates were expressed as a percentage increase or decrease.

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Table S5. The effect of consuming micronutrient-fortified biscuits compared to unfortified biscuits on post-intervention prevalence difference in micronutrient deficiencies after 26 weeks of intervention in adolescent girls in Ghana: A per-protocol analysis

Outcome	Overall sample (n= 559)		Pre-menarche (n= 282)		Post-menarche (n= 277)	
	Prevalence difference[1] (95% CI)	P-value	Prevalence difference[1] (95% CI)	P-value	Prevalence difference[1] (95% CI)	P-value
Anaemia						
Model 1	2.11 (-6.01, 10.22)	0.61	-2.20 (-13.49, 9.10)	0.70	6.52 (-5.11, 18.16)	0.27
Model 2	3.21 (-4.39, 10.80)	0.41	-2.58 (-13.33, 8.16)	0.64	7.87 (-2.72, 18.46)	0.15
Iron deficiency (PF < 15µg/L)						
Model 1	2.27 (-2.89, 7.44)	0.39	1.66 (-4.32, 7.64)	0.59	2.90 (-5.57, 11.37)	0.50
Model 2	1.05 (-3.06, 5.16)	0.62	2.50 (-2.41, 7.41)	0.32	0.02 (-6.34, 6.37)	0.99
Iron deficiency (TfR >8.3)						
Model 1	2.36 (-5.92, 10.65)	0.58	5.37 (-6.26, 17.00)	0.37	-0.72 (-12.52, 11.07)	0.90
Model 2	2.15 (-5.43, 9.74)	0.58	3.40 (-7.42, 14.22)	0.54	1.48 (-9.19, 12.14)	0.79
Iron deficiency (PF <15µg/L or TfR >8.3)						
Model 1	1.32 (-6.95, 9.58)	0.75	3.31 (-8.30, 14.93)	0.58	-0.72 (-12.48, 11.04)	0.90
Model 2	1.14 (-6.33, 8.61)	0.77	1.42 (-9.30, 12.13)	0.80	1.71 (-8.65, 12.06)	0.75
IDA (anaemia with PF <15µg/L or TfR >8.3)						
Model 1	3.96 (-2.92, 10.85)	0.26	2.88 (-6.70, 12.47)	0.56	5.07 (-4.81, 14.96)	0.31
Model 2	4.18 (-1.84, 10.19)	0.17	2.78 (-6.05, 11.62)	0.54	5.25 (-2.83, 13.34)	0.20
VAD (RBP < 0.7µmol/L)						
Model 1	1.93 (-1.73, 5.58)	0.30	5.93 (-0.31, 12.17)	0.06	-2.17 (-5.87, 1.53)	0.25
Model 2	1.92 (-1.62, 5.46)	0.29	6.61 (0.69, 12.52)	0.03	-2.09 (-5.79, 1.60)	0.27
P-interaction (biscuits*menarche)		0.03				
Low or VAD (RBP < 1.05 µmol/L)						
Model 1	-0.33 (-8.32, 7.66)	0.94	5.72 (-5.76, 17.19)	0.33	-6.52 (-17.59, 4.54)	0.25
Model 2	-0.54 (-7.90, 6.83)	0.89	6.63 (-4.20, 17.46)	0.23	-7.79 (-17.68, 2.10)	0.12
P-interaction (biscuits*menarche)		0.05				

[1]All results are in percentages, reflecting percentage point difference between fortified compared to unfortified biscuits group. Model 1 was a fixed-effects model and included the biscuits group and the study design effect (menarche status at enrolment). Model 2 adjusted for baseline micronutrient biomarkers (haemoglobin, PF, TfR and RBP) and the girl's baseline age, and HAZ

Table S6. The effect of consuming micronutrient-fortified biscuits compared to unfortified biscuits on post-intervention prevalence difference in micronutrient deficiencies after 26 weeks of intervention in adolescent girls in Ghana: A sub-group analysis among anaemic girls at baseline, following intention to treat

Outcome	Overall sample (n= 253)		Pre-menarche (n= 132)		Post-menarche (n= 121)	
	Prevalence difference[1] (95% CI	P-value	Prevalence difference[1] (95% CI)	P-value	Prevalence difference[1] (95% CI)	P-value
Anaemia						
Model 1	6.36 (-5.86, 18.59)	0.31	0.02 (-17.64, 18.08)	0.98	11.90 (-4.86, 28.67)	0.16
Model 2	8.46 (-3.12, 20.03)	0.15	1.78 (-15.71, 19.27)	0.84	14.85 (0.19, 29.50)	0.047
Iron deficiency (PF < 15µg/L)						
Model 1	0.61 (-8.28, 9.49)	0.89	-0.83 (-12.66, 10.99)	0.89	1.90 (-11.21, 15.02)	0.78
Model 2	11.70 (-5.90, 8.25)	0.74	3.05 (-7.04, 13.13)	0.55	0.34 (-9.19, 9.87)	0.94
Iron deficiency (TfR >8.3)						
Model 1	3.30 (-8.93, 15.53)	0.60	3.78 (-13.94, 21.50)	0.68	2.86 (-14.04, 19.75)	0.74
Model 2	6.77 (-4.25, 17.80)	0.23	6.82 (-9.01, 22.66)	0.40	5.28 (-9.64, 20.20)	0.49
Iron deficiency (PF <15µg/L or TfR >8.3)						
Model 1	-0.33 (-12.43, 11.77)	0.96	-1.06 (-18.66, 16.54)	0.91	0.32 (-16.35, 16.98)	0.97
Model 2	3.21 (-7.63, 14.04)	0.56	1.98 (-13.65, 17.61)	0.80	2.63 (-11.63, 16.89)	0.72
IDA (anaemia with PF <15µg/L or TfR >8.3)						
Model 1	7.81 (-3.92, 19.54)	0.19	2.22 (-14.66, 19.11)	0.80	12.86 (-3.41, 29.12)	0.12
Model 2	10.38 (0.01, 20.74)	0.050	5.35 (-10.26, 20.96)	0.50	13.46 (0.07, 26.85)	0.049
VAD (RBP < 0.7µmol/L)						
Model 1	3.50 (-1.67, 8.67)	0.18	5.62 (-4.76, 15.99)	0.29		
Model 2	3.76 (-0.53, 8.06)	0.086	6.99 (-1.61, 15.60)	0.11		
Low or VAD (RBP < 1.05 µmol/L)	-3.03 (-14.99, 8.93)	0.62				
Model 1	-3.15 (-14.99, 8.70)	0.60	1.28 (-16.50, 19.60)	0.89	-7.14 (-22.97, 8.68)	0.38
Model 2	-3.94 (-14.78, 6.90)	0.48	4.00 (-12.41, 20.41)	0.63	-9.20 (-23.71, 5.31)	0.21

[1]All results are in percentages, reflecting percentage point difference between fortified compared to unfortified biscuits group. Model 1 was a fixed-effects model and included the biscuits group and the study design effect (menarche status at enrolment). Model 2 adjusted for baseline micronutrient biomarkers (haemoglobin, PF, TfR and RBP) and the girl's baseline age, and HAZ.

Table S7. The effect of consuming micronutrient-fortified biscuits compared to unfortified biscuits on selected outcomes after 26 weeks of intervention in adolescent girls in Ghana: A per-protocol analysis

Secondary outcomes	Adjusted for Design Effect		Adjusted for Child-level factors
	Mean ± S. E	β (95% CI)	β (95% CI)
Anthropometric indicators			
Height-for-age z-score			
Unfortified biscuits	-0.88 ± 0.03	Ref.	Ref.
Fortified biscuits	-0.88 ± 0.03	0.00 (-0.05, 0.05)	0.00 (-0.05, 0.05)
Body mass index-for-age z-score			
Unfortified biscuits	-0.60 ± 0.03	Ref.	Ref.
Fortified biscuits	-0.57 ± 0.03	0.04 (-0.02, 0.10)	0.04 (-0.02, 0.10)
Cognition			
Composite fluid Cognition			
Unfortified biscuits	29.12 ±0.72	Ref.	Ref.
Fortified biscuits	29.61 ± 0.72	0.49 (-0.67, 1.65)	0.41 (-0.75, 1.56)
Executive function			
Unfortified biscuits	34.85 ± 0.59	Ref.	Ref.
Fortified biscuits	35.20 ± 0.60	0.35 (-0.69, 1.39)	0.30 (-0.74, 1.33)
Attention			
Unfortified biscuits	32.59 ± 0.35	Ref.	Ref.
Fortified biscuits	31.94 ± 0.35	-0.65 (-1.52, 0.21)	-0.73 (-1.59, 0.12)
Working memory			
Unfortified biscuits	37.67 ± 1.40	Ref.	Ref.
Fortified biscuits	38.72 ± 1.39	1.05 (-1.39, 3.49)	1.01 (-1.43, 3.44)
Menarche*biscuits			
Pre-menarche (Ref= unfortified)	39.04 ± 1.38	3.56 (0.17, 6.96)	3.59 (0.20, 6.98)
Post-menarche fortified (Ref= unfortified)	37.35 ± 1.43	-1.47 (-4.96, 2.01)	-1.58 (-5.07, 1.90)
P-interaction		0.053	0.051
Processing speed			
Unfortified biscuits	36.40 ± 0.72	Ref.	Ref.
Fortified biscuits	36.30 ± 0.73	-0.10 (-1.59, 1.39)	-0.16 (-1.65, 1.32)
Episodic memory			
Unfortified biscuits	45.13 ± 0.48	Ref.	Ref.
Fortified biscuits	44.98 ± 0.48	-0.16 (-1.05, 0.73)	-0.22 (-1.11, 0.67)
Menarche*biscuits			
Pre-menarche (Ref= unfortified)	45.63 ± 0.47	-1.33 (-2.58, -0.07)	-1.42 (-2.67, -0.16)
Post-menarche fortified (Ref= unfortified)	44.48 ± 0.49	1.01 (-0.27, 2.28)	0.97 (-0.29, 2.24)
P-interaction		0.008	0.007
Academic performance			
Overall performance			
Unfortified biscuits	57.55 ± 1.47	Ref.	Ref.
Fortified biscuits	57.90 ± 1.47	0.35 (-1.26, 1.96)	0.27 (-1.29, 1.82)

table continues

Secondary outcomes	Adjusted for Design Effect		Adjusted for Child-level factors
	Mean ± S. E	β (95% CI)	β (95% CI)
English Language			
Unfortified biscuits	55.92 ± 1.44	Ref.	Ref.
Fortified biscuits	55.61 ± 1.44	-0.31 (-2.19, 1.57)	-0.37 (-2.17, 1.44)
Mathematics			
Unfortified biscuits	57.86 ± 2.04	Ref.	Ref.
Fortified biscuits	59.03 ± 2.04	1.17 (-0.71, 3.05)	1.14 (-0.69, 2.97)
General Science			
Unfortified biscuits	59.07 ± 1.44	Ref.	Ref.
Fortified biscuits	59.03 ± 1.44	-0.04 (-2.09, 2.01)	-0.16 (-2.17, 1.85)

Model 1 was a fixed-effects model and included the biscuits group and the study design effect (menarche status at enrolment). Model 2 adjusted for baseline micronutrient biomarkers (haemoglobin, PF, TfR and RBP) and the girl's baseline age, and HAZ

Table S8. The effect of consuming micronutrient-fortified biscuits compared to unfortified biscuits on selected outcomes after 26 weeks of intervention in adolescent girls in Ghana: A sub-group analysis for anaemic girls at baseline, following intention-to-treat analysis

Secondary outcomes	Adjusted for Design Effect		Adjusted for Child-level factors
	Mean ± SE	β (95% CI)	β (95% CI)
Anthropometric indicators			
Height-for-age z-score			
Unfortified biscuits	-0.99 ± 0.03	Ref.	Ref.
Fortified biscuits	-0.94 ± 0.03	0.04 (-0.03, 0.12)	0.04 (-0.03, 0.12)
Body mass index-for-age z-score			
Unfortified biscuits	-0.62 ± 0.04		Ref.
Fortified biscuits	-0.59 ± 0.04	0.03 (-0.06, 0.13)	0.03 (-0.06, 0.13)
Cognition			
Composite fluid Cognition			
Unfortified biscuits	29.17 ± 0.79	Ref.	Ref.
Fortified biscuits	29.84 ± 0.83	0.67 (-0.98, 2.32)	0.52 (-1.07, 2.11)
Executive function			
Unfortified biscuits	34.40 ± 0.65	Ref.	Ref.
Fortified biscuits	34.95 ± 0.69	0.55 (-0.99, 2.09)	0.46 (-1.05, 1.97)
Attention			
Unfortified biscuits	31.78 ± 0.46	Ref.	Ref.
Fortified biscuits	31.87 ± 0.49	0.09 (-1.15, 1.34)	0.05 (-1.17, 1.28)
Working memory			
Unfortified biscuits	38.44 ± 1.99	Ref.	Ref.
Fortified biscuits	39.44 ± 2.02	1.00 (-2.69, 4.69)	0.80 (-2.89, 4.47)
Menarche*biscuits			
Pre-menarche (Ref= unfortified)	40.75 ± 2.01	5.32 (0.09, 10.55)	5.06 (-0.15, 10.28)
Post-menarche fortified (Ref= unfortified)	37.13 ± 2.06	-3.33 (-8.58, 1.93)	-3.47 (-8.69, 1.76)
P-interaction		0.010	0.012
Processing speed			
Unfortified biscuits	36.21 ± 0.94	Ref.	Ref.
Fortified biscuits	35.90 ± 1.00	-0.32 (-2.51, 1.88)	-0.31 (-2.46, 1.84)

table continues

Secondary outcomes	Adjusted for Design Effect		Adjusted for Child-level factors
	Mean ± SE	β (95% CI)	β (95% CI)
Episodic memory			
Unfortified biscuits	45.43 ± 0.54	Ref.	Ref.
Fortified biscuits	44.62 ± 0.57	-0.82 (-2.14, 0.50)	-0.83 (-2.12, 0.47)
Academic performance			
Overall performance			
Unfortified biscuits	56.59 ± 1.58	Ref.	Ref.
Fortified biscuits	58.71 ± 1.63	2.12 (-0.35, 4.59)	2.08 (-0.26, 4.43)
English Language			
Unfortified biscuits	54.99 ± 1.52	Ref.	Ref.
Fortified biscuits	55.82 ± 1.58	0.83 (-1.98, 3.64)	0.92 (-1.77, 3.60)
Mathematics			
Unfortified biscuits	56.65 ± 2.23	Ref.	Ref.
Fortified biscuits	60.51 ± 2.28	3.86 (0.93, 6.79)	3.87 (1.07, 6.67)
General Science			
Unfortified biscuits	58.49 ± 1.63	Ref.	Ref.
Fortified biscuits	60.05 ± 1.69	1.56 (-1.55, 4.67)	1.36 (-1.63, 4.34)

Model 1 was a fixed-effects model and included the biscuits group and the study design effect (menarche status at enrolment). Model 2 adjusted for baseline micronutrient biomarkers (haemoglobin, PF, TfR and RBP) and the girl's baseline age, and HAZ.

Appendix 2

Study protocol for primary study "Ten2Twenty-Ghana"

Based on "Ten2Twenty-Ghana: Study Design and Methods for an Innovative Randomised Controlled Trial with Multiple-Micronutrient Fortified Biscuits Among Adolescent Girls in North-Eastern Ghana"

6

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Curr Dev Nutr. 2021; 5:1–20.
https://doi.org/10.1093/cdn/nzaa184

We aim to contribute to existing knowledge gaps by designing the innovative research titled "*Ten2Twenty-Ghana*" using a mixed-methods approach for data collection. The primary research question is:

1. What is the effect of consuming multiple-micronutrient fortified biscuits (MMB) compared to unfortified biscuits (UB) 5 days weekly for 26-weeks on biomarkers of micronutrient status of adolescent girls and how is the intervention effect related to its timing (before or after menarche)?

Secondary research questions include:

1. How do the intervention (RCT) and its timing relate to changes in psychosocial health, cognitive functioning, academic performance, and fertility of the adolescent girls?
2. How do the intervention and its timing relate to changes in the adolescent girls' vertical growth and body composition?

Overall, we hypothesised that a fortified food intervention programme using MMB would improve micronutrient status in the short to medium term with changes in the secondary outcomes in the medium to long-term (**Figure 1**).
The study also includes 3 additional research questions, including:

1. What is the dietary intake and its determinants of adolescent girls in different pubertal stages (before/after menarche)?
2. What affordable, evidence-based, population-specific food-based dietary guidelines (FBDGs) can fulfil or best meet adolescent girls' nutrient requirements in Ghana?
3. How do the mother's nutritional status and her participation in household decision-making influence the adolescent girl's nutrition and health?

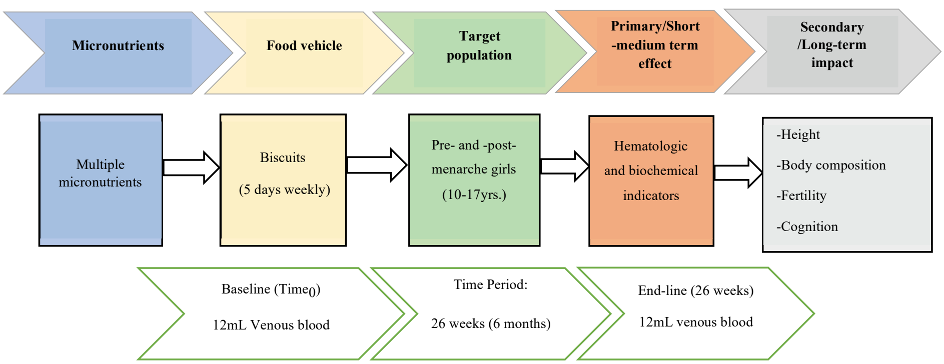

Figure 1. A logical framework for the effect of a multiple-micronutrient fortified biscuits intervention for adolescent girls

Methods

Study Area

We conducted the study in the Mion District, in North-Eastern Ghana. The district is located in the eastern corridor of the Northern Region of Ghana between Latitude 90 – 35" North, 00 – 30" West and 00 – 15" East. The district shares boundaries with the Tamale Metropolis, Savelugu Municipal and Nanton District to the west, Yendi Municipal to the east, Nanumba North and East Gonja districts to the south and Gushegu and Karaga districts to the north. The district capital is Sang; the largest community in the district. The district covers a surface area of 2714.1 sq. km and has a population density of 30.1 persons per square kilometre [1]. The area has a typically tropical climate with 2 main seasons: a dry season (November–March) characterised by high temperatures and a single rainy season (April–October). According to the 2010 Ghana Population and Housing Census [1], the Mion district had a population of 81,812 with the majority (91.1%) living in rural locations. In 2010, the average household size in the district was 9.3 persons per household. According to the Ghana Statistical Service (GSS), about 20% of the district's female population was aged 10-19 years [1]. The district's main ethnic groups are Dogombas and Konkombas, and about 61.8% of the district population professes Islam. Over 90% of the people depend on agriculture for their livelihood. In 2010, the district's literacy rate was 28.7%, for both sexes, implying a very high illiteracy rate in the district [1].

