

The Iron(y) of Industrial Fortification

Aligning industry-led fortified dairy-based food products with population nutritional needs in Côte d'Ivoire and Nigeria

Katie L. Ayling

Propositions

1. Too much emphasis is placed on the assumption that most anaemia cases result from iron deficiency (this thesis).

2. Evaluating the effectiveness of voluntary fortification programmes is required for population nutrition and health impact (this thesis).

3. Food companies have the responsibility to align with public health goals and positively influence population nutrition and health (this thesis).

4. Conflict of interest is inherent in nutrition science, just as the pursuit of profit is inherent in commercial companies, and both require ethical management.

5. Health by stealth is the most effective strategy to improve nutrition.

6. Female reproduction remains the greatest driver of gender inequality.

7. Wealth without societal responsibility is a failure of humanity.

8. The ability to think beyond one's basic needs is a privilege.

Propositions belonging to the thesis, entitled:

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Thesis

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Chapter 1

GENERAL INTRODUCTION

INTRODUCTION

The triple burden of malnutrition at the global level

A healthy diet is a basic human right, yet the global food system does not ensure universal access to it. According to the World Health Organisation and Food and Agriculture Organisation, a healthy diet is one that is adequate, balanced, moderate and diverse; supports normal growth and development; prevents all forms of malnutrition; and reduces the risk of diet-related noncommunicable diseases (1). In 2024, an estimated 2.6 billion people (32% of the global population) could not afford a healthy diet, with sub-Saharan Africa and Southern Asia most affected, where more than 70% of the population was unable to do so (2). This lack of access contributes to a triple burden of malnutrition, where undernutrition, micronutrient deficiencies, and overweight or obesity coexist within the same population. Sub-Saharan Africa and Southern Asia continue to experience high prevalences of stunting in children under 5 years old and anaemia in women of reproductive age, while the prevalence of overweight and obesity are rising rapidly and, in many settings, now exceed those of undernutrition (3,4). Sub-Saharan Africa is particularly vulnerable to these overlapping challenges because its fast-growing and predominantly young population concentrates a large share of the population in nutritionally sensitive life stages, while simultaneously increasing the ratio of dependents to the working-age population and placing additional pressure on food, health, and social systems (5).

Historically, nutrition programmes in low-and-middle income countries (LMICs) focused primarily on achieving energy sufficiency. Over time, this focus expanded to reducing undernutrition and micronutrient deficiencies because of their substantial contributions to morbidity and mortality. An estimated two billion people are deficient in key vitamins and minerals, particularly vitamin A, iodine, zinc and iron (6), accounting for approximately 3% of global disability-adjusted life years (DALYs) and an estimated US$2.1 trillion in annual productivity losses (7). As these burdens persist, nutrition priorities have continued to evolve towards ensuring access to healthy, diverse diets rather than energy adequacy alone.

The prevalence of overweight and obesity has now surpassed undernutrition globally (8). This shift is increasingly evident in LMICs, where rates of overweight and obesity have risen rapidly alongside persistent undernutrition and micronutrient deficiencies. These trends have contributed to a growing burden of non-communicable diseases, such as type two diabetes and coronary heart disease (8,9). High Body Mass Index (BMI) accounts for approximately 9% of global DALYs (10), and without effective prevention, obesity could cost the

global economy over US $4 trillion annually by 2030 (11). In response to this convergence of nutritional challenges, LMICs are increasingly adopting integrated "double-duty" and "triple-duty" actions that aim to address undernutrition, micronutrient deficiencies, and overweight and obesity simultaneously (12).

The food industry's role in transforming the food system

The High-Level Panel of Experts (HLPE) defines food systems as the interconnected processes of producing, processing, distributing, and consuming food that collectively shape diets and nutrition outcomes (13). The triple burden of malnutrition reflects structural inequalities within these systems, which often fail to provide affordable, nutritious foods while simultaneously enabling the widespread availability of unhealthy dietary patterns (13–15).

Achieving equitable access to a healthy diet requires system-wide transformation of food production, marketing, and consumption patterns through coordinated action among governments, civil society, and the private sector (16). Large corporations and supermarket networks dominate global supply chains, shaping what is produced, marketed, and consumed and thereby exerting substantial influence on population diets and health (13,17,18). Through their roles in production, distribution, reformulation, fortification, and marketing, food companies influence nutrition across the supply chain, the food environment, and consumer behaviour (13). The *EAT–Lancet 2.0* report highlights that these corporations now hold unprecedented power over global diets, with major implications for health, sustainability, and equity (14).

Global policy frameworks increasingly recognise the private sector as both a key partner and a shared stakeholder in achieving healthier and more sustainable food systems. The Sustainable Development Goal 2 (Zero Hunger), the WHO Global Nutrition Targets 2025, the UN Food Systems Summit (2021), and the EAT–Lancet Commissions (2019, 2025) collectively call on the food industry to reformulate products, support food fortification, and align marketing practices with public-health and sustainability goals **(Supplement Table 1).**

Voluntary (market-driven) food fortification

Global food product reformulation efforts mainly target reductions in sugar, salt, and unhealthy fats. While such efforts are important for addressing aspects of the triple burden of malnutrition related to non-communicable diseases, reducing micronutrient deficiencies in LMICs remains a critical priority and can be addressed through fortification (19), particularly as urbanisation and industrialisation increase. Market-driven fortification refers to the voluntary addition of micronutrients to foods by manufacturers within government-set

regulatory limits. Unlike mandatory fortification of staple foods implemented through public programmes, market-driven fortification is industry-led, primarily motivated by commercial objectives, with potential public-health benefits when aligned with national nutrition priorities (20). When appropriately designed and governed, market-driven fortification can complement public initiatives by broadening access to nutrients that are lacking in the diet and contributing to reductions in micronutrient deficiencies. Effective implementation requires alignment with national nutrition goals, clear regulatory frameworks, and robust quality assurance systems, as outlined in the WHO/FAO food fortification guidelines (19,21,22).

A framework for aligning food Industry action with public health interests

This thesis is tailored to the context of FrieslandCampina, an international commercial dairy company, that distributes milk-based products in West Africa, particularly in Nigeria and Côte d'Ivoire. For this thesis, we developed a framework to guide the design and implementation of market-driven food fortification programmes, with the aim of aligning private-sector action with public-health goals.

The framework illustrated in **Figure 1** consists of three interconnected themes: (1) understanding population nutritional needs, (2) product (re)formulation and food fortification for population and industry needs, and (3) evaluation of product and programme effectiveness on nutrition and health outcomes. These themes are informed by two external approaches: (1) the HLPE food systems framework**,** identifying leverage points for industry engagement in food system transformation, and (2) the WHO/FAO food fortification guidelines, providing technical guidance for designing, monitoring and evaluating fortification initiatives. In addition, we applied a "reverse thinking" principle, whereby desired public-health outcomes, such as improved population nutrition, are defined first and used to work backwards to determine how private-sector actions, including fortification, can contribute to their achievement (23).

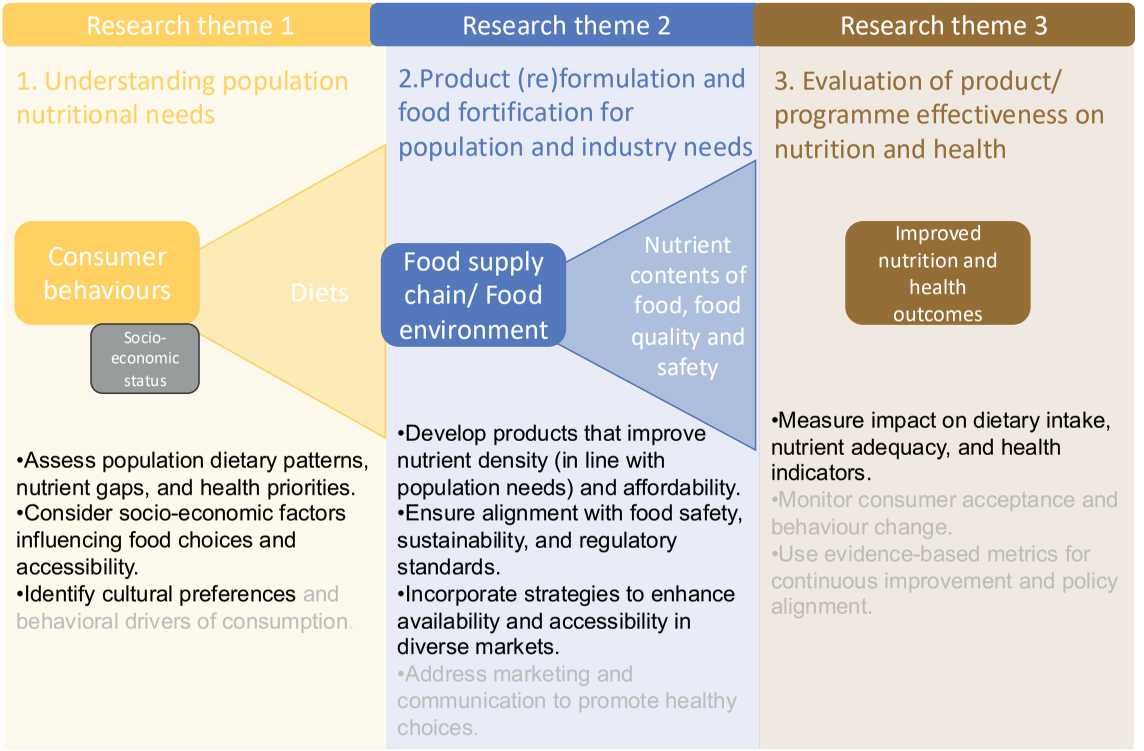


Figure 1: Tailoring reformulation of commercial food and beverages to population nutritional needs. Adapted from the HLPE food systems framework (13), this figure applies a "reverse thinking" approach to market-driven food fortification (23) and illustrates how commercial food and beverage reformulation can be aligned with population nutritional needs using the FAO/WHO food fortification guidelines (19). The framework comprises three research themes (1) Understanding population nutritional needs in Côte d'Ivoire and Nigeria; (2) Product (re)formulation and food fortification for population and industry needs; and (3) Evaluation of product and programme effectiveness on nutrition and health outcomes. Grey text appears in the HLPE food systems framework but is not included in this thesis.

Theme 1: Understanding population nutritional needs

According to WHO/FAO, reliable data on the nutritional needs of a population are essential to ensure that fortification addresses actual nutrient gaps rather than applying generic or inappropriate solutions. This requires collecting (i) biochemical or clinical indicators of nutrient status, which identify the prevalence and severity of specific micronutrient deficiencies; (ii) information on dietary patterns and the composition of the usual diet; and (iii) detailed data on micronutrient intakes, including the distribution of usual intakes across the population. Together, these data establish the magnitude of deficiencies, identify priority nutrients and target groups, and guide the formulation of fortification levels that are both safe and effective.

In sub-Saharan Africa, including Côte d'Ivoire and Nigeria, national nutrition and/or health surveys inform government policy and intervention design, but data quality and availability vary (24). Nigeria has recent, comprehensive health and dietary intake data, whereas Côte d'Ivoire relies largely on limited health and demographic indicators, resulting in substantial gaps in our understanding of micronutrient inadequacies and dietary patterns, see **Box 1** and **2** below. Both countries, however, have multisectoral nutrition policies broadly aligned with global recommendations, prioritising fortification and supplementation among children under five years, adolescents, and women of reproductive age.

Box 1: Côte d'Ivoire

No national nutrition survey has been conducted in Côte d'Ivoire, and available health data are primarily derived from the Demographic and Health Survey (DHS) 2021. Among children under five years of age, approximately 23% are stunted, 14% underweight, 68% are anaemic and 3% overweight (25). Among children and adolescents aged 5-19 years, thinness has declined to approximately 7.5% among boys, while overweight and obesity are increasing and was projected to affect 24% of girls in 2019 (26). Similar trends are observed for women of reproductive age (15-49 years), for whom underweight has declined to approximately 13%, whereas overweight and obesity have increased to 18% and anaemia affects around 61% (25,26).

Together, these indicators reflect a clear triple burden of malnutrition, characterised by the coexistence of undernutrition, micronutrient deficiencies, and rising overweight. Among children aged 6–23 months, only 24% receive a diversified diet in accordance with recommendations (25). To date, no published studies have assessed dietary intake, among school-aged children (6–12 years) or women of reproductive age (15-49 years) in Côte d'Ivoire, representing a significant knowledge gap for targeted nutrition interventions.

Box 2: Nigeria

Nigeria has nationally representative health data from the DHS 2024 (27) and nutrition data from the National Food Consumption and Micronutrient Survey (NFCMS) 2021 (28,29). Among children under five years old, approximately 40% are stunted, 27% underweight, 58% are anaemic and 1% overweight (27). Among women of reproductive age (15-49 years), underweight has declined to approximately 13%, while overweight and obesity have increased to 30% and anaemia remains highly prevalent affecting approximately 47% of women (27,26).

Micronutrient deficiencies remain widespread (29). Iron deficiency affects 21% of children and 10% of women, vitamin A deficiency 31% of children and 12% of women, and zinc deficiency 35% of both children and women. High prevalences of deficiency were reported for folate (95% women) and vitamin B2 (79% women), while deficiencies of vitamin B1 (2% women) and vitamin B12 (3% of children and 2% of women) were less common. In contrast, a median urinary iodine concentration of 293 µg/L among women suggests possible excessive intake.

Dietary intake data (28) similarly indicate widespread inadequacies, particularly for minerals and selected vitamins. High prevalences of inadequate intake were observed for calcium (children: 92%; women: 95% of women) and folate (children: 63%; women: 90%). Moderate inadequacies were reported for B vitamins, including thiamin (children: 32%; women: 65%), riboflavin (children: 59%; women: 80%), and vitamin B12 (children: 52%; women: 54%). By contrast, inadequate vitamin C intake was uncommon in children (5%) but affected over half of women (53%). A similar pattern was observed for iron (children: 8%; women: 45%), zinc (children: 3.5%; women: 26%), and vitamin A (children: 12%; women: 26%).

Anaemia in Nigeria is multifactorial, reflecting not only iron deficiency but also deficiencies of other micronutrients, infections, and inflammatory conditions (29). Dietary diversity remains limited; only 6% of children aged 6-23 months received a minimum acceptable diet, and only 30% of women consumed foods from at least five or more of the ten recommended food groups, indicating restricted access to animal-source and nutrient-dense foods (27).

In both Nigeria and Côte d'Ivoire, the prevalence of stunting, persistent micronutrient deficiencies, and rising rates of overweight and obesity illustrate the triple burden of malnutrition. To better understand these patterns, nutrition research has evolved from nutrient-level analyses towards dietary-pattern approaches that capture how changing environments, such as urbanisation, and behaviours influence dietary quality. Such approaches help explain the nutrition transition, whereby traditional diets are progressively replaced by energy-dense, nutrient-poor foods, leading to both undernutrition and excess weight (2,30,31). In Nigeria, one study among urban adults identified three dietary patterns; "High (food and energy) intakes and vegetables," "Low (food and energy) intakes," and "Dairy and sugar", with the first associated with the highest diet quality (32). Understanding such patterns supports the design of targeted double- and triple-duty interventions that address multiple forms of malnutrition within evolving food systems (12).

Côte d'Ivoire lacks data on nutrient intakes and dietary patterns, a common issue in LMICs due to the resource-intensive nature (time, cost and personnel) of conducting multiple-day 24-hour dietary recalls, the most widely used method to collect dietary intake data (33–35). Multiple recalls capture within-person variability (36–38) but increase cost and analytical complexity. In addition, multiple methodological approaches are used to assess nutrient inadequacy, including the estimated average requirement (EAR) cut-point and the probability of adequacy (37–39); as well as to estimate usual nutrient intakes, such as the Iowa State University (ISU) method, the National Cancer Institute (NCI) method, the Statistical Program to Assess Dietary Exposure (SPADE), and the Multiple Source Method (MSM) (40,41). Simplifying dietary intake assessment methods through digital tools (e.g. INDDEX24 mobile application as used in the NFCMS 21), statistical advances such as the NCI-1day method (42) or by modelling (e.g. NutriR) (43) may improve data collection and analysis efficiency, however, their implications for data quality and the accuracy of nutrient inadequacy estimates remain uncertain.

Anaemia remains a severe health issue in both Côte d'Ivoire and Nigeria, affecting 68% of Ivorian and 58% of Nigeria children under 5 years old; and 61% of Ivorian and 47% of Nigerian women of reproductive age (25,27–29). Iron deficiency is the leading cause, accounting for nearly two-thirds of anaemia cases worldwide (44). Despite sustained intervention efforts, progress in reducing anaemia has been limited (26). This limited impact may reflect, first, inconsistent measurement methods i.e. (venous vs capillary sampling) (45–48) and second, incomplete understanding of anaemia aetiology particularly in high-infection-burden settings (49–53). Combining iron interventions with malaria

prevention, deworming, and multiple micronutrient (MMN) fortification (iron, folate, calcium, vitamin A, zinc, and iodine) has proven cost-effective in reducing malnutrition and anaemia (54).

Children aged 5–15 years are often excluded from national data collection and nutrition programmes, despite well-established evidence that nutrition during this life stage strongly influences health, cognitive development, and education outcomes (55,56). Knowledge and behaviours learnt in childhood can also influence health and economic well-being in adulthood (57–59). School feeding initiatives offer an opportunity to address micronutrient deficiencies at scale, while supporting local food systems and educational output (60,61). In Nigeria, the Renewed Hope National Home-Grown School Feeding Programme (RH-NHGSFP) aims to provide at least 33% of children's recommended dietary allowances per meal and may serve as a platform for fortified foods (62).

In summary, Nigeria benefits from more extensive dietary data, whereas Côte d'Ivoire faces major information gaps, and in both countries children aged 5-15 years remain underrepresented. Strengthening fortification strategies, particularly through market-driven initiatives, targeting the high burden of anaemia represents an opportunity to complement existing public health programmes and to reach a larger proportion of the population. School feeding programmes provide an additional channel for impact by specifically targeting children aged 5-15 years.

Theme 2: Product (re)formulation and food fortification for population and industry needs

Effective fortification depends not only on identifying nutritional needs but also on developing products that are safe, acceptable, affordable, and feasible for large-scale implementation. This theme focuses on four interrelated components: selection of an appropriate food vehicle, assurance of nutrient stability and bioavailability, compliance with safety and regulatory requirements, and achievement of sensory acceptability to support consumer uptake and potential public-health impact (19).

Within the framework of this thesis, the selection of dairy as an *appropriate food vehicle* was examined due to its relevance for FrieslandCampina, and its high nutrient density providing quality protein and key micronutrients such as calcium, riboflavin, and vitamin B_{12}. However, dairy consumption is typically low in low-income countries, averaging approximately 70–75 g/day, compared with a global average of 172 g/day and around 394 g/day in high-income countries, limiting its potential as a stand-alone vehicle (14). Consumption is higher

among children, for whom even moderate dairy intake can reduce micronutrient deficiencies (63), and animal-source foods are associated with greater dietary diversity and lower stunting risk (64–67). Given that dairy is not typically consumed on its own in this context, integrating dairy with a cereal staple such as *pap*, a culturally relevant and widely consumed maize-based porridge, offers a practical approach to market-driven fortification by combining dairy's nutrient density with an accessible staple to increase reach, particularly among children.

The reformulation of existing commercial products in Côte d'Ivoire and Nigeria is guided by WHO/FAO principles to determine nutrient selection, chemical form, and fortification levels, while ensuring nutrient stability, sensory quality, and processing compatibility, to enable safe, scalable, and acceptable products for the target population. Priority nutrients (iron, zinc, vitamin A, folate, and B vitamins) are selected based on nutrient deficiencies and inadequacies identified from Nigeria's National Food Consumption and Micronutrient Survey (29) and a sub-national study in school-aged children (68). In this process, it was recognized that in Côte d'Ivoire further assessment is required to confirm nutritional issues.

The nutrients selected are permittable and their levels and claims in the product are guided by the *regulations* set by the local regulators in each country, namely Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC), whereas in Côte d'Ivoire, Codex Alimentarius standards, are applied in the absence of specific national guidance (19,69). Regulations do not typically prescribe the chemical form for fortified nutrients; however, bioavailability can vary substantially and can be influenced by the food vehicle. The choice of chemical form and food matrix may also affect taste, texture, and colour or interact with processing steps such as pasteurisation, homogenisation, or packaging. Iron and dairy-based matrices are particularly challenging in this regard (70,71). *Technological feasibility* and sensory quality must therefore be ensured so that the product acceptability is not compromised. Market-driven fortification therefore depends on achieving a balance between nutritional efficacy, technological practicality, and consumer appeal within the West African context.

Theme 3: Evaluation of product/programme effectiveness on nutrition and health

Monitoring and evaluation are essential to ensure that fortification programmes achieve their intended population reach, deliver measurable nutritional impact, remain safe, and maintain alignment between public-health and commercial goals. In Nigeria, for example, data on compliance and effectiveness of mass fortification are captured through national nutrition and health surveys (29).

Although the population coverage of seven fortified food staples was difficult to assess, compliance was high for iron and zinc fortification in wheat and semolina flour (100%), however lower for vitamin A in vegetable oil (31%) and wheat flour (26%) (29).

Globally, several mass fortification initiatives have demonstrated substantial public-health benefits. Wheat flour fortified with iron and folic acid has been associated with a 24% reduction in anaemia through improved iron stores and a 41% lowered odds of neural-tube defects; salt iodisation has reduced goitre risk by 74%; and vegetable oil fortification with vitamin A significantly reduced vitamin A deficiency (73–76). Despite such successes, limited monitoring capacity remains a major constraint to sustaining and scaling mass fortification programmes.

This challenge also extends to voluntary (market-driven) fortification, for which evidence on effectiveness is scarce. Reviews of private-sector collaborations show that well-designed market-driven fortification can meaningfully reduce deficiencies when appropriately designed and monitored (75,76). Notable examples include multiple micronutrient fortification in young children's dairy-based products (75) and iron fortification of bouillon cubes in West Africa (77). Expanding high quality evidence on market-driven fortification effectiveness particularly in LMICs is essential to strengthen accountability and demonstrate the private sector's contribution to population nutrition.

OBJECTIVES OF THIS THESIS

The overall aim of this thesis is to understand the diet and nutritional needs of Ivorian and Nigerian populations in order to guide the (re)formulation of fortified dairy-based food products and assess their effectiveness on health in a real-world school-feeding context.

The specific research objectives are:

1. To identify nutrient inadequacies and dietary patterns among school-aged children (SAC) and women of reproductive age (WRA) in Côte d'Ivoire (Research Theme 1)
2. To assess the effectiveness of a multi-nutrient fortified food product in a school feeding programme in Nigeria (Research Themes 2 and 3).

THESIS OUTLINE

This thesis investigates how market-driven food fortification can contribute to improving population nutrition and health in Côte d'Ivoire and Nigeria, with a particular focus on aligning industry-led product development with public-health priorities. This research is guided by a framework comprising three interconnected themes: (1) understanding population nutritional needs, (2) product (re)formulation and food fortification for population and industry needs, and (3) evaluation of product and programme effectiveness on nutrition and health outcomes.

Research Theme 1 focuses on generating evidence on dietary intake, nutrient inadequacies, and nutritional status through a cross-sectional dietary intake survey among Ivorian school-aged children (6-12 years old) and women of reproductive age (18-49 years old) in four urban settings: Bouaké, Daloa, Abengourou and Yopougon. This theme is addressed in **Chapters 2–4**, which evaluate nutrient intake gaps and nutritional status, examine methodological assumptions in usual intake estimation (e.g. with the modelling tool NutriR), and characterise dietary patterns and their associations with nutritional, diet quality and socio-demographic outcomes.

Research Theme 2 focuses on the translation of identified population nutritional needs into a technologically feasible and commercially acceptable fortified dairy-based product within a real-world industry context. This theme addresses key practical constraints in food fortification, especially the selection of iron compounds and their bioavailability, explored through bench-scale testing of alternative iron compounds and fortification levels in dairy matrices. The work was enabling and translational rather than hypothesis-driven and was conducted through applied product development within a commercial food company. It informed the design of the intervention evaluated in subsequent chapters (and research theme 3) and is therefore documented in the **Appendix**.

Research Theme 3 evaluates the effectiveness of a multi-nutrient fortified dairy-based product delivered through a school feeding programme in Nigeria. This theme is addressed in **Chapters 5 and 6**, which present the study protocol and statistical analysis plan, as well as the results of a randomised, double-blind trial assessing impacts on iron status outcomes among school-aged children (5-8 years). The co-primary outcomes were haemoglobin (g/L) and serum ferritin (µg/L). The secondary outcomes included soluble transferrin receptor (sTfR, mg/L), iron deficiency, anaemia and iron-deficiency anaemia as well as other nutrient biomarkers (vitamin A, vitamin B12, iodine).

Finally, **Chapter 7** synthesises findings across all themes, discusses implications for public health nutrition and industry-led fortification strategies, and reflects on the opportunities and limitations of aligning market-driven approaches with population nutrition needs.

Supplement Table 1: Summary of public health authorities calls to action that include industry to improve nutrition and health outcomes

Framework/initiative	Focus	Call to action for industry
UN/WHO (Sustainable Development Goal (SDG) 2) (78)	SDG 2: Zero Hunger—End hunger, achieve food security, improved nutrition, and promote sustainable agriculture by 2030. Also calls for action on all forms of malnutrition, including undernutrition, overweight, and micronutrient deficiencies (targets 2.1–2.2).	Private sector is called to support multisectoral partnerships for food security, sustainable agriculture, and improving access to safe, nutritious, and sufficient food for all; contribute to healthy diets by investing in sustainable food production, distribution, and responsible marketing.
WHO (Global Nutrition Targets 2025) (79)	WHO's Global Nutrition Targets 2025: Six measurable targets including: 40% reduction in child stunting, 50% reduction of anaemia in women of reproductive age, 30% reduction in low birth weight, no increase in childhood overweight, 50% increase in exclusive breastfeeding, and reducing childhood wasting.	Calls on industry to: • align product reformulation and marketing with targets: reduce added sugars, saturated fats, and sodium; • support food fortification; • contribute to public-private accountability to improve population-level nutrition outcomes.
UN Food Systems Summit (2021) (60)	Landmark summit prioritizing transformation of food systems for the SDGs. Calls to action for private sector (and all stakeholders) to reformulate products, invest in sustainable supply chains, reduce food loss and waste, improve food environments, and collaborate for nutrition/SDG targets.	Emphasizes industry's responsibility to support healthy food environments, sustainable and equitable supply chains, and to partner in delivering SDG nutrition targets—particularly via "game-changing solutions" (e.g., reformulated products, healthy school meals, reduction of food loss/waste).
HPLE framework 2020 (13)	The High Level Panel of Experts framework (2020) provides a comprehensive policy framework linking food security, nutrition, and SDGs, and sets principles for food systems transformation—including governance, sustainability, equity, and resilience	Specific recommendations for industry • to improve the affordability and accessibility of healthy diets; • to support equitable food environments; • to practice transparency and accountability in food systems; • and to align with public health and environmental policy goals for transformation.