6

We purposively selected the Mion district as it is relatively new; carved out of Yendi Municipal Assembly in 2012. Hence, data on nutrition and health in the district is scanty. Moreover, the district is mainly rural (about 91%), and our secondary analysis [2] suggests a very high prevalence (64.6%) of anaemia among adolescent girls in the rural northern savannah agro-ecological zone. Lastly, the district capital is only about an hour drive from Tamale, the regional capital and location of the University for Development Studies (UDS), which coordinated the fieldwork. **Figure 2** is a map of the district with locations of the selected communities where the study was conducted.

Study Design

The study started with an extensive cross-sectional survey (*n*=1057), 2 months before a follow-up double-blind placebo randomised controlled trial (RCT). Herein, we refer to the cross-sectional survey as the "*survey*", and we describe the methods for the survey and RCT in this manuscript. For ethical reasons, a non-targeted approach that did not distinguish both anaemic and non-anaemic girls was used to include a random sub-sample (*n*=620) of girls from the RCT survey. The non-targeted approach was also justified by the high prevalence of anaemia (64.6%) among female adolescents in rural Northern Savannah agro-ecological zone of Ghana [2]. The non-targeted approach was previously used in similar efficacy trials and proved to be effective [3–5]. The girls were randomised into two parallel treatment arms receiving nutrition/health education (5 different occasions) with a 5-day weekly MMB or UB for 26 weeks. Similar studies [5–10] reported significant effects of

MMFs on children and adolescents' micronutrient status when consumed between 5-7 days for 3-12 months with an average duration of 6 months; this informed our decision to administer the treatment 5 days weekly for 6 months. **Figure 3** is a schematic overview of the RCT. To estimate mean nutrient intake and the proportion of the population at risk of nutrient inadequacy, a random sub-sample (n=310) of subjects from the RCT (including both pre-and post-menarche girls) was selected for a quantitative 24-hour dietary recall (24hR). Out of the first 24hRs, a random sample of 100 girls was selected for a second 24hR; allowing us to adjust for the random day-to-day variation in dietary intake. For triangulation purposes, we also conducted one focus group discussion in each of the RCT arms at the end-line. Likewise, in the extensive cross-sectional survey, we conducted qualitative in-depth interviews (n=30) and 2 focus group discussions.

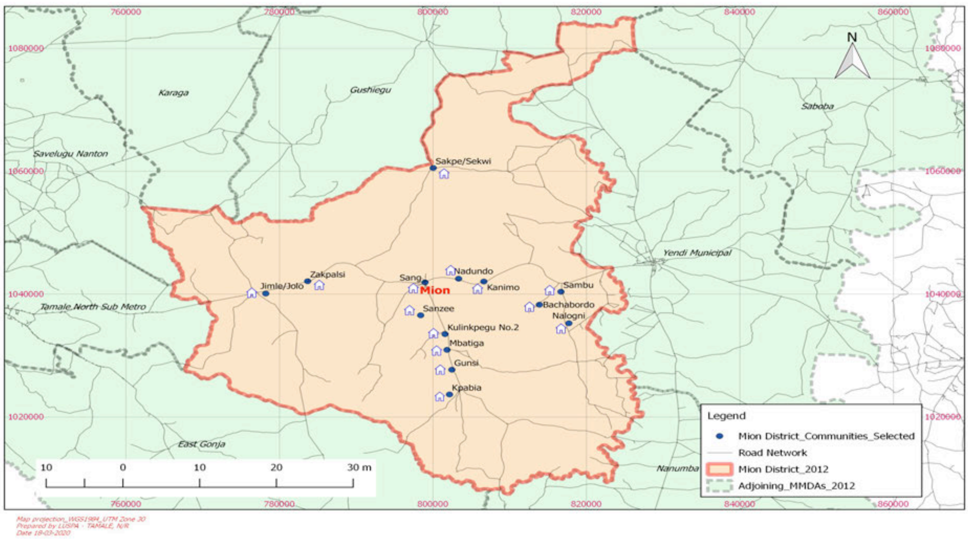

FIGURE 2. Map of Mion District, Ghana, with the communities included in the Ten2Twenty-Ghana study

Study Population

The target population for the study included pre-menarche and post-menarche adolescent girls. The girls were seemingly healthy, non-pregnant and non-lactating adolescents aged 10-17 years, residing in the Mion district in the Northern Region of Ghana. To be enrolled into the survey, girls had to meet all the inclusion criteria, and those who did not meet the exclusion criteria of the RCT were eligible to participate in the RCT (**Table 1**).

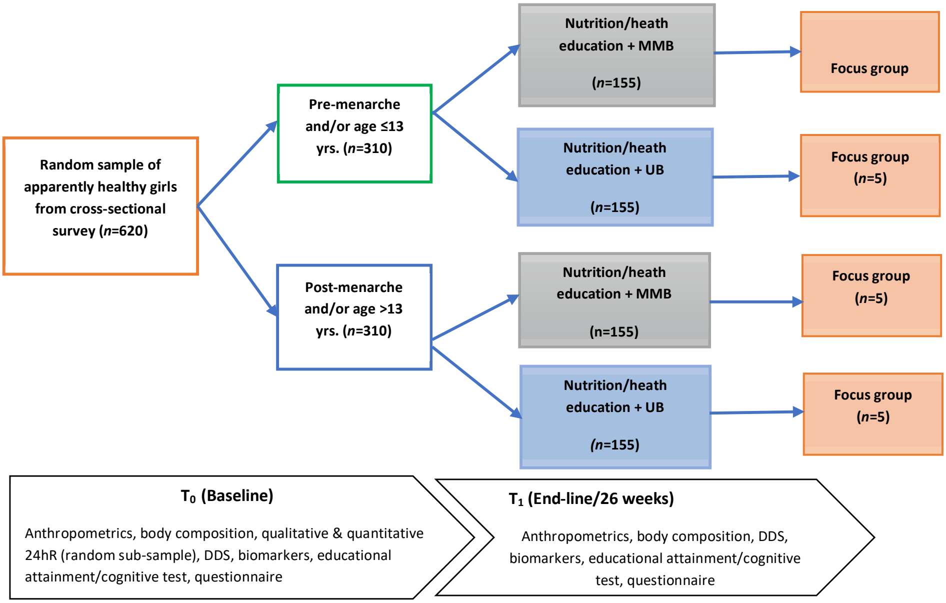

Figure 3. Design of a 26-week double-blind, randomised placebo-controlled trial (RCT) among adolescent girls in Ghana; MMB, multiple-micronutrient fortified biscuits; UB, unfortified biscuits

Table 1: The Inclusion and Exclusion Criteria for Ten2Twenty-Ghana Study

No	Inclusion criteria for the survey	Exclusion criteria for RCT
1	Aged 10 to 17 years (verified by birth certificate, health- record, insurance card, school register, or another formal document)	Severely anaemic (Hb < 80 g/L [11])[1]
2	Apparently healthy without any visible sign(s) of poor health	History of medical/surgical events that may significantly affect RCT outcomes
3	Non-pregnant	Sign(s) of chronic infection or metabolic disorder
4	Non-lactating	Clinical sign(s) of vitamin A deficiency and/or iodine deficiency
5	No incompatible mental status	Severely underweight (BAZ[2] < – 3SD)
6	Willing to participate	Taking medical drugs or nutrient supplements at the time of enrolment
7	Informed consent of parent or guardian obtained for survey	Participating in another food-, supplement-, and/or drug study
8		Not willing to consume biscuits from Monday to Friday for 26 weeks
9		Any known food allergy to biscuits
8		Afraid or not willing to donate approximately 12 mL of blood on 2 different occasions
9		Refusal of parents or guardian
10		[3]Second informed consent from parent or guardian obtained for RCT

[1]Hb: Haemoglobin; those who were severely anaemic were referred to a hospital; [2]BAZ: Body Mass

Index- for-age z-scores; [3]Ethical approval requirements demanded that we obtain 2 different informed consents for the extensive survey and the RCT

Sample Size Estimation

The survey sample (n=1040) was estimated to detect a minimum mean difference of 0.30 in maths and verbal skills z-scores between stunted and non-stunted adolescent girls [12] using the RMASS programme (http://www.rmass.org/) [13]. The RCT sample size calculation was based on 80% power, a one-sided hypothesis at 5% significance level for 3 variables: haemoglobin (Hb), serum ferritin (SF), and serum retinol. The standard deviation (SD) for Hb in this population was 12.9 g/L (for both anaemic and non-anaemic girls) and 8.4 g/L (for only anaemic girls) [2]. Therefore, to detect a difference in mean Hb of 3.83 g/L between the MMB and UB groups required 141 girls per group for a non-targeted approach and 122 girls per group for only anaemic girls. Based on an SD of 20.1 µg/L for SF from a previous study [14]; 57 girls per group were required to detect a mean difference of 9.5 µg/L for SF between MMB and UB groups. Lastly, the SD of serum retinol from a previous study was 0.29 µmol/L; hence, 23 girls per group were required to detect a mean difference of 0.22 µg/L between MMB and UB groups. Expected mean differences for Hb (3.83 g/L), SF (9.4 µg/L) and serum retinol (0.11 µmol/L) are biologically plausible [5]; which are within the range of our estimates. We considered the larger estimate (n =141) of the 3 variables (Hb, SF and retinol) and considering a maximum attrition rate of 10% during follow-up, a minimum sample of 155 girls/group was considered for the RCT. With pre-menarche and post-menarche girls randomised into the parallel arms of the RCT, the study had in total 4 groups implying a total of 620 adolescent girls were required for the RCT (310 pre-menarche and 310 post-menarche).

Lastly, we estimated the sample size for the 24hR with the one random sample formula considering a 95% confidence interval, an estimated width of 10.13mg and SD of 28.9 mg for iron intake, as well as an estimated width of 50.5 µg RE and SD of 113.2 µg RE for vitamin A intake [15]. For both iron and vitamin A intake, the estimated sample was 130 girls and considering an attrition rate of 20%; this was rounded up to 150 girls for each menarche/age cohort. Using the rule of thumb recommended by Rothman [16], a sub-sample of 50 per cohort from the first recalls was included in a repeated recall to allow for adjustment for random day-day variation in intake at the population level. Focus groups typically have 6-12 members plus a moderator [17], but Wyatt *et al.* [18] indicate that focus groups with 4-6 children are most effective in yielding valuable information because duplicate responses are less common, and smaller groups are easier to control. According to Wyatt *et al.*, children may be reluctant to talk in larger groups. Hence, we sampled 5 girls for the composition of each focus group in the survey and the RCT end-line. Finally, Rothman's [16] rule of thumb was used to decide on 50 RCT non-enrolled girls as a control group for the RCT enrolled girls for the body composition analysis.

Screening and Sampling for RCT

In Mion District, where the study was carried out, there are 70 Primary and 11 Junior High Schools. The latter were excluded due to an already ongoing iron and folate supplementation project called GIFTS (**G**irls' **I**ron **F**olate **T**ablet **S**upplementation) among adolescent girls in Junior High Schools [19]. While the Ghana Education Service (GES) has zoned the Mion district into 6 clusters, for the Ten2Twenty-Ghana project, 2 clusters were excluded based on their in-accessibility (remoteness). The remaining 4 clusters had 41 Primary Schools, and we ranked these schools in descending order based on the size of their girl child enrolment; using secondary data on enrolment obtained *a priori* from the GES in Mion. We purposively selected all the urban primary schools (n=4) and the larger rural primary schools (n=15) for screening until the minimum sample required for the survey (n=1040) was met. In each school, we screened all the girls using a 16-item screening questionnaire including personal and household identification and demographics, menarche status, pregnancy and lactation status, health condition, use of any medical drug, iron supplements and participation in a study with drugs, supplements or food. Subsequently, girls who met the survey's inclusion criteria were invited to participate after obtaining their assent and their parents/guardians' informed consent. During the enrolment of subjects into the RCT, we added participants who were not post-menarche in the survey using age >13 years; ensuring that we had enough sample for randomization into the post menarche group of the RCT. The cut-off age (>13 years) was chosen in conformity with the average age at menarche in Ghana from the literature [20–22]. Thus, the post-menarche group in the present study includes post-menarche girls at screening or were expected to become post-menarche during the RCT.

Probability proportion to size was used to select a random sub-sample of girls from the survey who did not meet the exclusion criteria of the RCT in 2 stages. Firstly, we generated random numbers (between 0-1) by school and menarche group in Microsoft Excel (MS Excel, version 2016). The random numbers were sorted in ascending order (lowest to highest); the first set of participants from the menarche group of each school was enrolled until the sample required for the school's menarche group was met. Any girl who dropped out during the enrolment was replaced with the next girl in the list from the same school and menarche group in the ascending order till the sample requirement was met. Subsequently, a second set of random (between 0-1) numbers was generated for the girls enrolled in the RCT in MS Excel. All enrolled subjects with random numbers < 0.5 were assigned to a yellow colour code whiles subjects with random numbers ≥ 0.5 were assigned to a red colour code.

The first step of the probability proportional to size approach described for the RCT was again used to randomly select 155 girls from each of the RCT menarche groups for the first 24hR. For the repeated 24hR, another random selection process like the preceding was employed in selecting 100 girls from the sample for the first 24hR. At the RCT end-line, the probability proportion to size approach was once more used to select 50 RCT non-enrolled girls for body composition assessment.

In-depth interviews are known to be labour-intensive, and most studies utilising in-depth interviews are based on fewer than 50 cases [17]; explaining our decision to randomly sample 30 girls with at least 1 girl from each participating school. Additionally, in the extensive survey, we randomly selected 2 clusters, and from each cluster, one school was randomly selected for a focus group discussion. We next selected 5 girls randomly from each of the selected schools to compose the focus group. Lastly, we randomly selected 4 out of the 19 participating schools for 4 focus group discussions at the RCT end-line. The 4 selected schools were next randomised for one focus group in each arm of the RCT (2 yellow groups and 2 red groups); the first 5 girls in each of the biscuit groups constituted the school's focus group. All the randomizations were done using random number generations in MS Excel. **Figure 4** describes participants' flow, as per CONSORT guidelines including reasons for non-enrolment in the RCT.

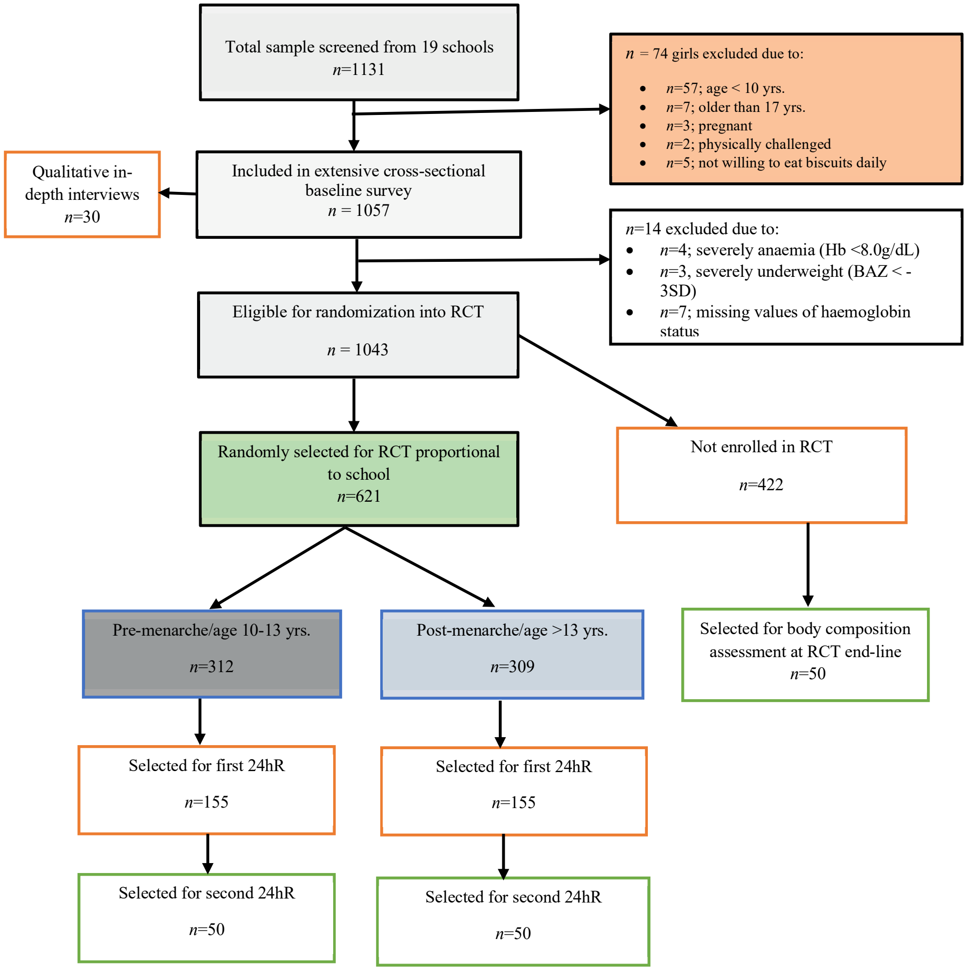

Figure 4. Flowchart for study population selection for the RCT and 24hR

Run-in to the RCT

At the RCT baseline, all participants were dewormed against intestinal parasites with a single dose of mebendazole 400 mg chewable tablets. Malaria rapid diagnostic cassettes (First Response; Premier Medical) were used to screen for current or recent malaria. Participating girls who were found to have malaria during the run-in or the intervention period were treated promptly with artemether-lumefantrine (80mg/480mg) by the guidelines of the Ghana Health Services (GHS) [23] and were referred to the local health facilities when necessary. We repeated the malaria screening and treatment at the midpoint and end-line of the RCT. To assess the sensitivity and specificity of the malaria rapid test kits (RDT), we undertook malaria microscopy for about 11% (68 out of 621) of the girls at the mid-point malaria screening. During the run-in period (5 days), all enrolled subjects received unfortified biscuits procured from the open market. The run-in biscuits' nutrient content was similar to the UB (**Table 2**) in the RCT and was similar in size (50g) to the MMB and UB for the RCT. The run-in was necessary for *a prior* data on the girls' compliance, the feeding set-up practice in each school and the completion of a daily case report form by the teachers and supervisors.