Supplement Table 1: Continued

Framework/initiative	Focus	Call to action for industry
EAT–Lancet Commission 2020, 2025 (14)	Proposes "planetary health diet" and policy actions to deliver healthy, sustainable diets for all. Sets science-based target ranges for food group intakes to protect both human health and environmental sustainability.	Calls on the food industry to: • align products and portfolios with planetary health and nutrition goals; • reformulate foods; • take responsibility for product marketing and supply chain sustainability; • support regenerative agriculture; • make healthy, nutrient-dense foods more accessible and affordable; • avoid lobbying against public health policies
ATNI (Access to Nutrition Initiative) and SUN (Scaling Up Nutrition) (80,81)	Global initiatives monitoring and supporting business commitment to nutrition	Their reports primarily benchmark and advise industry on best practices for product portfolios, marketing to children, labelling, and responsible business leadership aligned with public health and SDG goals.

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Chapter 2

Evaluation of nutrient intake gaps and nutritional status in urban Ivorian school-aged children and women of reproductive age: associations with socio-economic status

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Abstract

Little is known about dietary intake and nutrient adequacy of the Ivorian population. This study estimated the inadequacy for energy and nutrients in Ivorian school-aged children (6-12 years) and women of reproductive age (15-49 years) and explored the differences between age, sex and socio-demographics groups. A cross-sectional dietary intake study was conducted with Ivorian school-aged children (n=443) and women of reproductive age (n=441) from different households across four cities, recruited by two-stage random sampling. Dietary intake data were collected by multiple pass 24-hour recall, with a non-consecutive repeat recall day in a sub-sample (12% of subjects). By adjustment for within-person variation, usual daily nutrient intake was calculated, and the prevalence of nutrient adequacy was assessed using the Estimated Average Requirement (EAR) cut-point method (except for iron in women of reproductive age, for which the probability of adequacy method was used with the full requirement distribution). Differences between age, sex and socio-demographic variables in mean intake and prevalence of inadequacy was determined based on confidence intervals, where possible. The results show that intake of most nutrients was adequate for both school-aged children and women of reproductive age, except for calcium (99% and 96% inadequacy, respectively), riboflavin (53% and 79% inadequacy) and zinc (51% and 53% inadequacy). Older children (11-12 years) experienced higher prevalence of inadequacies than younger children (6-10 years), and for women of reproductive age, the youngest age group (15-17 years) experienced higher prevalence of inadequacies than older age groups (18-49 years). No differences were found between sexes among children. Most nutrient inadequacies showed a negative linear relationship with socio-economic status for school-aged children, where the highest inadequacies were seen in the lowest socio-economic status groups. Most school-aged children (69%) and women of reproductive age (56%) met their energy requirements. Protein intake was often below the Acceptable Macronutrient Distribution Range (AMDR) (53% children and 45% women). Ivorian nutrition policies and interventions should generally focus on improving calcium, riboflavin and zinc intake, in addition to vitamin A and folate intake specifically in older children and young women.

INTRODUCTION

Globally, undernutrition is still a key concern, as it contributes to an estimated 45% of child deaths under the age of 5 years (1). In the Côte d'Ivoire , there appears to be a shift in malnutrition with age and time, as thinness decreases and overweight and obesity are on the rise. The Côte d'Ivoire has a predominantly young population: children under 15 years old make up 42% of the 22 million Ivorian people. According to the USAID Demographic Health Survey (DHS) 2021, around 20% of Ivorian children under 5 years of age are stunted, 68% are anaemic and 3% overweight (2). For children and adolescents (5-19 years), thinness has shown a downtrend to now affecting an estimated 7.5% of boys, whereas overweight and obesity has steadily increased over time and is predicted to affect 24% of girls in 2019 (3) . Similar trends are shown for women of reproductive age (20 years and older): underweight has declined to around 7%, whereas overweight and obesity have steadily increased to 39%. In addition, the prevalence of anaemia in women of reproductive age is also remarkably high at approximately 60% (2). These nutritional issues can for a large part be addressed by adequate dietary intake.

To the best of the authors' knowledge no studies have investigated dietary intake in Ivorian school-aged children (6-12 years) and women of reproductive age (18 years and above). Identifying nutrient intake inadequacies (nutrients consumed below recommended levels) is important to better tailor nutrition interventions and ultimately to improve the health status of a population. This is especially important in the case of Côte d'Ivoire , where there appears to be a transition in malnutrition with age and time, from undernutrition to overnutrition.

Diets in low- and middle-income countries (LMIC) are often characterised as monotonous and staple-based, leading to low dietary diversity and high micronutrient deficiencies (4). Available data suggests that this also applies to the diets of Ivorian children and women (2). The 2021 DHS reports low diet diversity for children aged 6-23 months, with only 24% meeting the minimum diversity criteria (five out of eight food groups) and for women aged 15-29 years, only 29% met the minimum (five out of ten food groups). A scoping review of 30 subnational studies, including 15 from Abidjan (2000-2019), found high prevalence of iron and riboflavin deficiencies based on blood measurements in 10-19 year olds, as well as inadequate dietary intake of iron, riboflavin and vitamin A. These nutrient inadequacies were attributed to low consumption of animal foods, fruits and vegetables (5).

The purpose of this study was to fill the knowledge gap regarding dietary intake and to identify inadequate nutrients in the diet of school-aged children (6-12 years old) and women of reproductive age (18-49 years old) in four Ivorian cities: Bouaké, Daloa, Abengourou and Yopougon. Differences in nutrient intake inadequacy between sex, age, and socio-demographic groups were also assessed.

METHODS

A cross-sectional study was conducted to assess the usual dietary intake of Ivorian school-aged children and women of reproductive age. The study received ethical approval from the National Ethics and Research Committee of Côte D'Ivoire (CNER-CI). Consent for study participation was obtained from study participants for women and by parent and/or care-giver in the case of the children.

Participants

The study was conducted in February 2021 with 443 school-aged children (6-12 years) and 443 women of reproductive age (15-49 years) in four cities of Côte d'Ivoire: Bouaké, Daloa, Abengourou and Yopougon. Participants were selected by a two-stage custom random sampling technique. First, neighbourhoods were selected by simple random sampling from a list of neighbourhoods in each of the cities, according to the General Population and Housing Census RGPH 2014. The number of selected neighbourhoods in a city was based on the probability proportional to size of the city. Households were then selected by using the random walk method. Participants were selected from the household if they were healthy and between the age of 6-12 or 15-49 years old. Only one child or woman was selected per household; hence, women and children did not reside in the same households. The required number of participants selected for the study was calculated based on a response rate of 90%, precision rate of 0.05, and iron as the most critical nutrient (6). The prevalence of iron inadequacy was estimated conservatively at 50%.

Tools and procedure

Before the study, field investigators were trained on the study's tools and procedures, and a pilot study was conducted with 20 participants in Yopougon (participants were not included in the final sample). Feedback from the training and pilot study was incorporated into the tools used in the study.

All information collected in the study was through face-to-face interviews by a field investigator with the participants (6-12 years old and 15-49 years) and their

main care-givers (for all children 6-12 years). Data collection was conducted with an electronic survey tool developed in KoboCollect (versions 1.25.1, KoboToolbox, USA). First, height and weight were measured using a standardized scale, height board and measuring tape. Then, a questionnaire was administered on demographic and socio-economic data. Socio-economic information included the main care-giver's education level and occupation, living situation, number of people living in the household and daily household expenditure on food.

Dietary intake information was collected via a standard multiple pass 24-hour recall as previously described and validated for other low- and middle-income countries (6). Participants were asked in a four-step approach to detail all foods and beverages consumed over the previous 24-hour period in and outside their home from the time of waking up the day preceding the interview to the time of waking up on the day of the interview. Details on each ingredient, their quantity and frequency of consumption were collected. Portion sizes of foods and beverages were estimated based on a photographic food atlas. The photographic food atlas was created by following the protocol from Michael Nelson *et al* (7). A second 24-hour recall was re-administered to 12% of the participants on non-consecutive days with at least 7 days and at most 18 days between the two recalls (6).

Calculations and statistical analysis

Nutrient intake data were generated by the software tool Compl-eat™ (Wageningen University, Wageningen, the Netherlands, Version 1.0) (8). For this, the detailed information on consumed foods and beverages from the 24-hour recall was entered. Conversion factors and edible portion sizes were applied to calculate the actual amount of food consumed. Nutrient values of foods and beverages were taken from the West African Food Composition Table (WAFCT, 2019) (9). Vitamin A-fortified oils specific to Côte d'Ivoire were available, however they were not used due to significant vitamin A loss during cooking (10). When foods, beverages or certain nutrients were not available in the WAFCT, the French Food Composition Table (CIQUAL, 2020) was used, followed by scientific literature when available (11). For foods and meals prepared out of home and common local recipes (example sauces), the study team created standard recipes via investigation with local food vendors.

Energy and nutrients included for analysis were macronutrients: protein, fat, and carbohydrates; fibre; and 11 micronutrients: calcium, iron, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12 and vitamin C. These nutrients were chosen as they are a public health concern for children and women in the West-African region and their composition and requirement

information was available (12,13). Data was cleaned by first calculating the z-scores of energy intake, after which the 24-hr recalls with z-scores higher or lower than ± 2.58 (99% confidence interval), and the highest and lowest 1% of the amounts of recalled foods were identified. These records were checked for discrepancies with the original electronic questionnaires and corrected where possible. If no discrepancies were found, the plausibility of each record was discussed with a team of experts. Ten identified records for school-aged children were judged implausible and their data were excluded, leaving a final number of 488 observations (final subject number= 434, with 54 subjects having a 2nd day record). For women of reproductive age, seven records were judged implausible and their data were excluded, leaving a final number of observations of 486 (final subject number= 434, with 52 subjects having a 2nd day record).

Daily nutrient intakes were used to estimate the usual nutrient intake distribution and adequacy of nutrient intake of the study population with the Statistical Program to Assess Dietary Exposure (SPADE; version 4.1.1, RIVM, The Netherlands) in R Studio (Packages: SPADE.RIVM). Twelve percent of the participants had a repeat 24h recall, which allowed adjustment of nutrient intake distributions for inter-person variation. The estimates of usual nutrient intake, obtained through bootstrapped resampling techniques (200 iterations), were then used to calculate the prevalence of nutrient adequacy with corresponding 95%-confidence intervals.

Nutrient adequacy was calculated by comparing average nutrient intakes to nutrient reference values. The nutrient reference values used were the Average Requirement (AR) provided by *Allen et al.* (2020), as these are the most up-to-date and appropriate for use as Côte d'Ivoire does not have their own set of nutrient intake requirements (14). By exception, iron intake adequacy in women of reproductive age, where the requirement distribution is skewed, was evaluated by using the full requirement distribution of the European Food Safety Authority (EFSA) (15). Moderate levels of bioavailability were assumed for iron (10%) and zinc (25%) (16), based on the Human Development Index classification of Côte d'Ivoire as "Moderate" (17). Although average nutrient requirements are available for vitamin D, they assume no sun exposure, so we did not calculate vitamin D inadequacy. The inadequacy of iodine intake was not calculated, as insufficient information was collected on iodine fortified foods, especially on iodine fortified salt as an ingredient in meals or recipes.

The reference value for energy intake was calculated for each individual by using the Estimated Energy Requirement (EER) based on age, sex, body weight, height and physical activity level (PAL) set by IOM, where PAL was assumed to be 'low activity' for all participants. Intakes of macronutrients were also evaluated as a percentage of the total daily energy intake, and inadequacy and excessive intake were classified as less than the lower and higher than the upper limit of the Acceptable Macronutrient Distribution Range (AMDR) respectively. AMDR used for macronutrients are according to IOM, 2005 (18).

Anthropometric parameters (height and weight) were compared to healthy ranges as defined by the age and gender-specific WHO ranges. Anthropometric data of children was analysed by using the World Health Organization (WHO) Anthro Plus Software (version 1.0.4) (19). The WHO defines obesity for children (5-19 years) as BMI-for-age greater than 2 standard deviations (SD) above the WHO growth reference median, overweight as BMI-for-age greater than 1 SD above the median, while thinness is defined as a BMI-for-age greater than 2 SD below the median and stunting as height-for-age greater than 2 SD below the median (20). For adults, BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Then, individuals were categorized based on the BMI cut-off values recommended by WHO as underweight (<18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2) and obese (≥30 kg/m^2) (21).

Differences in nutrient inadequacies by demographic characteristics (sex and age categories) were then analysed by evaluating the confidence intervals. A difference was considered significant in cases where the mean usual intake or prevalence of nutrient inadequacies did not fall within the 95%-confidence intervals of the comparison groups. In instances where sample sizes within variable groups were insufficient to permit bootstrapping, the linear relationship between nutrient inadequacies and SES variable groups was assessed by chi-square tests for trend. Differences between population groups (school-aged children and women of reproductive age) were also assessed. For continuous variables (for example: age) independent samples t-tests were used, and categorical variables (for example: sex, nutritional status, sociodemographic status) were compared using chi-square tests.

RESULTS

Demographics

In total, 45% of children (6-12 years) were male and 55% female **(Table 1).** In all age groups, most participants lived in Abidjan (36%), followed by Bouaké (29%), Daloa (26%) and Abengourou (9%). The socio-demographic information of children and women was similar.

Nutritional status of Ivorian children and women

Table 1 presents the nutritional status of the Ivorian children and women participating in our study. Overall, for Ivorian children, 1.4% were obese, 5.7% were overweight, 11% were thin, and 2.5% were stunted. More female compared to male children were overweight (p=0.02) (**Supplement Table 1**). Overall for Ivorian women; 17% were obese, 32% were overweight, and 7% were underweight. Younger women were often more underweight (p=0.00) and older women more overweight and obese (p=0.00) (**Supplement Table 2**).

Table 1: Demographic, socio-economic and nutritional status characteristics of school-aged children (6-12 years) and women of reproductive age (15-49 years)

Characteristic		School-aged children (n=434) %	Women of reproductive age (n=434) %
Sex	Male	45	0
	Female	55	100
Household size, n	mean ± sd	7 ± 4	7 ± 4
Number of dependent children, n	mean ± sd	4 ± 2	3 ± 3
Household Type, %	Monogamous	85	72
	Polygamous	10	22
	Other	5	6
Food Household Expenditure (per day), %	< 2000 Fr	26	21
	2000-4000 Fr	54	61
	> 4000 Fr	19	18
Living situation, %	Shack or common commune	48	45
	Apartment	35	36
	Villa	16	20
Marital Status, % [1]	Single	47	48
	Partnership	37	33
	Married	15	17
	Separated/ Divorced	0	1
	Widow	2	2

Table 1: Continued

Characteristic		School-aged children (n=434) %	Women of reproductive age (n=434) %
Education, % [1]	None	16	25
	Primary	52	24
	Secondary	28	38
	Tertiary	4	14
Occupation, % [1]	Student or not employed	44	36
	Informal sector	36	50
	Manager or salary	20	15
Ethnicity, %	Akan	42	46
	Gour	4	4
	Krou	7	7
	Mande du nord	17	13
	Mande du sud	20	16
	Non Ivorian	7	7
	other	4	7
Nutritional status, % [2]	Normal weight [a]	82	44
	Thin or underweight [b]	11	7
	Stunted [c]	3	NA
	Overweight [d]	6	32
	Obese [e]	1	17

[1] Characteristics refer to the mother or care-giver of the child [2] For children z-scores cut-offs for [a] BMI/age between -2SD and +1 SD, [b] BMI/age <– 2SD [c] ht/age <-2SD [d] (BMI/age >+ 1SD), [e] BMI/age >+ 2SD and for adults the Body Mass Index (BMI) (kg/m^2) cut-offs were used.

Abbreviations: NA; not applicable, FR, West African Franc, conversion rate in 2021 was 0.0015 euro, BMI; body mass index, SD; standard deviation.

Energy, nutrient intake and inadequacies in Ivorian children (6-12 years)

Table 2 shows the usual intake distributions of energy, macronutrients, and fibre, and their comparison to recommended requirements for children. Energy intake was mostly above the estimated requirement, with 31% of children not meeting their requirement. Carbohydrate intake was mostly within the respective AMDR for total energy (56%), whereas fat intake often exceeded the upper bound (33% of children) and protein was often under the lower bound (53% of children).

Table 2: Distribution of usual intake of energy, macronutrients and fibre of Ivorian children (6-12 years)

Nutrient (unit)	Age group (years)	Mean ± SD[1]	5th Percentile	10th Percentile	25th Percentile	50th Percentile	75th Percentile	90th Percentile	95th Percentile	% < requirement[2]/ AMDR[3]	% > AMDR[3]
Energy (kcal)	6-12	2120 ± 766	1400	1538	1785	2084	2417	2749	2962	31	69
	6	1982 ± 1212	1302	1434	1672	1951	2258	2576	2767	33	67
	7-11	2117 ± 959	1414	1547	1794	2085	2410	2725	2928	31	69
	11-12	2239 ± 1217	1500	1640	1894	2203	2548	2893	3099	32	68
Protein (g)	6-12	53 ± 26	35	38	44	52	61	71	77	53	0
	6	49 ± 44	33	35	41	48	56	65	71	56	0
	7-11	53 ± 29	35	38	44	52	61	70	76	54	0
	11-12	57 ± 46	37	40	47	55	65	75	82	49	0
Fat (g) [4]	6-12	74	47	56	67	74	79	92	106	34	33
	6	70	49	59	67	69	69	87	103	40	27
	7-11	74	47	56	71	74	77	92	105	30	35
	11-12	78	45	54	71	79	82	95	109	40	32
Carbohydrate (g)	6-12	74	47	56	67	74	79	92	106	17	27
	6	280 ± 169	166	188	227	273	325	381	421	13	27
	7-11	298 ± 138	182	204	243	290	345	402	442	16	23
	11-12	315 ± 212	197	217	257	306	363	426	466	19	34
Fibre (g) [4]	6-12	25	16	19	22	25	28	30	34	NA	NA
	6	24	17	20	22	23	23	28	33	NA	NA
	7-11	25	16	19	24	25	27	30	34	NA	NA
	11-12	27	16	19	24	28	29	31	36	NA	NA

[1] Standard deviation is calculated by the formula = ((Upper bound confidence interval - Lower bound confidence interval) / 2) / 1.96 * √ (sample size)

[2] Estimated energy requirement; EER males (6-12 years)= 88.5−(61.9×age)+PA×[(26.7×weight (kg))+(903×height (m))]+20 or 25 and EER females (6-12 years)= 135.3−(30.8×age)+PA×[(10.0×weight (kg))+(934×height (m))]+20 or 25; [3] AMDR used for macronutrients are according to IOM, 2005; protein (10-30%), fat (25-35%) and carbohydrate (45-65%); [4] Bootstrapping could not be performed due high within-individual variance and low between individual variance, therefore no SD could be given, as a confidence interval was not provided.

Abbreviations: AMDR ; Acceptable Macronutrient Distribution Range, SD ; standard deviation

Figure 1 shows that calcium, riboflavin and zinc intake did not meet the EAR for most children aged 6-12 years, with 99%, 53% and 51% of children having inadequate intakes, respectively. Children had moderate intake inadequacies for vitamin A (33%), folate (28%), vitamin B6 (24%) and vitamin C (24%). The intake of iron, thiamin, niacin and vitamin B12 met the EAR for ≥ 85% of children. Supplement Table 3 details the mean nutrient intake and prevalence of nutrient inadequacy per age group and sex. The prevalence of nutrient intake inadequacies was similar between male and female children.

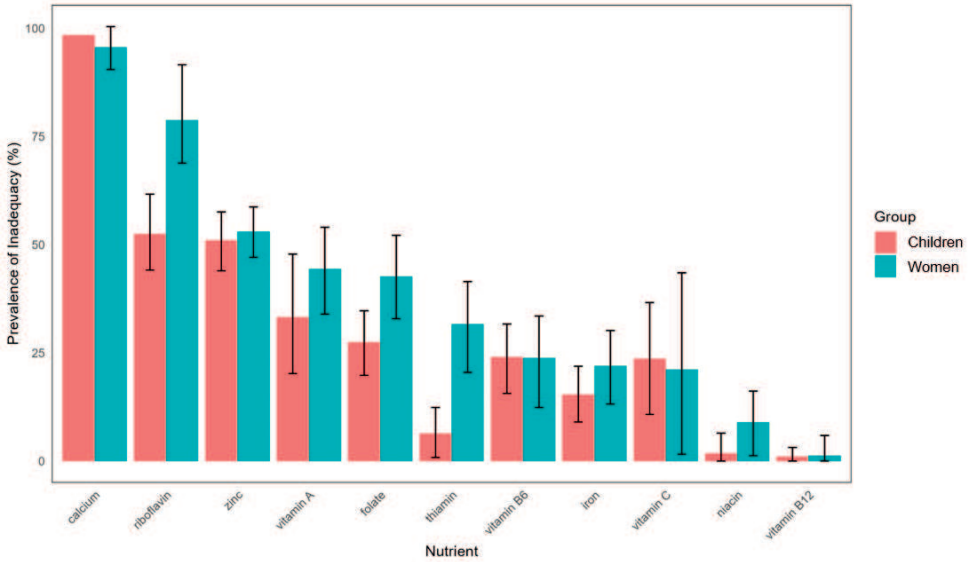


Figure 1. Prevalence of nutrient inadequacy of Ivorian school-aged children (6-12 years) and women of reproductive age (15-49 years). Error bars indicate the 95% confidence intervals.

As seen in **Figure 2,** the prevalence of inadequacy was highest for children aged 11-12 years old for all nutrients. Differences between the oldest age group and the two younger age groups were found for all nutrients except niacin, vitamin B12 and calcium. Differences between the eldest and middle age group were also found for niacin.

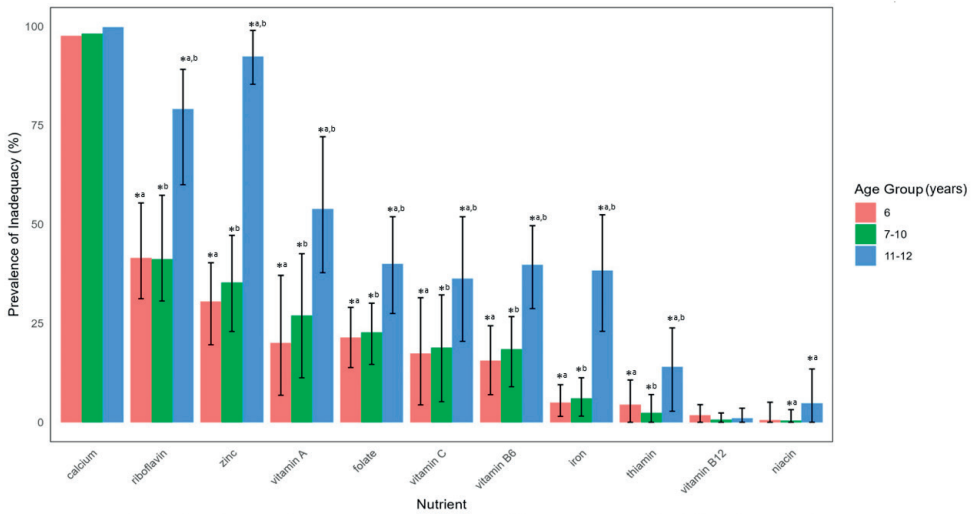


Figure 2. Prevalence of nutrient intake inadequacy by age group of Ivorian school-aged children (6-12 years). Error bars indicate the 95% confidence intervals. Significant differences between age groups, determined using confidence interval method, are indicated by a, b. Bars that share the same letter are significantly different from each other ($p < 0.05$).

Energy, nutrient intake and inadequacies in women of reproductive age (15-49 years)

Table 3 reports the usual intake distributions of energy, macronutrients and fibre and their comparison to recommended requirements for women. Energy intake of 46% of women was below their energy requirement. Carbohydrates were mostly within the AMDR boundaries, whereas protein was often under the lower bound (45% of women) and fat exceeded the upper boundary for fat intake (35% of women). For micronutrients, **Supplement Table 4** details the mean nutrient intake and prevalence of nutrient inadequacy per age group.

Figure 1 indicates that a large proportion of women did not meet the EAR for intake of calcium (96%), riboflavin (79%), zinc (53%), vitamin A (44%) and folate (43%). Intake of thiamin, vitamin B6, iron and vitamin C was inadequate for 20-30% of women, whereas intake of niacin and vitamin B12 was mostly sufficient.

As seen in **Figure 3,** the prevalence of inadequacy was highest for women aged 15-17 years old for most nutrients. Differences were observed between the youngest age group and the two older age groups for calcium, riboflavin, zinc, vitamin A and folate inadequacies. For vitamin B6, a difference was observed only between the youngest and the oldest age groups.

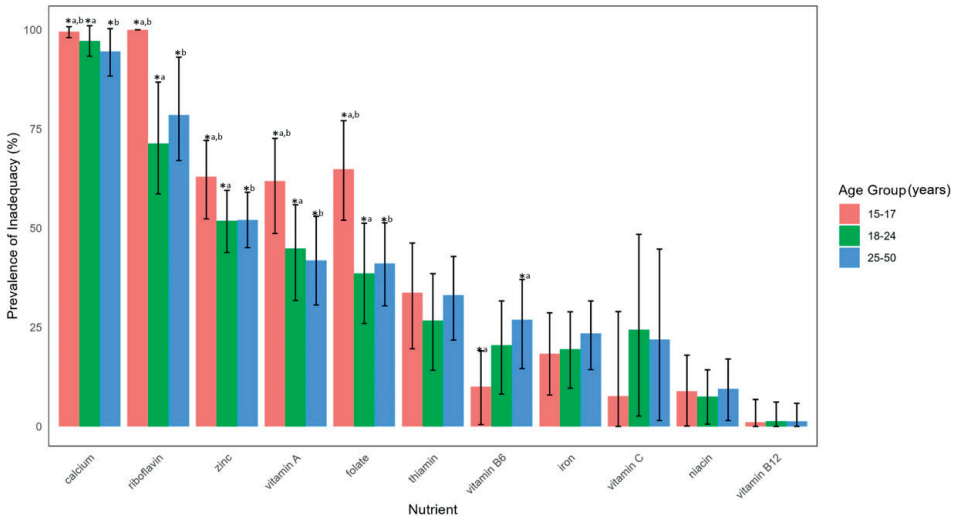


Figure 3. Prevalence of nutrient intake inadequacy by age group of Ivorian women of reproductive age (15-49 years). Error bars indicate the 95% confidence intervals. Significant differences between age groups, determined using confidence interval method, are indicated by a, b. Bars that share the same letter are significantly different from each other ($p < 0.05$).

Table 3: Distribution of usual energy and macronutrient intake of Ivorian women of reproductive of age (15-49 years)

Nutrient (unit)	Age group (years)	Mean ± SD[1]	5th Percentile	10th Percentile	25th Percentile	50th Percentile	75th Percentile	90th Percentile	95th Percentile	% < requirement2/ AMDR[3]	% > AMDR[3]
Energy (kcal)	15-49	2672 ± 1358	1305	1525	1962	2543	3240	3981	4471	46	54
	15-17	2871 ± 3125	1461	1660	2135	2728	3468	4254	4734	38	63
	18-24	2802 ± 2047	1373	1609	2080	2672	3379	4164	4682	39	61
	25-49	2600 ± 1577	1274	1484	1903	2474	3153	3880	4346	50	50
Protein (g)	15-49	71 ± 38	38	43	53	67	84	103	115	45	2
	15-17	72 ± 67	39	43	54	68	86	104	116	55	0
	18-24	71 ± 50	38	43	54	68	85	104	117	48	0
	25-49	70 ± 46	37	43	53	67	84	102	114	42	2
Fat (g)	15-49	94 ± 68	57	64	76	92	110	129	141	20	35
	15-17	104 ± 123	66	72	85	101	121	141	153	15	43
	18-24	101 ± 91	62	69	82	98	116	136	149	20	36
	25-49	91 ± 75	55	62	73	88	106	124	135	21	33
Carbohydrate (g)	15-49	370 ± 200	162	195	261	350	458	571	645	19	22
	15-17	397 ± 589	183	212	284	375	488	608	681	10	23
	18-24	388 ± 320	170	206	277	367	476	596	675	18	23
	25-49	360 ± 237	157	189	253	341	446	557	629	20	21
Fibre (g)	15-49	33 ± 20	14	17	23	31	41	51	59	NA	NA
	15-17	35 ± 41	15	18	25	33	43	55	62	NA	NA
	18-24	34 ± 30	14	18	24	32	42	54	61	NA	NA
	25-49	32 ± 22	13	16	22	30	40	50	57	NA	NA

[1] Standard deviation is calculated by the formula = ((Upper bound confidence interval - Lower bound confidence interval) / 2) / 1.96 * √ (sample size)

[2] Requirement= Estimated energy requirement, EER=354−(6.91×age (years))+PA×[(9.36×weight (kg))+(726×height (m))]; [2] AMDR used for macronutrients are according to IOM, 2005; protein (10-35%), fat (20-35%) and carbohydrate (45-65%).

Abbreviations: AMDR ; Acceptable Macronutrient Distribution Range, SD ; standard deviation, NA ; non-applicable

Comparisons of nutrient adequacy by socio-economic status (SES)

Table 4 and 5 summarise the prevalence of nutrient intake inadequacy by SES variables for school-aged children and women of reproductive age, respectively. Overall, the four different SES variables (living situation, household expenditure, occupation and school education) did not show similar trends in prevalence of nutrient inadequacies between school-aged children and women of reproductive age.

For school-aged children nutrient inadequacy roughly followed the expected negative linear relationship with SES, where the lowest SES group, had higher prevalence of nutrient inadequacies, except for folate. As seen in Table 4, most nutrient inadequacies (8 nutrients) showed a strong negative relationship with household expenditure, followed by living situation (7 nutrients), education (6 nutrients) and then occupation (3 nutrients).

For women of reproductive age, the trend for nutrient inadequacy across the four SES variables was not as expected. Majority of nutrients showed an unexpected positive relationship with SES variables, where the highest SES groups experienced higher nutrient inadequacies, especially for living situation. This relationship was strong for 5 nutrients, as seen in Table 5, where the p-value < 0.0167. A strong negative relationship (as expected) was also seen with nutrient inadequacies and occupation (5 nutrients), followed by household expenditure (4 nutrients), education (2 nutrients) and living situation (1 nutrient) ($p<0.00$).

Table 4. Prevalence of nutrient inadequacy across socio-economic levels of household expenditure, school education, occupation and living situations for Ivorian School-aged Children (6-12 years).

	Household Expenditure				School education				Occupation				Living Situation			
SES level	**Lowest**	**Middle**	**Highest**	**p value**	**Lowest**	**Middle**	**Highest**	**p value**	**Lowest**	**Middle**	**Highest**	**p value**	**Lowest**	**Middle**	**Highest**	**p value**
Calcium	100	99	97	<0.00*	100	98	100	0.71	96	99	97	0.12	99	99	90	<0.00
Folate	11	29	30	<0.00*	27	27	34	0.02	30	22	36	0.05	33	23	19	<0.00
Iron	19	18	7	<0.00*	28	15	0	<0.00	18	14	23	0.05	24	3	10	<0.00
Niacin	9	3	0	<0.00*	2	4	3	0.70	3	2	3	0.83	6	0	4	0.13
Vitamin A	41	38	0	<0.00*	46	34	21	<0.00	39	33	36	0.29	36	20	34	0.46
Riboflavin	46	46	45	0.95	73	55	42	<0.00	61	44	46	<0.00	67	42	37	<0.00
Thiamin	15	7	4	<0.00*	17	7	12	0.02	8	5	11	0.06	15	0	0	<0.00
Vitamin B12	8	1	3	<0.00*	0	3	1	0.76	3	0	0	<0.00	1	5	0	0.62
Vitamin B6	26	28	6	<0.00*	34	28	25	<0.00	30	18	19	<0.00	33	21	7	<0.00
Vitamin C	35	20	34	0.82	23	23	16	<0.00	20	22	19	0.50	28	28	0	<0.00
Zinc	53	47	34	<0.00*	67	46	59	0.0	49	47	49	0.84	45	36	38	0.04

p value: Comparison of the percent of children below the EAR across the three socio-economic levels were conducted by chi-square trends * indicates p<0.0167. For household expenditure, Lowest= <2000 Fr, Middle= 200-400 Fr, and Highest= >4000 Fr; for School Education, Lowest= no education, Middle= primary, and Highest= secondary; for Occupation, Lowest= not employed or student, Middle= informal sector, and Highest= salary or manager; for Living Situation, Lowest= shack or commune, Middle= apartment, and Highest= villa. FR, West African Franc, conversion rate in 2021 was 0.0015 euro.

Table 5. Prevalence of nutrient inadequacy across socio-economic levels of household expenditure, school education, occupation and living situations for Ivorian Women of Reproductive Age (15-49 years).

	Household Expenditure				School education				Occupation				Living Situation			
SES level	**Lowest**	**Middle**	**Highest**	**p value**	**Lowest**	**Middle**	**Highest**	**p value**	**Lowest**	**Middle**	**Highest**	**p value**	**Lowest**	**Middle**	**Highest**	**p value**
Calcium	92	97	96	0.01	93	93	98	0.00	99	94	95	0.00	97	91	100	0.03
Folate	58	54	42	<0.00*	48	38	57	0.01*	59	51	36	<0.00*	41	64	70	<0.00*
Iron	22	19	13	<0.00*	21	10	15	0.03	23	18	11	<0.00*	13	26	24	<0.00*
Niacin	25	7	0	<0.00*	7	19	11	0.05	8	10	9	0.55	8	18	11	0.12
Vitamin A	53	35	50	0.31	16	61	58	<0.00*	61	37	60	0.84	30	64	52	<0.00*
Riboflavin	89	85	90	0.47	84	90	91	<0.00*	92	83	89	0.14	82	87	100	<0.00*
Thiamin	44	32	37	0.04	40	22	24	0.00*	35	34	27	0.02	24	45	42	<0.00*
Vitamin B12	4	7	6	0.30	4	9	7	0.08	5	4	16	<0.00*	3	3	12	<0.00*
Vitamin B6	21	15	13	<0.00*	11	14	14	0.16	23	15	7	<0.00*	16	16	12	0.13
Vitamin C	27	12	35	0.01*	23	9	8	<0.00*	31	16	8	<0.00*	21	6	2	<0.00*
Zinc	56	48	60	0.25	51	40	55	0.25	58	49	35	<0.00*	43	59	61	<0.00*

p value: Comparison of the percent of children below the EAR across the three socio-economic levels were conducted by chi-square trends, * indicates p<0.0167. Bonferroni correction was applied to adjust for multiple group comparisons. For household expenditure, Lowest= <2000 Fr, Middle= 200-400 Fr, and Highest= >4000 Fr; for School Education, Lowest= no education, Middle= primary, and Highest= secondary; for Occupation, Lowest= not employed or student, Middle= informal sector, and Highest= salary or manager; for Living Situation, Lowest= shack or commune, Middle= apartment, and Highest= villa. FR, West African Franc, conversion rate in 2021 was 0.0015 euro.

DISCUSSION

In summary it was found that both under- and over- nutrition was prevalent in children, whereas overnutrition was a major issue for women. High levels of micronutrient inadequacies were observed in both children and women, with calcium deficiency affecting over 90%, and riboflavin and zinc deficiencies affecting more than 50%. Additionally, 20-45% of both groups showed inadequacies in vitamin A, folate, vitamin B6, and vitamin C. Thiamin inadequacies were also prevalent among women. In addition, most nutrients appeared to have higher prevalence of inadequacies in older children (11-12 years) and younger women (14-17 years).

Nutrients with high prevalence of inadequacies found in this study were similarly seen in other studies on children from Côte d'Ivoire and Nigeria; a scoping review of Ivorian adolescents (10-19 years) and cross-sectional dietary intake study in Nigerian children (4-13 years) (5,22). Calcium and riboflavin showed the highest prevalence of inadequacies and are found in high amounts in animal products. Consumption of dairy, especially for calcium, and of meat, nuts and green vegetables, for riboflavin, should be further explored and potentially increased in this population group.

High prevalence of inadequate intakes of iron, zinc and vitamin A are expected in LMIC, and this indeed was the case for our study, except for iron. The Nigerian study found all three nutrients (iron, zinc and vitamin A) to be inadequate in over half of the children (22). These nutrients are generally prioritised by national and international stakeholders in LMIC national policies and programmes. Large-scale food fortification is a cost-effective example of this. In Côte d'Ivoire , the fortification programme "Ivoirien de la Promotion des Aliments Fortifiés [PIPAF]" has been implemented since 2007. Wheat is fortified with electrolytic iron and folic acid, refined vegetable oil with vitamin A and salt with iodine. These nutrients are also the focus of other nutrition initiatives as part of the country's National Multisector Nutrition Plan (2016-2020). Initiatives include iron and vitamin A supplementation of children under 5 years (23). In the present study, zinc inadequacy was found to be problematic for over half of children (6-12 years old), increasing to even 92% for older children and 53% for women. Notably zinc is not currently included in the fortification initiatives in Côte d'Ivoire . The national mandatory fortification of vitamin A in cooking oils was found to have high compliance (97%) to the national fortification policy however compliance was much lower for iron fortified wheat (32%) (23). Despite the high compliance of vitamin A fortification standards and the high consumption of cooking oils, vitamin A inadequacy remained moderate in the present

study (affecting approximately 20-45% of children and women) and was higher than might be expected; this may be partly attributed to degradation of vitamin A during cooking (10).

This study found a higher prevalence of nutrient inadequacies in women compared to children, consistent with existing literature from Nigerian and South African families living in poor resource settings. In such contexts, patriarchal social structures often dictate intra-household food distribution, with adult males receiving priority, followed by male children, then female children and finally adult women (24,25). However, this pattern does not fully explain the observed differences in nutrient inadequacies between age groups. Older children (11-12 years) and younger women (15-17 years), both in adolescence, had the highest nutrient inadequacies. This could be due to increased nutrient requirements during later childhood and adolescence.

Interestingly, there was no difference found between male and female children in energy and nutrient inadequacies. However female children had a higher prevalence of overweight and obesity ($p=0.02$), and over 50% of adult women were overweight and obese. This discrepancy may be explained by under-reporting of energy intake among females in this study, a common issue in overweight or obese populations (26). These findings align with the Global Nutrition Report, which reports a higher prevalence of overweight or obesity among Ivorian female children, adolescents and women compared to their male counterparts (3).

Health and nutrition disparities linked to socio-economic status are well-documented in literature (27). Identifying the SES factors contributing to these disparities within a population is crucial for developing targeted nutrition and health interventions. Similar to the literature, our study showed that lower SES was linked to higher nutrient inadequacies in children. Most nutrients showed a strong negative relationship with household food expenditure, living situation and/or school education.

Among women, fewer nutrients showed a consistent negative relationship with SES, primarily with household food expenditure (similarly to children) and occupation. In contrast, several nutrients showed an unexpected positive or non-linear (U-shaped or inverted U-shaped) relationship with SES variables, most notably for living situation, where nutrient inadequacy was often higher in intermediate or higher SES categories. These findings suggest that more than one pattern of association between SES and nutrient inadequacy is present among women, indicating that higher SES does not uniformly translate into better

nutrient adequacy. Further investigation of dietary patterns among women with higher SES may therefore be warranted. In contrast to these findings, the dietary intake study in Ibadan Nigeria, showed no significant differences between SES groups, where SES was categorised based on the Market Research Society of India (MSRI) (22). Further research may also be needed to identify more comprehensive variables for measuring SES in Côte d'Ivoire (example Multidimensional Poverty Index (MPI)) (27).

This study is limited by the fact it did not include participants from rural areas. Current literature in the region suggests rural populations face a higher burden of child undernutrition than urban populations (28,29). Secondly, the gold standard to assess usual nutrient intake is to conduct three repeat 24-hour recalls on non-consecutive days (30,31). For feasibility reasons this was not followed in this study, only one 24-hour recall was performed and a second repeat for 12% of the study population. Physical activity levels were also not collected and low physical activity was assumed for all participants. In addition to this, Côte d'Ivoire does not have their own nutritional food composition table, thus instead the West African and French composition tables was used. This limits the matching of food items with accurate nutritional composition. Future research should also consider the nutrient status of the population (via collection of blood and urine markers) to further confirm the findings in this study or those that could not be included in this study (example vitamin D and iodine).

This study was the first study to investigate nutrient inadequacies in urban Ivorian school-aged children and women of reproductive age. Targeting the nutrients identified as inadequate could improve the effectiveness of nutrition policies, programmes and interventions for these groups in Côte d'Ivoire. Effective programmes addressing calcium, riboflavin and zinc inadequacies in LMIC should be considered (32). Nutrition education, particularly through schools could help older children and younger women to make healthier food choices. School feeding programmes are an example of interventions that can be tailored to this age group and prioritise missing foods and nutrients. In 2017-2018 school feeding programmes were implemented in 12% of Ivorian schools (33). This presents great potential for lasting dietary improvements, particularly preparing young women for child bearing years, where nutritional requirements increase and directly impact the health of the unborn child. Since national fortification is already implemented in Côte d'Ivoire (iron, folic acid, vitamin A and iodine), the fortification level, bioavailability and degradation factors (example phytates in wheat, cooking of oil) should be evaluated, along with compliance to national fortification policies.

CONCLUSION

Overnutrition is a problem for Ivorian urban women of reproductive age and undernutrition still remains a prevalent issue in Ivorian school-age children. Majority of children and women had inadequate intakes of calcium, riboflavin and zinc. Nutrition policies, programmes and interventions for Ivorian school-aged children and young women should focus on improving these nutrients. Examples include school education and feeding programmes on food groups that are rich in the nutrients of concern, and improvement of current nutrient fortification programmes and consideration of including new nutrients such as zinc.

SUPPLEMENTARY MATERIAL

Supplement Table 1. The demographic, socio-economic and nutritional status characteristics of school-aged children (6-12 years) by sex

Characteristics		Total (%)	Male (%)	Female (%)	P-value 1
Age Group	6 years	21	7	14	0.0
	7-10 years	119	59	60	
	11-12 years	60	34	26	
Age	mean ± sd	9 ± 2	9± 2	9 ± 2	0.03
# household	mean ± sd	7 ± 4	7 ± 3	8 ± 4	0.54
# dependent children	mean ± sd	4 ± 2	4 ± 2	4 ± 2	0.46
Marital Status	Single	47	44	49	0.40
	Partnership	37	40	35	
	Married	15	15	14	
	Separated/Divorced/widowed	2	1	3	
Household Expenditure	<2 000 Fr	26	27	26	0.92
	2000-4000 Fr	54	53	55	
	>4000 Fr	19	20	19	
Education	None	16	18	14	0.66
	Primary	52	50	54	
	Secondary	28	28	28	
	Tertiary	4	5	4	
Occupation	Student or not employed	44	41	46	0.46
	Informal sector	36	37	36	
	Manager or salary	20	22	18	
Living situation	Shack or common commune	35	52	46	0.38
	Apartment	48	34	36	
	Villa	16	14	18	
Household Type	Monogamous	85	84	86	0.32
	Polygamous	10	10	11	
	Other	5	6	3	
Ethnicity	Akan	42	44	40	0.78
	other	4	4	4	
	Gour	7	5	8	
	Krou	17	17	17	
	Mande du nord	20	19	21	
	Mande du sud	7	7	6	
	Non Ivorian	4	3	5	

Supplement Table 1. Continued

Characteristics		Total (%)	Male (%)	Female (%)	P-value 1
Nutritional status [1]	Normal weight (BMI/age < 1SD)	82	84	81	0.46
	Thin (BMI/age – 2SD)	11	13	9	0.27
	Underweight (wt/age – 2SD)	10	13	8	0.19
	Stunted (ht/age -2SD)	3	3	2	0.52
	Overweight (BMI/age + 1SD)	7	4	10	0.02
	Obese (BMI/age + 2SD)	3	1	2	0.17

[1] Significant differences were calculated by chi-square tests between males and females

Abbreviations: FR, West African Franc, conversion rate in 2021 was 0.0015 euro

Supplement Table 2. The demographic, socio-economic and nutritional status characteristics of school-aged children (6-12 years) and women of reproductive age (15-49 years) by age group

Characteristic		Total SAC, n=434	6 years, n=47	7-10 years, n=258	11-12 years, n=129	P-value3	Total WRA, n=43	15-17 years, n=40	18-24 years, n=100	25-50 years, n=294	P-value2
Gender, n (%)	Male	45	28	45	52	0.0	0	0	0	0	0.6
	Female	55	72	55	48		100	100	100	99	
Household size	mean ± sd	4	7± 4	7 ± 4	8 ± 3	0.5	7 ± 4	7± 3	7± 5	6± 4	0.2
Number of dependent children	mean ± sd	2	4± 2	4 ± 2	4± 3		2.7 ± 2.7	1.4 ± 2.1	2.3 ± 3.2	3 ± 2.6	0.0
Marital Status, n (%)	Single	46	53	47	44	0.8	48	93	70	34	0.0
	Partnership	37	38	36	38		33	0	22	41	
	Married	15	9	15	16		17	8	8	21	
	Seperated/Di-vorced	0	0	0	0		1	0	0	2	
	Widow	2	0	2	2		2	0	0	2	
Household Expenditure, n (%)	<2 000 Fr	26	30	26	25	1.0	21	15	25	20	0.5
	2000-4000 Fr	55	51	55	54		61	73	57	61	
	>4000 Fr	19	19	19	21		18	13	18	19	
Education, n (%)	None	15,8	17	15	17	0.1	25	10	15	30	0.0
	Primary	52,4	57	57	40		24	3	16	29	
	Secondary	27,3	23	24	38		38	88	50	27	
	Tertiary	4,5	2	5	5		14	0	19	14	
Occupation, n (%)	Student or not employed	44	49	44	40	0.1	36	90	69	17	0.0
	Informal sector	36	21	36	43		50	8	24	64	
	Manager or salary	20	30	20	17		15	3	7	19	

Supplement Table 2. Continued

Characteristic		Total SAC, n=434	6 years, n=47	7-10 years, n=258	11-12 years, n=129	P-value3	Total WRA, n=43	15-17 years, n=40	18-24 years, n=100	25-50 years, n=294	P-value2
Living situation, n (%)	Shack or common commune	48	32	35	36	0.8	45	38	50	44	0.7
	Apartment	35	47	49	47		36	38	33	36	
	Villa	16	21	15	17		20	25	17	20	
Household Type, n (%)	Monogamous	85	13	11	8	0.3	22	13	35	19	0.0
	Polygamous	5	87	85	85		72	80	60	75	
	Other	10	0	4	7		6	8	5	6	
Ethnicity, n (%)	Akan	42	40	42	43	0.5	46	55	38	48	0.2
	other	4	4	2	8		4	3	5	4	
	Gour	7	4	6	9		7	8	5	8	
	Krou	17	19	17	17		13	8	18	12	
	Mande du nord	20	19	23	15		16	13	20	16	
	Mande du sud	6	9	6	6		7	8	3	8	
	Non Ivorian	4	4	5	3		7	8	11	5	
Nutritional status [1], n (%)	Normal weight (BMI/age < 1SD)	82	75	85	80	0.2	44	68	60	35	0.0
	Thin (BMI/age – 2SD)	11	9	10	13	0.6	NA	NA	NA	NA	NA
	Underweight (wt/age – 2SD)	10	2	11	0	0.1	5	10	8	3	0.0
	Stunted (ht/age -2SD)	3	0	4	2	0.2	NA	NA	NA	NA	NA
	Overweight (BMI/age + 1SD)	10	17	5	7	0.0	32	13	22	38	0.0

Supplement Table 2. Continued

Characteristic	Total SAC, n=434	6 years, n=47	7-10 years, n=258	11-12 years, n=129	P-value3	Total WRA, n=43	15-17 years, n=40	18-24 years, n=100	25-50 years, n=294	P-value2
Obese (BMI/age + 2SD)	7	6	0	2	0.0	12	0	7	15	0.0

[1] Nutritional status is measured differently for children and adults; z-scores for children and Body Mass Index (BMI) (kg/m2) for adults, [2] Significant differences were calculated by chi-square tests

Abbreviations: SAC- school-aged children; WRA- women of reproductive age, Not Assessed (NA), FR, West African Franc, conversion rate in 2021 was 0.0015 euro

Supplement Table 3. The usual mean energy and nutrient intake, distribution and prevalence of inadequacy, with confidence intervals for Ivorian school-age children

Nutrient (unit)	Age group (years)	Sex	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Energy (kcal)	6-12	F/M	2121	2049	2184	1400	1539	1786	2087	2415	2748	2960		31	-	-
	6	F/M	1979	1856	2097	1307	1430	1664	1953	2258	2565	2755		33	-	-
	7-10	F/M	2119	2047	2190	1414	1549	1796	2085	2409	2729	2929		31	-	-
	11-12	F/M	2241	2105	2356	1502	1646	1901	2204	2550	2892	3101		32	-	-
	6-12	M	2138	2040	2227	1381	1528	1789	2108	2446	2788	3005		36	-	-
	6	M	1946	1804	2082	1260	1390	1628	1921	2231	2533	2721		36	-	-
	7-10	M	2148	2043	2242	1413	1560	1818	2122	2444	2774	2983		35	-	-
	11-12	M	2329	2149	2506	1576	1716	1991	2298	2642	2979	3180		37	-	-
	6-12	F	2100	1995	2197	1422	1550	1784	2063	2379	2698	2905		27	-	-
	6	F	2037	1831	2238	1393	1506	1730	2007	2310	2613	2813		32	-	-
	7-10	F	2083	1980	2199	1417	1542	1775	2046	2361	2668	2861		28	-	-
	11-12	F	2160	1963	2318	1458	1588	1836	2127	2444	2783	2988		25	-	-
Protein (g)	6-12	F/M	53.4	51.0	55.8	34.7	37.9	44.0	51.9	61.0	70.9	77.4		52	-	-
	6	F/M	49.4	45.5	53.0	32.7	35.4	40.9	48.1	56.2	64.9	71.0		56	-	-
	7-10	F/M	53.3	50.6	56.1	35.1	38.2	44.4	51.9	60.8	70.1	76.2		54	-	-
	11-12	F/M	56.8	52.8	60.9	36.8	40.3	47.0	55.3	65.1	75.4	82.0		49	-	-
	6-12	M	53.8	50.6	57.1	34.9	38.2	44.4	52.4	61.4	71.3	77.8		55	-	-
	6	M	47.9	42.9	52.8	31.9	34.7	40.0	46.8	54.5	62.4	67.6		50	-	-
	7-10	M	54.0	50.4	57.8	35.9	39.1	45.2	52.7	61.2	70.6	76.7		56	-	-
	11-12	M	59.9	54.0	66.0	40.4	43.6	50.3	58.4	68.0	78.2	84.3		54	-	-
	6-12	F	52.9	49.1	56.6	34.4	37.6	43.7	51.3	60.4	70.3	76.8		52	-	-
	6	F	52.0	45.4	58.0	34.2	37.0	42.9	50.6	59.4	68.9	75.3		56	-	-
	7-10	F	52.5	48.6	56.5	34.3	37.4	43.5	51.0	60.1	69.6	75.7		54	-	-
	11-12	F	53.9	48.3	59.5	34.8	38.0	44.4	52.4	61.7	72.1	78.7		49	-	-