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Table 2: Nutrient Content of biscuits for Ten2Twenty-Ghana RCT

No.	Nutrient	Product name	Nutrient content of fortified biscuits (mg) per serving (51.3g)	[1]Nutrient content (mg) of unfortified biscuits per serving (51.3g)
1	Vitamin A	Dry vitamin A palmitate	0.504	0.10
2	Vitamin D	Dry vitamin D3	0.005	0.00
3	Vitamin E	Dry vitamin E	6.00	0.00
4	Vitamin K	Dry vitamin K1	0.05	0.00
5	Thiamine	Thiamine mononitrate	1.20	0.43
6	Riboflavin	Riboflavin	1.20	0.23
7	Niacin	Niacinamide	14.00	3.03
8	Vitamin B6	Pyridoxine Hydrochloride	1.60	0.00
9	Folic acid	Folic acid	0.311	0.11
10	Vitamin B12	Vitamin B12	0.002	0.001
11	Ascorbic acid	Ascorbic acid	70.00	0.00
12	Calcium	Calcium carbonate	150	0.00
13	Cupper	Cupper Gluconate	0.20	0.00
14	Iodine	Potassium Iodide	0.04	0.00
15	Iron	Ferrous Fumarate	4.05	1.03
16	Magnesium	Magnesium oxide	52.50	0.00
17	Selenium	Sodium Selenite	0.012	0.00
18	Zinc	Zinc Oxide	2.38	1.45

[1]Obtained from the lab division of Mass Industries, Tema-Ghana; the nutrient content reflects the fortification level of wheat flour in Ghana as by law

Biscuits formulation

Van Stuijvenberg *et al.* [24] argue that biscuits are convenient food vehicles since they do not require any preparation by consumers, are relatively easy to distribute and have a long shelf life. According to van Stuijvenberg *et al.* [24], biscuits are snacks rather than a meal and are unlikely to replace meals at home. Subjects in the treatment arm of the RCT received MMB enriched with 11 vitamins

(vitamins B_1, B_2, B_6, B_{12}, A, D, K_1, E, niacin, folate and ascorbic acid) and 7 minerals (Zn, Ca, Fe, Cu, I, Se and Mg) as shown in Table 2. On the other hand, participants in the control arm received UB similar in appearance to the MMB. The UB were simply wheat flour without any additional micronutrients. However, the wheat flour was fortified as by law in Ghana (Table 2). The average weight of a pack of each of the biscuits was 51.3 ± 3.2g. A pack of each biscuit contained between 8-10 pieces of biscuits with the average weight of a piece being 5.6 ± 0.5g.

The micronutrient mix (fortificant) used for the fortification was procured from DSM Nutritional Products (South Africa), and the biscuits were produced by Mass Industries (Tema, Ghana) through the coordination of the Obaasima project. The Obaasima project is a scheme developed by the project "*Affordable and Nutritious Foods for Women (ANF4W)*" which is a partnership between the German Development Cooperation (GIZ) and the private sector in Ghana (http://obaasimaghana.com/campaign.php). Both biscuits (MMB and UB) provided 477.3Kcal per 100g (244.85Kcal for the 51.3g pack of biscuits) (*Sight and Life; personal communication*) and hence varied only in micronutrient content (Table 2). We estimated the energy requirement of the girls using their mean bodyweight (35.8 ± 7.3 kg) and the FAO/WHO/UNU algorithms with the software Optifoood. Energy intake from the biscuits was then estimated to be about 10% of the girls' required energy intake per day. We sent 3 packs of each of the biscuits to Wageningen University and Research (WUR, The Netherlands) for independent and confirmatory analysis of Fe, Ca, and Mg's nutrient content. The packaged biscuits' shelf life was indicated to be 12 months (*Sight and Life; personal communication*); and no organoleptic changes were expected during this period.

When received, the MMB and the UB's original packages were distinguishable, so we re-packaged them to ensure blinding of the first author, the field team, and subjects. Both the MMB and UB were re-packaged in clear zip-lock bags with yellow and red stickers to distinguish between them two weeks before the RCT baseline plasma sample collection. The re-packaging was done cautiously, opening the biscuit pack, and instantly pouring the entire content into a zip-locked bag. **Figure 5** illustrates the original packaged and re-packaged biscuits. The re-packaging was done in an enclosed room in batches for 2-4 weeks of feeding and was coordinated by the project field supervisor from the UDS; he kept the seal to the colour codes and was no longer blinded.

Administration of the Biscuits

We recruited and trained one teacher from each school to administer the biscuits to the girls. Four (4) trained field research assistants with a nutrition background were each assigned to a cluster of schools; they supervised the teachers and participated in at least 2 feeding sessions weekly in each school. Separate classrooms were used for the different biscuit colours during feeding sessions. The girls consumed the biscuits *ad libitum* as a snack during one of the school break periods, Monday through to Friday in the teacher and/or field assistant's presence. During the school holiday period, the girls, together with their teacher, agreed on a convenient time to come to school for the feeding.

For girls who failed to turn up during a feeding session, the teacher and/or field assistant visited them to administer the biscuits. Girls could eat during the weekend (Saturday and Sunday) to make up for any loss day of feeding during the week. A maximum of 2 days lost in feeding during a week was allowed, and they could not carry forward a previous week's feeding to the next week.

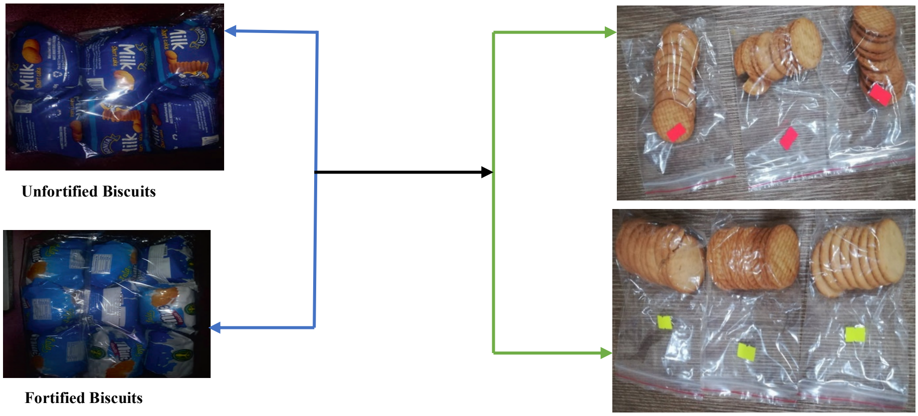

Figure 5. Re-packaged biscuits for Ten2Twenty-Ghana RCT

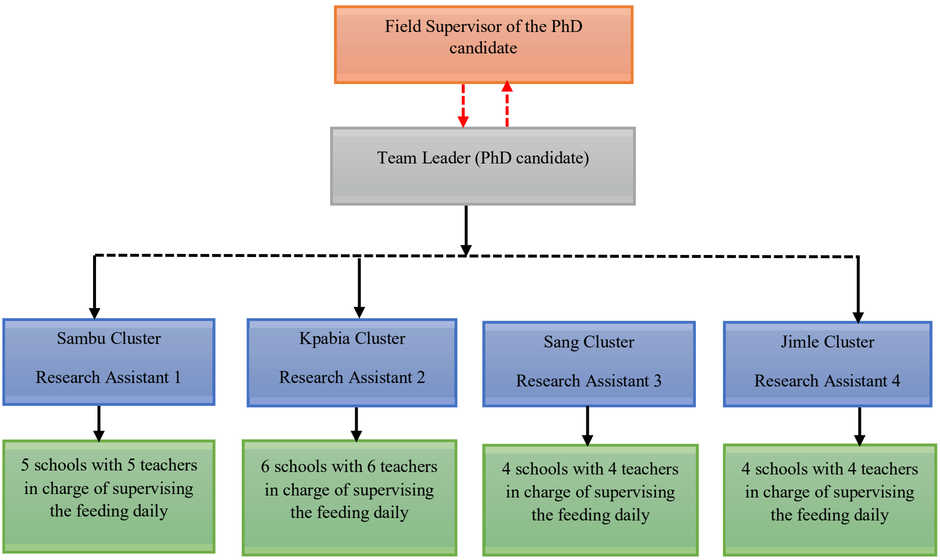

Figure 6. Field supervision plan in the Ten2Twenty-Ghana RCT project

Each girl was given a laminated sheet of her assigned colour code (Yellow or Red) in bold letters to hand out to collect the biscuits each day. The teacher who supervised the daily feeding also had a daily case report form containing girls' list in each school's colour code. The case report form captured attendance and leftovers (if any) were counted and recorded in the daily case report form as pieces leftover. The daily case report forms also captured any adverse events (AEs) and severe adverse events (SAEs) during the feeding. The RCT management and supervision team included the field supervisor, the first author, and 4 trained research assistants recruited from the UDS. Each research assistant was assigned to supervise a cluster of schools (**Figure 6**).

Use of Co-intervention

As biscuits are generally dry to consume, 500mL filtered and packaged sachet water produced by the Nyankpala Campus of the UDS was provided daily alongside the girls' biscuits to help wash down the biscuits. Additionally, nutrition and health education were provided to all students in the selected schools. The education component included modules on anaemia, dietary diversification, personal and environmental hygiene and sanitation, malaria in Ghana, and sexual and reproductive health education. The nutrition/health education was provided as a complement to improve the girls' awareness and knowledge about their health, nutrition, and reproductive health. Subjects received the nutrition and health education modules (**Table 3**) on 5 different occasions for the entire duration of the study through lectures, group discussions and demonstrations; each session lasted about an hour. Colour picture aids were used to aid the sensitisation. The sensitisation was conducted by Community Health Nurses who stay and work in each of the communities. The nurses were recruited and given a one-day training with the district health advocacy team directly supervising them.

Table 3: Modules for Nutrition and Health Education in the Ten2Twenty-Ghana RCT

Module 1: Water, Hygiene and Sanitation (WASH)	Module 2: Anaemia, Malaria and Dietary Practices	Module 3: Sexual and Reproductive Health Education Part 1	Model 4: Sexual and Reproductive Health Education Part II	Recap of all modules
Food and water hygiene	Anaemia: causes, signs/symptoms & prevention	Menstruation and menstrual hygiene	Sexually transmitted diseases (STDs): types, causes and prevention	A re-cap of all topics discussed; group discussions and questions and answers session
Household and environmental hygiene	Malaria: causes, symptoms, consequences & prevention	Sexual behaviour		
Personal hygiene and good grooming	Healthy dietary practices for children and adolescents	Teenage pregnancy: causes and consequences		

Data Collection Methods

We used a mixed-methods data collection technique applying both quantitative and qualitative data collection methods for triangulation in the study. A pre-tested questionnaire was used to collect data on various social, economic, and health-related topics to provide comprehensive information on the girls' social and economic trajectories. The questionnaire was pre-tested in a pilot survey, in November 2017 in the neighbouring Yendi Municipality. The data collection methods included one-on-one interviews, anthropometry, haemoglobin (Hb) status assessment, the collection of venous blood for plasma, a quantitative 24hR, in-depth interviews, and focus group discussions. **Table 4** is a summary of the data collected in the extensive survey and RCT. We first describe the data collected in only the RCT and then data collected in both the extensive survey and/or RCT end-line.

Table 4: Details of the data collected in the Ten2Twenty-Ghana Research Project

Data	Data Collection Period Survey (Nov. /Dec. 2018) n=1057	RCT Baseline (Jan-March 2019) n=621	RCT End-line (Sept. 2019) n=588
Individual characteristics			
Age (Date of birth)	√		
Birth order	√		
Girl's education	√		
Religion	√		
Ethnicity	√		
Maternal data			
Anthropometry of the biological mother	√		
Final decision-making index	√		
Fertility and labour history calendar	√		
Household characteristics			
Parental education & occupation	√		
HH rooster (sex, age structure, religion, education, occupation, & literacy)	√		
HH wealth index (International wealth index)	√		
Psychosocial outcomes			
Self-reported health-related quality of life (HRQoL)	√		√
Subjective health complaints	√		√
Life satisfaction	√		√
Self-esteem	√		√
Self- efficacy	√		√
Body image	√		
Children's depression inventory			√
Cognitive skills and academic performance			
NIH toolbox for cognition	√		√
[1]Seconday data on academic performance and school attendance	√		√
Reproductive health and sexuality			
Age at menarche (recall)	√		√
8-item Pubertal Development Scale (PDS)	√		√
Relationship (boyfriend)	√		
Age at first sex (if applicable)	√		
Marital status	√		
Age at marriage if married or ever married	√		

table continues

Data	Data Collection Period Survey (Nov. /Dec. 2018) n=1057	 RCT Baseline (Jan-March 2019) n=621	 RCT End-line (Sept. 2019) n=588
Number of biological children if any	√		
Age at first birth (if any)	√		
Dietary intake and nutritional status			
Dietary diversity score (single qualitative 24hR)	√		√
Household food security (FIES)	√		√
1-month food frequency questionnaire (FFQ)	√		√
Frequency of the consumption of energy drinks			√
Quantitative 24hR repeated with a sub-sample on non-consecutive days (USDA Standard multiple-pass procedure)		√	
Anthropometry	√		√
Body composition (bio-electric impedance)	√		√
Biomarkers of nutritional status			
Hb (HemoCue)	√		√
Plasma micronutrient status (ferritin, TfR, RBP, zinc, folate)		√	√
Inflammation biomarkers (CRP and AGP)		√	√
Qualitative data collection			
Focus group	√		√
In-depth interviews	√		

HH, household; Hb, haemoglobin; TfR, plasma transferrin receptor; CRP, c-reactive protein; AGP, alpha glycoprotein; [1]The data was collected for the overall sample from the cross-sectional survey (n=1057) at both time points.

Data collected in only the RCT

Except for venous blood, and the quantitative 24hR, data from the larger survey informed the baseline data of subjects enrolled in the RCT including Hb, the secondary outcomes and covariates. The RCT data are grouped into primary outcome data, secondary outcome data and covariates (**Table 5**).

Plasma Samples: Hb assessment for the survey was by finger prick using a HemoCue 301 (Ängelholm, Sweden; 0.1g/dL precision) 2 months preceding the RCT. At baseline and end-point of the RCT, a phlebotomist from the Tamale Teaching Hospital (TTH) collected approximately 10ml venous blood (non-fasting state) from each subject, for biomarkers of micronutrient status into two (4ml each) Na-Heparin Vacutainers (Becton-Dickinson Diagnostics). The biomarkers being assessed include plasma ferritin (SF), soluble transferrin receptor concentration (TfR), retinol-binding protein (RBP), C-reactive protein (CRP) and alpha-glycoprotein (AGP), zinc, folate, and vitamin B_{12}. In the RCT's end-line, we assessed Hb status in the field using a small portion (~ 2ml) of the venous blood with the HemoCue 301. The blood samples were kept in a cool opaque box containing freezer packs (~ 0^0C) in the field and during transport from the field. The venous blood was centrifuged in Rotofix 32A centrifuge at 4000RPM for 5 minutes at the end of each field day at room temperature. The centrifuging was done at the emergency services laboratory of the TTH. We pipetted and stored 2.5mL plasma in duplicate 1.25 mL cryptogenic vials at -20°C (Thermo Fisher Scientific) at the Public Health Laboratory of the TTH, Ghana.

Table 5. Outcomes and covariates assessed in Ten2Twenty-Ghana RCT

Primary Outcomes	Secondary Outcomes	Covariates
Changes and differences in micronutrient status between biscuit groups in: • Hb status • Plasma ferritin (SF) • Plasma soluble transferrin receptor concentration (TfR), • Retinol-binding protein (RBP) • Plasma zinc • Plasma folate • Vitamin B_{12}	Changes and differences between biscuit groups in anthropometric indicators (e.g., attained height, height-for-age z-score, body-mass-index-for-age z-score) and body composition (fat mass, fat-free mass, muscle mass, skeletal muscle mass, body cell mass, total body water, extra-cellular water, and intracellular water)	Inflammation biomarkers (C-reactive protein and alpha-glycoprotein)
Quantitative dietary intake for a subset (n=310)	Changes and differences between biscuit groups in cognitive skills and academic performance, perceptions, and aspirations (qualitative)	Dietary diversity score, dietary patterns, household food security Demographics (age, education, religion, ethnicity, household composition) and socio-economic covariates (household wealth index, parental occupation, and education)

Plasma samples were subsequently transported 2 months after the RCT on dry ice to Wageningen University (WUR, The Netherlands) for storage in liquid nitrogen gas (-88°C). One-hundred microliters (100μL) of the plasma samples were then pipetted into Micronic tubes and shipped on dry ice to the VitMin Lab (Willstätt, Germany) for the analysis of SF, Term, RBP, CRP and AGP using a combined sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique [25]. All measurements were done in duplicate, and where the CV (inter-assay) was >10%, measurements were repeated, and obvious outliers were removed. The CV for the various indicators were SF, 2.3%; TfR, 3.6%; RBP, 3.6%; CRP, 5.8%; and AGP, 8.1%. Certified quality control samples from the CDC/Atlanta and Bio-Rad Liquicheck controls (Bio-Rad) were used to calibrate the results. Plasma samples from the 2-time points (RCT baseline and end-line) were analysed at the same time. Plasma zinc is analysed with an atomic absorption spectrometer (AAS) while folate and B_{12} are analysed with high-performance liquid chromatography (HPLC) later.

Quantitative 24-hour dietary recall (24hR): We assessed the current intake of a sub-sample of the girls enrolled in the RCT with a quantitative 24hR using the USDA standard multiple-pass procedure [26]. To enable adjustment for random day-to-day variation in dietary intake, we repeated the quantitative 24hR in a sub-sample (n=100) of the girls with a first quantitative 24hR

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on non-consecutive days to avoid dependency of intake. Trained interviewers conducted the dietary interviews at home, a month preceding the RCT. We randomly assigned subjects to all days of the week and interviewers to account for differences in intake between days and interviewers. No interviewer could interview the same subject twice.

In the standard multiple-pass procedure, the girl was first asked to mention all foods and drinks, including snacks that she consumed in and outside the home (including school) the previous day. She was then asked to describe the ingredients and cooking methods of any mixed dishes. The primary caregiver and/or the person who prepared home meals the preceding day was asked to help the girl list and estimate ingredients for mixed dishes prepared at home. We recorded the actual weight of a duplicate portion of each food, beverage and ingredients of mixed dishes using a digital kitchen scale (Soehnle Plateau, model 65086) precisely to 2g with a maximum capacity of 10kg. In the absence of duplicate portions in the household, amounts were estimated in as their monetary value equivalents, weight-to-weight with other foods (e.g., amount of sugar estimated with refined corn flour), in volumes, food models (small, medium, or large) or as household units in priority order. The research team agreed a *prior* on models for food such as onion, tomatoes and garden eggs which were carried alongside. We estimated the total volume of each mixed dish cooked at the respondent's household and the volume of this dish consumed explicitly by the girl to determine the proportion of the dish she consumed. The amount of ingredients consumed from mixed dishes by the girl was estimated by multiplying the proportion consumed with the total amount of ingredients used to prepare the dish. We also recorded each food ingredient's frequency of intake (for mixed dishes) or food item for the last seven days preceding the interview day. For shared bowl eating, the girl's usual intake for such dishes and the number of persons who ate from the shared bowl were recorded in the logbook. The 24hR ended by probing the girl for likely forgotten foods; notably, fruits, sweets and snacks consumed on the recall day. Standard recipes and school feeding recipes were generated to estimate grams of ingredients consumed from mixed dishes eaten outside the home or from the school feeding programme. Estimates of these recipes were obtained by averaging three recipes of different vendors and different school feeding matrons/cooks. The vendors and school feeding matrons/cooks were each selected from different localities and schools. Moreover, we developed conversion factors to convert monetary values, weight-weight measures, volumes, food models and household units to their weight (gram) equivalents following Gibson and Ferguson [26]. Lastly, we conducted a market survey in 4 different markets in each of the study clusters. We determined the mean price per 100g of edible food for each listed food in the 24hRs using the average price and weight of foods obtained from each of the surveyed markets.

Data Collected in the Extensive Survey and/or RCT End-Line

Anthropometry and Body Composition: Height and weight were measured in duplicates to the nearest 0.1 decimal with the Seca stadiometer and digital weighing scale, respectively in the survey and RCT end-line. Standard anthropometry guidelines were followed [27] in the assessment.

Height and weight would be transformed into height increment, attained height, Z-scores (height-for-age, body-mass-index-for-age) and body-mass index (BMI). The Z-scores will be computed with the WHO AnthroPlus software with the WHO growth reference for 10-19 years adolescent girls. In the survey and RCT-end-line, we also assessed body composition with bio-electric impedance using the Bodygram Plus Analyser (Akern, Germany) [28]. In the body composition assessment, subjects laid in a backward position with their arms by their side on a field camp bed for 3-5 minutes to ensure uniform distribution of body fluids before the assessment. The girls' feet and wrists were wiped with non-alcoholic wipes before plastering the bio-electric electrodes on them for the appraisal. The electrical resistance (Rz) of the tissues and capacitive resistance of the cell membranes (XC) in whole numbers were recorded on a form and later inputted into Bodygram Plus Analyser's software (Akern, Germany) for the computation of body composition. Body composition estimates were to the nearest 0.1 decimal. They included fat mass (kg), fat-free mass (kg), muscle mass (kg), skeletal muscle mass (kg), body cell mass (kg), total body water(L), extra-cellular water(L) and intracellular water (L). The programme also computes indices and percentages to the total body weight for these estimates.

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Food Security and other Dietary Data: In the survey and RCT end-line**,** we assessed the girls' dietary patterns with a qualitative 1-month food frequency questionnaire (1-month FFQ). A 10-food group indicator [29] was adopted for the FFQ. Likewise, we assessed the frequency of the consumption of energy drinks using a list of energy drinks a *prior* collected through a market survey (*Abdul-Razak Abizari, University for Development Studies; 2018, unpublished data*) at the RCT end-line. A single qualitative 24hR was as well used to assess the dietary diversity score (DDS) of the girls using the 10-food group indicator [29] in the survey and RCT end-line. Furthermore, the girls' households' food security status was assessed with the Food Insecurity Experience Scale (FIES) [30].

Fertility and Marriage: In the survey and RCT end-line, age at menarche was assessed by recall, and pubertal development stage by a 5-item Pubertal Development Scale (PDS) questionnaire [31, 32]. A semi-structured questionnaire assessed relationships (sexual) of the girls.

Psychosocial Outcomes: Psychosocial health outcomes were assessed in the survey and RCT end-line with validated scales including self-reported health-related quality of life (HRQoL) using KIDSCREEN-27 [33, 34], subjective health complains [35], self-esteem [36], self-efficacy [37] and life satisfaction [35, 38]. Furthermore, the assessment included body image of subjects using the Stunkard figure rating scale [39] in the survey. Finally, we included and assessed depression using the children's depression inventory [40] at the RCT's end-line.

Cognitive Skills and Academic Performance: The data included secondary data collected from the schools on the school attendance of the girls and of their grades in English Language, Mathematics and General Sciences in the academic year before the study (September 2017 to July 2018) and at the end of the RCT (September 2018 to July 2019). The academic data was collected for the overall sample (n=1057) from the survey at both time points.

At both time points (survey and RCT end-line), we assessed the cognitive function of the girls with the National Institute of Health toolbox cognition battery (NIH-TCB) [41, 42]. The NIH-TCB is a recognised and standardised test tool for measuring cognitive function. The test is computerised on iPad, and the scores are automated at the end of each test. The tests appeared as games the girls had to play, but since our subjects were generally from a rural setting, we recognised that they might be limited in playing computer games. Hence, they could point to the right answer on the screen, with the interviewer clicking for them instead. We assessed five domains of cognitive function, which were found, based on literature, to be relevant to adolescents' neurological development [43, 44]. The 5 domains included Episodic Memory with the Picture Sequence Memory Test, Working Memory with the List Sorting Working Memory Test, Attention with the Flanker Inhibitory Control Attention Test, Processing Speed with Pattern Comparison Processing Speed Test and Executive/Shifting function with the dimensional Change Card Sort Test. A set of unscored trial tests preceded the actual tests; the unscored test allowed the girls to practice before the actual test.

Labour, Time Use and Aspirations: In the survey, we adopted the *"Young Lives"* questionnaire [45, 46] to assess the time use, labour participation and earnings of the girls. A life history calendar [47] mapped the labour participation of the girls. Lastly, the questionnaire included the girls' expectations and aspirations for marriage, family formation, education, and work.

Maternal and household-related covariates: In the survey, the anthropometric assessment included the height and weight (nearest 0.1 decimal) of the girls' mothers for whom body-mass index and maternal height would be used. The data also included the mothers' participation in household decision making using the demographic and health survey 8-item final decision-making index [48]. Life history calendars [47] also captured data on the mothers' fertility and labour participation.

Moreover, we enumerated household members with a household rooster, including their sex, relationship to the index girl, age-group, education, occupation, and literacy, ensuring that we can compute various household-related indices. Finally, the international wealth index [49] captured data on the household's socio-economic status.

Focus Group Discussions: In the survey and RCT end-line, focus group discussions were conducted by 2 trained research assistants who had previous experience with focus groups. They were trained to probe, listen, and record in writing and use a digital recorder the expressions of the girls. One of them moderated the discussions while the other recorded the discussions both digitally and in a notebook. Topics for discussion included the knowledge, attitudes, and practices (KAP) of the girls regarding relationships, reproductive health, risk behaviours and dietary habits. The discussions also delved into the aspirations, expectations, and life satisfaction of the girls. In the survey, the focus groups also explored the *KAP* of the girls with regards to their body image. A visual storytelling technique was incorporated into the focus group discussions. The girls' data generated in the focus group discussions included digital records, notes, and worksheets used for sketches.

In-depth Interviews: According to Mack *et al.* [50], in-depth interviews are optimal for collecting data on individuals' personal histories, perspectives, and experiences, mainly when sensitive topics are being explored. Boyce & Neale [51] also posit that the approach provides detailed information about a person's thoughts and behaviours and in exploring new issues in-depth. We used in-depth interviews to explore rich insights into the girls' lives and understand their motivations, expectations, aspirations for the future, their life satisfaction, relationships, risk behaviours, and the challenges confronting them in their everyday lives. We also solicited information on their usual dietary patterns and the reasons for adherence to these dietary patterns.

The Internal Validity of the Data

Several measures were taken to ensure the internal validity of the data. We recruited and trained field research assistants as well as supervisors with relevant field experience who could speak at least one of the key local dialects (Dagbani or Likpakpa) fluently. The training included 5 days for the survey, 3 days for the 24hR, 1 day for the focus group discussion and a 3-day refresher for the end-line. Owing to the sensitive nature of questions regarding menarche, relationships, and sexuality, all interviewers administering the one-to-one questionnaire were ladies; recruited from the UDS. In the field, supervisors checked and validated all questionnaires for consistency and completeness. A Microsoft Access template was designed and used for the data entry. The data template was coded numerically, such that implausible values in coded categorical data were impossible. All data entry clerks received a 5-day training by an ICT expert who oversaw the data entry. The entries were merged into a single MS-Access file and the data was exported into different SPSS templates based on data themes. Data cleaning was done in the SPSS templates in the field. The entries of 449 out of 1057 (42.5%) and 202 out of 589 (34.3%) questionnaires were verified entirely in all the data files in the survey and RCT end line respectively.

Statistical Analysis Plan

Data analysis would be conducted with SAS 9.4 (SAS Institute Inc., Cary NC.) and IBM SPSS (version 25) where necessary. Frequencies and percentages are used to describe baseline summary statistics for categorical data while means and standard deviations (mean ± SD) will be used for continuous normally distributed data. Skewed continuous data would be presented as the median and interquartile range (IQR). Data normality will be visually explored with histograms with normality curves, boxplots, and Q-Q plots. Baseline differences in proportions between biscuit groups will be determined with Chi-square or Fisher's exact test as appropriate. On the other hand, one-way ANOVA, or its non-parametric version (Mann-Whitney U-test) will be used to determine differences in means between biscuit groups for descriptive population statistics. Summary statistics will be presented for socio-demographics, anthropometric, and micronutrient indicators at baseline by biscuit group in the RCT to describe the study population.

The RCT data analysis follows the intention-to-treat approach with a sensitivity analysis following per-protocol (compliance ≥ 80%). Compliance is defined by the amount (gram) of biscuits consumed expressed as a percentage of the expected total amount that should have been consumed for the entire RCT. The effects of the fortified biscuits on micronutrient status will be analysed using Linear Mixed Models (LMM) with Maximum-Likelihood Estimations. Linear mixed models are more robust in handling unbalanced and missing data; the models are also better able to handle the assumption of independence and homogeneity of slopes in the data [52]. As our study population was selected from 4 clusters, 19 different schools and different classes, linear mixed model analysis is preferred over the analysis of covariance (ANCOVA) to adjust random hierarchical variables related to the cluster, school, and class of the girls. Similarly, we will use LMM analysis to examine the intervention's effect on cognition, body composition and the psychosocial health outcomes (HRQoL, self-efficacy, self-esteem, and life satisfaction) of the subjects. A "*Step-up strategy*" [53] will be used in building the LMMs. The analysis of body composition includes the effect of the fortified compared to the unfortified biscuits; and as well as the effect of being enrolled and not enrolled in the RCT. However, for dichotomous/categorical outcome variables, Cox Proportional Hazard models will be used to examine the incidence rate and prevalence risk ratio. Cox and Poisson models with robust variance are reportedly better alternatives than logistic regression [54, 55]. We hypothesise that the fortified biscuits would significantly affect micronutrient status, and the secondary outcomes; hence a one-sided hypothesis at 5% significance and 95% confidence interval will be used in the analysis. We will adjust for a set of identified socio-demographic and socio-economic confounding variables in all associations in the statistical analysis. A confounder will be defined as any variable that differs significantly between the biscuit groups at baseline or any variable contributing at least a 10% change in the crude effect estimates after adjustment [16, 56]. All missing data will be imputed if more than 5% of data are missing using multiple imputation methods in SAS, assuming that the data are missing at random [57]. Although no interim analysis was planned, the decision to conduct interim analysis was dependent on reports from the field on AEs and SAEs. The data safety monitoring committee had access to reports of the AEs and SAEs and could request for an interim report.

Plan for Analysis of Quantitative 24-Hour Dietary Recall Data

Compl-eat software (www.compleat.nl) of WUR will be used to estimate individual nutrient intake. Nutrient intake will be adjusted for random day-to-day variation in intake using the Statistical Program to Assess Dietary Exposure (SPADE) [58]. To determine the population at risk of nutrient inadequacy, we will use the harmonised average requirements (H-ARs) proposed by Allen *et al.* [59]. Optifood linear programming [60, 61] will be used to develop and evaluate affordable alternative FBDGs that can fulfil or best meet adolescent girls' nutrient requirements. Pubertal timing may influence the dietary habits/patterns of adolescent girls; for instance, mid-adolescent, compared to early adolescent girls, consumed fewer protein, and vitamin-rich foods in Indian [62]. As well, the nutrient requirements, notably iron for post-menarche girls, are higher than pre-menarche girls. Hence, in the formulation of the FBDGs, stratified analysis by menarche status would be conducted. Lastly, principal component analysis (PCA) will be used to identify dietary patterns for the girls.

Analysis of qualitative data

We will use the *inductive thematic analysis* approach [63] in analysing all qualitative data from the in-depth interviews and focus groups discussions. The analyses will focus on the similarities and differences in the themes within transcripts. This method provides a rich and detailed account of data and the themes emerging from the data [64]. Analyses include transcriptions of digitally recorded discussions, field notes, and worksheets from the girls in the focus groups. We would conduct open-ended coding on each text unit (e.g., sentence or paragraphs) and coding the "raw" participant data such as quotes. The different categories will be sorted into potential themes and all the relevant coded data extracts will be collected within the identified themes. Coding and categorizing will be done with ATLAS ti (Version 8.0; Scientific Software Development, Berlin, Germany) data management software which will facilitate the retrieval of coded chunks of transcripts.

Ethics approval and consent to participate: The protocol was approved in January 2019 by the Navrongo Health Research Centre Institutional Review Board (NHRCIRB323). The RCT was prospectively registered with the Netherlands Trials Register (https://www.trialregister.nl/trial/7487) with registration number NL7487 in February 2019. A Data safety monitoring committee comprising of 3 independent persons with relevant experience in nutrition trials reviewed the trial's safety monthly during implementation. Before the study, a stakeholder meeting was held with the Mion District Assembly, the GES and GHS, and all heads of the selected schools in the district capital-Sang. Written permission was next obtained from the GES in the district. We also undertook a community entry sensitisation with all the opinion leaders, the School Management Committee (SMC), Parent-Teacher Association (PTA) and teachers of all the selected schools. Lastly, in the survey and RCT, we invited parents of the eligible girls for sensitisation and education about the study at the school; their signed/thumb-printed informed consent for their girl-child's participation was then sought. Data collected remains confidential, and study results would be reported in aggregated form so that participants would remain anonymous. Only members of the RCT team had access to participant's records. RCT assistants also signed a written statement to maintain the confidentiality of any personal information from trial participants to which they may become acquainted.

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Chapter 7

General Discussion

In sub-Saharan Africa, adolescent girls are an important target group for nutrition interventions due to the high prevalence of micronutrient deficiencies, the threat of early pregnancy, and the risk of intergenerational transfer of dis(advantages). Aside from dietary inadequacies, increased physiologic requirements following menarche and socio-cultural and economic (SCE) deprivations may drive girls' nutritional problems, but, to date, adolescents have largely been neglected in the public health agenda, Ghana not being an exception. The dearth of data on the trends and SCE determinants of malnutrition during adolescence impedes progress in the design of targeted policies and interventions that can improve adolescents' nutrition in a holistic manner. For the design of appropriate interventions, it is also imperative to understand the age and sex-specific risks and correlates of the nutrition and health status of adolescents; however, data from different settings present conflicting results on the association between malnutrition and adolescent sex. Food fortification is potentially a useful nutrition strategy for adolescent girls but there is a paucity of data on the efficacy and timing (regarding menarche) of such interventions, especially in resource-poor settings.

This thesis primarily aimed to examine the interrelations between nutritional, social, and economic trajectories when optimising female adolescents' nutrition for better health, education, family formation, and labour participation in Ghana. The thesis achieved its aim with 5 interrelated studies, including a systematic narrative review of the literature on the SCE determinants and consequences of adolescent undernutrition in low and lower-middle-income countries, analyses of secondary cross-sectional data in Ghana, and a randomised placebo-controlled trial (RCT) among adolescent girls in north-eastern Ghana. This chapter includes a summary and discussion of the main findings, methodological considerations, the public health and policy implications of the findings and suggestions for future research.

Summary of Main Findings

Table 1 presents the main findings of this thesis. In general, our study shows a steady burden of undernutrition with an existing and upcoming dual-burden of overnutrition among non-pregnant adolescent girls in Ghana. Anaemia is a severe public health problem, irrespective of sex, with no meaningful improvements since 2003 among girls. Adolescent boys were more likely to be stunted and thin, and to have pre-hypertension/hypertension. A broad range of individual-level factors (age, marital status, parity (of the girl), dietary habits, lifestyle (TV watching), autonomy in the household and education), household factors (availability of maize stock in the household, relations, socio-economic status, and size of the household) and broader community/environmental factors (ecological zone and type of residence) were associated with the nutritional status of adolescents. Our primary study "Ten2Twenty-Ghana" showed that multiple-micronutrient fortified biscuits (MMB) consumption did not improve iron status and anaemia risk but seemed to decrease the prevalence of deficient/low vitamin A status for post-menarche girls. Only in anaemic subjects, did we find evidence that MMB consumption improves mathematics score and the working memory of pre-menarche girls. In anaemic subjects, we found evidence that MMB consumption improves some aspects of cognition, i.e., mathematics score and the working memory of pre-menarche girls.

Table 1. Summary of the main findings in this thesis

Objective	Main Results
Chapter 2: *Type*: A systematic narrative review **_Population_: Articles on undernutrition among adolescents aged 10-19 years in low and lower-middle-income countries (LLMICs)**	
To provide a synthesis of the SCE determinants and consequences of undernutrition among adolescents in LLMICs	• A broad range of context-specific socio-cultural and economic factors were found • Using the socio-ecological framework, most determinants were operating at the individual and household-level, while very few at the broader community-level; most studies did not examine community-level determinants • Reported consequences of adolescent undernutrition were mainly related to education and cognition
Chapter 3: *Type*: Secondary analyses of cross-sectional data from an impact evaluation of the Ghana School Feeding Programme in 2014 **_Population_: Rural School-aged children (SAC) (6-9 years) and adolescents (10-19 years)**	
To determine the severity of anaemia and contextual factors associated with anaemia and Hb status among rural SAC and adolescents in Ghana	• Anaemia is a severe public health problem in adolescence, irrespective of sex • Farm diversity, availability of maize stock in the household, household asset index and agro-ecological zone were the main predictors of Hb and anaemia
Chapter 4: *Type*: Secondary analysis of cross-sectional data from 3 nationwide Ghana Demographic and Health Surveys (GDHS) conducted in 2003, 2008 and 2014 **_Population_: Non-pregnant adolescent girls aged 15-19 years**	
To examine the trends-over-time and the factors associated with malnutrition among adolescent girls in Ghana	• Between 2003 and 2014 stunting decreased slightly by 1.5% points and thinness by less than 1% point but overweight increased by 4.3% (95% CI, 0.74, 7.84) points • Anaemia increased by 18.1% points in 2008 with a slight increase (2.82%, 95% CI, -1.76, 7.41) in 2014 compared to 2003 • A low level of education was positively associated with stunting • Increasing age was positively associated with stunting but inversely associated with anaemia • Girls who had ever given birth were more likely to be anaemic compared to those who never did • HWI and household size were inversely associated with overweight/obesity • Urban dwelling girls were less likely to be stunted

table continues

Chapter 5: ***Type:*** **Comparative secondary analysis of data from the 2014 GDHS** ***Population:*** **Non-pregnant adolescent girls and adolescent boys aged 15-19 years**	
To assess the association between malnutrition, pre-hypertension/hypertension (PHH) and sex among adolescents.	• Boys were about twice more likely to have PHH, more than twice likely to be stunted and thin, but less likely to be overweight/ obese compared to girls • Girls may be more at risk of the detrimental effects of low education level on stunting and PHH • Autonomy index was inversely associated with stunting and positively associated with overweight/obesity among girls • HWI was positively associated with overweight/obesity among girls and inversely associated with stunting and PHH for adolescent boys • Improvement in household WASH was inversely associated with stunting for boys •
Chapter 6: ***Type:*** **A 26-week double-blind placebo RCT with MMB and UB** ***Population:*** **Adolescent girls aged 10-17 years (*n*=621) classified into two groups (pre-and post-menarche)**	
To determine the efficacy of MMB consumption compared to unfortified biscuits (UB) on the girls' micronutrient status, height, and cognition, and the effect of timing of intervention (before or after menarche).	• In MMB compared to UB, we found • No change in plasma levels of ferritin (PF), transferrin receptor (TfR) and retinol-binding protein (RBP) • No effect on anaemia and micronutrient deficiencies in the total study population • A significant interaction effect between biscuits and menarche for the post-intervention vitamin A deficiency (*P*=0.04) and deficient/ low Vitamin A status (*P*=0.03); VAD increased by 6.2% points among pre-menarche girls, but VAD/low vitamin A decreased by ~10% among post-menarche girls • Marginal increases in cognition [β=0.26, 95% CI (-0.85, 1.38)], academic performance [β=0.46, 95% CI (-1.00, 1.92)], HAZ [β=0.04, 95% CI (-0.08, 0.16)] and BAZ [β=0.04, 95% C.I (-0.02, 0.10)] • An increase in post-intervention mathematics score of anaemic girls [β=4.22, 95% CI (1.41, 7.03)] • A significant increase in working memory post-intervention [β=5.38, 95% CI (0.07, 10.68)] in pre-menarche girls who were anaemic at baseline

LLMICs, low and lower-middle-income countries (LLMICs); SAC, School-aged children; PHH, pre-hypertension/hypertension; HWI, household wealth index; WASH, household water, hygiene, and sanitation; MMB, multiple-micronutrient fortified biscuits; UB, unfortified biscuits; PF, plasma ferritin; TfR, transferrin receptor concentration, RBP, retinol-binding protein; HAZ, height-for-age z-score; BAZ, body-mass-index-for age z-score

Discussion of Key Findings

In this section, we reflect on the key findings in this thesis, related to the triple burden of malnutrition and of cardiovascular risk among adolescent girls in Ghana; the factors associated with malnutrition among Ghanaian adolescent girls and boys, the efficacy of multiple-micronutrient fortified foods on micronutrient status, cognition, and height and the role of menarche in micronutrient status.