Supplement Table 3. Continued

Nutrient (unit)	Age group (years)	Sex	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Fat (g)	6-12	F/M	74.4	-	-	47.1	55.9	66.6	73.9	79.2	92.2	106.0		34	-	-
	6	F/M	70.5	-	-	49.9	59.2	66.6	69.0	69.0	87.5	101.8		40	-	-
	7-10	F/M	74.3	-	-	46.9	55.7	71.4	73.9	76.5	91.7	105.3		30	-	-
	11-12	F/M	77.7	-	-	45.5	53.9	71.5	79.2	81.9	95.9	110.1		40	-	-
	6-12	M	73.8	-	-	66.6	66.6	69.0	73.9	79.2	79.2	81.9		32	-	-
	6	M	67.8	-	-	66.6	66.6	66.6	67.8	69.0	69.0	69.0		29*	-	-
	7-10	M	73.9	-	-	71.4	71.4	71.4	73.9	76.5	76.5	76.5		27	-	-
	11-12	M	80.1	-	-	79.2	79.2	79.2	79.2	81.9	81.9	81.9		41	-	-
	6-12	F	75.1	-	-	40.3	46.0	57.7	72.3	89.6	107.9	119.7		36	-	-
	6	F	75.3	-	-	41.0	46.4	57.7	72.7	89.7	108.0	120.1		45*	-	-
	7-10	F	74.7	-	-	40.3	45.9	57.6	71.8	89.2	107.1	118.2		33	-	-
	11-12	F	75.6	-	-	39.9	45.9	57.8	72.8	90.3	109.3	121.4		40	-	-
Carbohy-drate (g)	6-12	F/M	299.0	288.0	309.8	181.3	203.3	242.8	290.8	346.1	405.6	446.2	-	17	-	-
	6	F/M	279.9	260.8	296.1	165.5	187.4	225.7	273.2	325.8	381.4	417.9	-	13	-	-
	7-10	F/M	298.6	287.1	309.5	182.3	204.3	243.4	290.0	344.9	403.3	443.5	-	16	-	-
	11-12	F/M	315.6	296.2	337.4	197.6	218.3	258.5	306.3	364.0	425.4	466.2	-	19	-	-
	6-12	M	304.2	289.1	319.1	169.7	194.1	238.7	295.5	360.0	426.5	470.5	-	17	-	-
	6	M	279.3	257.8	300.4	154.5	176.6	218.1	271.5	331.1	392.5	430.3	-	13	-	-
	7-10	M	305.9	289.4	319.9	172.1	196.9	241.4	297.3	360.3	426.7	469.3	-	18	-	-
	11-12	M	328.6	301.7	359.4	191.3	214.7	262.8	319.1	386.9	455.1	496.5	-	19	-	-
	6-12	F	292.7	276.3	308.7	196.2	213.8	246.4	286.4	332.4	379.4	410.4	-	16	-	-
	6	F	280.9	250.5	304.1	190.0	205.7	236.8	275.7	319.3	362.7	392.9	-	14	-	-
	7-10	F	289.7	273.8	307.1	195.1	212.3	245.0	283.8	329.0	374.2	403.7	-	15	-	-
	11-12	F	303.5	276.8	331.3	202.9	221.2	256.1	297.8	344.4	394.4	425.6	-	19	-	-

Supplement Table 3. Continued

Nutrient (unit)	Age group (years)	Sex	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Calcium (mg)	6-12	F/M	371	-	-	195	233	321	380	389	479	567	680	99	-	-
	6	F/M	375	-	-	190	225	305	389	392	508	594	680	98	-	-
	7-10	F/M	372	-	-	198	234	322	383	386	480	568	680	98	-	-
	11-12	F/M	365	-	-	195	238	337	377	377	452	538	960	100	-	-
	6-12	M	361	-	-	172	200	257	336	437	555	636	680	97	-	-
	6	M	366	-	-	176	201	260	340	445	563	642	680	96	-	-
	7-10	M	362	-	-	173	202	258	337	437	556	638	680	97	-	-
	11-12	M	354	-	-	168	194	253	330	429	542	623	960	100	-	-
	6-12	F	382	-	-	374	374	377	383	386	389	392	680	100	-	-
	6	F	390	-	-	389	389	389	389	392	392	392	680	100	-	-
	7-10	F	384	-	-	380	380	380	383	386	386	386	680	100	-	-
	11-12	F	376	-	-	374	374	374	377	377	377	377	960	100	-	-
Iron (mg)	6-12	F/M	12.9	12.3	13.5	7.6	8.7	10.4	12.4	14.8	17.6	19.7	5.0	15	9	22
	6	F/M	12.0	11.1	13.0	7.0	7.9	9.6	11.6	13.9	16.5	18.4	5.0	5	1	9
	7-10	F/M	12.9	12.3	13.5	7.7	8.7	10.4	12.4	14.8	17.5	19.7	8.0	6	2	11
	11-12	F/M	13.7	12.8	14.6	8.5	9.4	11.1	13.2	15.7	18.6	20.8	12.8	38	23	52
	6-12	M	12.9	12.2	13.7	7.0	7.9	9.8	12.3	15.4	18.9	21.4	5.0	14	6	21
	6	M	11.8	10.7	13.1	6.5	7.3	8.9	11.2	14.0	17.1	19.2	5.0	7	2	13
	7-10	M	13.0	12.2	13.8	7.1	8.1	9.9	12.3	15.4	18.9	21.3	8.0	10	2	16
	11-12	M	14.1	12.9	15.4	7.8	8.7	10.7	13.3	16.7	20.4	22.8	11.2	30	16	41
	6-12	F	12.9	11.9	13.9	9.0	9.7	11.0	12.6	14.5	16.4	17.7	5.0	17	7	29
	6	F	12.4	10.8	14.0	8.7	9.3	10.6	12.1	13.9	15.7	16.9	5.0	1	0	8
	7-10	F	12.7	11.8	13.9	8.9	9.6	10.9	12.5	14.3	16.2	17.4	8.0	1	0	9
	11-12	F	13.3	11.9	14.7	9.3	10.0	11.4	13.1	15.0	17.1	18.4	12.8	46	21	72

Supplement Table 3. Continued

Nutrient (unit)	Age group (years)	Sex	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Zinc (mg)	6-12	F/M	6.8	6.6	7.0	4.6	5.0	5.8	6.7	7.7	8.7	9.5	4.6	51	44	58
	6	F/M	6.4	6.0	6.8	4.2	4.6	5.4	6.3	7.2	8.2	8.9	4.6	31	20	40
	7-10	F/M	6.8	6.5	7.0	4.6	5.1	5.8	6.7	7.6	8.7	9.4	6.2	35	23	47
	11-12	F/M	7.2	6.8	7.5	5.0	5.4	6.2	7.1	8.1	9.1	9.9	8.9	92	85	99
	6-12	M	6.8	6.5	7.1	4.3	4.7	5.6	6.6	7.9	9.2	10.1	4.6	50	42	58
	6	M	6.3	5.8	6.8	4.0	4.4	5.1	6.1	7.3	8.4	9.2	4.6	32	19	44
	7-10	M	6.9	6.5	7.2	4.3	4.8	5.6	6.7	7.9	9.2	10.0	6.2	38	24	51
	11-12	M	7.4	6.8	7.9	4.7	5.2	6.1	7.2	8.5	9.8	10.6	9.9	91	82	100
	6-12	F	6.8	6.4	7.2	5.1	5.4	6.0	6.7	7.5	8.2	8.7	4.6	52	40	64
	6	F	6.5	5.9	7.2	4.9	5.2	5.8	6.5	7.2	7.8	8.3	4.6	27	7	46
	7-10	F	6.7	6.3	7.1	5.1	5.4	6.0	6.7	7.4	8.1	8.6	6.2	32	9	55
	11-12	F	7.1	6.4	7.6	5.3	5.7	6.3	7.0	7.8	8.5	9.0	8.9	94	82	104
Vitamin A (RAE)	6-12	F/M	582	503	658	145	195	306	477	726	1069	1359	245	33	20	48
	6	F/M	635	502	743	155	208	323	510	784	1180	1535	245	20	7	37
	7-10	F/M	582	496	681	145	196	307	478	725	1060	1349	320	27	11	43
	11-12	F/M	538	424	646	137	184	289	452	679	991	1230	480	54	38	72
	6-12	M	590	484	685	116	156	258	440	742	1193	1581	245	38	19	55
	6	M	657	495	808	132	177	288	491	827	1320	1693	245	24	5	42
	7-10	M	591	477	685	117	157	260	443	742	1191	1608	320	34	12	54
	11-12	M	514	383	651	104	140	227	384	660	1056	1356	480	61	42	78
	6-12	F	572	447	706	218	262	362	509	712	964	1140	245	27	9	50
	6	F	596	368	774	232	274	377	532	738	999	1198	245	13	0	44
	7-10	F	570	427	768	220	263	363	507	711	956	1127	320	18	0	43
	11-12	F	560	381	723	208	252	352	496	697	953	1137	480	47	22	78

Supplement Table 3. Continued

Nutrient (unit)	Age group (years)	Sex	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Thiamin (mg)	6-12	F/M	1.0	0.9	1.0	0.5	0.6	0.8	0.9	1.1	1.4	1.5	0.5	6	1	12
	6	F/M	0.9	0.8	1.0	0.5	0.6	0.7	0.9	1.1	1.3	1.4	0.5	4	0	11
	7-10	F/M	1.0	0.9	1.0	0.6	0.6	0.8	0.9	1.1	1.3	1.5	0.5	2	0	7
	11-12	F/M	1.0	0.9	1.1	0.6	0.7	0.8	1.0	1.2	1.4	1.6	0.7	14	3	24
	6-12	M	1.0	0.9	1.0	0.5	0.6	0.8	1.0	1.2	1.4	1.6	0.5	5	0	13
	6	M	0.9	0.8	1.0	0.5	0.6	0.7	0.9	1.1	1.3	1.4	0.5	6	0	13
	7-10	M	1.0	0.9	1.0	0.5	0.6	0.8	1.0	1.2	1.4	1.6	0.5	3	0	8
	11-12	M	1.1	1.0	1.2	0.6	0.7	0.9	1.0	1.3	1.5	1.7	0.7	10	0	22
	6-12	F	0.9	0.9	1.0	0.6	0.6	0.7	0.9	1.1	1.3	1.4	0.5	7	0	18
	6	F	0.9	0.8	1.0	0.6	0.6	0.7	0.9	1.1	1.3	1.4	0.5	2	0	12
	7-10	F	0.9	0.9	1.0	0.6	0.6	0.7	0.9	1.1	1.3	1.4	0.5	2	0	10
	11-12	F	1.0	0.8	1.1	0.6	0.6	0.8	0.9	1.1	1.3	1.5	0.7	17	0	34
Riboflavin (mg)	6-12	F/M	0.9	0.8	1.0	0.4	0.5	0.7	0.8	1.0	1.3	1.5	0.6	53	44	62
	6	F/M	0.8	0.7	0.9	0.4	0.4	0.6	0.8	0.9	1.2	1.4	0.6	41	31	55
	7-10	F/M	0.9	0.8	0.9	0.4	0.5	0.7	0.8	1.0	1.2	1.5	0.8	41	31	57
	11-12	F/M	1.0	0.9	1.1	0.5	0.6	0.8	0.9	1.1	1.3	1.6	1.1	79	60	89
	6-12	M	0.9	0.8	1.0	0.4	0.4	0.6	0.8	1.1	1.5	1.8	0.6	53	43	62
	6	M	0.8	0.7	0.9	0.3	0.4	0.5	0.7	1.0	1.3	1.6	0.6	47	34	57
	7-10	M	0.9	0.8	1.0	0.4	0.4	0.6	0.8	1.1	1.5	1.8	0.8	49	36	60
	11-12	M	1.0	0.8	1.2	0.4	0.5	0.6	0.9	1.2	1.6	1.9	1.1	67	53	81
	6-12	F	0.9	0.8	1.0	0.7	0.7	0.8	0.9	1.0	1.0	1.1	0.6	52	37	71
	6	F	0.8	0.7	0.9	0.6	0.7	0.7	0.8	0.9	1.0	1.0	0.6	32	11	67
	7-10	F	0.9	0.8	0.9	0.7	0.7	0.8	0.9	0.9	1.0	1.1	0.8	32	11	67
	11-12	F	0.9	0.8	1.1	0.7	0.8	0.8	0.9	1.0	1.1	1.2	1.1	90	58	105

Supplement Table 3. Continued

Nutrient (unit)	Age group (years)	Sex	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Niacin (mg)	6-12	F/M	15.9	14.9	16.7	8.6	9.7	12.0	15.1	18.9	23.1	26.1	6.0	2	0	6
	6	F/M	14.7	13.3	16.1	8.1	9.1	11.2	14.0	17.4	21.3	23.9	6.0	1	0	5
	7-10	F/M	15.8	14.8	16.8	8.6	9.7	12.0	15.0	18.8	22.9	25.7	6.0	0	0	3
	11-12	F/M	16.9	15.3	18.2	9.0	10.3	12.8	16.1	20.2	24.7	27.7	9.0	5	0	13
	6-12	M	16.1	14.8	17.3	8.9	10.1	12.4	15.4	19.0	23.0	25.8	6.0	1	0	7
	6	M	14.6	12.8	16.5	8.2	9.2	11.3	14.0	17.3	20.8	23.1	6.0	0	0	7
	7-10	M	16.2	14.7	17.7	9.1	10.3	12.5	15.5	19.0	23.0	25.7	6.0	0	0	4
	11-12	M	17.5	15.3	19.6	10.0	11.2	13.7	16.8	20.7	24.9	27.6	9.0	2	0	15
	6-12	F	15.6	14.2	16.9	8.2	9.3	11.6	14.7	18.6	23.2	26.5	6.0	3	0	9
	6	F	14.9	12.5	17.1	8.0	9.0	11.1	14.1	17.8	22.1	25.1	6.0	1	0	5
	7-10	F	15.4	14.0	16.8	8.1	9.3	11.5	14.5	18.4	22.8	25.8	6.0	1	0	4
	11-12	F	16.3	14.2	18.0	8.4	9.6	12.1	15.4	19.4	24.4	27.8	9.0	7	0	18
Vitamin B6 (mg)	6-12	F/M	1.3	1.3	1.4	0.6	0.8	1.0	1.3	1.6	2.0	2.3	0.6	24	16	32
	6	F/M	1.3	1.1	1.4	0.6	0.7	0.9	1.2	1.5	1.9	2.2	0.6	16	7	24
	7-10	F/M	1.4	1.3	1.4	0.7	0.8	1.0	1.3	1.6	2.0	2.3	0.9	18	9	27
	11-12	F/M	1.4	1.3	1.5	0.7	0.8	1.0	1.3	1.7	2.2	2.5	1.2	40	29	50
	6-12	M	1.4	1.3	1.5	0.6	0.7	0.9	1.3	1.7	2.1	2.4	0.6	23	10	35
	6	M	1.2	1.1	1.3	0.5	0.6	0.8	1.1	1.5	1.9	2.1	0.6	19	7	30
	7-10	M	1.4	1.3	1.5	0.6	0.7	1.0	1.3	1.7	2.1	2.4	0.9	20	7	32
	11-12	M	1.5	1.4	1.7	0.7	0.8	1.1	1.4	1.9	2.3	2.7	1.2	33	15	47
	6-12	F	1.3	1.2	1.4	0.7	0.8	1.0	1.3	1.6	2.0	2.2	0.6	25	13	37
	6	F	1.4	1.2	1.6	0.8	0.8	1.0	1.3	1.6	2.0	2.3	0.6	10	0	23
	7-10	F	1.3	1.2	1.5	0.7	0.8	1.0	1.3	1.6	1.9	2.2	0.9	16	2	30
	11-12	F	1.3	1.1	1.5	0.7	0.8	1.0	1.2	1.6	1.9	2.2	1.2	46	30	63

Supplement Table 3. Continued

Nutrient (unit)	Age group (years)	Sex	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Folate (ug DFE)	6-12	F/M	228	216	240	105	126	164	214	274	345	396	110	28	20	35
	6	F/M	207	189	225	92	111	148	196	252	316	361	110	21	14	29
	7-10	F/M	227	215	241	106	127	165	213	273	342	393	160	23	15	30
	11-12	F/M	245	223	268	122	141	180	230	295	369	422	210	40	27	52
	6-12	M	231	214	250	93	114	155	213	287	371	431	110	29	22	35
	6	M	204	180	229	81	99	136	188	254	328	377	110	25	16	33
	7-10	M	233	214	251	95	116	158	215	288	372	430	160	26	19	33
	11-12	M	258	228	294	111	132	178	239	320	409	467	210	38	27	48
	6-12	F	224	206	241	126	142	174	215	264	316	352	110	26	10	41
	6	F	213	181	240	121	136	166	205	251	300	334	110	15	1	31
	7-10	F	221	202	244	125	141	173	212	260	311	344	160	19	2	33
	11-12	F	234	200	263	131	148	182	225	275	332	368	210	41	20	64
Vitamin B12 (ug)	6-12	F/M	5.5	4.9	6.1	1.9	2.4	3.5	4.8	6.7	9.1	11.2	1.0	1	0	3
	6	F/M	4.5	4.0	5.2	1.5	1.9	2.8	4.0	5.6	7.7	9.5	1.0	2	0	4
	7-10	F/M	5.4	4.9	5.9	1.9	2.5	3.5	4.8	6.5	8.9	11.0	1.0	1	0	2
	11-12	F/M	6.3	5.4	7.5	2.5	3.1	4.2	5.7	7.7	10.2	12.4	1.5	1	0	4
	6-12	M	5.7	5.0	6.6	1.5	2.0	3.1	4.9	7.4	10.6	13.1	1.0	2	0	5
	6	M	4.7	4.0	5.6	1.2	1.6	2.5	4.0	6.1	8.7	10.6	1.0	3	0	7
	7-10	M	5.8	5.0	6.5	1.6	2.1	3.2	4.9	7.4	10.6	12.9	1.0	1	0	4
	11-12	M	6.8	5.4	8.5	2.0	2.6	3.9	5.8	8.7	12.3	14.8	1.5	2	0	7
	6-12	F	5.1	4.4	5.9	2.6	2.9	3.7	4.8	6.1	7.6	8.6	1.0	0	0	2
	6	F	4.2	3.4	5.0	2.2	2.5	3.1	4.0	5.0	6.2	6.9	1.0	0	0	2
	7-10	F	4.9	4.2	5.6	2.5	2.9	3.7	4.7	5.9	7.2	8.0	1.0	0	0	1
	11-12	F	5.9	4.6	7.3	3.1	3.6	4.5	5.7	7.0	8.6	9.7	1.5	0	0	2

Supplement Table 3. Continued

Nutrient (unit)	Age group (years)	Sex	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Vitamin C (mg)	6-12	F/M	71.8	65.0	78.3	25.3	31.6	44.8	64.5	90.5	121.4	143.8	25.0	24	11	37
	6	F/M	63.2	53.9	72.4	22.2	27.4	39.2	56.9	80.0	106.9	125.9	25.0	17	4	31
	7-10	F/M	71.3	64.7	78.4	25.4	31.8	45.1	64.1	89.8	119.7	140.9	40.0	19	5	32
	11-12	F/M	79.6	67.7	90.0	28.9	36.0	50.8	71.8	100.6	133.8	156.5	60.0	36	20	52
	6-12	M	72.7	64.5	81.5	24.9	31.5	45.1	65.4	92.1	123.4	146.2	25.0	21	3	40
	6	M	62.5	51.2	74.5	21.0	26.6	38.4	56.3	79.4	106.3	124.7	25.0	17	0	36
	7-10	M	73.2	64.2	83.2	25.6	32.3	46.1	66.2	92.2	123.3	145.0	40.0	18	0	37
	11-12	M	82.8	66.9	99.1	30.9	37.7	53.6	75.1	104.6	138.2	160.6	60.0	33	10	55
	6-12	F	70.6	59.5	80.4	25.7	31.7	44.6	63.3	88.7	118.6	140.7	25.0	26	7	46
	6	F	64.4	49.5	78.0	23.7	28.9	40.7	58.1	81.2	107.8	128.3	25.0	18	0	40
	7-10	F	68.9	58.2	79.5	25.3	31.2	44.0	62.0	86.7	115.3	135.8	40.0	20	0	41
	11-12	F	76.5	59.9	90.1	27.8	34.4	48.7	69.1	95.9	129.0	151.9	60.0	39	16	63

Bootstrapping (n.boot = 200) of calcium could not be fully completed in SPADE. The number of converged bootstrap samples was 131, which is not enough for safe bootstrap intervals. This issue is likely due to the highly skewed distribution of calcium intake data. No significant differences between sexes were found using the confidence interval method.

Abbreviations: LB- lower bound; UB- upper bound; EAR- Estimate Average Requirement; p- prevalence; <EAR- inadequacy; SPADE- Statistical Program to Assess Dietary Exposure; AMDR- Acceptable Macronutrient Distribution Range

Supplement Table 4. The usual mean nutrient intake, distribution and prevalence of inadequacy, with confidence intervals for Ivorian women of reproductive age

Nutrient	Age group	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Calcium (mg)	15-49	457	429	486	235	269	337	431	548	679	767	1100	96	90	100
	15-17	467	409	515	244	274	346	439	561	694	780	1100	100	98	101
	18-24	464	425	499	236	270	344	438	555	690	784	860	97	93	101
	25-49	454	422	488	233	267	334	428	544	673	761	750	95	88	100
Iron (mg)	15-49	16.8	15.9	17.6	7.4	8.8	11.7	15.7	20.6	26.1	29.7	11.2	22	13	30
	15-17	17.6	15.9	19.3	8.1	9.4	12.4	16.5	21.7	27.3	30.9	11.2	18	8	29
	18-24	17.3	16.1	18.5	7.6	9.1	12.2	16.2	21.2	26.9	30.8	11.2	19	10	29
	25-49	16.4	15.5	17.5	7.3	8.7	11.5	15.4	20.2	25.5	29.1	11.2	23	14	32
Zinc (mg)	15-49	9.3	8.8	9.8	4.4	5.1	6.6	8.7	11.3	14.3	16.2	9.9	53	47	59
	15-17	9.4	8.4	10.3	4.5	5.1	6.7	8.7	11.4	14.3	16.2	9.9	63	52	72
	18-24	9.3	8.7	10.0	4.3	5.1	6.7	8.7	11.3	14.4	16.4	8.9	52	44	60
	25-49	9.3	8.7	9.9	4.4	5.1	6.6	8.7	11.3	14.2	16.2	8.9	52	45	59
Vitamin A (RAE)	15-49	643	581	706	177	229	354	550	828	1172	1420	630	44	34	54
	15-17	620	515	733	168	222	347	526	793	1138	1384	630	62	49	73
	18-24	627	551	708	173	224	348	533	803	1139	1394	490	45	32	56
	25-49	652	574	728	179	232	357	561	841	1187	1433	490	42	31	53
Thiamin (mg)	15-49	1.2	1.1	1.2	0.5	0.6	0.8	1.1	1.5	1.8	2.1	1.0	32	20	41
	15-17	1.3	1.1	1.4	0.6	0.7	0.9	1.2	1.5	1.9	2.2	1.0	34	20	46
	18-24	1.2	1.1	1.3	0.5	0.7	0.9	1.2	1.5	1.9	2.2	0.9	27	14	39
	25-49	1.2	1.1	1.2	0.5	0.6	0.8	1.1	1.4	1.8	2.0	0.9	33	22	43

Supplement Table 4. Continued

Nutrient	Age group	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Riboflavin (mg)	15-49	1.1	1.0	1.2	0.6	0.7	0.9	1.1	1.3	1.5	1.7	12.0	79	69	92
	15-17	1.2	1.0	1.3	0.7	0.8	0.9	1.1	1.4	1.6	1.8	12.0	100	100	100
	18-24	1.1	1.0	1.2	0.6	0.7	0.9	1.1	1.3	1.6	1.8	1.3	71	59	87
	25-49	1.1	1.0	1.1	0.6	0.7	0.8	1.0	1.3	1.5	1.7	1.3	79	67	93
Niacin (mg)	15-49	21.3	20.1	22.6	9.6	11.4	15.1	20.1	26.2	32.8	37.1	12.0	9	1	16
	15-17	22.7	20.2	25.1	10.6	12.3	16.3	21.4	27.8	34.7	39.0	12.0	9	0	18
	18-24	22.2	20.3	24.1	10.0	11.9	15.9	21.0	27.2	34.1	38.8	11.0	7	1	14
	25-49	20.8	19.4	22.4	9.4	11.1	14.7	19.6	25.6	32.1	36.3	11.0	10	1	17
Vitamin B6 (mg)	15-49	1.8	1.7	1.9	0.9	1.0	1.3	1.7	2.2	2.6	3.0	1.1	24	12	34
	15-17	1.9	1.7	2.1	1.0	1.1	1.4	1.8	2.3	2.8	3.1	1.1	10	0	19
	18-24	1.9	1.7	2.0	0.9	1.1	1.4	1.8	2.2	2.8	3.1	1.3	20	8	32
	25-49	1.7	1.6	1.8	0.8	1.0	1.3	1.7	2.1	2.6	2.9	1.3	27	15	37
Folate (ug DFE)	15-49	298	277	319	134	158	208	277	365	464	531	330	43	33	52
	15-17	306	264	344	140	162	214	283	375	476	541	330	65	52	77
	18-24	303	274	332	135	159	213	282	370	473	544	250	39	26	51
	25-49	295	272	319	133	157	205	275	362	459	527	250	41	30	51
Vitamin B12 (ug)	15-49	7.3	6.7	8.0	2.8	3.5	4.8	6.7	9.2	12.0	13.9	2.0	1	0	6
	15-17	7.3	6.1	8.4	2.9	3.5	4.8	6.7	9.2	12.0	13.8	2.0	1	0	7
	18-24	7.3	6.4	8.2	2.8	3.4	4.8	6.7	9.1	12.0	14.0	2.0	1	0	6
	25-49	7.3	6.6	8.1	2.8	3.5	4.8	6.7	9.2	11.9	13.8	2.0	1	0	6

Supplement Table 4. Continued

Nutrient	Age group	Mean	Mean LB	Mean UB	P5	P10	P25	P50	P75	P90	P95	EAR	p< EAR	LB p < EAR	UB p< EAR
Vitamin C (mg)	15-49	103	95	112	61	68	82	100	121	143	158	63	21	2	44
	15-17	101	86	115	60	66	80	97	118	140	153	63	8	0	29
	18-24	101	91	113	59	66	81	98	119	141	156	80	24	3	48
	25-49	104	95	114	61	68	82	101	122	144	159	80	22	1	45

Abbreviations: LB- lower bound; UB- upper bound; EAR- Estimate Average Requirement; p- prevalence; <EAR- inadequacy; SPADE- Statistical Program to Assess Dietary Exposure; AMDR- Acceptable Macronutrient Distribution

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Chapter 3

Assuming the "best-fit" shape of the usual nutrient intake distribution (with nutriR) does not affect prevalence estimates of nutrient intake inadequacy: results from a dietary intake survey in Ivorian school-age children

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Abstract

Background: Common methods to assess nutrient intake inadequacy are the EAR cut-point method and the Probability of Adequacy (PA). It has been postulated that the shape of the intake distribution can have a significant impact on estimates of inadequacy. The "Nutritional intake functions for R" ("nutriR") package has been developed to address this.