A Triple Burden of Malnutrition for Adolescent Girls in Ghana

The 2019 UNICEF report on "*the state of the world's children*" suggests an emerging trend of a triple burden of malnutrition including protein-energy undernutrition, "hidden-hunger" or micronutrient deficiencies and overweight among children aged 5-19 years [1]. In conformity with previous studies [2, 3], our findings also indicate that adolescent girls in Ghana suffer from this triple burden of malnutrition. Stunting was about 8% among the adolescent girls in 2003, declining by only 1.5% points in 2014. Overweight/obesity among the girls increased by 4.3% points in 2014 from 10.0% in 2003. Likewise, Ofori-Asenso and colleagues [4] also observed an increasing trend in overweight and obesity prevalence in the period 1998–2016 with women being more overweight (27.8% vs 21.8%) and obese (21.9% vs 6.0%). In the randomised trial, about one-third of the girls were either vitamin A deficient or had a marginal status, with vitamin A deficiency (VAD, 9.3%)) being a mild (<10%) public health issue, a lower severity compared to Alicke *et al.* [5] in southern Ghana (36.0%, in 2017) but higher as reported in the 2017 Ghana micronutrient survey report for 15-19 years girls (0.6%) [6]. The prevalence of iron deficiency (ID) was mild (10.8% based on plasma ferritin) and higher compared to Alicke *et al.* (4.0%) but lower compared to the Ghana micronutrient survey (20.9%). Using transferrin receptor concentration (TfR) resulted in a much higher prevalence of ID in our study (50.1%) with about a quarter of the girls having iron deficiency anaemia (IDA). Anaemia was of severe public health relevance, showing no improvement over the years (chapter 4) or through interventions (chapter 6) as also observed by Kassebaum and colleagues for females of all ages between 1990 and 2010 [7]. Overall, the study population in our randomised trial was younger (age range 10-17, mean 12.8 ± 2.0) compared to that in Alicke *et al.* and the 15-19 years girls in the Ghana micronutrient survey report.

The co-existence of undernutrition and overnutrition reflects persistent food insecurity and poverty alongside a nutrition transition with an increasingly sedentary lifestyle [8]. Despite improvements in socio-economic conditions in the last three decades, food insecurity remains a national challenge with a new trend of urban poverty and food insecurity on the rise in Ghana [9]. Dietary inadequacies, resulting mainly from monotonous plant-based diets partly account for the burden of anaemia and micronutrient deficiencies [10, 11]. The consumption of animal-sourced foods in rural Ghana is inadequate [12, 13], mainly because of the high cost of animal-sourced foods [14]. Although we could not thoroughly examine the role of dietary intake or household food security on anaemia, the availability of maize stock in the household which was used as a proxy of food security, was associated with anaemia among school-aged children (Chapter 3).

The nutrition transition presently occurring in Ghana partly accounts for the emerging trend of overnutrition. Dietary patterns in Ghana are shifting from traditional foods towards intake of more refined foods, ready to eat fast-foods, poor-nutrient, and energy-dense snacks with little consumption of fruits and vegetables, particularly in the urban settings [15]. Over half of rural Ghanaian adolescent girls aged 10-19 years have inadequate fruit intake [16]. There is increasing processing and packaging in the local food environment, and the consumption of more processed food [15, 17]. There is presently a proliferation of fast-food franchises and restaurants across the country especially in big cities [18]. The effect of fast-food consumption on the rising of obesity is well established in both developed and developing countries [19, 20]. Reardon *et al.* [21] observed a positive association between the rise of the double-burden of malnutrition and the increase in ultra-processed food consumption in Africa.

Sedentary behaviour, characterised by increasing time and frequency behind televisions, computers, smartphones, and tablets, is on the rise in SSA children and adolescents, and this trend is particularly seen in urban areas [16, 22]. In Ghana, physical activity is reportedly lower among adolescent girls and women compared to their male peers [23–25]. Cultural and gender roles often limit girls to household chores compared to boys who have more opportunity for leisure time and outdoor physical activity than girls in settings as Ghana [26].

The co-existence of undernutrition and overnutrition is a real public health challenge. Low socio-economic groups with lower food expenditure have unhealthier food choices as they are more likely to consume cheaper processed and energy-dense foods compared to more expensive nutrient-rich foods [27]. Evidence suggests that in many LMICs, increasing numbers of lower socio-economic groups struggle with undernutrition, while obesity and over-nutrition are increasing [4, 28]. Adolescent overweight/obesity poses an emerging cardiovascular risk for adolescents. It is a risk factor for obesity in adulthood, and diabetes, hypertension, asthma, lower extremity venous oedema, and obstructive sleep apnoea in later life [29, 30]. From an intergenerational perspective, undernutrition among girls during adolescence has consequences for foetal growth restriction, ultimately leading to small gestational age and low birth weight infants [31]. Obese mothers are also at risk of gestational diabetes, give birth to children with higher birth weight, who are more likely to have obesity and type-2 diabetes later in life [32, 33].

An Existing and Upcoming Burden of Cardiovascular Risk

In our analysis of the 2014 GDHS data, about one-fifth of the adolescent boys and girls had PHH (Chapter 5). There is little data on the prevalence of hypertension among adolescents in SSA. However, one study estimated that about 33% of adolescent boys and girls aged 13-17 years in South Africa have PHH [34]. The prevalence of hypertension from our analysis (0.2%) was lower compared to cross-sectional data by Alicke *et al.* [5] (9.0%) and Afrifa–Anane *et al* [23] (4.0%) in Ghana. While Alicke and colleagues did not indicate the prevalence of pre-hypertension, the prevalence was higher in the study of Afrifa–Anane and colleagues (25.8% vs. 32.3%) compared to ours. A more recent study, also in southern Ghana found about 34% of adolescents with pre-hypertension and about 5.4% with hypertension [35]. Whereas the mean age of adolescents in the study of Alicke *et al* was like our study population, the mean age of participants in that of Afrifa–Anane *et al* was higher (20.0 ± 2.7 vs. 16.9 ± 1.7 years). In both studies, the study populations were urban (Accra and Kumasi Metropolis) with a higher socio-economic status, a factor that was associated with PHH also in our study. It must be stated that a comparison of blood pressure across studies is difficult as blood pressure varies due to a large number of factors including measurement technique, the accuracy of the equipment and multiple subject factors such as anxiety, and the preparation of the subject before measurement, for example, if the subject had voided urine just before, and has been sitting or laying down 15 minutes before measurement, then blood pressure readings are lower [36].

In conformity with previous studies [35, 37], overweight/obese adolescents were about twice more likely to have PHH compared to their peers with normal nutritional status. In our analysis, adolescent boys were 75% less likely to be overweight/obese compared to girls, which explains that the association between overweight/obesity and PHH was not statistically significant in the stratified model for boys. Overall, the positive association between overweight/obesity and PHH emphasises the need for interventions to curtail overweight/obesity among adolescents.

Factors Associated with Malnutrition Among Ghanaian Adolescent Girls

In Chapters 3 and 4, we identified a set of factors associated with adolescent girls' nutritional status, which we grouped following the socio-ecological framework in Chapter 2 (Figure 1). Our study illustrated that adolescents' nutritional status is influenced by a complex interplay of individual, household, and environmental factors. Most of the correlates of the adolescent girls' nutritional status were individual-level factors, with a few being household-level factors. We identified only agro-ecological zone and type of residence as the community-level factors associated with malnutrition in the secondary analysis (Chapters 3-5). Our analysis could not include several other factors from the socio-ecological framework in Chapter 2 as they were not available in the dataset we analysed, including the workload and time spent by the girl in work, dietary intake, and household food security. While parental education and occupation were modelled in the GSFP (Ghana School Feeding Programme) data analysis (Chapter 3), we could not include them in our analyses of the GDHS data (Chapters 4 and 5). Our population for analyses in these chapters were a sub-set of the fertile age (15-49 years) population surveyed in the GDHS; hence, they were assumed to be parents/guardians, and no data on parental occupation and education were available for them. Nonetheless, we computed and explored the '*mean household years of education*' in our analysis, which though an important covariate of systolic blood pressure, was dropped because it correlated with many other variables at the individual and household level. The correlation was notably strong for the household wealth index

(Rho=0.73; *P*<0.0001) which was a significant correlate in virtually all our statistical models. Indeed, education, as presented in our discussion in Chapter 4 is a proxy for socio-economic status [38].

In Chapters 4 and 5, we created an index of autonomy and empowerment, using data on the perception and acceptance of domestic violence and property ownership. In Chapter 4, the index was limited to only the perception and acceptance of domestic violence, referred to as "*attitude towards wife-beating*" in the GDHS. However, the index additionally included property ownership, available only for the 2014 GDHS in Chapter 5. In Chapter 4, the use of the "*attitude towards wife-beating*" data ensured a uniform measure for the 3 rounds of the survey. It must be stated that a third index relating to final household decision-making in the GDHS was not included in any of the empowerment indexes since the data was limited to only married adolescents. While education suffices as a proxy of empowerment, we found no multicollinearity between the empowerment indexes and education in our analysis. According to Santoso *et al.* [39], empowerment in one domain does not imply empowerment in another; for instance, being educated does not imply having the autonomy to make decisions for oneself or the household. The indexes assessed the autonomy dimension of women empowerment. In the 2014 GDHS report (p. 303), the "*attitude towards wife-beating*" is used as a second indicator of empowerment, in addition to participation in final household decision-making [40], measuring a construct of autonomy.

Individual-level factors: In our study, individual-level factors (Figure 1) influencing the nutrition of the adolescent girl included age, marital status, parity (of the girl), dietary habits, lifestyle (TV watching), autonomy and education (Chapters 3-5). The possible mechanisms for the associations were discussed in Chapters 3-5. While increasing age was associated with being anaemic in our analysis of the GSFP data in Chapter 3, it was rather associated with reduced anaemia in our analysis of the 2003-2014 GDHS data in Chapter 4. The study population in Chapter 3 were mostly pre-menarche with a mean age of 11.2 ± 1.3 years while the study population in Chapter 4 were mostly post-menarche with a mean age of 16.9 ± 1.6 years. The difference might partly be that older girls acquire immunity over time and are more resistant to infections [41, 42]. Moreover, the data from the GSFP had a wider age range (6-17 years) to detect an association compared to the GDHS data (15-19 years).

In our analyses, the only dietary-related variables included the frequency of the consumption of fruits and vegetables in the last week, limited to the 2008 and 2014 GDHS. Fruits and vegetable consumption may partly indicate healthy dietary behaviours. The consumption of fruits and vegetables, especially vitamin A and iron-rich fruits and vegetables, is associated with improved micronutrient intake and better health outcomes [43]. In our study, neither the frequency of consuming fruits nor vegetables was an independent determinant of overweight/obesity or anaemia. However, girls with a higher frequency of fruit consumption were significantly less likely to be thin (Chapter 4 and 5).

Religion was not an important predictor of nutritional status. Also, ethnicity and marital status had inconclusive associations with stunting and being overweight/obese. Rather, food beliefs and eating habits may be influenced by religious or ethnic beliefs [44]. However, data were lacking on food beliefs and nutritional practices of adolescents and further research to understand the effect of food beliefs in the nutrition of adolescents may be desired. While married girls are more likely to have emotional support, support for health services and food access, the physiologic and socio-economic deprivations associated with adolescent marriage [10, 45–47] outweigh any possible benefits in the girl's nutrition and health. Moreover, any possible benefits of adolescent marriage largely depend on the partner's socio-economic status.

Household-level factors: Household factors associated with nutritional status in our analysis included the socio-economic status (HWI) and size of the household, household dietary diversity and food security (Figure 1). High socio-economic status is inversely associated with overweight/obesity in high-income countries [48, 49]; however, the reverse is true for LMICs. A recent systematic review on childhood and adolescent overweight/obesity in SSA indicated a positive association between high HWI and overweight/obesity [50]. While a higher HWI may improve household food security and dietary diversity [51, 52], it may also mean increased access to high calorie, fast food, and a more sedentary lifestyle in general in LMICs [53]. This probably explains that increasing HWI was associated with reduced undernutrition including stunting and anaemia but also associated with being overweight/obese in our analysis.

Large household size has long been recognized as a determinant of inadequate dietary intake and undernutrition [54], which is partly a result of competing needs for resources that may be limited including food and health care. Competing needs also imply a higher risk of micronutrient problems, a result of poor diet quality and access to health. Data on household food security in our study was limited to the availability of maize stock in the household, and it was positively associated with a reduced odds of anaemia for SAC (Chapter 3). The association between household food security and dietary diversity with nutritional outcomes has been well established [51, 52].

Community-level factors: Several studies, including systematic reviews, have clearly shown that adolescent overweight/obesity is more prevalent in urban than in rural settings of SSA [44, 50]; however, place of residence was not an independent predictor of overweight/obesity in our analysis of the GDHS data (Chapter 4). The emergence of fast food, poor-nutrient and energy-dense snacks may account for the rise of overweight/obesity even in rural settings. According to Andam *et al.* [17], imported processed products are more prevalent in traditional retail outlets than in modern retail outlets in Ghana; implying residency may not limit access to ultra-processed, poor-nutrient and energy-dense foods. Nonetheless, residing in an urban setting was an independent predictor of a lower prevalence of chronic undernutrition for adolescent girls, as expected. Our analysis of the GSFP data (Chapter 3) showed that residing in the Guinea/Sudan savannah agro-ecological zone of Ghana was associated with a higher likelihood of anaemia but this association did not hold as an independent predictor of anaemia in our analysis of the GHDS data in Chapter 4. In the GDHS data, the forest zone had the highest prevalence of anaemia in 2008, which partly explains that the prevalence risk of anaemia peaked in 2008. The finding may relate to the prevalence and type of disease vectors during the survey. The forest zone of Ghana has a tropical climate in which malaria exposure is higher [6]. A recent study reported that anaemia in children and women (fertile age) was associated with sub-clinical inflammation (a biomarker of infections) in the middle and southern belts (forest zone), but not the northern belt of the country [55].

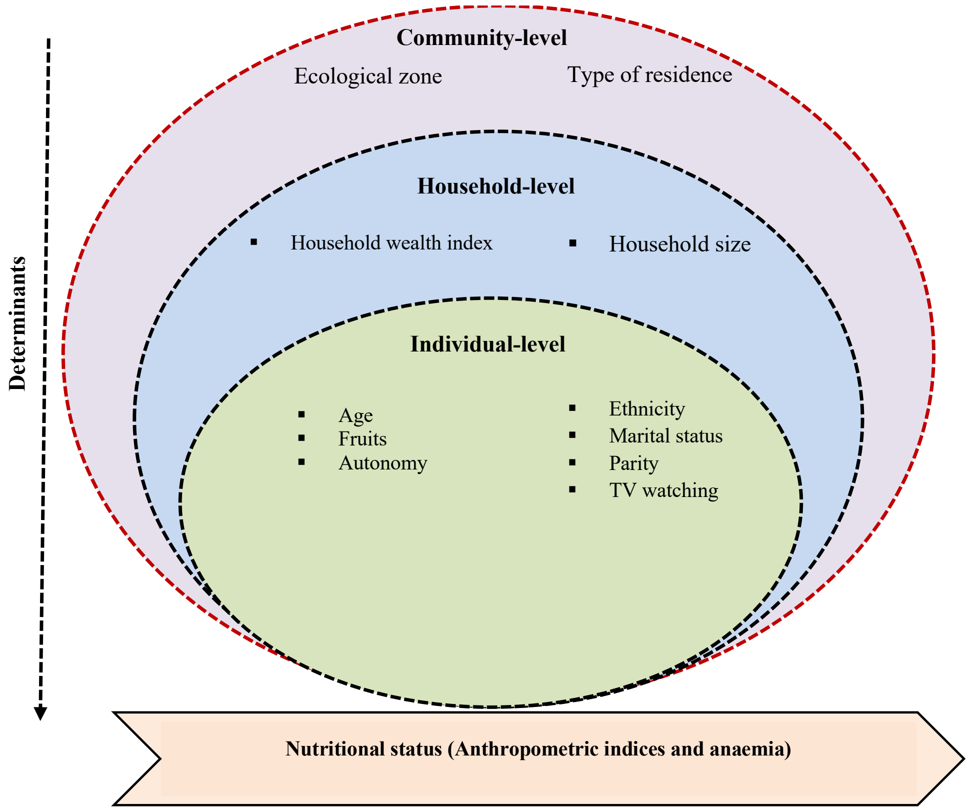

Figure 1: Factors influencing the nutritional status of adolescent girls in Ghana

The Vulnerability of Adolescent Boys, Sex-Specific Correlates of Malnutrition for Adolescents, and their Intersections

Our findings suggest that adolescent boys are also vulnerable to undernutrition and poor cardiovascular health (Table 2). Surprisingly, the prevalence of anaemia was slightly higher for adolescent boys compared to their female peers in our secondary analysis of data from an impact of evaluation of the GSFP (Chapter 3), but we could not verify this further with the 2014 GDHS, which had no haemoglobin status data for adolescent boys. The results from the 2014 GDHS data (Chapter 5) showed that adolescent boys were more than twice likely to be stunted and thin, and about twice likely to have PHH.

While the literature including our systematic review (Chapter 2) is generally inconclusive on the sex difference in nutritional status during adolescence, there is increasing evidence that adolescent boys like male infants and young children are more vulnerable to stunting and thinness compared to their female [5, 23, 56–59]. Hall and colleagues [60] long-established that adolescent boys in multiple Africa countries including Tanzania, Mozambique, Malawi, and Ghana were more anaemic compared to their female peers. Recently, one study in rural Gambia also found adolescent boys more anaemic compared to girls [56]. Despite the paucity of data on adolescents' cardiovascular risk, two cross-sectional studies in the Greater Accra and Ashanti regions of Ghana reported a higher prevalence of PHH among adolescent males than their female colleagues [5, 23].