Objective: To assess the impact of assuming the "best-fit" shape of nutrient intake distributions (using nutriR) on prevalence estimates of inadequate nutrient intake, as compared to observed nutrient intake distributions with the EAR cut-point or PA method.

Methods: A cross-sectional dietary intake survey was conducted among 443 Ivorian school-aged children (6-12 years) by 24-hour recall, with non-consecutive repeats for 12% of the population. Six methods to estimate prevalence of intake inadequacy for 11 nutrients were compared: methods 1-3 used single 24h recall data to either apply the EAR cut-point method (1), the PA method (2), or the nutriR method (3); and methods 4-6 applied the same methods after correction for within-person variation using the Statistical Program to Assess Usual Dietary Exposure (SPADE), which generates estimates of usual daily nutrient intake.

Results: Nutrient intake distributions were right-skewed (skewness average 1.0, range: 0.4-2.0) and showed large variation (CV average 40%, range: 20-60%). The prevalence estimates of inadequate nutrient intake were similar across all methods, with an average difference of ±5 percent-point (range: 0-27 percent-point). The smallest average differences, 0.4 and 0.3 percent-point, were observed with usual intake data between nutriR and EAR cut-point (method 6 vs 4), and nutriR and PA (method 6 vs 5), respectively. Larger differences (~12 percent-point) were observed when comparing single 24h recall data (methods 1-3) with usual intake (method 4-6).

Conclusions: Assuming nutrient intake distributions with the shape of "best-fit" to the observed intake distribution did not affect prevalence estimates of nutrient inadequacy in this population of Ivorian school-children.

INTRODUCTION

Assessing the prevalence of nutrient inadequacy at a population level remains complex. Ideally, it is estimated from joint distributions of usual intake and requirement of the nutrient in the population. However, this is impractical, and thus univariate distributions of usual intakes and requirements are commonly used. The two most predominantly used methodologies to do so are the Estimated Average Requirement (EAR) cut-point method and the Probability of Adequacy (PA) approach (1–3). The EAR cut-point method uses one input (the central tendency) from the requirement distribution, while the PA considers the entire distribution of the requirement. For this reason, the PA method is more accurate and can be used when the full requirement distribution is known. In most cases the mean and coefficient of variation (CV) of the requirement are known and a normal distribution is assumed. The EAR cut-point method is a simplification of the PA method and assumes symmetry of the requirement distribution around the average. The EAR cut-point method appears to be used more frequently due to its simplicity and not requiring information on the full requirement distribution (1,4,5).

Recently, it has been suggested that not only the shape or symmetry of the requirement distribution, but also the shape of the intake distribution, can influence estimates of nutrient adequacy (6). Intake distributions, characterised by the variance and skewness of usual nutrient intake, typically follow a log or gamma distribution rather than a normal distribution. When the observed intake distribution is unknown, a normal distribution is often assumed for ease (7).

To address this, it has been recommended to consider the shape of the nutrient intake distribution when calculating the prevalence of inadequacy. The open access R package "nutriR" was developed to assist with this, among other functions (6). Passarelli *et al* 2022 reported differences in nutrient inadequacies (sometimes as large as 50%) between normally distributed EAR cut point method and "best-fit" shape (lognormal or gamma distributed) PA method. What remains unknown however is the differences in nutrient inadequacy between the "best-fit" shape and observed intake distributions and it is unclear in which situations either should be used.

The 24-hour recall is one of the most commonly accepted and applied instruments in population-based nutrition studies (8–10). Collection of a second-day recall, at least in a subsample, is recommended to allow differentiation of within- and between-person variation. Statistical methods can then be used to adjust for within-person variation, yielding population estimates of usual intake

distributions (long-term intake) (2). In contrast, a one-day recall or averaging dietary intake across multiple days may lead to over- or underestimation of nutrient intake inadequacy due to biased estimates especially in the tails of the distribution (2,11).

This study aims to assess the impact of assuming the "best-fit" shape of nutrient intake distributions (using nutriR) on prevalence estimates of inadequate nutrient intake of Ivorian school-aged children (6-12 years), as compared to observed nutrient intake distributions using the standard EAR cut-point or PA method. An additional aim was to compare results using intake data from a single 24h recall, or usual intake after correction for within-person variation.

METHODS

Study design: A cross-sectional dietary intake survey was conducted to assess the usual nutrient intake of Ivorian school-age children and estimate the prevalence of nutrient inadequacy. The study received ethical approval from the National Ethics and Research Committee of Côte d'Ivoire (CNER-CI), Abidjan. Verbal and written consent for study participation was obtained from the study participant's parent or care-giver. All statistical analyses were performed with R Studio (version 4.3.1 R Studio Team, 2023).

Participants: The study was conducted in February 2021 on 443 school-age children (6-12 years) in four cities of Côte d'Ivoire : Abengourou, Abidjan (Yopougon), Bouaké and Daloa. Participants were selected by a two-stage custom random sampling technique. First, neighborhoods were randomly selected based on the General Population and Housing Census (RGPH) 2014, considering the probability proportional to the size of the city. The household within a selected neighborhood was then determined using the random walk method. One participant was selected from the household if they were healthy and between the ages of 6 and 12. The required sample size was determined using a 90% response rate, a precision rate of 0.05 (±5% margin of error), and iron as the most critical nutrient, given the high prevalence of anaemia in Ivorian children and women, and suspected high prevalence of iron-deficiency anaemia (9,12). The prevalence of iron intake inadequacy was estimated at 50%.

Dietary Assessment: Dietary intake was assessed with a multiple pass 24-hour recall, a method previously described and validated in many low- and middle-income countries (9). Face-to-face interviews were conducted by field investigators using an electronic survey tool, Kobo-collect, engaging both participants

and their primary caregiver. A second 24-hour recall was administered to a convenience sample comprising 12% (n=55) of participants on non-consecutive days, with a minimum of 7 and a maximum of 18 days between the two recalls.

In a systematic four-step approach, participants were prompted to provide comprehensive details about all foods and beverages consumed over the preceding 24 hours, both inside and outside their homes, from the morning before the interview to the morning of the interview. Details on each ingredient, its quantity, and frequency of consumption were collected. Portion sizes were estimated using a photographic food atlas developed according to the protocol by Michael Nelson *et al.* in 1998 (13). Conversion factors and edible portions were applied to determine the actual amount of food consumed. For foods and meals prepared out of home standard recipes were created by the study team with the help of local food vendors.

The detailed data on food and beverage consumption from the 24-hour recalls were used to calculate the daily nutrient intake of participants with the software tool Compl-eat™ (Wageningen University, Wageningen, the Netherlands, Version 1.0) (14). Nutrient values of foods and beverages were taken from the West African Food Composition Table (WAFCT, 2019), and when not available the French Food Composition Table (CIQUAL, 2020) was used, followed by scientific literature (15,16). Eleven nutrients were included for analysis: calcium, iron, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12 and vitamin C. Data was cleaned by first calculating the z-scores of energy intake, after which the 24-hr recalls with z-scores higher or lower than ± 2.58 (99% confidence interval), and the highest and lowest 1% of the amounts of recalled foods were identified. These recalls were checked for discrepancies with the original electronic questionnaires and corrected where possible. If no discrepancies were found, the plausibility of each case was discussed with a team of experts. Ten recalls were judged implausible and were excluded, resulting in 434 participants included for analysis and 54 of these participants had a second day recall.

One-day and usual nutrient intake distributions: Nutrient intake distributions were calculated in two ways: (1) One-day intake; and (2) the usual intake estimated by using the statistical tool Statistical Program to Assess Dietary Exposure (SPADE). SPADE estimates the usual intake distributions through a three-step process: first, transforming observed intake data to approximate normality; second, applying a shrinkage process that reduces the within-person variation at the transformed scale; and third, back-transforming the data to the original scale, ensuring the final intake distribution exhibits only between-person variation.

To address distribution fitting challenges, error handling procedures such as applying dynamic starting values, excluding zero intakes, or capping extreme values at the 1st and 99th percentiles were applied as needed. During back-transformation, pseudo-sampling was employed to enhance the accuracy and robustness of the usual intake estimation, generating multiple intake values per individual to improve distributional stability. This was implemented using the default input backtrans.nr = 2, or specifically backtrans.nr =3, for method 6, to generate pseudo sampling output required for nutriR input. The 1-part SPADE model was used as foods or their components were consumed daily. Indicators of skewness and variance (including within-person and between-person variance) were reported and compared for each nutrient and age-group. Additionally, the mean intake was compared to the mean of the requirement and the best-fit shape of the intake distribution was determined using nutriR for each nutrient.

The theoretical "method of choice" to calculate prevalence of nutrient inadequacy was also determined between the EAR cut-point, PA and nutriR method. This was based on the assumptions and observations of the three methods in literature regarding their appropriate use in assessing nutrient inadequacy (1,2,4,5)**.** The two types of nutrient intake distributions (one-day and usual intake) were then used to calculate the prevalence of nutrient inadequacy.

Prevalence of nutrient inadequacy: The prevalence of nutrient inadequacy was estimated by either using one-day intake combined with the EAR cut-point method (method 1), the PA method (method 2) and the nutriR (method 3), or by using usual intake combined with the same three methods: EAR cut-point (method 4), PA (method 5), and nutriR (method 6; **Figure 1**).

The EAR cut-point method compares the mean nutrient intake to the Estimated Average Requirement (EAR) value. This method uses the formula:

$$\text{Prevalence} = \sum \frac{(\text{intake}_{\,i} < \text{EAR})}{\text{n (total population)}}$$

where intake$_i$ represents the intake for the individual (*i*), to determine the proportion of the population with intake below the EAR. The calculation was performed manually in R Studio for method 1 (one-day intake) and using the SPADE software for method 4 (usual intake). The PA method, which estimates the expected risk that the distribution of intakes in the population does not meet the EAR distribution of requirements, was also employed. The PA calculation uses the formula:

$$\mathrm{PA}_{(\text{population})} = \frac{1}{\mathrm{m}} \sum_{\mathrm{i\ or\ p}=1}^{\mathrm{m}} \mathrm{pnorm}\left(\frac{\mathrm{Intake}_{(\mathrm{i\ or\ p})} - \mathrm{EAR}}{\mathrm{SD\ of\ EAR}} \right)$$

where intake *(i)* is individual intake, or when this is not available as is the case with SPADE output (method 5), the intake at each percentile *(p)* of the distribution is used, *(m)* is total population or percentiles, and *pnorm* function is the cumulative distribution function for a normal distribution. This calculation was performed manually in R Studio for methods 2 (one-day intake) and 5 (usual intake). Additionally, PA was also estimated using the nutriR package in R Studio for methods 3 (one day intake) and 6 (usual intake). This program estimates the shape of "best fit" to the intake distribution (normal, log or gamma) and based on two distribution variables (measure of central tendency and variability) calculates the risk of not meeting the requirement distribution (6). For both the PA and nutriR methods, the prevalence of nutrient inadequacy for the 6-12 year old age group was manually calculated using the weighted average values from the three different age groups (6, 7-10 and 11-12 years) and the age-specific EAR values.

The EAR values used in this study are the Harmonized Average Requirements (H-AR) provided by Allen *et al. (2020),* which are the most up-to-date and internationally applicable dietary reference values, due to the lack of local reference values in Côte d'Ivoire (17). These H-AR are taken from either EFSA or IOM and are defined for all 11 nutrients included in this study. Nutrients without a defined H-AR were left out of the study. Energy, carbohydrate, fibre, and fat inadequacies were not calculated as assumptions made for the EAR cut-point and PA method do not hold for these nutrients, specifically because the requirement is not independent from intake.

Moderate levels of bioavailability were assumed for iron (10%) and zinc (25%) (17), based on the Human Development Index classification of Côte d'Ivoire as "Moderate" (18). Although an EAR has been defined for vitamin D, it assumes no sun exposure, and therefore we did not calculate vitamin D intake inadequacy here. The assumed values for variation of the requirement (CV), were taken from EFSA or IOM, based on where the H-AR had been taken from (19,20). The three age groups used for this study (6 years; 7-10 years and 11-12 years) are based on the age groups underlying the H-AR (17) .

The average differences in prevalence estimates of nutrient inadequacy (in absolute percent-points), as calculated by each of the six methods described above, were then compared as follows: 1) One-day intake (methods 1-3) vs usual intake (methods 4-6); 2) EAR cut-point vs PA (methods 1 vs 2 and 4 vs 5); 3) nutriR vs EAR cut-point (methods 1 and 4 vs 3 and 6); 4) nutriR vs PA (methods 2 and 5 vs 3 and 6).

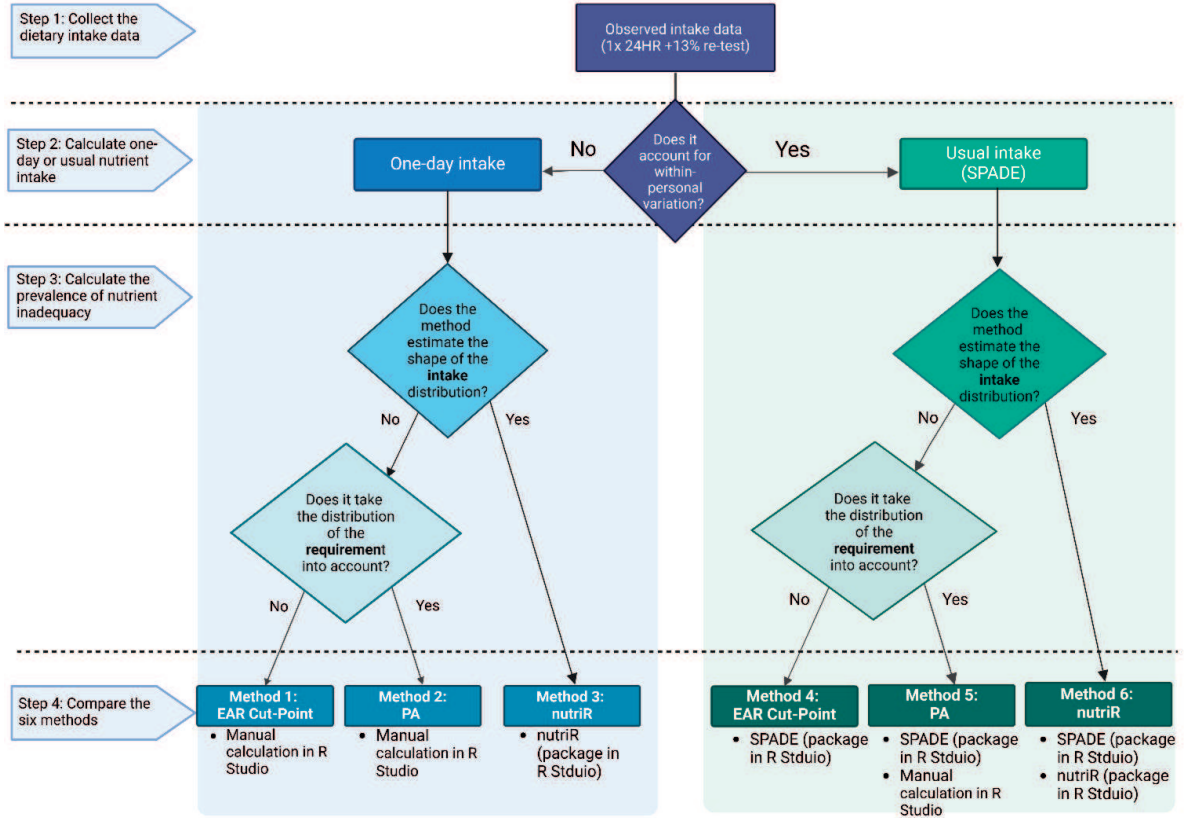


Figure 1. Flow chart with the four steps of analysis used to assess prevalence of nutrient inadequacy of Ivorian school-aged children (6-12 years old) and the comparison between six different methods.

Abbreviations: SPADE, Statistical Program to Assess Dietary Exposure; EAR, Estimated Average Requirement; PA, Probability of Adequacy; nutriR, Nutritional intake functions for R.

RESULTS

As reported in **Table 1,** 45% of children were male and 55% female**.** Forty-seven (11%) children fell in the age group of 6 year old, 258 (59%) in the age group 7-10 years, and 129 (30%) in the age group 11-12 years. Most children were of normal weight (82%) and lived in Abidjan (35%), followed by Bouke (29%), Daloa (27)% and Abengourou (9%).

Table 1. Demographic, socio-economic and nutritional status characteristics of Ivorian school age children by age group (n=434)

Characteristics		Total (n=434)	6 years (n=47)	7-10 years (n=258)	11-12 years (n=129)
Sex, Female, (%)		55	72	55	48
City, (%)	Abengourou	9	15	9	5
	Abidjan	35	28	33	43
	Bouaké	29	32	29	29
	Daloa	27	26	29	23
Ethnicity, (%)	Akan	42	40	42	43
	Gour	4	4	2	8
	Krou	7	4	6	9
	Mande du nord	17	19	17	17
	Mande du sud	20	19	23	15
	Non Ivorian	7	9	6	6
	Other	4	4	5	3
Nutritional Status [1]					
Normal weight (BMI-for-age -2 SD < Z < +1 SD)		82	75	85	80
Thin (BMI-for-age Z< – 2SD)		11	9	10	13
Underweight (wt-for-age Z< – 2SD)		10	2	12	0
Stunted (ht-for-age Z< -2SD)		3	0	4	2
Wasted (wt-for-age Z< -2SD)		10	2	11	0
Overweight (BMI-for-age Z> + 1SD)		7	17	5	7
Obese (BMI-for-age Z> + 2SD)		2	6	0	2
Household size, n[2]		7 ± 4	7 ± 4	7 ± 4	8 ± 3
Living situation, (%) Apartment		35	32	35	36
Common commune or shack		48	47	50	47
Villa		16	21	15	17
Household food expenditure (per day), (%)					
< 2000 Fr[3]		26	30	27	25
2000-4000 Fr[3]		54	51	55	54
>4000 Fr[3]		19	19	19	21
Dependent children, n[2]		4 ± 2	4 ± 2	4 ± 2	4 ± 3

Table 1. Continued

Characteristics	Total (n=434)	6 years (n=47)	7-10 years (n=258)	11-12 years (n=129)
Main caregiver's marital Status, (%)				
Single	47	53	47	44
Partnership	37	38	36	38
Married	15	9	15	16
Separated/ divorced/ widow	2	0	2	2
Education of the main caregiver, (%)				
None	16	17	15	17
Primary	52	57	57	40
Secondary	28	23	24	38
Tertiary	4	2	5	5
Occupation of the main caregiver, (%)				
Student or not employed	44	49	44	40
Informal sector	36	21	36	43
Manager or Salaried employment	20	30	20	17

[1]WHO Growth Reference data for 5-19 years old (28) [2]Values are means ± standard deviations; [3] West African Francs, conversion rate in 2021 was 1 Fr = 0.0015 euro

Description of usual observed nutrient intake distributions

As reported in **Table 2**, the usual mean (± SD) intake as generated by SPADE was higher than the EAR in all cases, except for calcium (6-12 years), zinc (11-12 years) and riboflavin (11-12 years). The usual mean intake was closest to the EAR for zinc, riboflavin, folate, vitamin B6, iron, vitamin C, thiamin and vitamin A. Vitamin B12 and niacin had usual mean intakes far above (at least double) the EAR.

On average, the skewness across the 11 nutrients was 1.0 (range: 0.4-2.0). The skewness in the distribution of usual intakes was highest for vitamin A, followed by vitamin C, vitamin B12, vitamin B6, riboflavin, niacin, folate, iron, thiamin, calcium and zinc. On average, the CV across the 11 nutrients was 0.4 (range: 20-60%). The variation in the distribution of usual intake was highest for vitamin A, followed by vitamin C, vitamin B12, vitamin B6, folate, niacin, riboflavin, thiamin, iron, zinc and calcium. All usual nutrient intake distributions showed a right-skew. None of the nutrient intake distributions were normally distributed, and for five of the nutrients the best fit was the gamma distribution (zinc, thiamin, vitamin C, folate and vitamin B12), for the remaining six the lognormal distribution was preferred (**Supplementary Figure 1**).

Table 2. The distribution of usual vitamin and mineral intake of Ivorian children (6-12 years)

Nutrient (unit)	Age group (years)	Usual Mean intake ± SD1	EAR2	Mean intake/ EAR	10th Percentile	25th Percentile	50th Percentile	75th Percentile	90th Percentile	Skewness3	CV(%)[4]
Calcium (mg)	6-12	370 ± 69.9	-	0.5[5]	286	321	364	413	463	0.6	19
	6	379 ± 71.0	680	0.6	294	330	372	422	473	0.6	19
	7-10	372 ± 70.2	680	0.5	286	322	365	414	465	0.5	19
	11-12	365 ± 68.3	960	0.4	282	317	359	406	455	0.6	19
Iron (mg)	6-12	12.9 ± 3.4	-	1.6[5]	9.0	10.5	12.5	14.8	17.4	0.8	26
	6	11.9 ± 3.0	8.0	1.5	8.4	9.8	11.5	13.6	16.0	0.8	26
	7-10	12.7 ± 3.3	8.0	1.6	8.8	10.3	12.3	14.6	17.0	0.8	26
	11-12	13.7 ± 3.5	12.8[6]	1.1	9.6	11.2	13.3	15.7	18.3	0.8	25
Zinc (mg)	6-12	6.8 ± 1.3	-	1.1[5]	5.2	5.9	6.7	7.7	8.6	0.4	20
	6	6.3 ± 1.2	4.6	1.4	4.8	5.5	6.2	7.1	7.9	0.5	19
	7-10	6.7 ± 1.3	6.2	1.1	5.1	5.8	6.6	7.5	8.4	0.4	19
	11-12	7.2 ± 1.4	8.9	0.8	5.6	6.3	7.1	8.1	9.0	0.4	19
Vitamin A (RAE)	6-12	578 ± 375	-	1.8[5]	218	322	486	728	1050	1.9	65
	6	643 ± 412	320	2.0	249	364	539	808	1163	1.8	64
	7-10	588 ± 380	320	1.8	221	327	495	743	1069	1.9	65
	11-12	535 ± 345	480	1.1	203	300	452	676	968	2.0	65
Thiamin (mg)	6-12	1.0 ± 0.3	-	1.9[5]	0.6	0.8	0.9	1.1	1.3	0.7	30
	6	0.9 ± 0.3	0.5	1.8	0.6	0.7	0.9	1.0	1.2	0.7	30
	7-10	0.9 ± 0.3	0.5	1.9	0.6	0.7	0.9	1.1	1.3	0.7	30
	11-12	1.0 ± 0.3	0.7	1.5	0.7	0.8	1.0	1.2	1.4	0.7	29

Table 2. Continued

Nutrient (unit)	Age group (years)	Usual Mean intake ± SD1	EAR2	Mean intake/ EAR	10th Percentile	25th Percentile	50th Percentile	75th Percentile	90th Percentile	Skewness3	CV(%)4
Riboflavin (mg)	6-12	0.9 ± 0.3	-	1.1[5]	0.5	0.7	0.8	1.1	1.3	1.0	34
	6	0.8 ± 0.3	0.8	1.0	0.5	0.6	0.8	0.9	1.1	1.0	33
	7-10	0.9 ± 0.3	0.8	1.1	0.5	0.7	0.8	1.0	1.3	1.0	34
	11-12	1.0 ± 0.3	1.1	0.9	0.6	0.7	0.9	1.1	1.4	1.0	33
Niacin (mg)	6-12	15.9 ± 5.5	-	2.6[5]	9.7	12.0	15.0	18.9	23.2	1.0	35
	6	14.6 ± 5.0	6.0	2.4	9.0	11.1	13.8	17.3	21.2	1.0	34
	7-10	15.6 ± 5.4	6.0	2.6	9.5	11.7	14.7	18.5	22.7	1.0	34
	11-12	16.9 ± 5.7	9.0	1.9	10.4	12.8	16.1	20.0	24.5	1.0	34
Vitamin B6 (mg)	6-12	1.3 ± 0.5	-	1.5[5]	0.8	1.0	1.3	1.6	2.0	1.1	39
	6	1.3 ± 0.5	0.6	2.1	0.7	0.9	1.2	1.5	1.9	1.1	39
	7-10	1.3 ± 0.5	0.9	1.5	0.7	1.0	1.2	1.6	2.0	1.1	39
	11-12	1.4 ± 0.5	1.2	1.2	0.8	1.0	1.3	1.7	2.1	1.1	38
Folate (ug DFE)	6-12	227 ± 87	-	1.4[5]	129	165	214	276	343	0.9	38
	6	204 ± 78	110	1.3	116	149	191	246	308	1.0	38
	7-10	222 ± 84	160	1.4	126	161	209	269	335	0.9	38
	11-12	246 ± 90	210	1.2	143	181	234	297	367	0.9	37
Vitamin B12 (mg)	6-12	5.4 ± 2.7	-	5.4	2.5	3.5	4.9	6.8	9.0	1.2	50
	6	4.4 ± 2.2	1.0	4.4	2.0	2.8	3.9	5.5	7.2	1.2	50
	7-10	5.2 ± 2.5	1.0	5.2	2.4	3.4	4.7	6.5	8.5	1.2	49
	11-12	6.3 ± 2.9	1.5	4.2	3.1	4.2	5.8	7.9	10.2	1.2	46

Table 2. Continued

Nutrient (unit)	Age group (years)	Usual Mean intake ± SD1	EAR2	Mean intake/ EAR	10th Percentile	25th Percentile	50th Percentile	75th Percentile	90th Percentile	Skewness3	CV(%)4
Vitamin C (mg)	6-12	71.7 ± 36.6	-	1.8[5]	32.2	45.5	64.7	90.2	120	1.3	51
	6	62.1 ± 32.1	25.0	2.5	28.0	39.5	55.7	78.1	105	1.3	52
	7-10	69.5 ± 35.4	40.0	1.7	31.2	44.1	62.7	87.6	117	1.2	51
	11-12	79.5 ± 38.9	60.0	1.3	37.1	51.5	72.4	99.5	131	1.2	49

[1] Intake data is usual nutrient intake via SPADE [2] EAR are H-AR values from Linsey Allen *et al* 2020, [3] Skewness measures the lack of symmetry in the distribution, 0= unskewed, >0 positively skewed and <0 is negatively skewed, [4] CV%= coefficient of variation, or the ratio of the standard deviation to the mean, is a measure of relative variability; if the CV% = 100 then the sd= mean, if > 100 it means the sd>mean and if <100 it means the sd < mean, [5] No EAR is available for the age group 6-12 years, the EAR of the middle age group 7-10 years was for the Mean intake/EAR calculation in this table, [6] male EAR was used for iron, this differs to the female EAR=11.2mg/day

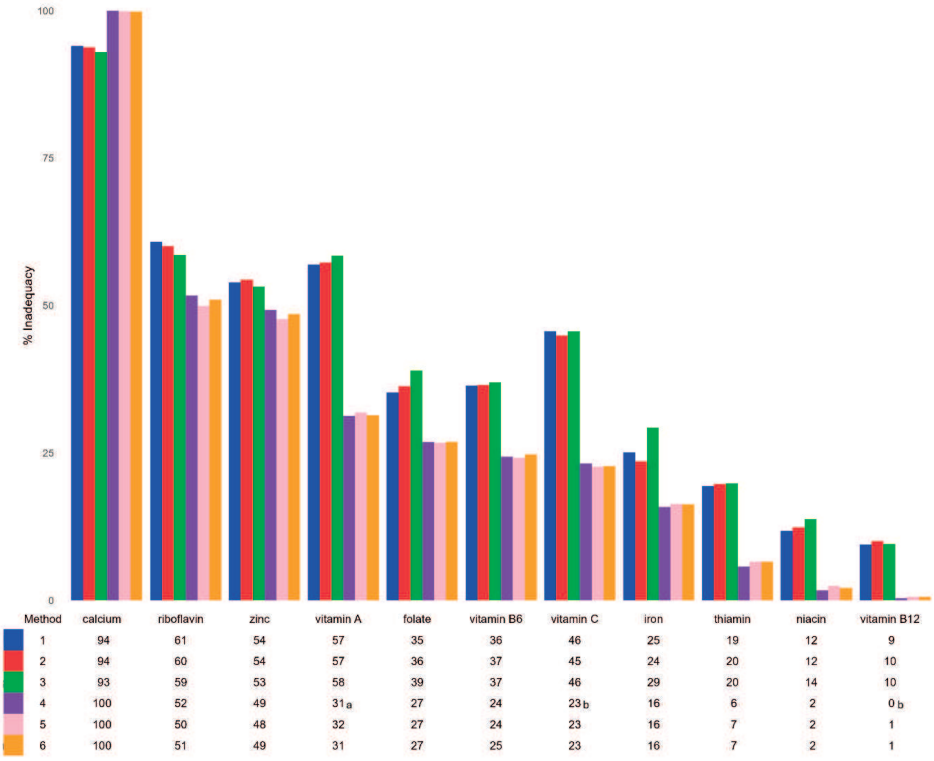
Abbreviations: SD, standard deviation; SPADE, Statistical Program to Assess Dietary Exposure; EAR, Estimated Average Requirement; PA, Probability of Adequacy; nutriR, Nutritional intake functions for R; H-A, Harmonized Average Requirements; DFE, Dietary Folate Equivalents; RAE, Retinol Activity Equivalent

For all nutrients, since the observed intake distribution was available, the PA or EAR cut-point method is preferred over nutriR. For five out of the eleven nutrients, the PA method is theoretically preferred over the EAR cut-point method, mainly because the mean nutrient intake values were not close to the EAR (vitamin B12 and niacin) or because the mean intake was in between the EAR and the Recommended Intake (RI) (zinc, riboflavin and folate). For the remaining six nutrients (vitamin A, vitamin C, thiamin, vitamin B6, calcium and iron), the EAR cut-point method was expected to perform equally well as the PA method. Based on our findings and the current functionalities of nutriR, we consider it the method of choice when the observed distribution is not available and the intake is not normally distributed, although best practices for its application remain to be fully established**.**

Comparison of the prevalence of nutrient inadequacy between the six methods

Figure 2 shows that the highest prevalence of nutrient inadequacy was found for calcium when using usual intake (methods 4-6: 100%), whereas it was somewhat lower when using one-day intake (methods 1-3: 93-94%). The opposite was seen for the remaining nutrients, where estimates based on one-day intake (methods 1-3) showed higher prevalence of inadequacy than estimates based on usual intake (methods 4-6). In descending order of prevalence of nutrient inadequacy, the prevalence of riboflavin intake inadequacy ranged between 50-52% using usual intake methods 4-6, this was higher when using one-day intake 59-61%. The prevalence of zinc inadequacy ranged between 48-49% using usual intake methods 4-6 and similarly this was higher using one-day intake methods 1-3 (range 53-54%). For vitamin A, folate, vitamin B6 and vitamin C prevalence of inadequacy ranged between 23-32% using usual intake methods 4-6 and was higher using one-day intake methods 1-3 (35-58%). For the other nutrients, the prevalence of nutrient inadequacy was ≤16% when using usual intake methods 4-6 and was higher at < 30% when using one-day intake methods 1-3.

The differences in prevalence estimates of nutrient inadequacy between the six methods ranged between 0-27 percent-point (**Figure 3**).



Method	calcium	riboflavin	zinc	vitamin A	folate	vitamin B6	vitamin C	iron	thiamin	niacin	vitamin B12
1	94	61	54	57	35	36	46	25	19	12	9
2	94	60	54	57	36	37	45	24	20	12	10
3	93	59	53	58	39	37	46	29	20	14	10
4	100	52	49	31 [a]	27	24	23 [b]	16	6	2	0 [b]
5	100	50	48	32	27	24	23	16	7	2	1
6	100	51	49	31	27	25	23	16	7	2	1

Figure 2. The prevalence of nutrient intake inadequacy (%) calculated based on one-day intake (Methods 1–3) or usual intake (corrected for within-person variation by SPADE) (Methods 4–6).

Method 1 = EAR cut-point method using one-day nutrient intake, Method 2 = PA method using one-day nutrient intake, Method 3 = nutriR using one-day nutrient intake, Method 4 = EAR cut-point method using usual nutrient intake, Method 5 = PA method using usual nutrient intake, Method 6 = nutriR using usual nutrient intake.

[a] Error handling applied dynamic starting values based on the mean and variance of the data to improve the fitting process for the gamma distribution.

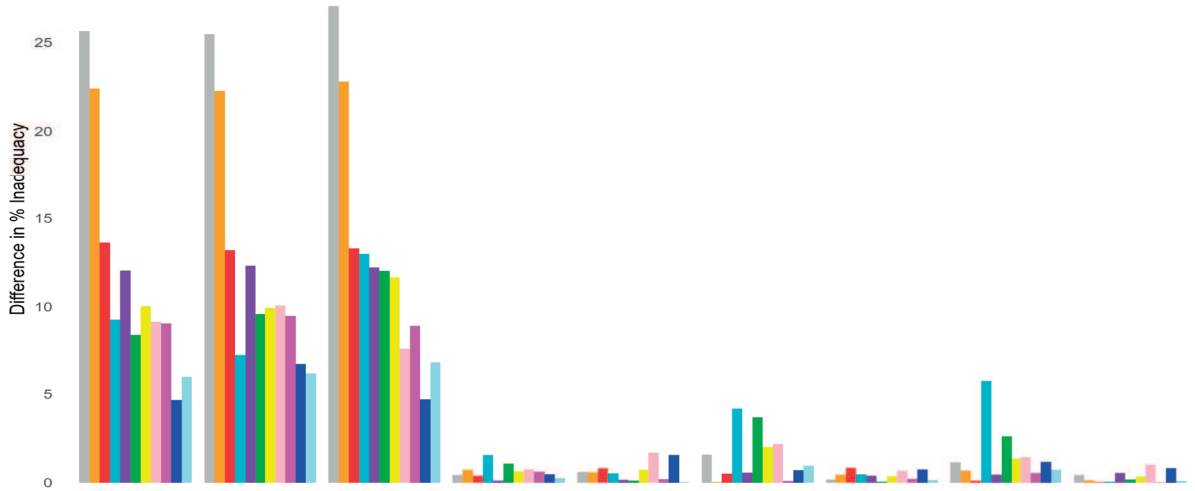
[b] Error handling applied where zero values were removed to avoid issues during distribution fitting and extreme outliers were capped at the 1st and 99th percentiles to mitigate their impact on the fitting process.

Abbreviations: SPADE, Statistical Program to Assess Dietary Exposure; EAR, Estimated Average Requirement; PA, Probability of Adequacy; nutriR, Nutritional intake functions for R.

One-day vs. usual intake – The largest differences were seen between using one-day intake (methods 1-3) and usual intake (methods 3-6). A mean difference of 11.9, 12 and 12.7 percent-point in estimated prevalence of intake inadequacy was found between the usual and one-day intake for the EAR cut-point method (method 1 and 4), PA (method 2 and 5) and nutriR (method 3 and 6), respectively (**Figure 3**). The within-person variation in intake differed per nutrient; nutrients in order of highest to lowest within-person variation were vitamin C, vitamin A, folate, vitamin B12, calcium, riboflavin, niacin, zinc, vitamin B6, iron and thiamin. In **Supplementary Figure 2**, an example is provided for zinc and vitamin A to illustrate the impact of using one-day or usual intake in nutriR on estimated prevalence of zinc intake inadequacy, showing differences in percent-points of 11.5 (gamma distribution), and of vitamin A inadequacy, showing a difference in 16.1 percent points (lognormal distribution). The prevalence of nutrient inadequacy using the three methods with usual intake (methods 4-6) was consistently lower than with one-day intake (methods 1-3) for all nutrients except for calcium.

EAR cut-point vs PA – For children aged 6-12 years, minor differences in nutrient inadequacy were found between the PA and EAR cut-point method, either when the one-day or when usual intake was used (both average differences were: 0.6 percent-points). The variation in prevalence of inadequacy for all 11 nutrients ranged between 0.1 and 1.7 percent-points (**Figure 3**). Similarly, minor differences were observed across all age groups, except for the 6-year-old group. In this group, when usual intake was used, the differences between PA and EAR cut-point method were more pronounced, with an average difference of 7.2 percent-points and a range of -1.6-20.4 percent-points for all 11 nutrients.

The effect of nutriR – The smallest differences in prevalence estimates of nutrient inadequacy between methods were seen when the nutrient intake distribution was assumed to follow the shape of "best-fit" (with nutriR) vs. observed intake. NutriR using one-day intake (method 3) differed on average by 1.5 percent-points to the EAR cut-point (method 1) and also by 1.5 percent-points to the PA (method 2). NutriR using usual intake (method 6) differed on average by 0.4 percent-points when compared to the EAR cut-point (method 4) and 0.3 percent-points when compared to the PA (method 5). Using vitamin A as an example of a nutrient with one of the highest variation (CV= 65%) and skewness (skewness = 1.9) of the usual intake distribution (**Supplementary Figure 3**) illustrates the small effect nutriR had on the prevalence estimate of inadequacy when assuming the usual intake to follow a log normal distribution (<1% percent-point difference).



	(1) One-day vs Usual intake EAR cut-point, PA and Nutri-R			(2) EAR cut-point vs PA One-day and Usual intake		(3) Nutri-R vs EAR cut-point One-day and Usual intake		(4) Nutri-R vs PA One-day and Usual intake	
Nutrient	1 vs 4	2 vs 5	3 vs 6	1 vs 2	4 vs 5	1 vs 3	4 vs 6	2 vs 3	5 vs 6
Vitamin A	25.6	25.5	27.0	0.4	0.6	1.6	0.2	1.2	0.4
Vitamin C	22.4	22.3	22.8	0.7	0.6	0.0	0.4	0.7	0.1
Thiamin	13.6	13.2	13.3	0.4	0.8	0.5	0.8	0.1	0.0
Iron	9.3	7.2	13.0	1.6	0.5	4.2	0.5	5.7	0.1
Vitamin B6	12.1	12.3	12.2	0.1	0.2	0.6	0.4	0.4	0.5
Folate	8.4	9.6	12.0	1.1	0.1	3.7	0.1	2.6	0.2
Niacin	10.0	9.9	11.7	0.6	0.7	2.0	0.4	1.4	0.4
Riboflavin	9.2	10.1	7.6	0.8	1.7	2.2	0.7	1.4	1.0
Vitamin B12	9.1	9.5	8.9	0.6	0.2	0.1	0.2	0.5	0.0
Zinc	4.7	6.7	4.7	0.5	1.6	0.7	0.7	1.2	0.8
Calcium	6.0	6.2	6.8	0.2	0.0	1.0	0.1	0.7	0.1
Average (%)	11.9	12	12.7	0.6	0.6	1.5	0.4	1.5	0.3
Range (%)	4.7-25.6	6.2-25.5	4.7-27	0.1-1.6	0-1.7	0-4.2	0.1-0.8	0.1-5.7	0-1

Figure 3. Percent-point differences in the prevalence (%) of nutrient intake inadequacy between the 6 methods per nutrient, and the overall averages and ranges comparing (1) One-day (Methods 1–3) vs Usual intake (Methods 4–6), (2) EAR cut-point (Methods 1 and 4) vs PA (Methods 2 and 5), (3) Nutri-R vs EAR cut-point (Methods 1 vs 3 and 4 vs 6), and (4) Nutri-R vs PA (Methods 2 vs 3 and 5 vs 6) in Ivorian school-aged children (6–12 years).

Abbreviations: EAR, Estimated Average Requirement; PA, Probability of Adequacy; nutriR, Nutritional intake functions for R.

The effect of nutriR combined with age categorization – Differences in the prevalence of nutrient intake inadequacy between nutriR and other methods were much larger when age of the children was grouped into one category (6-12 years) instead of three age-specific nutrient requirement groups (6 years, 7-10 years, and 11-12 years). Across all nutrients, the categorization of age into one group (6-12 years) resulted in an average difference of 5 percent-points when using the nutriR method with usual intake data (**Table 3**).

Table 3: Differences in prevalence estimates of usual nutrient intake inadequacy when using nutriR with one age category (6-12 years) and a non-age specific requirement for 7-10 years vs. age-specific requirements with a weighted average.

	Prevalence of nutrient inadequacy		**Difference**
Nutrients	One age-category[1]	Three age-categories[2]	
	%	%	%
Calcium	99.8	99.8	0.0
Riboflavin[3]	43.9	51.0	3.4
Zinc	35.3	48.7	-13.4
Vitamin A[3]	25.3	31.4	- 6.2
Folate	23.2	26.9	3.7
Vitamin B6	20.1	24.7	- 4.6
Vitamin C	18.4	22.8	- 4.3
Iron[3]	5.1	16.3	-11.2
Thiamin	3.2	6.6	3.4
Niacin	0.6	2.1	1.5
Vitamin B12[3]	0.5	0.6	0.1
Average (percent-points)			**5.1**

[1] 6-12 years using one H-AR based on the middle age group (7-10 year old), [2] 6 years old, 7-10 year old, and 11-12 year old; age-specific H-AR's are used with weighted averages based on sample size of the group, [3] Based on log normal distribution; for all other nutrients a gamma distribution was used.

Abbreviations: H-AR, Harmonized Average Requirements; nutriR, Nutritional intake functions for R

DISCUSSION

Assuming nutrient intake distributions with the shape of "best-fit" using the nutriR tool had minor effects on the prevalence estimates of nutrient inadequacy across 11 nutrients in Ivorian school-aged children, as compared to using observed nutrient intakes. Similarly, minor differences in nutrient inadequacy were found between the EAR cut-point and PA methods. The largest differences in prevalence estimates of nutrient intake inadequacy were found between using one-day and usual intake; within the 6-year-old age group; and between using age-specific requirement values and a non-age specific requirement.

In this study we found that despite skewed and varied nutrient intake data, there was a small difference in prevalence of inadequacy between the EAR cut-point method (using observed intake and only central tendency to describe the requirement distribution), the PA method (using observed intake and the entire requirement distribution) and nutriR (using the central tendency and dispersion to describe the intake distribution and assuming the "best-fit" gamma or log-normal distribution and the entire requirement distribution). This was consistent across all nutrients. The variation and skewness of nutrient intakes found in our study were comparable to those found in Passarelli *et al* (2022) where datasets from 31 countries were used (6).

Passarelli *et al*. (2022) reported that for nutrients with high variation and skewness in intake distribution, the difference in estimated prevalence by applying nutriR could be as high as 50% (mostly between 0-25%) compared to intake data with an assumed normal distribution using the EAR cut-point method. However, when the correct nutrient intake distribution was assumed, with nutriR, the differences between the "best-fit" distribution shape using PA method and EAR cut-point method were small (Figure 5d from Passarelli *et al* 2022). This is in line with the minor differences found in our study between using nutriR where the shape of "best fit" either a gamma or log normal distribution (and not the normal intake distribution) was compared to the observed intake distribution using the EAR cut-point and the PA method (7).

The similarity seen between using the observed intake distributions (using both the EAR cut-point method 4 and PA method 5) and using just two distribution variables for central tendency and dispersion (nutriR method 6) suggests that nutriR can effectively estimate nutrient inadequacy prevalence, when the correct "shape" (log normal or gamma) is chosen. NutriR may therefore be useful to calculate nutrient inadequacies in situations where simplicity is important, a median usual intake and variation are only available; and when the main research question is beyond the calculation of nutrient intake inadequacy for a population from individual dietary intake data, e.g. to investigate effects of modelling, comparisons across a large number of datasets (e.g. Passarelli *et al* 2022) or when using household food consumption and availability data (e.g. Passarelli *et al* 2024) (6,21).

In such cases mentioned above where the nutriR methodology would be applicable, it should be noted that currently the nutriR package is limited in that it does not allow calculated weighted averages for age groups with different EAR values. This requires additional manual steps outside of the program, as was necessary for our study with the age range (6-12 years) comprising three

different EAR values, while the sample size of these three age categories were not similar. We found that not using age-specific EAR values in nutriR led to an average under- or over-estimation of the prevalence of usual nutrient intake inadequacy by 5.1 percent-point (range: 0.0 – 13.4 percent-point). Passarelli *et al.* (2022) estimated nutrient inadequacy with nutriR using 5-year age groups, therefore an EAR value not specified for age may have been used, resulting in an under or overestimate of nutrient inadequacy (6).

In practice, when the observed distribution intake is known, as was the case for the current study, and the main objective is to estimate nutrient inadequacy in one population group using dietary intake data, the use of observed data would be preferable over nutriR. Despite the minor difference observed, it is more accurate to use the observed intake distribution, and also simpler to use. The added complexity of using nutriR with observed intake data is that the nutrient intake distribution needs to be corrected for the within-person variation before use of nutriR requiring a tool such as SPADE. These tools also calculate prevalence of inadequacy using the EAR cut-point method, making nutriR redundant in this specific case.

The largest differences in estimated nutrient intake inadequacy (on average ~12% and as large as 27%) were found between methods using one-day intake and usual nutrient intake, for which within-person variation was taken into account. This was expected and described previously by Carriquiry (1998) and others (1,2,9). Our study once more emphasizes the importance of collecting more than one day of intake data to allow removal of within-person variation, for example with a statistical program such as SPADE. Other methods do also exist to calculate usual nutrient intake, such as the Iowa State University (ISU), National Cancer Institute, (NCI) and Multiple Source Method (MSM), and minor differences between methods have been reported with sample sizes >400 subjects (22,23). Additionally, the sample size of individual age groups should also be considered when interpreting usual nutrient intake data and selecting between the EAR cut-point or PA methods. In our study, the 6-year-old group had a much smaller sample size, resulting in higher variability and skewness of usual intake data for some nutrients. This variability was better accommodated for by the PA method than the EAR cut-point method. However, this discrepancy was not observed when a weighted average was used for the total 6-12 year age group.

Multiple day 24 hour recalls in LMIC are difficult to conduct but information derived with it is impactful and needed (24). This study included one 24-hour recall and a second repeated recall for 12% of a convenience sample. This is not in accordance with the gold standard of two 24-hour recall surveys on non-

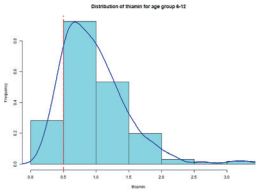
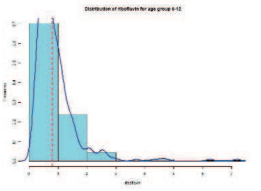
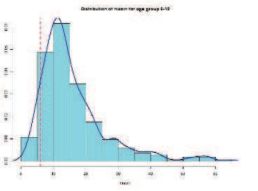
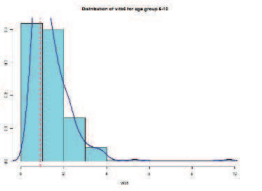
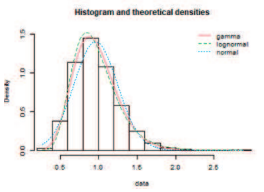
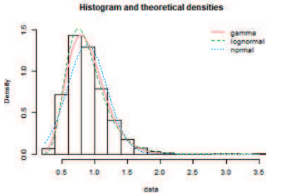
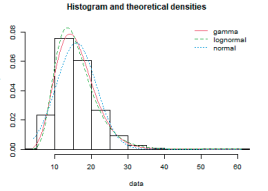
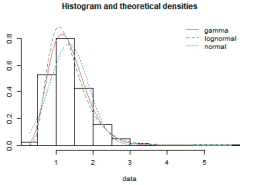
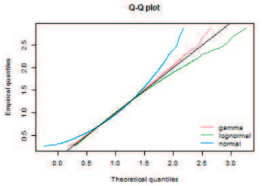
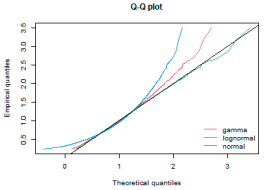
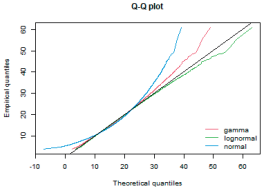
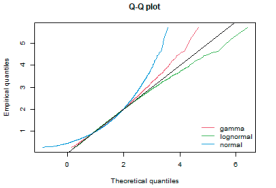
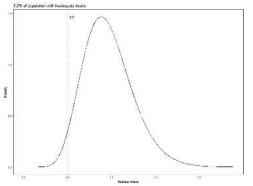
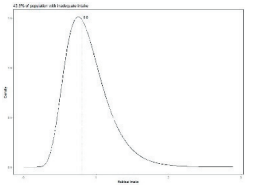
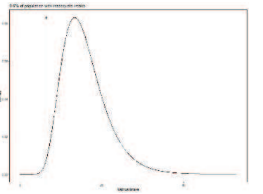
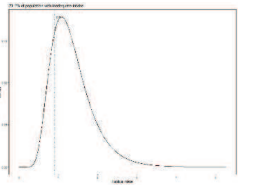
consecutive days or three 24-hour recall surveys on consecutive days (25,26). The development of statistical tools such as the NCI-1day are important to help explore how to best utilise data that can feasibly be collected (e.g., one 24-hour recall) or tools such as nutriR that utilise data that have already been collected (e.g. household food consumption, expenditure surveys or food supply data). NCI-1day calculates usual nutrient intake with one 24-hour recall, based on the within-person variation found in the NHANES survey (27). Given the impact within-person variation can have on the estimations of nutrient inadequacy, more effort should be made in understanding it across countries and population groups. Passarelli *et al* (2022) have made global comparisons of usual nutrient intake distributions. If these distributions could be taken into account in the NCI-1day method, this would lead to more valid estimations across different population groups, outside of The United States of America. The same may be true for household food consumption, food supply and expenditure surveys in LMIC where dietary intake surveys have not been conducted. Passarelli *et al* 2024, improved the validity of such data using nutriR, where the median nutrient intake from the publicly available Global Dietary Database (containing household food consumption surveys and food balance sheets) was combined with the newly established variability values of each nutrient distribution to predict global prevalence estimates of nutrient intake inadequacies (21).