In brief, the literature indicates that males are more susceptible than females to malnutrition and poor health from conception [61]. Although the fragility of males from conception is not well understood, they seem to show a greater response to nutritional supplementation during the prenatal period [62]. Maturational delay in boys compared to girls may also explain the difference [63]. It has also been hypothesised that the burden of anaemia may affect the body weight of boys than girls since boys put on more muscle which requires more iron [63]. Also, boys have a greater susceptibility to environmental constraints than girls [64]; this includes a higher predisposition to infections such as helminths [65]. Besides overweight/obesity, stunting is said to be associated with higher rates of later arterial hypertension for children and adolescents [66], which may partly explain that boys were more likely to have PHH in our analyses. Social attitudes about the resilience of boys worsen the biological deficit [67]; cultural expectations of masculinity in a setting as Ghana can make boys seem invulnerable even when they genuinely need support. Finally, the gender roles of boys which involves more labour may also greatly reduce body weight with implications for thinness in settings where dietary intake is inadequate.

The level of education was associated with nutritional status and PHH for both adolescent boys and girls. However, a low level of education was a stronger predictor of stunting for girls compared to boys, emphasising the need to strengthen programmes to reduce school dropout rates for girls, which is still a problem, especially in rural settings in Ghana. Besides education, our findings suggest that intervention programmes that would directly empower girls and influence their education, marital status and lifestyle may improve their nutrition and health. Overall, education is a proxy of socio-economic status and empowerment [38], and more educated girls would be more empowered in job opportunities and autonomy in the household [68]. The greater susceptibility of boys to environmental constraints than girls [64] may explain that more factors at the household and community level were associated with malnutrition and PHH among boys compared to girls (Table 2). The negative association between increasing household WASH (water, hygiene, and sanitation) index and stunting for boys may yet be affirming the susceptibility of boys to a poor household environment.

Nutritional problems for girls are perpetuated by gender bias in nutrition, education, and aspirations [69]. While evidence on intergenerational effects for adolescent boys is presently sparse, it has largely been established that improvements in the nutrition and health of the adolescent girl can break intergenerational cycles of malnutrition and deprivation [1, 31, 70, 71]. Overall, an all-inclusive approach including boys and girls is desired for a holistic improvement in nutrition and health for national development.

Table 2: Sex-specific correlates of malnutrition and pre-hypertension/hypertension among Ghanaian Adolescent Boys and Girls aged 15-19 years

Level of Factor	Predictors of Malnutrition and Pre-Hypertension/Hypertension					
	Malnutrition			Pre-hypertension/Hypertension		
	Boy	Girl	Both	Boy	Girl	Both
Community	Agro-ecological zone			Agro-ecological zone		
Household	WASH Household size		Household wealth index	Sex of household head Relationship to the household head Household wealth index	Household has agricultural land	
Individual	Sex Ethnicity	Menstruated in last 6 weeks Fruit intake Vegetable intake Relationship to the household head Marital status Empowerment	Education level	Sex	Has a child	Education level

WASH, water, sanitation, and hygiene

The efficacy of multiple-micronutrient fortified foods on micronutrient status

We found no evidence that MMB consumption improves hematologic biomarkers. However, there was evidence from a stratified analysis that menarche and baseline vitamin A status modified the effect of the intervention on vitamin A status and Hb.

Our findings are contrary to the results of a meta-analysis by Das *et al.* [72] which showed that food fortification with vitamin A, iron and multiple micronutrients for children significantly increased hematologic markers and serum micronutrient concentrations. However, our findings corroborate a more recent systematic review and meta-analysis by Eichler and colleagues [73] who concluded that the consumption of fortified dairy and cereal foods only results in a minor increase in Hb with no differences in anaemia risk for 5-15 years children. Similarly, Salam *et al* [74] in another recent review and meta-analysis also concluded that they were uncertain of the effect of multiple-micronutrient fortified foods on Hb (mean difference: -0.10 g/dL, 95% CI: -0.88, 0.6).

The efficacy of a micronutrient intervention, especially iron fortification, largely depends on the choice and dose of the iron compound and other intrinsic or extraneous factors. In the present study, ferrous fumarate was the fortificant used for iron, while dry vitamin A palmitate (retinyl palmitate) was used for vitamin A, both of which have been suggested by the WHO/FAO for the fortification of wheat flour [75]. Nevertheless, Stuijvenberg *et al.* [76] argue that the bioavailability of ferrous fumarate in biscuits without a vitamin-C-fortified drink is inadequate. Although 70mg of ascorbic acid was included in our MMB, vitamin C is known to be unstable and easily lost during processing and storage [75]. In the design of our study, the co-intervention was a pack (500mL) of filtered water; while we considered the inclusion of a citrus-based drink as a co-intervention, cost implications, and the possibility of indirectly promoting the consumption of sugary beverages and a brand (company), implied this was not appropriate.

The lack of an effect on haematological indicators in our study may relate to the fortification level of the biscuits. Zimmermann and colleagues [11] hypothesised that 23mg additional iron per day is required to significantly improve the iron status of children consuming mainly cereal-legume-diets as in our study population. Similarly, Tee *et al.* [77] found that weekly supplementation with 120mg but not 60mg iron increased Hb concentrations among adolescent girls. Accordingly, the 4.1mg iron per serving from the MMB was unlikely sufficient to improve the iron status of our study population. However, the present study assessed the effect of an existing multiple-micronutrient fortified food product (MMB) on the market, designed to meet only 15% and 30% of the recommended dietary allowance of iron and vitamin A respectively for women aged 19-35 years. Our study showed that such a product is not able to improve the micronutrient status of adolescent girls within 6 months.
VAD alters the absorption, storage, release, or transport of iron to the bone marrow, increasing the risk of iron deficient-erythropoiesis and anaemia [78]. Hence, adequate vitamin A status improves the absorption and mobilization of hepatic iron stores [79]. The improvement in vitamin A status yet decline in the Hb of post-menarche girls in our study suggests that vitamin A does not exert any influence on Hb for girls in our setting. Indeed, the association between vitamin A status and hematologic biomarkers has been inconsistent in the literature. While some authors have shown that interventions that control VAD improve iron status and control anaemia induced by either ID or infection [79, 80], other authors have equally shown that vitamin A interventions have no effect

on iron status and anaemia among children and adolescents [81, 82]. The inconsistency may be attributed to intrinsic or extraneous factors within the population that can influence both vitamin A and iron status indicators.

One intrinsic factor that may influence vitamin A and iron status is underlying infections and inflammation. Infections affect vitamin A and iron metabolism, increasing PF and TfR concentrations and decreasing plasma retinol concentrations [83, 84]; notably, PF increases during infection, giving false-negative results [83]. According to Nairz and Weiss [42], persistent infection, autoimmune disease, or malignancy sequesters iron from infectious agents toward storage, subsequently resulting in anaemia of chronic inflammation. At both time points in the randomised trial, C-reactive protein (CRP) and alpha-glycoprotein (AGP) were significantly correlated with the micronutrient biomarkers. In our study, over a third of the girls had sub-clinical inflammation (SCI) at baseline; more than three-quarters of SCI was in the late convalescence phase, signifying chronic infection and inflammation. The prevalence remained high at the end-line despite deworming all subjects at baseline. Further, about three-fifth of subjects with post-intervention anaemia also had SCI at either time point (results *not shown*). In a national survey, SCI and VAD each accounted for about 10% of anaemia among reproductive-age women in Ghana [55]. Considering the above, efficacy trials that include iron and vitamin A may need to exclude subjects with SCI. The prevalence of inflammation also emphasizes the need to strengthen school children's mass deworming against schistosomiasis and soil-transmitted helminths.

Micronutrient deficiencies other than iron (i.e., folate, riboflavin, vitamin A, B_6, B_{12}, zinc, copper) are known causes of anaemia [85, 86], but the MMB included these nutrients. Non-nutritive factors such as parasitic infections (e.g., malaria, helminths) [42], discussed above, or genetic factors (e.g., haemoglobinopathies) [87] may partly account for the modest effects on iron and anaemia. Although the prevalence of sickle cell traits among Ghanaian adolescents is unknown, about a third of infants and young children, and non-pregnant reproductive-age women in Ghana have α-thalassemia, with more than a tenth having sickle cell disorder [6]. Sickle cell traits may be associated with increased TfR [87]. But as we did not assess haemoglobin variants, we are unable to examine the extent to which these conditions contributed to elevated TfR in our population.

Dietary inadequacies among our study population may also partly explain the modest effects of the intervention. Vitamin C [88] and dietary zinc depletion inhibit iron absorption [89]. Although we are yet to analyse data on dietary and nutrient adequacy for our population, dietary inadequacies are likely prevalent. Less than a fifth of the girls' households were food secured at baseline and dietary patterns were mainly plant-based with poor consumption of animal source foods except for anchovies, cooked as part of recipe soups and sources. A study among 5-13 years school-aged children in northern Ghana confirms the existence of dietary inadequacies of multiple micronutrients [12]. TfR has been suggested as an alternative biomarker of iron status in high inflammation contexts [90].

However, both ID and inflammation increase TfR values [83]. TfR concentration was positively correlated with both CRP and AGP at both time points in our study, and the correlation was notably moderate-to-strong for AGP at baseline (*r*=0.75, *results not shown*). In conformity with Righetti *et al.* [91], the prevalence estimates of ID and IDA were particularly higher when using or including TfR. Consequently, caution may be needed when estimating ID and IDA prevalence in a context with probable high SCI like Ghana.

In our population of adolescent girls from north-eastern Ghana, adjusting PF, TfR and retinol-binding protein (RBP) for biomarkers of inflammation with the internal regression correction approach and Thurnham *et al.* [83] correction had little influence on the intervention effects. Excluding subjects with SCI reduced the sample size (*n*=243, 39.1%), and our findings (*results not shown*) were not different from the internal regression correction, and Thurnham correction approaches.

7

Multiple-Micronutrient Fortified Foods, Cognition, and Height

Emerging evidence indicates that the adverse effects of undernutrition on cognitive performance are not only limited to childhood but manifest during adolescence as well [90, 92–94]. However, our randomized trial found inconclusive evidence that the consumption of MMB improves cognition and anthropometric indices. Evidence of an effect was limited to anaemic subjects for whom MMB consumption improved working memory and mathematics score. Our findings support the results of three previous meta-analyses [72–74] which concluded that multiple-micronutrient fortified foods consumption only results in minor improvements in anthropometric indices and cognition of children and adolescents.

The modest effects of the MMB on iron and vitamin A status in our study may explain the modest improvements in cognition and the anthropometric indices of the girls. When decreased, brain iron stores may impair the activity of iron-dependent enzymes, necessary for the synthesis, function, and degradation of neurotransmitters such dopamine, serotonin, and noradrenaline [95]. Iron deficiency, apart from provoking important physiological repercussions, also adversely affects adolescents' cognitive ability and behaviour [10]. Iodine deficiency can also impair adolescents' cognitive function [96], but the MMB used in our study included iodine. However, we could not ascertain the role of iodine in the cognition of our subjects since we did not measure their iodine status.

Besides the lack of effect of the MMB on micronutrient status, existing infections can lead to neurocognitive dysfunction and reduced school performance for children [97]. Overall, the factors associated with cognition in children and adolescents are multi-factorial, with past (stunting) and current (e.g., ID) nutritional factors and non-nutritional factors such as parasitic infections, socio-economic status, and school environment being implicated [90]. The lack of effect on micronutrient status and the similarity in the energy content of the MMB and UB may explain the non-significant difference between the MMB and UB groups in height-for-age z-score. Additionally, improvements in linear growth relate to prolonged exposure to improved conditions of nutrition and health [63]. The 26-week duration of our intervention, although sufficient to observe a trend, was plausibly too short to find an effect for height. Short duration of intervention is mentioned as a reason for non-significant improvements in the cognition and anthropometric indices of children and adolescents following the consumption of fortified cereal foods [73]; a continuous consumption of fortified foods alongside regular treatment of infections may be more beneficial. The cognition of adolescents has often been assessed as a secondary objective in intervention studies with multiple-micronutrient fortified foods; hence, the minimum duration required for detecting an effect has not been well established. Nonetheless, based on the three systematic reviews and meta-analysis mentioned above, a minimum intervention duration of a year would suffice to detect an effect.

Menarche and Micronutrient Status

In Chapter 7, we explained how menarche may influence vitamin A and iron status. In brief, increased nutrient requirements after menarche plausibly induces a compensatory absorption of vitamin A for post-menarche girls, explaining the improvement in their vitamin A status in our study. Our finding suggests the need for intervention programmes among post-menarche girls to compensate for the additional vitamin A for growth and puberty and to prevent VAD. However, iron losses, associated with blood loss and the pro-inflammatory nature of menstruation [10, 98] probably have more negative effects on iron absorption than any possible benefit from a compensatory absorption during menstrual loss. The increased iron demand and loss may be greater than erythropoiesis, explaining the decline in haemoglobin for post-menarche girls despite their improved vitamin A status. Also, the observed reduction in haemoglobin implies that bone marrow erythropoiesis could not match the pace of blood volume expansion. This also possibly explains the modest increase in TfR in post-menarche girls in our study. One of the reasons stated in the literature for the compromised iron status of adolescent girls is the loss of iron with menstruation in addition to the rapid growth [99]. According to Hallberg *et al.* [100], the average menstrual blood loss in 15-year-old girls is ~28 mL per period, corresponding to a daily loss of ~0.4 mg iron. The role of menarche in the efficacy of micronutrient interventions warrants further investigation.

Methodological Reflections

This section reflects on the methodological challenges related to the studies in chapters 3–6, which may potentially influence the conclusions in this thesis, specifically internal and external validity issues. Conclusions may be biased, because of errors in the study (internal validity); this can also lead to a bias in the external validity (a generalization of the findings) of the study. In the following paragraphs, we discuss possible bias sources in the secondary analyses and RCT.

Internal validity

The internal validity of the study relates to random errors or systematic errors in the study, including selection bias, measurement errors (information bias), and confounding [101, 102], which we discuss in the succeeding paragraphs.

Analyses of Secondary Data

Study Design Strengths and Limitations: Chapters 3-5 were based on cross-sectional data from the baseline of an impact evaluation of the GSFP [103] and three rounds (2003, 2008 and 2014) of the GDHS [40, 104–106]. Hence, inferences of possible causalities are speculative, and our findings therein are limited to the description of observed associations. Further, the GDHS data is limited to respondents of fertile age (15-49 years). A key strength of the GDHS data is that it allows for examining health and nutritional status changes over time using repeated cross-sectional designs as in Chapter 4 of this thesis.

Selection bias: In the multistage sampling of the GDHS, all eligible household members in a stratum were interviewed. Respondents from the same stratum may be similar and thus serially correlated, but SURVEY statistical procedures allow for correct estimation of variances/standard errors by Taylor Series Linearization [107], which we used in our analysis of the GDHS. The national coverage and high response rates (typically ≥ 95% in the GDHS) also ensure that the study population is representative of the target population.

Measurement error: It is worth noting that some portions of the GSFP and GDHS data, such as household dietary diversity and the frequency of consuming fruits and vegetables in the last week, may be subject to information bias, including socially desirable answers and recall bias. However, the core DHS questionnaire is standardised and pre-tested to ensure comparability across populations and over time [108]. Besides, adaptations were made in the GDHS to fit the local context [40, 104–106]. Further, standard data collection procedures and interviewer training ensured reliability. Likewise, the data in Chapter 3 utilised validated scales and tools, which were also pre-tested and adapted, minimising any possible information bias, influencing our findings.

Confounding: In Chapters 3-5, the analyses for factors associated with malnutrition and PHH were exploratory; we thoroughly modelled several potential explanatory variables available in the data using the socio-ecological framework proposed in chapter 2. Nonetheless, we could not account for all possible explanatory variables in our framework as they were missing from the data. Notably, dietary intake data were not available and thus unaccounted for in the statistical models. Accordingly, we cannot rule out the effect of residual confounding in our statistical models.

Statistical modelling: In the statistical analysis, three hierarchical models were built: model 1(individual only), model 2 (individual plus household) and model 3 (individual, household, and community). The individual-level model was first built from a univariate analysis using the criteria $P \leq 0.25$; household variables meeting the same criteria (in model 1) were included in model 2 and likewise community-level variables for model 3. In the final models, only significant predictors with a good model fit were retained and the independent effects of each predictor, after adjusting for other significant predictors were presented. Though our approach fails to present the unadjusted effects of each predictor, it acknowledges that there is a complex interplay of determinants that contribute to the observed associations. Accordingly, we tested for interactions among predictors in the final models using pairwise interaction terms; only significant interaction effects were presented.

Randomised Controlled Trial

To the best of our knowledge, Ten2Twenty-Ghana is the first randomised trial with multiple-micronutrient fortified foods in a sub-Saharan Africa population of adolescent girls where anaemia and IDA were prevalent. The study design of Ten2Twenty-Ghana ensures that causal associations can be described. We employ the Cochrane tool of assessing bias in the RCT in the following paragraphs. **Selection bias:** The RCT was limited to only the primary schools where the girls were primarily pre-menarche; junior high school girls were excluded, a result of the GIFTS (**G**irls' **I**ron **F**olate **T**ablet **S**upplementation) programme among adolescent girls in junior high schools [113]. Consequently, the post-menarche group was under-represented, but we included girls who were close to attaining their menarche (age >13 years), following the mean age at menarche for girls in Ghana [109–111]. While the preceding may introduce a selection bias, the rigorous randomisation ensured that the girls were equally distributed in all groups, and any remaining bias was non-differential with little influence on our findings and conclusions.

Non-response of subjects may also lead to selection bias, as healthier or more knowledgeable subjects may be selected, or the reverse [102]. However, less than 1% of the subjects screened were reluctant to participate in the RCT during the screening; reluctant subjects did not want to eat biscuits for six months as they alleged that they were allergic to biscuits.

Performance bias: Blinding of subjects and the research team was achieved by repackaging the biscuits into clear zip-lock bags with yellow and red stickers to distinguish between them. When received, the MMB and the UB's original packages were distinguishable, hence the re-packaging which ensured that the blinding of the first author, the field team, and subjects. We discussed the re-packaging process in detail in appendix 2 [112] of Chapter 6. There was unlikely any differential treatment between groups in the care provided to subjects, or in exposure to factors other than the intervention.

A lack of treatment effect in trials may result from poor adherence to treatment [113, 114]. Except for two communities (schools) where most girls failed to meet the per-protocol criteria (adherence ≥80%), adherence in our study was high (≥ 90%), and no difference between the treatment groups was found for adherence. Adolescents have a likeness for snacks [115], and biscuits are often consumed as snacks and are unlikely to replace meals at home [76]. More so, biscuits are handy and easy to manage in the field. Hence our choice of biscuits as the food vehicle in the present study. Non-adherence was mostly related to the fear that the intervention was a family planning programme as it targeted only girls. We, however, managed to defuse the family planning myth through engagements with several stakeholders, including the schoolteachers, parent-teacher association, school management committee, community leaders and parents (mothers/fathers). In one case, where one of our girls got pregnant, she attributed missing her menstrual period to the intervention (biscuits). Nevertheless, we quickly defused the misinformation by visiting and engaging the girl, her parents, and the community leaders with the study's medical practitioner. The preceding highlights the need for a thorough stakeholder engagement when implementing a community health and nutrition intervention programme in a setting like ours. In our randomised trial, we found no differences in the conclusions when conducting a per-protocol analysis; besides, the baseline characteristics for the subjects when following intention-to-treat or per-protocol criteria did not differ, which suggests that multiple imputations of missing data for subjects lost to follow-up in the intention-to-treat analysis did not bias our conclusions.