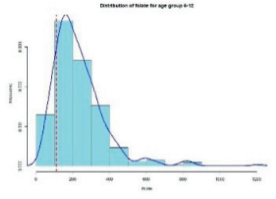
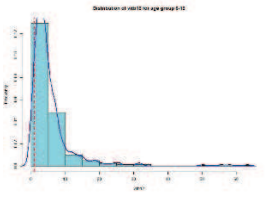
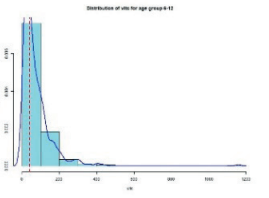
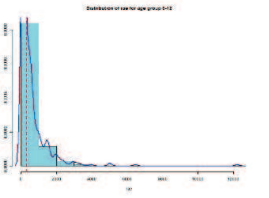
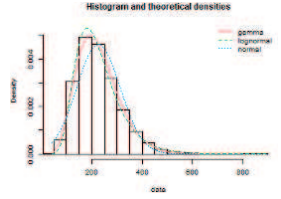
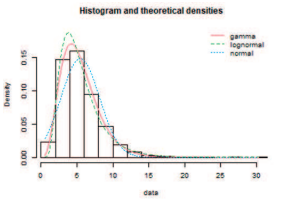
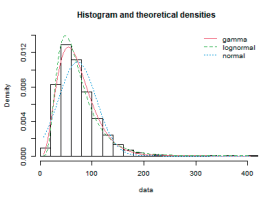
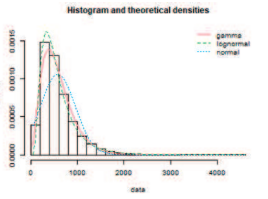
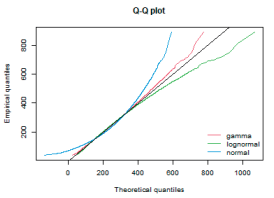
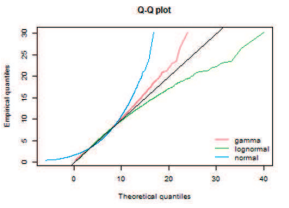
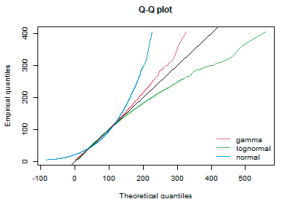
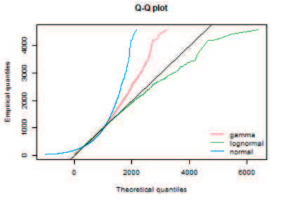
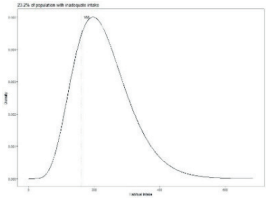
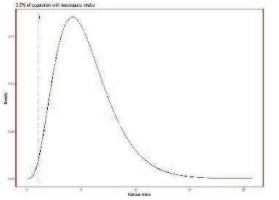
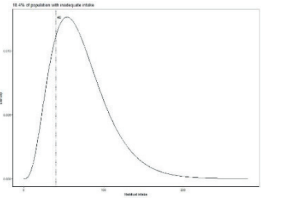
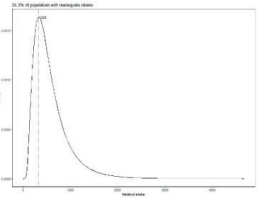
Several limitations should be considered in interpreting these findings. Firstly, dietary intake data were self-reported, recall bias and underreporting may have occurred. Nutrient inadequacies should be validated with biomarkers. Twenty-four hour recalls were only repeated for 12% of a convenience sample, which may be insufficient to estimate within-person variation accurately. Sampling relied on convenience rather than fully random, potentially introducing selection bias. Additionally, the random walk method used for household selection may have introduced clustering. The prevalence of iron inadequacy presented in this study may be inaccurately estimated because the EAR for boys aged 11-12 years has been applied to both sexes. This was due to the high ratio of within-person to between-person variance for males across several nutrients which violated the assumptions of the SPADE subgroup analysis. Finally, estimation of nutrient intake was limited by the use of a food composition table that is not country specific, as is the case for many African countries. Food items were difficult to match in many cases, and therefore their nutrient composition may not be representable.

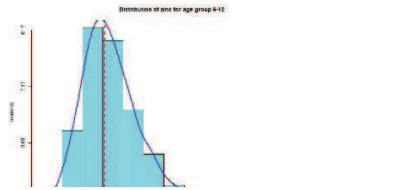
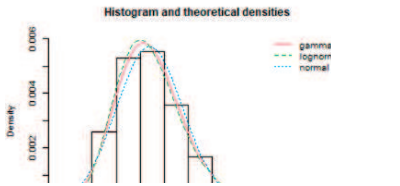
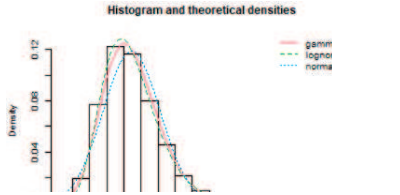
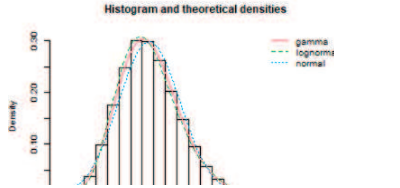
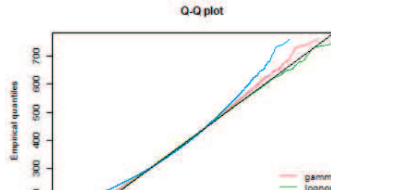
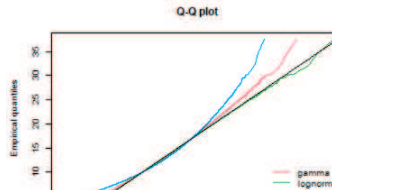
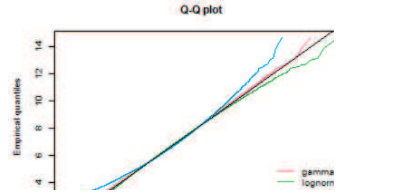
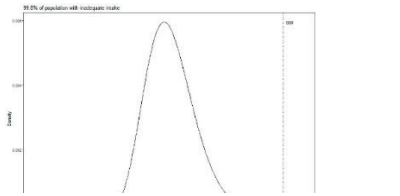
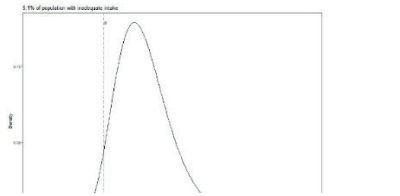
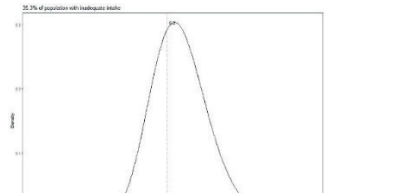
In conclusion, nutriR produced similar prevalence estimates of nutrient inadequacy compared to observed usual intake data with high variability and skewness. This suggests that nutriR is redundant when observed dietary intake data

are available, such as in this study for Ivorian school-aged children, but also that it is a valid tool for estimating nutrient inadequacy in case of data limitations. The tool may be improved for future use by allowing for age categories (e.g. 6-12 years) to match with age-specific EAR values. This study again underlines the importance of correcting for nutrient intake data for within-person variation before calculating the prevalence of nutrient inadequacy.

SUPPLEMENTARY MATERIAL

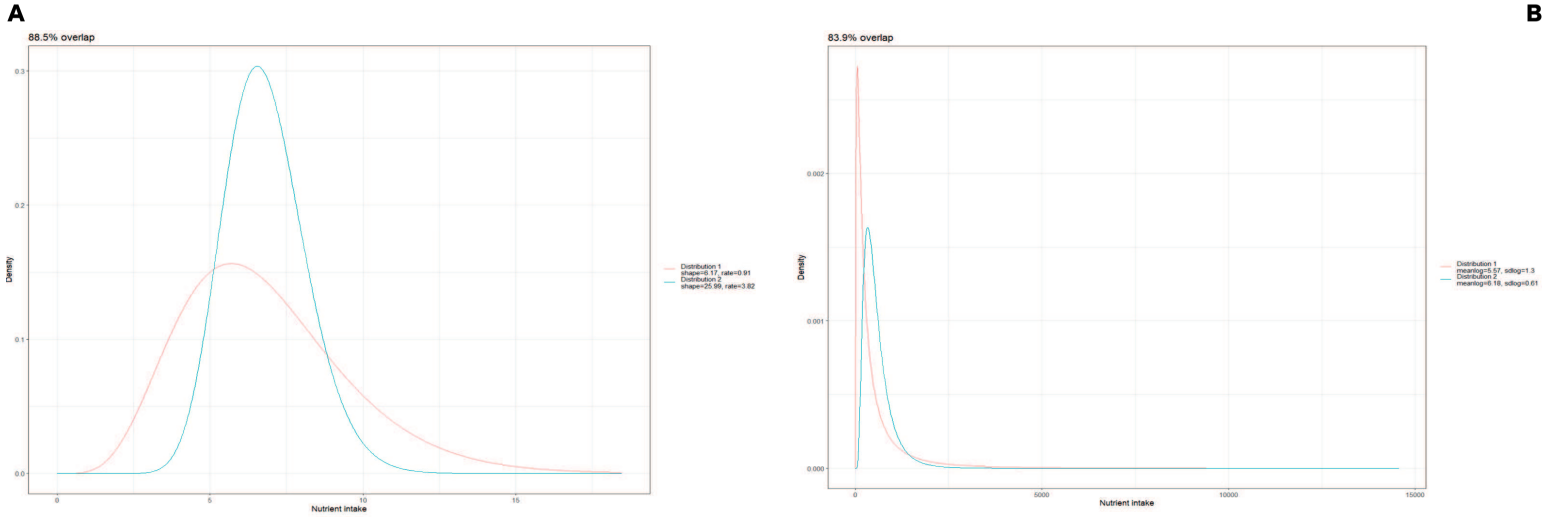
Nutrient	Thiamin	Riboflavin	Niacin	Vitamin B6
(a) One-day distribution				
(b) Usual distribution (Histogram and theoretical density graph)				
(c) Shape fit in nutriR (Q-Q Plot)				
(d) Prevalence of IA (%) using nutriR (PA method with best-fit shape)				
	3.2% of population with inadequate intakes	43.9% of population with inadequate intakes	0.6% of population with inadequate intakes	20.1% of population with inadequate intakes

Nutrient	Folate	Vitamin B12	Vitamin C	Vitamin A
(a) One-day distribution				
(b) Usual distribution				
c) Shape fit in nutriR				
(d) Prevalence of IA (%) using nutriR (PA method with best-fit shape)				
	23.2% of population with inadequate intakes	0.5% of population with inadequate intakes	18.4% of population with inadequate intakes	25.3% of population with inadequate intakes

Nutrient	Calcium	Iron	Zinc
(a) One-day distribution			
(b) Usual distribution			
(c) Shape fit in nutriR			
(d) Prevalence of IA (%) using nutriR (PA method with best-fit shape)			
	99.8% of population with inadequate intakes	5.1% of population with inadequate intakes	35.3% of population with inadequate intakes

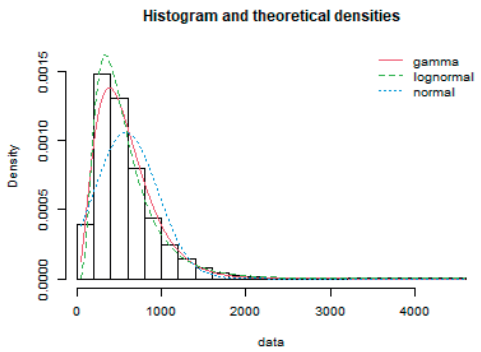
Supplementary Figure 1. NutriR output of the nutrient intake distributions of 11 nutrients from children aged 6-12 years from Côte d'Ivoire, with (a) one-day intake (used for methods 1-3); (b) usual intake (used for methods 4-6); (c) the best fit shape; and (d) the prevalence of inadequacy (%) (method 6)

Abbreviations: SPADE, Statistical Program to Assess Dietary Exposure; EAR, Estimated Average Requirement; PA, Probability of Adequacy; nutriR, Nutritional intake functions for R

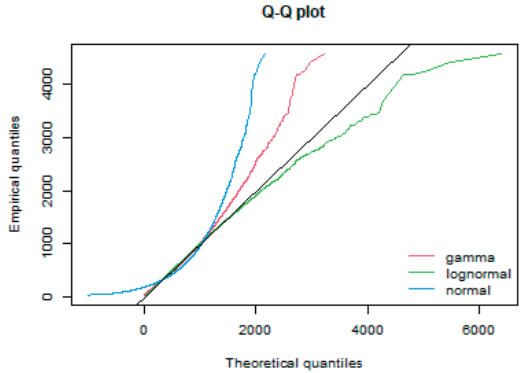


Supplementary Figure 2: The effect of using distributions of usual intake vs one-day intake on the estimation of the prevalence of zinc and vitamin A intake inadequacy of Ivorian school-age children (6-12 years)[1] in nutriR. Panel A: Gamma-distribution of one-day zinc intake (red line, distribution 1), with an estimated intake inadequacy of 46.1%, and the gamma distribution of usual zinc intake (blue line), with an estimated prevalence of zinc intake inadequacy of 35.3% (difference:11.5 percent-point). Panel B: Log-distribution of one-day vitamin A intake (red line), with an estimated intake inadequacy of 55.8%, and the log-distrbution of usual zinc intake (blue line), with an estimated prevalence of vitamin A intake inadequacy of 25.3% (difference: 16.1 percent-point). [1]non age specific EAR's have been, instead the 7-10 years age group EAR was used.

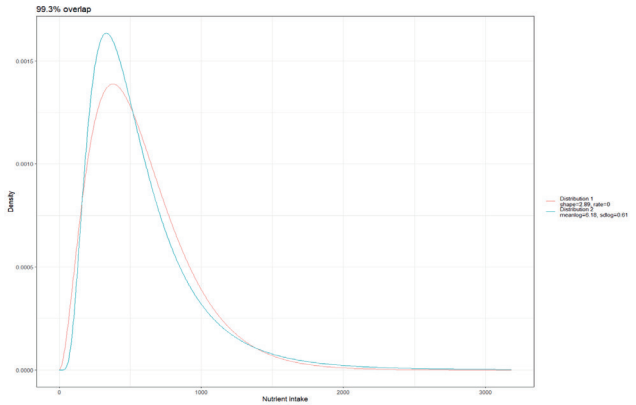
Abbreviations: nutriR- Nutritional intake functions for R.



(a) Distribution of usual vitamin A intake is rightly skewed, skewness = 1.9 and coefficient of variation (CV) = 0.6 (60%).

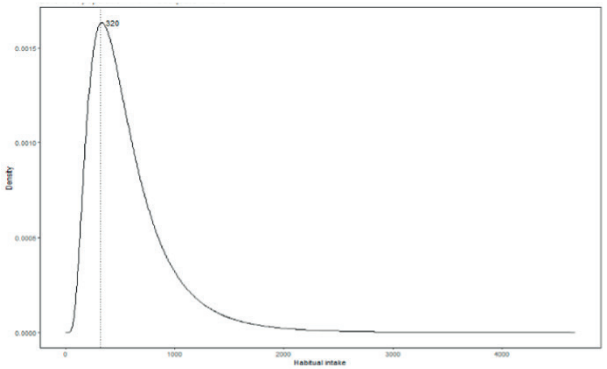


b) Usual vitamin A intake fits better to a lognormal distribution than to a normal or gamma distribution.



c) There is a 0.7 percent-point difference in prevalence of vitamin A inadequacy between the gamma and log distribution.

NutriR assumed lognormal distribution (non-age-specific[1]) = 25.3%



(d) *The difference in inadequacies between methods:*
Method 6: NutriR assumed lognormal distribution (age-specific[2]) = 31%
Method 5: PA method, using observed distribution (age-specific[2]) = 32%
Method 4: EAR cut-point method, using observed distribution (age-specific) = 31%

Supplementary Figure 3: The effect of using nutriR to adjust the usual vitamin A intake distribution (with high variation and skewness) to a log normal distribution on the prevalence of vitamin A intake inadequacy of Ivorian school-age children (6-12 years). [1] Using nutriR grouping children into one age group of 6-12 years with a lognormal intake distribution yields a prevalence of inadequacy of 25.3% (as shown in the (d) image). This is 6.2% lower than if three age-specific requirement groups were assessed separately and a weighted average was used (31.4%). See Table 3. [2] Age-specific refers to the Harmonized – Average Requirements (H-ARs) according to age groups that were used by running the analysis 3 times with the 3 different Average Requirements (ARs) for each specific age groups, after which a weighted average was calculated. See Supplementary Figure 3 for further explanation.
Abbreviations: SPADE, Statistical Program to Assess Dietary Exposure; EAR, Estimated Average Requirement; PA, Probability of Adequacy; NutriR, Nutritional intake functions for R

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Chapter 4

Dietary patterns and nutritional status of urban school-children and women of reproductive age in Côte d'Ivoire

Katie Ayling, Kim Maasen, Marleen de Jonge, Amoin Georgette Konan, Georges Tiahou, Mory Gbane, Ingeborg Bovee-Oudenhoven, Alida Melse-Boonstra, and Elise F. Talsma

Submitted for publication to a peer-reviewed journal.

Abstract

Background: Diet strongly influences nutritional status, contributing to the coexistence of undernutrition and excess weight in many low- and middle-income countries. In Côte d'Ivoire, however, dietary patterns and their relationship to nutritional status are not well characterised, limiting evidence-based approaches to double-duty nutrition interventions.

Objective: This study aimed to (1) identify dietary patterns among urban Ivorian school-children and adult females of reproductive age, (2) assess diet quality by pattern, and (3) characterise the socio-demographic factors and nutritional status of individuals within each pattern.

Methods: We conducted a cross-sectional dietary survey in four cities (Bouaké, Daloa, Abengourou and Yopougon). Dietary data were collected through two non-consecutive 24-hour recalls from 443 children (6–12 years) and 441 adult adult females (15–49 years). Dietary patterns were derived using latent class analysis. Associations with nutritional status, socio-demographic indicators, diet healthiness (Global Diet Quality Score), diet diversity (Diet Diversity Score) and nutrient adequacy (Probability of Adequacy) were examined using the step-3 bias-adjusted approach.

Results: Four dietary patterns were identified for each group. Two were found in both children and adult females ("Fish, oil and vegetables" and "Dairy and sweet"), while children had "Poultry and fruit" and "Refined, red meat and vegetable" patterns, and adult females had "Poultry, red meat and fruit" and "Low energy intake". The fish and dairy patterns, more common in Bouaké and Daloa, were associated with lower education levels (and with occupation, housing and dependent children among adult females only), yet showed higher diet quality, reflected by greater dietary diversity and nutrient adequacy. In contrast, the poultry (children) and low-intake (adult females) patterns showed poorer dietary diversity and nutrient adequacy. No associations were observed between dietary patterns and nutritional status.

Conclusions: Dietary patterns among urban Ivorian children and adult females were largely comparable. Promoting familiar, fish- and vegetable-based diets, alongside improved dietary diversity, may enhance nutrient adequacy and help reduce the double burden of malnutrition.

INTRODUCTION

Côte d'Ivoire, like many low- and middle-income countries, is now experiencing a 'triple burden' of malnutrition, characterised by the coexistence of undernutrition and micronutrient deficiencies, and rising rates of overweight and obesity (1,2). This burden is not uniform, varying across regions, age groups, and genders. More than half (51%) of adult females are overweight or obese, while adult females and children both continue to experience multiple micronutrient inadequacies (3). Among urban school-aged children (6–12 years), most (82%) have a healthy weight, yet recent evidence indicates increasing overweight in this group (4). These differing trends between adult females and children reflect broader regional shifts in sub-Saharan Africa, where rapid urbanisation and dietary change are driving complex and overlapping forms of malnutrition (5–8).

The high prevalence of overweight and obesity observed among adult females in Côte d'Ivoire likely reflects broader dietary changes accompanying economic and social transformation. Dietary change, commonly referred to as the nutrition transition, describes a shift from traditional, cereal-based diets to those with higher consumption of animal-sourced foods and vegetable oils (stage 3), followed by increased intake of energy-dense foods rich in fats, sugars and processed ingredients (stage 4) (9–11). Across sub-Saharan Africa, these shifts are evident as monotonous cereal-based diets give way to more diverse patterns that include animal sourced foods, fruits and vegetables, and processed or out-of-home foods (12,13). Such changes typically accompany economic growth and urbanisation and are concurrent with lower physical activity (14). Importantly, they do not affect everyone equally, with greater differentiation by socio-economic status than urban-rural residence (12). Despite these regional trends, relatively little is known about Côte d'Ivoire. In particular, few studies have characterised dietary behaviours across population groups to identify shared and group-specific drivers of malnutrition within the same socio-economic and food system context. Understanding how these dietary shifts manifest within and between groups is essential for addressing emerging nutritional inequalities (9–11).

Dietary pattern analysis offers a useful approach for addressing this evidence gap. By examining combinations of foods rather than individual nutrients, this approach provides insight into how overall diets relate to socio-demographic factors, urbanisation, and exposure to processed, energy-dense foods (15,16). In Côte d'Ivoire, where adult females and children face distinct but overlapping malnutrition challenges, understanding dietary patterns may clarify how urbanisation and changing dietary behaviours contribute to both undernutrition and

excess weight. In this study, we aimed to (1) identify dietary patterns of urban Ivorian school-aged children and adult females, (2) examine the similarities and differences between these patterns, (3) assess diet quality by pattern, and (4) characterise the socio-demographic factors and nutritional status of individuals within each pattern. Together, these analyses aim to generate evidence to inform equitable nutrition policies in rapidly urbanising settings.

METHODS

Study design and participants

We conducted a cross-sectional study in February 2021 to assess dietary intake among Ivorian school-aged children and adult females of reproductive age. Data were collected from 443 school-aged children (6-12 years) and 441 adult females of reproductive age (15-49 years) in four cities of Côte d'Ivoire: Bouaké, Daloa, Abengourou and Yopougon. The study received ethical approval from the National Ethics and Research Committee of Côte D'Ivoire (CNER-CI). Informed consent was obtained from all adult females participants and from a parent or care-giver for each child.

Sampling

Participants were selected using a two-stage random sampling procedure. First, neighbourhoods were selected by simple random sampling from a list of neighbourhoods in each city, according to the General Population and Housing Census RGPH 2014. The number of selected neighbourhoods per city was determined using probability proportional to city size. Within each neighbourhood, households were selected by using the random walk method. Eligible participants were apparently healthy, as assessed by physician, and were either children (6-12 years) or adult females (15-49 years). Only one participant was selected per household; hence, adult females and children did not reside in the same households.

Data collection

Trained field investigators conducted face-to-face interviews with the participants (and with care-givers for all children). Data were collected using an electronic survey tool KoboCollect (versions 1.25.1, KoboToolbox, USA). Height and weight were measured using a standardized height board and scale. Demographic and socio-economic characteristics, including care-giver's educational level and occupation, housing type, number of people living in the household and daily household expenditure on food were collected by questionnaires. Dietary intake was collected via a standard multiple pass 24-hour recall (17).

Portion sizes of foods and beverages were estimated based on a photographic food atlas (18). A second 24-hour recall was administered to 12% of the participants on non-consecutive days with at least 7 days and at most 18 days between the two recalls. Further details on dietary intake, socio-demographic measures, anthropometry and data cleaning are reported elsewhere (3).

Socio-demographic status

Socio-economic status was derived using Principal Component Analysis (PCA) in R Studio (packages: "Corr" and "ggcorrplot"), based on four commonly used indicators: housing type, household food expenditure, education and occupation. However, no component with an eigenvalue ≥ 1 was identified. Consequently, a composite SES score was not constructed, and the individual SES indicators were analysed separately.

Nutritional status

Nutritional status was assessed using anthropometric measures, compared with age-and sex-specific World Health Organization (WHO) standards, and calculated using the WHO AnthroPlus Software (version 1.0.4) (19). For children (5-19 years), obesity was defined as BMI-for-age > + 2 standard deviations (SD), overweight as >+ 1 SD, thinness as < -2 SD and stunting as height-for-age <- 2 SD (20). For adults, BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2) and categorized as underweight (<18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2) and obese (≥30 kg/m^2) (21).

Diet Quality

Diet Quality was evaluated using three indicators: (i) the Global Diet Quality Score (GDQS) (22), (ii) the Diet Diversity Score (DDS) (23), and (iii) nutrient adequacy.

(i) the Global Diet Quality Score (GDQS)

Individual foods based on the first day 24-hour recall were classified into the corresponding GDQS food groups, and reviewed by four experts (KA, KM, EFT and AMB). The GDQS is a validated, food-based measure of diet quality that reflects the risk of nutrient inadequacy and diet-related non-communicable disease (24,25). It includes 25 food groups: 16 are considered healthy (GDQS+), 7 unhealthy, and 2 unhealthy at excessive intakes (GDQS−), with total scores ranging from 0 to 49, where higher scores reflect healthier diets (24,25). The total daily consumption (grams/day) from each food group was calculated from the 24 hour dietary recall data. GDQS total, GDQS+ and GDQS− scores were then derived for adult females and children separately using the published scoring cut-offs. Population-level GDQS categories were defined as ≥ 23 points (low

risk of non-communicable diseases and nutrient inadequacy); 15 to 23 points (moderate risk), and < 15 points (high risk). We reported the mean GDQS and the prevalence of participants in each risk category for both groups (13).

(ii) the Diet Diversity Score (DDS)

Diet diversity was assessed using the DDS, which reflects the number of different food groups consumed over a 24-hour period and serves as an indicator of dietary variety and micronutrient adequacy. Individual foods from the first day 24 hour dietary recall were classified into the corresponding ten food groups: (1) grains, white roots and tubers, and plantains; (2) pulses (beans, peas, and lentils); (3) nuts and seeds; (4) dairy; (5) meat, poultry, and fish; (6) eggs; (7) dark green leafy vegetables; (8) other vitamin A-rich fruits and vegetables; (9) other vegetables; and (10) other fruits. Participants received one point for consuming more than 15 g from each food group, and the points were summed to obtain a total DDS (range 0-10). For adult females, we calculated Minimum Dietary Diversity for Women (MDD-W), which uses the same ten food groups but applies a validated cut-off of ≥5 food groups, representing the minimum dietary diversity associated with a higher likelihood of adequate micronutrient intake at a population level (26). We therefore reported both the mean DDS and proportion of adult women meeting MDD-W (≥ 5 food groups). For children, we reported the mean DDS and the proportion consuming ≥ 5 food groups for a descriptive comparison, while acknowledging that this cut-off has not been validated for children (23).

(iii) nutrient adequacy

Usual intakes of 11 micronutrients (calcium, iron, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12 and vitamin C) were estimated using the Statistical Program to Assess Dietary Exposure (SPADE; version 4.1.1, RIVM, The Netherlands) in R Studio (Packages: SPADE.RIVM). Usual intake distributions were adjusted for within-person variation using repeat 24-hour dietary recalls available for 12% of participants. Nutrient adequacy was then assessed using the Estimated Average Requirement (EAR) cut-point method by comparing usual intake distributions with the age- and sex- specific EARs. Micronutrients were selected for their public health relevance among children and adult females in the West-African region and the availability of reliable food composition and requirement data (27,28).

For sub group analyses by dietary pattern, SPADE's subgroup functionality was used to derive usual intake and adequacy within the same model, with 50 bootstrap iterations to obtain 95% confidence intervals. Nutrient reference values (EARs) used were the harmonized average requirement (H-ARs)

provided by Allen *et al* (2020), as Côte d'Ivoire lacks national nutrient intake recommendations (29). Iron adequacy among adult females of reproductive age was assessed using the full requirement distribution of the WHO, given the skewed nature of iron requirements in this group [30]. We assumed moderate levels of bioavailability for iron (10%) and zinc (25%) (30), consistent with Côte d'Ivoire's classification as "moderate" on the Human Development Index (31).