Attrition bias: A differential loss to follow-up in the treatment groups can bias study findings [102, 113]. In our RCT, the attrition rate (*n*=33, 5.3%) was less than the anticipated 10% attrition rate and did not differ between the biscuit groups. A little over half of the subjects lost to follow-up were post-menarche and had experienced their menarche a year earlier (12.8 years vs 13.7 years, P=0.06) compared to those who completed the study. They were more likely to have a CRP > 5mg/L (21.2% vs 5.4%, P=0.003), had a significantly higher BAZ (-0.4±1.0 vs -0.8 ± 0.8; P=0.04) and hence a higher prevalence of overweight/obesity (9.1% vs 1.0, P=0.01). Girls who were lost to follow-up mostly migrated to the urban areas in search of menial jobs. Interestingly, 2 of our subjects got pregnant, and one got married during the intervention, dropping out of school and the intervention. The preceding confirms that teenage marriage and pregnancy is prevalent in our target population.

Detection bias: One possible source of detection bias is measurement error related to the measurement of Hb by finger prick at baseline, and with venous blood at the end-line, which we addressed in our study design (Chapter 6). Haemoglobin concentration is higher in capillary blood as in finger prick compared with venous blood because venous blood is deoxygenated [116]. In the RCT, this bias probably resulted in a systematic overestimation of the baseline haemoglobin, which was by finger prick compared to the end-line haemoglobin, which was by venous blood. However, the bias was systematic and non-differential for the MMB and UB groups; hence its effect on the post-intervention differences in Hb between the MMB and UB was unlikely to bias our conclusions. Another possible source of measurement error was the choice of RBP for vitamin A status. Retinol is the recommended biomarker for assessing populations' vitamin A status. Yet we used RBP to assess the vitamin A status of subjects in the present study. RBP is an acute-phase protein, suppressed by protein-energy malnutrition, infection, and inflammation [117]. However, when combined with CRP, RBP has been shown to produce an unbiased VAD estimate [118]. Hence, while it was plausible the girls' RBP levels was not a true reflection of their vitamin A status, we presume adjusting for CRP produced an unbiased estimate for our study population. More so, Larson *et al*. [119] illustrated that the internal regression correction approach we used accounts for the severity of inflammation when estimating VAD prevalence in regions with high inflammation and malaria.

Further, the present study is apparently the first to use the National Institute of Health Test Cognition Battery (NIH-TCB) [120, 121] in a rural African setting. Although the NIH-TCB has not been validated for settings in rural Africa, we opted for it after a review of cognitive assessment tools, since it is validated for use in diverse study designs and is easier to use. The main limitation of the NIH-TCB is its automation on iPads which may be unfamiliar with subjects from a rural setting as in our study. Nonetheless, automated trial tests preceding the actual test ensured that the girls familiarised themselves with the clicking on the screens. Besides, interviewers assisted the girls in clicking when necessary, and we assessed 'interviewer' as a possible confounder in our data analysis.

Other bias: Although randomisation ensures that treatment groups are similar, it may be incomplete [122, 123]. A possible confounder for the efficacy of the MMB was malaria infection; malaria alters iron indicators' concentrations (notably TfR) independent of iron status [91, 124]. Nevertheless, subjects with malaria at baseline or during the intervention were promptly treated following the Ghana Health Service protocol [125]. Importantly, our mid-point validation of the malaria rapid test kit (*n*=68) showed poor sensitivity (sensitivity=13.3%; specificity=84.9%) of the test kit. Hence, we cannot rule out the effect of residual confounding from asymptomatic malaria despite the adjustment for malaria as a covariate of micronutrient status in the internal regression correction approach we used. However, it must be said that the bias was non-differential and less likely to influence our conclusions. Another possible confounder for the efficacy of the MMB compared to the UB is SCI which we discussed earlier.

External validity

The external validity of a study relates to the generalization of the findings to a wider population [101, 102]. We reflect on the generalizability of our findings from the secondary analysis and the RCT in the following paragraphs.

Secondary analysis of cross-sectional data: The study population from the impact evaluation of the GSFP (Chapter 3) is rural, limiting the generalisation of our findings to rural school-aged children and adolescents in Ghana. Although the study population in chapter 3 included only school-going school-aged children and adolescents, school enrolment in Ghana including the girl-child has been over 85% since 2013 [126]. Accordingly, most school-aged children and adolescents were included in the sampling frame. Additionally, the sample frame in chapter 3 included all districts in Ghana

[103]; the study population, therefore, represented all rural school-aged children and adolescents in Ghana and similar settings. The study population in the GDHS data analyses was selected using multistage sampling [127] and included adolescents aged 15-19 years from rural and urban areas throughout the country. The findings may thus be generalised to all 15–19-year-old adolescent girls (Chapter 4) and adolescent boys and girls (Chapter 5) in Ghana. Although the study population in chapter 3 can only be generalised to rural school-aged children and adolescents, it also fills the gap for 10–14-year-old adolescents in the GDHS.

Randomised controlled trial: The RCT population included rural adolescent girls aged 10-17 years and can thus be generalised to all rural adolescent girls aged 10-17 years in contexts as northern Ghana.

General Conclusions

Based on our findings and discussions on the secondary analysis of cross-sectional data and the randomised controlled trial, conducted in this thesis, we come to the following five conclusions:

1. Our findings point to a triple-burden of malnutrition for Ghanaian adolescent girls which include a continual burden of protein-energy undernutrition, an increasing burden of overnutrition, which was associated with cardiovascular risk, and a persistent burden of severe anaemia.
2. A broad range of context-specific socio-cultural and economic factors at several levels influence adolescent nutritional status.
3. In our RCT among rural Ghanaian adolescent girls, we found no evidence that the consumption of MMB improves micronutrient status, cognition, and height, but MMB consumption reduced the prevalence of deficient/low vitamin A status in post-menarche girls.
4. Our findings suggest menarche may influence the vitamin A status of girls but the effect of menarche on the efficacy of micronutrient interventions warrants further investigation.
5. Our findings suggest boys are equally affected by malnutrition and poor cardiovascular health.

Implications For Public Health Policy and Future Research

Despite the lack of effect of the MMB in our randomised trial, the burden of micronutrient problems (anaemia, IDA, and VAD) in addition to the prevalence of adolescent pregnancy in the present study implies that food fortification programmes remain relevant micronutrient intervention programmes. Evidence from girl-centred programs in LMICs suggests that longer program exposure and multi-component programs may be more effective [128]. Accordingly, continuous consumption of fortified foods compared to unfortified foods may be more beneficial. In 2017, UNICEF in partnership with the Ghana Health Service and Ghana Education Service initiated a phased Iron and Folic Acid (IFA) Supplementation programme called "*Girls' Iron-Folate Tablet Supplementation (GIFTS)*" for menstruating girls aged 10-19 years in some selected regions including Northern Ghana [129]. Although the GIFTS programme is commendable, poor adherence may limit its effectiveness. For instance, Godin *et al.* [130] assert that the average intake adherence for the GIFTS programme in Ghana is about half of the available tablets. Equally, Dubik and colleagues [131] found only a quarter of adolescent girls in the Tamale Metropolis of Ghana compliant (≥ 70% of the expected dose) in the GIFTS programme. The programme can be improved upon using fortified snacks, which may be more acceptable to adolescents compared to tablets. An increase in the iron levels in the Obaasima products is desirable to increase the amount of iron intake per serving.

While our finding suggests malaria screening may not be the best approach to take malaria into account in micronutrient intervention programmes, feasibility and logistic constraints imply that screening tools such as malaria rapid test kits remain a valuable tool. Our findings suggest menarche may influence the vitamin A status of girls but the effect of menarche on micronutrient status and the efficacy of micronutrient interventions warrants further investigation. Future efficacy trials need to either screen and exclude subjects with SCI at baseline or include them as an additional arm. Likewise, if the intervention focuses mainly on anaemia and iron status, subjects with VAD at baseline also need to be included as an additional arm or excluded from the intervention programme. However, from a public health perspective, the prevalence of inflammation in the randomised trial emphasises the need to strengthen the mass deworming of school children against schistosomiasis and soil-transmitted helminths in Ghana. Schools may be an effective platform for reaching this population through integrated deworming, water, hygiene, and sanitation programmes [132] since most adolescents including girls are presently in school [126]. Additionally, micronutrient interventions in settings as ours need to include regularly deworming of the participants. In LMICs, inflammation and infections are often related to poor WASH [133]. Continuous exposure to poor sanitary conditions, in particular constant, faecal-oral contamination, can lead to chronic inflammation which can damage intestinal villi [134]; sensitization and behaviour change programmes on WASH alongside micronutrient programmes may offer some benefit [135, 136].

Government policy efforts to improve food security are likely to reduce thinness and improve the health of adolescents [137]. However, Hawkes and colleagues [138] argue that a focus on undernutrition in countries undergoing a rapid nutrition transition (like Ghana) raises the risks of poor-quality diets, obesity, and diet-related non-communicable diseases. Accordingly, our findings emphasise the so-called "double-duty actions" to tackle under- and overnutrition holistically. Based on our socio-ecological framework (Chapter 2) and results on predictors of malnutrition (Chapters 3-5), these double-duty actions must target a complex set of determinants of malnutrition, ranging from individual- to household and community-level factors. Notably, for adolescent girls, our findings suggest girl-child education may reduce stunting and efforts to prevent teenage marriage and pregnancy may reduce anaemia. About one-tenth of the girls reported ever giving birth (Chapter 4), and two subjects in the RCT got pregnant (Chapter 6), emphasising that teenage marriage and pregnancy is still prevalent. Access to improved reproductive health care and education for adolescent girls should be also improved.

Public health interventions to tackle childhood and adolescent overweight/obesity are presently lacking in Ghana. Nonetheless, integrated interventions including diet, exercise, and behaviour change have been recommended for the prevention and management of childhood obesity [139]. A hypocaloric diet plus physical activity is effective in reducing weight and blood pressure [140]. Physical activity is a low-cost intervention, but behaviour change programmes are required to promote physical activity among adolescents, especially in urban areas. It is worth mentioning that the built environment needs improvement in both infrastructure and safety to promote physical activity. Behaviour change interventions promoting healthy eating and snacking among children and adolescents may also be helpful; such interventions must include the family. Children and adolescents are more likely to adhere to family dietary patterns and menus since their diet is largely from the home or influenced by family dietary patterns [141]. The basic educational system can be revised to include compulsory modules on physical activity and healthy diets in schools. School interventions aiming at peer-to-peer influence may target school clubs and associations. However, innovative programmes targeting out-of-school girls are also desirable to involve girls who have dropped out of school.

From a public health point of view, our findings highlight the need to include adolescent boys alongside girls in nutrition and health intervention programmes, for a holistic improvement in nutrition and health outcomes of adolescents. Finally, the inclusion of boys in micronutrient intervention programmes targeting girls in settings as ours may ensure trust and improve adherence with the treatment.

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Summary

Summary

In sub-Saharan Africa, adolescent girls are an important target group for nutrition interventions due to the prevalence of micronutrient deficiencies, the risk of having an early pregnancy and the risk of intergenerational transfer of dis(advantages). The present weak understanding of the socio-cultural and economic determinants (SCE), trends-over-time, contextual correlates of malnutrition and sex-specific analysis delays progress in the design of optimal targeted policies and programmes that can improve adolescents' nutrition holistically. In addition, although food fortification is potentially a useful strategy to improve nutrition among adolescent girls, knowledge of the efficacy and timing (regarding menarche) of such interventions, especially in resource-poor settings, is limited. This thesis primarily aims to examine the interrelations between nutrition, social, and economic trajectories during adolescence among girls in Ghana using a systematic narrative literature review on the SCE determinants and consequences of adolescent undernutrition, analyses of secondary cross-sectional data in Ghana, and a randomised placebo-controlled trial among adolescent girls in north-eastern Ghana. **Chapter 1** provides the background and rationale that informed the thesis and describes the specific research objectives for each chapter.

In chapter 2, we undertook a systematic review of published literature to provide a narrative overview of the SCE determinants and consequences associated with undernutrition among adolescents in low and lower-middle-income countries. We identified 98 articles from PubMed, SCOPUS and CAB-Abstracts on determinants and consequences of undernutrition as defined by stunting, underweight, thinness and micronutrient deficiencies. At the individual level, significant determinants included age, sex, birth order, religion, ethnicity, educational and literacy level, working and marital status. At the household level, parental education and occupation, household size and composition, income, socio-economic status, and resources were associated with undernutrition. Few determinants were found at the broader community level, which included the residence, sanitation, school type, and seasonality, mainly because most studies did not consider the community or environmental factors. The sex-specific differences in undernutrition for adolescents were inconclusive. The consequences of adolescent undernutrition were mainly related to education and cognition. Most studies were cross-sectional in design, limiting inferences of causality to description of associations, and very few studies were found for sub-Saharan Africa indicating clear research gaps that informed the next parts of this study.

In **chapter 3,** we describe a secondary data analysis of baseline data of an impact evaluation of the Ghana School Feeding Programme indicating that anaemia is a severe public health problem among rural school-aged children (6-9 years; n=323) and adolescents (10-17 years; n=319) in Ghana, irrespective of sex. The mean haemoglobin was 113.8 ± 13.1g/L and the overall prevalence of anaemia was 52.3%, being higher among school-aged children (55.1%) compared to adolescents (49.5%). We identified child's age (β=2.21, P<0.001); farm diversity score (β=0.59, P=0.036); and

agro-ecological zone (*P*-trend <0.001) as the independent predictors of haemoglobin concentration of school-aged children. Household asset index (HAI) (*P*-trend=0.04) and agro-ecological zone (*P*-trend <0.001) were predictors of haemoglobin in adolescents. Agro-ecological zone and age were independent predictors of anaemia, but the effect of age was only significant for girls and not boys [prevalence odds ratio (POR) 1.35, 95% CI (1.04, 1.76) vs POR 1.14, 95% CI (0.88, 1.46)]. School-aged children in households with maize stock were less likely to be anaemic [POR 0.55, 95% CI (0.32, 0.97)] compared to their peers in households without maize stock. Household dietary diversity score (β=0.59, *P*=0.033) was associated with haemoglobin status for the full sample only. We concluded that protective strategies for anaemia may include context-specific interventions that aim to improve household farm diversity, availability of grain stock and socio-economic status through income generation programmes. Given the severity of anaemia in other agro-ecological zones compared to the forest zone, iron and folic acid supplementation may be prioritized in these zones for both school-aged children and adolescents, both for boys and girls.

Chapter 4 examines the trends-over-time and the factors associated with malnutrition among adolescent girls in Ghana using cross-sectional data from 3 nationwide Ghana Demographic and Health Surveys (GDHS) conducted in 2003 (*n*=983), 2008 (*n*=955) and 2014 (*n*=857). Stunting was about 8% among the adolescent girls in 2003, declining by only 1.5% points in 2014. Although only 2% of the girls were thin in 2003, the prevalence decreased by <1.0% point in 2014. However, overweight/obesity among the girls increased by 4.3% points in 2014 from 10.0% in 2003, after adjusting for significant predictors of overweight/obesity including household size, wealth index, and the marital status of the girl. Anaemia remained severe (2003: 44.3%; 2008: 62.1% and 2014: 47.3%) without a clear trend. A low level of education of the adolescent girl was positively associated with stunting. Increasing age was positively associated with stunting but inversely associated with thinness and anaemia. Girls who had ever given birth were more likely to be anaemic compared to those who never did. A lower level of household wealth and increasing household size were negatively associated with overweight/obesity. Urban dwelling girls were less likely to be stunted. We concluded that there is a steady burden of undernutrition and rising overnutrition, emphasising the need for the so-called double-duty actions to tackle malnutrition in all its forms in Ghanaian adolescent girls.

We also undertook a comparative analysis of data of non-pregnant adolescent girls (*n*=857) and adolescent boys (*n*=870) aged 15–19 years from the 2014 GDHS to assess and contrast the association between malnutrition, pre-hypertension/hypertension (PHH) and sex among adolescents (**Chapter 5**). Compared to adolescent girls, boys were more than twice likely to be stunted [Prevalence ratio (PR) 2.58, 95% C.I (1.77, 3.76)], underweight [PR 2.67, 95% C. I (1.41, 5.09)] and about twice likely to have PHH [PR 1.96, 95% C. I (1.47, 2.59)] but less likely to be overweight/obese [PR 0.85, 95% C.I (0.08, 0.29)]. Girls were more at risk of the detrimental effects of poor education on stunting and PHH. A higher empowerment index while inversely associated with stunting for girls [PR 0.82, 95% C.I (0.67, 0.99)] also increased their risk of overweight/ obesity [PR 1.31, 95% C.I (1.02, 1.68)]. A higher household wealth index (HWI) increased the likelihood of overweight/obesity for adolescent girls but was inversely associated with stunting and PHH for adolescent boys. Improvement in household water, hygiene, and sanitation (WASH) was associated with a lower risk of stunting by 15% for adolescent boys. Overall, our findings suggest a double-burden of malnutrition with existing and upcoming non-communicable disease burden for adolescents in Ghana. Our findings also highlighted the need to target adolescent boys alongside girls in nutrition and health intervention programmes.

Chapter 6 describes the implementation of our primary study, *Ten2Twenty-Ghana,* a 26-week randomised placebo-controlled trial with multiple-micronutrient fortified biscuits (MMB) compared to unfortified biscuits (UB) among adolescent girls in north-eastern Ghana. The study evaluated the effect of consuming MMB 5 days/week for 26 weeks compared to UB on micronutrient status, height, and cognitive performance of female adolescents. We also explored to what extent the intervention effect varied in girls that had or did not yet experience menarche. We found no effect of the intervention on plasma ferritin, transferrin receptor concentration and retinol-binding protein. MMB consumption did not affect anaemia, micronutrient deficiencies and anthropometric indices at the population level. After adjusting for the girl's baseline age, height-for-age z-score and baseline micronutrient status, vitamin A deficiency increased by 6.15% (95% C.I 0.72, 11.59) for pre-menarche girls in the MMB compared to the UB group, but deficient/low vitamin A status decreased substantially by 9.63% (95% C.I -18.94, -0.32) for MMB girls who were post-menarche compared to their UB peers. Only in anaemic subjects, we found evidence that MMB consumption improves mathematics score and working memory of pre-menarche girls. We concluded that MMB consumption did not improve iron status in our setting but decreased the prevalence of low vitamin A status in post-menarcheal girls.

Finally, **Chapter 7** discusses the main findings, methodological considerations, the public health and policy implications of the findings and suggestions for future research. Overall, our findings pointed to a triple-burden of malnutrition for Ghanaian adolescent girls which included a steady burden of protein-energy undernutrition, an increasing burden of overnutrition, which was associated with cardiovascular risk and a persistent burden of severe anaemia, affirming the need for double duty actions to holistically tackle malnutrition. Intervention programmes must tackle a broad range of context-specific socio-cultural and economic factors at several levels (individual, household, and community) that influence adolescent nutritional status. We concluded in our primary study that MMB consumption did not improve micronutrient status, cognition, and height, but reduced the prevalence of deficient/low vitamin A status for post-menarche girls. This thesis also suggests menarche may influence the vitamin A status of girls, but this warrants further investigation. Our findings also suggest boys are likewise affected by malnutrition and poor cardiovascular health; hence they should be included alongside girls in nutrition and health intervention programmes.

Acknowledgements

Acknowledgement

A journey that started in early 2017 has come to an end but yet becomes the start of another journey for me. This journey was the collaborative effort and support of several people around me. I am highly indebted and grateful to my daily supervisor, **Dr Inge D. Brouwer** for the opportunity given me to be part of the Ten2Twenty research project. You did not doubt my ability and competence and I simply cannot write enough to thank you. I have learnt a lot from you since 2014 when I first joined you in the food consumption and food-based dietary guidelines project in northern Ghana. Your support and guidance made me realize my dream of undertaking a PhD programme. You are undoubtedly a great mentor, and I am blessed to have been your student. Your many necessary questions and queries refined my output. You motivated and natured my capability, even when life became difficult for me at the start and ending of the project with the loss of my beloved and field supervisor.

My sincerest gratitude to my promotor **Prof. Edith J.M. Feskens** for her invaluable inputs that ensured the completion of this PhD. Despite, your tight and busy schedules as Chair of the Global Nutrition Group of Wageningen, you found time to discuss, read and advise me on my work. Your mentorship is invaluable, and I am still learning to "*write as if I pay a cent for every single word*".

I am also grateful to **Saskia J.M. Osendarp** of Micronutrient Forum for her inputs and support during the design of the study; you made the randomized trial possible by assisting us to secure support from Sight and Life for the biscuits. To **Prof. Hilde Bras**, many thanks for the critical inputs into my study design and secondary analysis; I wish you had stayed till the end of the project. To **Aulo Gelli and Elisabetta Aurino**, thanks for allowing me to work with you especially on your impact evaluation data of the Ghana School Feeding programme; I realize I still have a lot to learn from you both. To **Prof Hans Verhoef**, thanks for all the statistics I learnt in the last year and a half and all the critical plenty questions. I believe, I still have a lot of statistics to learn from you.

I am grateful to all staff members of the Division of Human Nutrition and Health at Wageningen University. My sincerest gratitude to the division secretariat: **Jasmijn Mater, Gea Brussen** and **Lucy Elburg** for arranging my visa, logistics for my data collection in Ghana and my stay in Wageningen. My special thanks to **Karin Borgonjen** for assisting me in the dietary data collection training and analysis. To **Ilse de Jager**, thanks for being my first immediate supervisor and helping me understand dietary data; I was honoured to learn from your PhD project.

Each time I was in Wageningen, I missed my family and friends in Ghana; yet again, I missed Wageningen which has become a second home to me, a few months after arriving home. To all my colleagues who made Wageningen a home for me, I am grateful to you: **Arli, Donya, Anita Tesfaye, Tsitsi**, **Maria, Inga, Erick, Santiago, Ibuku, Liangzi, Pol**. To the rest of my colleagues in the Ten2Twenty project (**Asrullah, Lowela, Ursulla**) and Edema-Steernberg Foundation colleagues

(**Kriste, Amy, Rachelle, Yvette, Christina**), it was nice meeting you all and having discussions with you.

To the Ghanaian community in Wageningen and the lovely **Hoek's family**, thank you all for making me feel loved and welcome in Wageningen. I cannot forget to say thank you to the **International Catholic Community (ICC)** and the **Africa Choir of the ICC** of Wageningen where I fellowshipped while in Wageningen; our togetherness and fellowship inspired me even when life was sometimes difficult for me.

I am grateful to the Ghana Education Service (GES) in the Mion District, the Ghana Health Services (Mion District) as well as all the communities, the girls and their parents who participated in the Ten2Twenty-Ghana Research Project. For their enthusiasm and commitment to the fieldwork, my sincerest appreciation to the Ten2Twenty-Ghana Field team (**Emmanuel, Kennedy, Ambrose, Salifu, Solomon, the two Fauzias, Esther, Millicent, Jennifer, Justina, Salamatu, Salimatu, Feruza, Hamida, Ukashetu, Azaratu, and Huzeifa**). My sincerest gratitude to all MSc students from Wageningen UR (**Vera Bunt, Judith ten Have, Adjoa A. Amoafo, Kris Woltering, Joseph Collins Kodom, Betül Uyar, Welmoed Sprong and Rosil Hesen**) who assisted us at different time points in the field.

Without the funding of our sponsors and donors, this research would not have been possible; we are grateful to the **Edema Steernberg Foundation, Judith Zwartz Foundation, Nutricia Foundation** and **Sight and Life-Switzerland** for their financial support.

To my beloved of blessed memory (**Jessica Suurikyibe Baissana-Azupogo**), I do not wish to repeat another emotional tribute to you. We journeyed together from 2012 until 3 weeks before my initial departure in March 2017 to Wageningen for the start of this PhD. Together, we started planning my journey and the months without me at home; unfortunate you could not live to see me start the project. The moments shared with you were joyful, but memories of the ending still bring me some pain. I wish you were there to witness this new chapter in my life, but God had a different plan. May you continue to rest in perfect peace.

To my newfound love-**Mavis**, and my daughter-**Eliane Wepea**, thank you for the sacrifices you made for me to complete this thesis; it never would have been possible without your sacrifices, understanding and support, especially in the last year when writing was demanding. Especially to my love, thanks for keeping the home while I was busy putting these pieces together. To my daughter, thanks for asking all the questions and always reminding me "*dada, we haven't played today*", hopefully, I have more time now to play with you daily. I am truly grateful to my mum **Ruth**; you did not only groom me, but you were there for me to care for my daughter, your granddaughter (Eliane) when I needed that most. Each time I was away in Wageningen, I was comfortable knowing that Eliane was

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in the safest care and home. The support and understanding of the rest of my family made it possible for me to focus on this PhD. To my eldest brother **David**, thanks for always being there for all of us. To the rest of the **Azupogo family,** many thanks for the love and support; your encouragement and support made it possible for me. May the peace and love of Christ continue to keep and unite us all.

I simply cannot thank everyone in this thesis. To everyone who contributed in diverse ways to the completion of this thesis. Many thanks for your support.

Dankjewel! **Die lei!** **Mm pooh-see-ah!**

Special Acknowledgement and Tribute to Prof Abdul-Razak Abizari

Prof. Abdul-Razak ABIZARI was not only a great mentor but a friend in the last 4 years before his demise. Many were the plans for research and academia we discussed in the last 2 years and were looking forward to. I first learnt of Wageningen University and Research in one of your lectures as your student in nutrition epidemiology during my BSc in the 2007/2008 academic year at the University for Development Studies. I told myself I would want to continue my education there, which I did in 2013. During my MSc, I returned to Ghana in July 2014 to participate in a food consumption survey for which you were the local principal investigator. With your support and that of my supervisors in the Netherlands, I learnt how to conduct, process and analyse dietary data from a quantitative 24-hour dietary recall. You mentored me in writing my first 2 scientific articles (published in PLoS One) and in writing rebuttals. I would never forget your statement "*always aim to publish in a high impact international journal; ensuring that you are not just a local champion*". This statement lingers in my mind whenever I am putting up an article.

When I went through a period of crisis, you encouraged me to keep on and look forward to the future. You mentored and guided me in undertaking the Ten2Twenty-Ghana field project, ensuring that all the technicalities were resolved. There were moments during my fieldwork when I felt some things were impossible doing, yet you always found a way out for me. I wouldn't forget having to re-package about 90,000 packs of biscuits for a trial which I almost gave up but you made it possible. You made difficult tasks look so simple; you added fun to work and it was always pleasing to be around you. I have lost a Great Mentor and a Friend and will forever miss you.
May God Grant Unto You Eternal Rest.

About the Author

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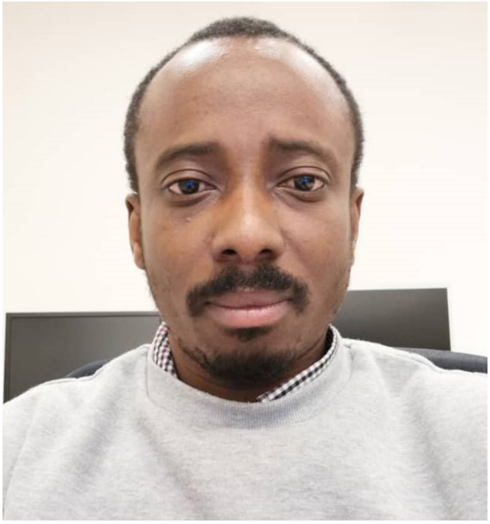

Fusta Azupogo was born on the 16th of September 1984 in Bolgatanga, in the Upper East Region of Ghana. He had his secondary education from 1998-2004 at Notre Dame Minor Seminary High School in Navrongo, Ghana. After his secondary education, he was admitted into the University for Development Studies (**UDS**) in 2005, where he successfully graduated in 2009 with a distinction in a 4-year bachelor's degree in Community Nutrition (BSc Community Nutrition).It was at UDS he developed a passion for nutrition, and to utilize his knowledge in contributing to solving the food and nutrition problems facing the developing world, particularly northern Ghana where he comes from.

In 2011, he worked as a research assistant in the Department of Family and Consumer Sciences, at the Nyankpala Campus of the UDS; he continued to work with the Department as an assistant Lecturer and since 2016 as a Lecturer. Fusta was first admitted to Wageningen University and Research to study the MSc Programme, Nutrition and Health in 2010 but as he could not afford the cost and without a fellowship, he deferred his admission until 2013 when he was awarded a NUFFIC fellowship for his study in Wageningen UR. His MSc thesis focused on the "*Development of Food-Based Dietary Recommendations for 6-8 Months Old Breastfed Infants in Karaga District-Ghana, Using Optifood-Linear Programming*".

He was awarded the Edema Steernberg Foundation and Judith Zwartz Foundation scholarship for his PhD programme within the Ten2Twenty Research Programme in November 2016 under the supervision of Prof. Edith J.M. Feskens and Inge D. Brouwer. He has peer-reviewed and published several scientific articles in some high impact international journals. Fusta is presently a member of the Nutrition Disparity Network of Wageningen University and the Ghana Academic of Nutrition and Dietetics. He can be contacted through his official (fazupoko@uds.edu.gh) and personal (azfusta@yahoo.co.uk) emails.

List of Publications

Peer-reviewed Journal Publications

1. **Azupogo, F.,** Abizari, A.-R., Aurino, E., Gelli, A., Osendarp, S. J. M., Bras, H., Feskens, E. J., & Brouwer, I. D. (2021). Trends and Factors Associated with the Nutritional Status of Adolescent Girls in Ghana: A Secondary Analysis of the 2003-2014 Ghana Demographic and Health Survey (GDHS) Data. Public Health Nutrition, 1–41. https://doi.org/10.1017/s1368980021003827
2. **Azupogo, F.,** Abizari, A.-R., Osendarp, S. J. M., Feskens, E. J., & Brouwer, I. D. (2021). Ten2Twenty-Ghana: Study Design and Methods for an Innovative Randomised Controlled Trial with Multiple-Micronutrient Fortified Biscuits Among Adolescent Girls in North-Eastern Ghana. Current Developments in Nutrition, 5(2), 1–20. https://doi.org/10.1093/cdn/nzaa184
3. **Azupogo, F.,** Abizari, A., Aurino, E., & Gelli, A. (2020). Malnutrition, Hypertension Risk, and Correlates: An Analysis of the 2014 Ghana Demographic and Health Survey Data for 15–19 Years Adolescent Boys and Girls. Nutrients, 12(9), 2737. https://doi.org/https://doi.org/10.3390/nu12092737
4. Boah, M., **Azupogo, F.,** Amporfro, D. A., & Abada, L. A. (2019). The epidemiology of undernutrition and its determinants in children under five years in Ghana. PLoS ONE, 14(7), 1–23. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0219665
5. **Azupogo, F.,** Abdul-Rahaman, F., Gyanteh, B., & Atosona, A. (2019). Hygiene and Sanitation Practices and the Risk of Morbidity among Children 6–23 Months of Age in Kumbungu District, Ghana. Advances in Public Health, 2019, 1–12. https://doi.org/10.1155/2019/4313759
6. **Azupogo, F.,** Seidu, J. A., & Issaka, Y. B. (2018). Higher vegetable intake and vegetable variety is associated with a better self-reported health-related quality of life (HR- QoL) in a cross-sectional survey of rural northern Ghanaian women in fertile age. BMC Public Health, 28(920), 1–13. https://doi.org/https://doi.org/10.1186/s12889-018-5845-3
7. **Azupogo, F.,** Aurino, E., Gelli, A., Bosompem, K. M., Ayi, I., Osendarp, S. J. M., Brouwer, I. D., & Folson, G. (2018). Agro-ecological zone and farm diversity are factors associated with haemoglobin and anaemia among rural school-aged children and adolescents in Ghana. Maternal & Child Nutrition, 15(1), 1–11. https://doi.org/10.1111/mcn.12643
8. Madjdian, D. S., **Azupogo, F.,** Osendarp, S., Bras, H., & Brouwer, I. (2018). Socio-cultural and economic determinants and consequences of adolescent undernutrition and micronutrient deficiencies in LLMICs: a systematic narrative review. Ann. N.Y. Acad. Sci., 1416, 117–139. https://doi.org/10.1111/nyas.13670
9. Abizari, A.-R., **Azupogo, F.,** & Brouwer, I. D. (2017). Subclinical inflammation influences the association between vitamin A- and iron status among schoolchildren in Ghana. PLoS ONE, 12(2), e0170747. https://doi.org/10.1371/journal.pone.0170747
10. Abizari, A.-R., **Azupogo, F.,** Nagasu, M., Creemers, N., & Brouwer, I. D. (2017). Seasonality affects Dietary Diversity of School-age Children in Northern Ghana. PLoS ONE, 12(8), 1–16. https://doi.org/http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0183206

Technical Reports

1. GAIN/USAID. (2017). Technical Brief: Karaga District, Ghana: Infant and Young Child Feeding and Food-Based Recommendations. Findings from an Optifood Analysis and Focussed Ethnographic Study.
2. Brouwer, I. D., Jager, I. de, Borgonjen, K., **Azupogo, F.,** Rooij, M., Folson, G., & Abizari, R. (2017). Background Technical Report. Development of Food-Based Recommendations using Optifood - Ghana. Development of food-based dietary recommendations for children, 6-23 months old, in Karaga District and Gomoa East District, Ghana (Issue May).

Conference Presentations

1. **Fusta Azupogo** and Inge D. Brouwer (2021). Effect of multiple-micronutrient fortified biscuits on cognitive function of adolescent girls in rural northern Ghana. *International Association for Adolescent Health (IAAH)12th World Congress on Adolescent Health. Meeting the Challenge of Global Change 18th-21st November 2021; Online poster*
2. **Fusta Azupogo**, Abdul-Razak Abizari, Inge D. Brouwer (2021). Efficacy of multiple-micronutrient fortified biscuits on micronutrients status and cognitive performance of adolescent girls in Ghana: A randomized control trial. *Agriculture, Nutrition and Health (ANH) Academy, 29th June-1st July 2021; Online, poster*
3. **Fusta Azupogo**, Abdul-Razak Abizari, Saskia J.M. Osendarp, Edith J. Feskens and Inge D. Brouwer (2020). Menarche and Pubertal Development in Relation to Anaemia and Anthropometric Indices. *Micronutrient Forum, 5th Global Conference, 2nd-11th November 20202. Online. Poster*
4. **Fusta Azupogo**, Fadilatu Abdul-Rahaman, Beatrice Gyanteh and Ambrose Atosona (2018). University for Development Studies (UDS)-Ghana/Dessert Research Institute-USA International Conference on Water, Sanitation and Hygiene (WASH). *UDS International Conference Centre, Tamale-Ghana. 17th-18th January 2018. Oral presentation.*

Overview of Completed Training Activities

Discipline-Specific courses	Organizing Institute	Year
Nutriscience: Global nutrition: from nutrients to whole diets	VLAG-Wageningen	2017
Healthy and Sustainable diets	VLAG-Wageningen	2017
Chemometrics	VLAG-Wageningen	2018
SPADE Training Programme	Global Nutrition Chair/ RIVM	2020
The Health Effects of Climate Change	EDX	2020
Global Adolescent health	Coursera	2020

Discipline-specific Conference and meeting	Organizing Institute	Year
UDS-DRI International WASH Conference	UDS/DRI-Tamale	2018
Adolescent Nutrition in Context of COVID and Beyond	Nutrition International-Webinar	2020
The Girls' Iron Folate Tablet Supplementation (GIFTS) Program: Impacts and Lessons Learned from Three Years of an Integrated Anemia Control Program Among Adolescent Girls in Ghana	USAID Advancing Nutrition-Webinar	2020
Nutrition Disparity Network: "Collective Action on Nutrition Disparity	Wageningen Nutrition Disparity Network-online	2020
Micronutrient Forum	Micronutrient Forum-Online	2020
Towards a healthy weight for all children – maintaining momentum in challenging times	UNICEF-Webinar	2020
Dietary Biomarker Symposium: Advances, Challenges, and Future Directions in Food Biomarker Research	Harvard T.H. Chan School of Public Health-Online	2020
International Conference on Diet and Activity Methods (ICDAM)	HNH Global Nutrition-Online	2021
Agriculture, Nutrition and Health(ANH) Academy Week	ANH Academy-Online	2021

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General courses	Organizing institute	Year
Logistic Regression	Erasmus Summer Programme-NIHES	2017
Regression Analysis	Erasmus Summer Programme-NIHES	2017
Efficient Writing Strategies	WGS-Wageningen	2018
Introduction to R	VLAG-Wageningen	2018
Research Data Management 1 & 2	WGS-Wageningen	2018
Advanced Multilevel Analysis	Utrecht Summer School-Utrecht	2018
Research Integrity	WGS	2020

Overview of Completed Training Activities

Other Activities	Organizing institute	Year
Preparation of research proposal	HNH Global Nutrition	
Paper Cafe HNH	HNH Global Nutrition	2018-2021
Edema-Steernberg Foundation Literature Group("Why We Eat What We Eat")	Global Nutrition and Sociology of Household Consumption	2017-2018
PaperClip Meetings (Discussion of drafts of each other's manuscripts)	HNH Global Nutrition	2017-2018
Edema-Steernberg meetings	HNH Global Nutrition	2018-2020
STAF/MR4 Meetings	HNH Global Nutrition	2021
Ten2Twenty meetings	HNH Global Nutrition	2020
Diet modelling Excel Solver Training	FAO	2021
Discussion and meetings on food-based dietary guidelines (FBDGs) for Ghana	FAO/Department of Public Health, University of Ghana	2020-2021
Diet modelling towards the formulation of FBDGs for the Ghanaian population	Ghana Technical Team on FBDGs for Ghana	2021

Colophon

Financial disclosure
The research described in this thesis was made possible through funding from the Edema Steernberg Foundation, the Judith Zwartz Foundation, Nutricia Research Foundation and Sight and Life Switzerland.
Financial support for printing the thesis was provided by Edema Steernberg Foundation

Cover Design	Bensile Bruce Aburinya
Printed by	Proefschriften.nl