Characterisation of dietary patterns in children and adult females

All foods and beverages consumed on the first recall day were classified into 25 food groups based on the Global Dietary Quality Score (GDQS) classification, as described above (22). Food groups with very few consumers (fewer than 10 consumers) were excluded (n=9), leaving 16 food groups. For each participant, total daily intake (g/day) was calculated for each food group. These intakes were then categorised into ordinal variables representing consumption levels (e.g. non-consumption, low intake, or high intake), because of the highly skewed nature of food group intakes, following the approach of Dalmartello *et al*. 2000, 2001 (32,33). Cut-offs for each category are provided in Supplement Table 1 (children) and Supplement Table 2 (adult females). These categorical variables were then used as observed input indicators in the latent class analysis (LCA).

For LCA, models with one to five classes were estimated. The optimal number of dietary patterns was determined based on model fit, considering lower values of Bayesian information criterion (BIC) and Akaike information criterion (AIC), parsimony and model interpretability. Additional diagnostic indices, such as entropy R^2, were also examined; however, entropy was not used as a primary selection criterion because it does not reliably identify the best-fitting model. Entropy reflects classification accuracy, ranging from 0-1 where values >0.6 are considered acceptable (34). The assumption of local independence between food groups was checked by examining bivariate residuals (BVRs). Pairs of food groups with a BVR >4 were reviewed, and residual correlations were permitted between food groups when theoretically justified, in order to improve model fit. Total energy intake (kcal) was included as a covariate, both as a predictor of class membership and as a direct effect on all food group indicators, to ensure that dietary patterns reflected composition rather than total energy intake (35). After selecting the best class model, we ran the model several times with 160 random start sets and 250 iterations to confirm that our solution was not due to reaching a local maximum, and then fixing the seeds.

Participants were assigned to dietary patterns based on their highest posterior probability of class membership. Each resulting pattern was described using

two complementary indicators: (i) the probability of consuming each food group and (ii) the class-specific mean daily intake (g/day) of each food group. To obtain bias-adjusted pattern-specific means, the means of food group intakes were estimated by treating food groups as distal outcomes in the bias-adjusted three-step approach with maximum likelihood (ML) estimation, robust standard errors, and class-dependent variances (36). Standard deviations of the means were derived from the standard errors.

Statistical analysis

All analysis were conducted in RStudio (version 4.3.1, R statistical software) and Latent Gold (version 6.1) (37). Food group intakes were summarised as means (±SD, g/ day) and as the percentage of consumers. Socio-demographic characteristics, nutritional status and diet quality indicators were described as frequencies (percentages) for categorical variables, and means (standard deviations) for continuous variables. All summary statistics were produced for the total sample and separately for each dietary pattern group.

Bivariate associations between dietary patterns and participant/household characteristics were examined within the LCA framework using Wald tests, with $p \leq 0.05$ considered statistically significant. For continuous distal outcomes (e.g. age, number of dependent children in the household, number of people living in the household, height, weight, nutrient inadequacy of the 11 nutrients, DDS and GDQS), we used Bolck-Croon-Hagenaars (BCH) method with robust standard errors and class dependent variances to account for classification error. For categorical distal outcomes (e.g. sex, occupation, education, living situation, relationship status, GDQS risk, BMI status) we used Maximum Likelihood (ML) estimation using the same bias-adjusted three step association (36). When overall Wald tests indicated significant differences across dietary patterns, pairwise comparisons were performed, with $p \leq 0.05$ considered statistically significant. Results are presented as pattern-specific means (±SD) for continuous variables and frequencies (percentages) for categorical variables.

For nutrient inadequacy, Wald tests could not be applied because SPADE produces estimates at the population level rather than the individual level. In this case, differences between patterns were evaluated by comparing 95% confidence intervals obtained as SPADE output for each dietary pattern group separately, with non-overlap interpreted as evidence of a statistical difference.

Sensitivity analysis

We evaluated the robustness and interpretability of the identified dietary patterns through three complementary analyses. First, we compared alternative

model solutions (two to four classes out of the five classes as these three had the best model fit) with and without total energy intake (kcal/day) included as a covariate (both as a predictor of class membership and as a direct effect on the food group indicators). This allowed us to assess whether adjusting for energy intake as a covariate influenced the number or composition of the dietary patterns. Second, we fitted a combined population model including both children and adult females in one dataset, and added participant type (i.e. child or woman) as random effect to check if there was higher level clustering (GCLasses=2), and compared two to four class solutions to examine the consistency of dietary patterns across groups. Third, we evaluated the effect of relaxing the local independence assumptions by comparing models with and without theoretically justified local dependencies between food groups, as identified by bivariate residuals (BVRs). Model fit, class interpretability, and stability of class membership probabilities were used to determine whether these specifications improved the overall solution.

RESULTS

Population characteristics

Participants were sampled across Abidjan (36%), Bouaké (29%), Daloa (26%), and Abengourou (9%). Among school-aged children, ten records were judged implausible and excluded, leaving 434 school-aged children (mean age: 9.1 ± 1.9 years; 55% female), of whom 54 completed a second recall. In this group, 82% had a normal BMI-for-age, 7% had overweight/obesity, 11% were thin or underweight and 3% were stunted. Among adult females, seven records were excluded as implausible, leaving 434 (mean age: 29.4 ± 8.8 years), of whom 52 completed a second recall. In this group, 44% had normal weight, 51% were overweight or obese, and 7% thin or underweight.

Identification of dietary patterns

For both children and adult females, a 4-cluster (dietary pattern) solution was selected based on a balance between statistical fit and interpretability (**Table 1**). Although the two-cluster models had the lowest BIC, the four-cluster models showed a more acceptable entropy, lower bivariate residuals, and yielded interpretable solutions.

Table 1. Comparison of model fit statistics among LCA models for dietary patterns with an increasing number of clusters.

Population group	Clusters	LL	BIC	AIC	AIC3	Npar	L^2	df	p-value	Boot-strap p	Max. BVR	VLMR	p-value	-2LL Diff	Boot-strap p	Class. Err.	Entropy R^2
Children	1-Cluster	-4681	9502	9408	9431	23	4096	412	1.2e-597	0	30					0	1
	2-Cluster	-4625	9536	9345	9392	47	3985	388	5.7e-588	0	11	111	0.00	111	0.00	0.12	0.59
	3-Cluster	-4583	9597	9308	9379	71	3899	364	1.6e-583	0	11	85	0.00	85	0.00	0.15	0.67
	4-Cluster	-4541	9660	9273	9368	95	3816	340	1.3e-579	0	11	83	0.00	83	0.00	0.18	0.67
	5-Cluster	-4511	9745	9260	9379	119	3756	316	2.1e-580	0	7	60	0.09	60	0.04	0.16	0.72
	4-Cluster-modified [1]	-4431	9596	9103	9224	121	8767	314	2.7e-1612	0	5					0.16	0.71
Adult females	1-Cluster	-4657	9460	9362	9386	24	4091	410	1.0e-597	0.30	94					0.0	1.0
	2-Cluster	-4576	9449	9249	9298	49	3928	385	2.1e-578	0.23	94	163	0.00	163	0.00	0.12	0.59
	3-Cluster	-4515	9480	9178	9252	74	3807	360	2.0e-567	0.19	21	121	0.01	121	0.00	0.14	0.66
	4-Cluster	-4479	9559	9155	9254	99	3734	335	5.5e-566	0.12	21	73	0.01	73	0.00	0.17	0.67
	5-Cluster	-4446	9645	9140	9264	124	3669	310	2.6e-566	0.09	12	65	0.02	65	0.02	0.15	0.74
	4-Cluster-modified [2]	-4273	9202	8762	8870	108	8482	326	5.8e-1544	0.15	5					0.08	0.79

In all models, the 7 food groups with no or low consumption were removed. Light yellow shows lowest BIC; Dark yellow indicates the final selected models.

[1] Energy (kcal) was added both as co-variate and as a direct effect on food groups. [2] Energy (kcal) was added both as co-variate and as a direct effect on food groups; in addition, residual correlation was allowed between food groups with a high BVR (nuts and seeds and other vegetables; red meat and fish).

Abbreviations: LCA, latent class analysis; LL, log-likelihood; BIC, Bayesian Information Criterion; AIC, Akaike Information Criterion; AIC3, adjusted Akaike Information Criterion; Npar, number of parameters; L^2, likelihood ratio statistic; df, degrees of freedom; p-value, probability value; Boots. trap p, bootstrap likelihood ratio test p-value; Max. BVR, maximum bivariate residual; VLMR, Vuong–Lo–Mendell–Rubin likelihood ratio test; −2LL Diff, difference in −2 log-likelihood; Class. Err, classification error; Entropy R^2, entropy-based measure of classification quality.

Characteristics of dietary patterns and diet quality

Among children, four distinct dietary patterns were identified and named (**Figure 1, Table 2**). The most common pattern, named "Dairy and sweet" (36% of children), was characterised by higher consumption of dairy, fruits other than vitamin A rich or citrus varieties, juice, and sweet foods as compared to the other patterns. This pattern showed intermediate diet healthiness (GDQS), highest diversity (DDS) and highest micronutrient adequacy (PA) compared to the other patterns. The intermediate GDQS reflected high consumption of GDQS "unhealthy" food groups, including refined grains, sweets, sugar-sweetened beverages, juice and white roots and tubers. The second largest, "Fish, vegetables and oil" pattern (25%), included higher consumption of dark green leafy vegetables, oils, fish, deep orange fruits and white roots and tubers. It showed the highest total healthiness (GDQS), the lowest diversity (DDS) and intermediate micronutrient adequacy compared to the other patterns. The "Poultry, eggs, fruits" pattern (23%), showed the lowest total GDQS, DDS and nutrient adequacy, reflecting low consumption of healthy foods (GDQS+) and high consumption of unhealthy foods (GDQS-). The smallest, "Refined, red meat and vegetables" pattern (16%), included higher consumption of red meat, refined grains, legumes, nuts and seeds, and vegetables, and showed intermediate GDQS, DDS and nutrient inadequacy.

Among adult females, four corresponding patterns were identified. The largest, the "Fish, vegetables and oils" pattern (32 %), was characterised by higher intakes of deep orange fruits, vegetables, fish, oils, and refined grains, and showed high GDQS, intermediate DDS and intermediate micronutrient adequacy compared to other patterns. The "Poultry, red meat, tubers and fruit" pattern (26%), included higher intake of poultry, citrus fruit, red meat, white roots and tubers, and intermediate GDQS, DDS, and nutrient adequacy. The "Dairy and sweet" pattern (24% of adult females) was characterised by higher intakes of fruits, legumes, eggs, high fat dairy, sweet foods and juice and showed the highest GDQS, DDS and nutrient adequacies compared to other patterns. Finally, the "Low energy intakes" pattern (18%), reflected generally lower intake across all food groups and the lowest mean energy intake (1242 kcal compared to an average of 2720 kcal in the overall population), the lowest GDQS, DDS and nutrient adequacy compared to other patterns.

In summary, across both children and adult females, the "Fish, vegetables and oil" pattern had the highest GDQS, while the "Dairy and sweet" pattern had the highest DDS and nutrient adequacy. The lowest GDQS, DDS and highest nutrient inadequacy was observed in the "Poultry and fruit" pattern for children and the "Low energy intake" pattern among adult females.

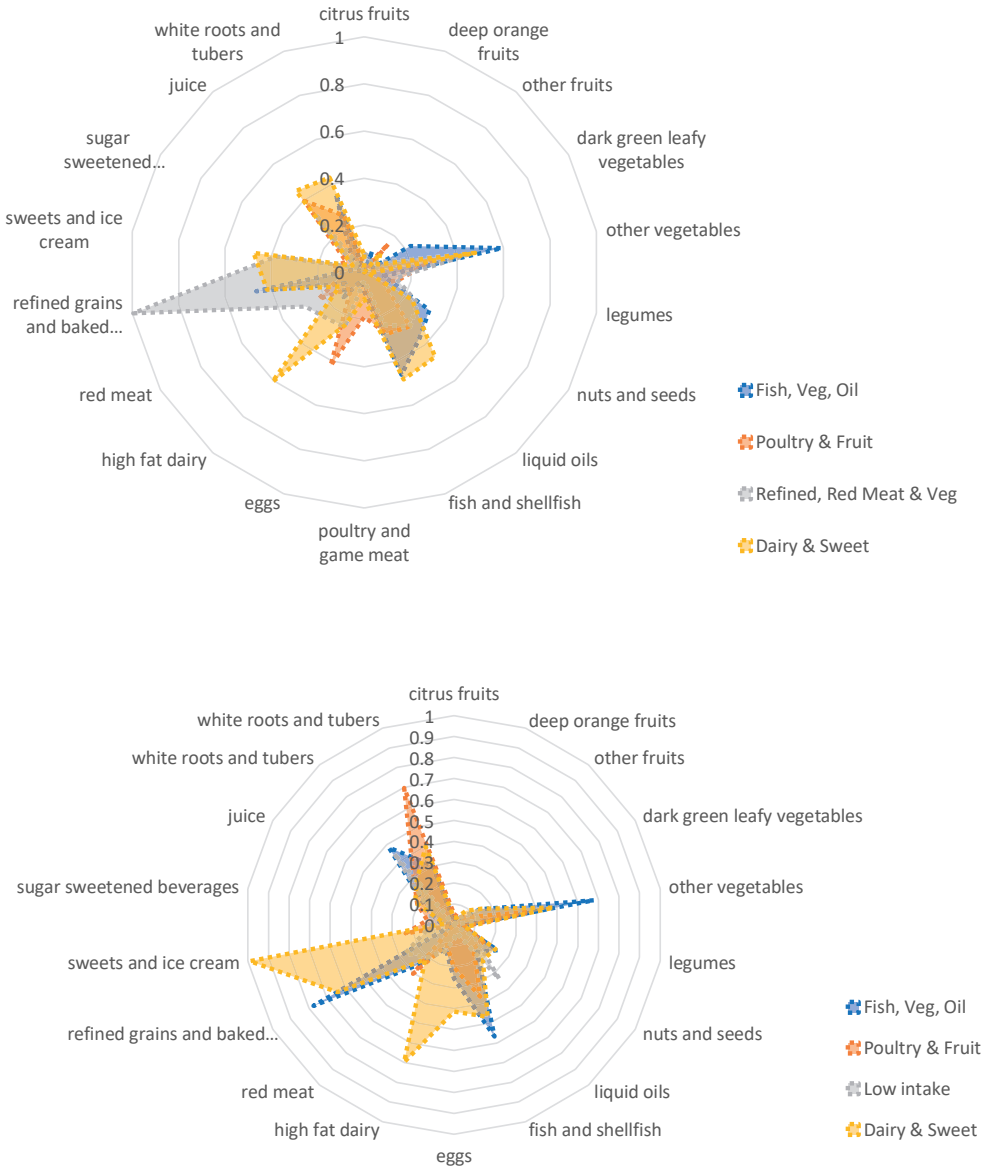


Figure 1. Dietary pattern classification based on the proportion of consumers in the highest intake category for each food group among children (top) and adult females (bottom). The figure highlights the food groups most frequently consumed at high levels within each dietary pattern. [1] Classification into intake categories and the definition of the proportion of consumers are described in the Methods section. The corresponding proportions for all intake categories, including non-consumers, are reported in Supplement Tables 1 and 2 for children and adult females, respectively.

Table 2 – Food group intake (g/day) and diet quality indicators by dietary patterns for children and adult females

			"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4[4]	Total	Wald (p-value)
Healthy food group intake (g/day)*	Proportion, n (%),	Children	156 (36)	108 (25)	100 (23)	71 (16)	435	
		Adult females	104 (24)	139 (32)	112 (26)	79 (18)	434	
	Total energy (kcal/day)	Children	2325 ± 74	2156 ± 93	1615 ± 81	2410 ± 129	2134 ± 38	50 (<0.001)
		Adult females	3170 ± 1920	3219 ± 1834	2727 ± 1176	1242 ± 485	2720 ± 1312	50 (<0.001)
	Citrus fruits (g/day)[1]	Children	0 ± 0	0 ± 0	**142 ± 232**	0 ± 0	32.6 ± 111	38 (<0.001)
		Adult females	0 ± 0	0 ± 0	**143 ± 343**	0 ± 0	37.1 ± 175	20 (<0.001)
	Deep orange fruits (g/day)[1]	Children	0 ± 0	**167 ± 324**	0 ± 0	0 ± 0	41.3 ± 161	29 (<0.001)
		Adult females	0 ± 0	**208 ± 747**	0 ± 0	0 ± 0	66.4 ± 422	11 (0.00)
	Other fruits (g/day)[1]	Children	**283 ± 1193**	0 ± 0	0 ± 0	0 ± 0	101 ± 715	9 (0.0031)
		Adult females	**106 ± 374**	0 ± 0	0 ± 0	0 ± 0.4	25.5 ± 183	9 (0.04)
	Dark green leafy vegetables (g/day)[1]	Children	0 ± 0	**50.1 ± 70.9**	0 ± 0	0 ± 0	12.4 ± 35.2	54 (<0.001)
		Adult females	24.2 ± 63.7	**58.7 ± 113**	0 ± 0	0 ± 0	24.5 ± 70.4	54 (<0.001)
	Cruciferous vegetables (g/day)[1]	Children	55.4 ± 415	0 ± 0	0 ± 0	0 ± 0	19.9 ± 249	3 (0.0950)
		Adult females	0 ± 0	0 ± 0	0 ± 0	**73.8 ± 137**	13.4 ± 58.6	23 (<0.001)
	Deep orange vegetables (g/day)[1]	Children	0 ± 0	0 ± 0	38.6 ± 193	0 ± 0	8.9 ± 92.3	4 (0.0450)
		Adult females	0 ± 0	0 ± 0	61.8 ± 512	0 ± 0	16 ± 261	2 (0.20)
	Other vegetables (g/day)[1]	Children	66 ± 154	116 ± 138	29.2 ± 27.2	**258 ± 307**	101 ± 145	150 (<0.001)
		Adult females	146 ± 249	**241 ± 326**	72 ± 80.5	28.4 ± 32.4	136 ± 200	121 (<0.001)
	Legumes (g/day)[1]	Children	0 ± 0	0 ± 0	76.8 ± 132	**235 ± 331**	56.1 ± 147	72 (<0.001)
		Adult females	**132 ± 550**	0 ± 0	0 ± 0	0 ± 0	31.7 ± 269	6 (0.01)
	Deep orange tubers (g/day)[1]	Children	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
		Adult females	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	

Table 2 – Continued

		"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4[4]	Total	Wald (p-value)
Nuts and seeds (g/day)[1]	Children	0 ± 0.1	53.6 ± 72.9	0 ± 0.1	**184 ± 249**	43.4 ± 112	87 (<0.001)
	Adult females	**108 ± 255**	84.9 ± 157	0 ± 0.1	0 ± 0.2	53.1 ± 137	76 (<0.001)
Whole grains (g/day)[1]	Children	11.8 ± 49.1	0 ± 0	0 ± 0	0 ± 0	4.2 ± 29.4	9 (0.0028)
	Adult females	0 ± 0	0 ± 0	19.9 ± 215	0 ± 0	5.1 ± 110	1 (0.33)
Liquid oils (g/day)[1]	Children	120 ± 109	**240 ± 224**	20.2 ± 45.3	3.3 ± 9	108 ± 132	302 (<0.001)
	Adult females	151 ± 185	**254 ± 297**	45.1 ± 79.6	6.8 ± 15.3	130 ± 196	175 (<0.001)
Fish and shellfish (g/day)[1]	Children	58.4 ± 50.9	**120 ± 118**	19.7 ± 59.2	17.9 ± 32.7	58.2 ± 75.4	129 (<0.001)
	Adult females	**122 ± 254**	**159 ± 206**	59 ± 90.1	19.5 ± 26.4	98.7 ± 111	196 (<0.001)
Poultry and game meat (g/day)[1]	Children	0 ± 0	0 ± 0	**142 ± 144**	0 ± 0	32.5 ± 68.8	97 (<0.001)
	Adult females	0 ± 0	0 ± 0	**130 ± 152**	0 ± 0	33.7 ± 77.5	82 (<0.001)
Low fat dairy (g/day)[1]	Children	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
	Adult females	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
Eggs (g/day)[1]	Children	33 ± 79.9	0 ± 0	**55 ± 80.7**	**51.9 ± 143**	33 ± 58.5	177 (<0.001)
	Adult females	**89.7 ± 115**	60.6 ± 76.5	0 ± 0	0 ± 0	40.9 ± 69.5	157 (<0.001)
High fat dairy(g/day)[1,2]	Children	**97.2 ± 217**	0 ± 0.2	0.1 ± 1	20.9 ± 70.1	38.4 ± 143	31 (<0.001)
	Adult females	**93.5 ± 144**	0 ± 0.4	0 ± 0.2	13.1 ± 23	24.8 ± 73	60 (<0.001)
Red meat (g/day)[1,2]	Children	22 ± 17.7	0 ± 0.1	43 ± 86.3	**80.3 ± 98.3**	30.9 ± 51.7	309 (<0.001)
	Adult females	24.1 ± 42.3	0 ± 0.1	**103 ± 101**	45.4 ± 33.6	40.8 ± 58.6	294 (<0.001)

Table 2 – Continued

			"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4[4]	Total	Wald (p-value)
Unhealthy food groups intake (g/day)*	Processed meat (g/day)[1]	Children	2.7 ± 40.8	0 ± -4.5	0 ± -4.3	0 ± -3.6	1 ± 24.5	1 (0.4100)
		Adult females	88.6 ± 920	0 ± 0	0 ± 0	0 ± 0	21.2 ± 450	1 (0.33)
	Refined grains and baked goods (g/day)[1]	Children	446 ± 296	493 ± 285	235 ± 160	**997 ± 380**	500 ± 276	288 (<0.001)
		Adult females	**857 ± 598**	**935 ± 500**	207 ± 203	396 ± 310	630 ± 440	332 (<0.001)
	Sweets and ice cream (g/day)[1]	Children	**56.2 ± 106**	0.6 ± 1.5	3.7 ± 11.2	14.3 ± 12.7	23.5 ± 66.6	102 (<0.001)
		Adult females	**68.3 ± 126**	0 ± 0.1	2.7 ± 9.5	0.8 ± 3.7	17.2 ± 63.3	37 (<0.001)
	Sugar sweetened beverages (g/day)[1]	Children	194 ± 480	0 ± 0	0 ± 0	0 ± 0	69.5 ± 288	25 (<0.001)
		Adult females	0 ± 0	0 ± 0	327 ± 512	0 ± 0	84.5 ± 261	46 (<0.001)
	Juice (g/day)[1]	Children	**236 ± 245**	0 ± 0	120 ± 120	0 ± 0	112 ± 159	238 (<0.001)
		Adult females	**219 ± 560**	0 ± 0	201 ± 314	0 ± 0	104 ± 268	89 (<0.001)
	White roots and tubers (g/day)[1]	Children	370 ± 423	**452 ± 528**	211 ± 159	1 ± 5	293 ± 236	689 (<0.001)
		Adult females	467 ± 675	300 ± 286	**759 ± 445**	84.2 ± 282	420 ± 375	209 (<0.001)
	Purchased deep fried foods (g/day)[1]	Children	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
		Adult females	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
Diet Quality	GDQS Total[1] (Range: 0-49)	Children	15.1 ± 3.2[a]	**17.2 ± 3.4[b]**	14.4 ± 3.4[a]	15.6 ± 3.8[a]	15.6 ± 2.9	36 (<0.001)
		Adult females	**16.7 ± 3.1[a]**	**16.5 ± 3[a]**	15.3 ± 2.9[b]	13.8 ± 2.5[c]	15.8 ± 2.7	36 (<0.001)
	GDQS plus [1] (Range: 0-32)	Children	5.8 ± 2.8[a]	**6.5 ± 3.3[b,d]**	4.7 ± 3[c,e]	4.9 ± 4[a,d,e]	5.6 ± 2.7	18 (<0.001)
		Adult females	6.4 ± 2.9[a]	6.2 ± 2.7[a]	5.1 ± 2.7[b]	2.4 ± 1.5[c]	5.3 ± 2.3	18 (<0.001)
	GDQS minus [1] (Range: 0-17)	Children	**9.2 ± 2[a]**	10.7 ± 1.2[b]	9.7 ± 2[c]	10.8 ± 2[b]	10 ± 1.6	55 (<0.001)
		Adult females	10.3 ± 2[b]	10.3 ± 1.5[a]	10.1 ± 1.8[a]	11.4 ± 1.9[c]	10.5 ± 1.6	55 (<0.001)
	GDQS category, High Risk (%)[3]	Children	43 (35–52)[a]	26 (16–36)[b]	**60 (49–71)[a]**	45 (32–73)[c]	43 (39–48)	17 (0.0073)
		Adult females	27 (18–35)[a]	32 (23–40)[a]	43 (33–53)[a]	**68 (56–38)[b]**	40 (36–45)	18 (<0.001)
	GDQS category, Moderate Risk (%)[3]	Children	56 (48–65)	73 (63–83)	40 (29–51)	48 (35–53)	55 (51–60)	
		Adult females	72 (62–81)	66 (57–75)	56 (46–66)	32 (20–83)	59 (54–63)	

Table 2 – Continued

		"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4[4]	Total	Wald (p-value)
GDQS category, Low Risk (%)[3]	Children	0 (-0.5–0.6)	1 (-1.1–4)	0 (0–0.1)	**6 (0.2–6)**	1 (0.3–2)	
	Adult females	**2 (-0.8–5)**	**2 (-0.3–5)**	0.6 (-1.2–3)	0 (0–2)	1 (0.3–2)	
DDS score [1]	Children	**4.2 ± 1.1[a]**	3.5 ± 1.2[b]	3.5 ± 1.2[c]	3.9 ± 1.4[a,b,c]	3.8 ± 1	27 (<0.001)
	Adult females	**4.6 ± 0.94[c]**	3.8 ± 0.92[a]	3.4 ± 0.87[b]	2.7 ± 0.69[d]	3.7 ± 0.85	27 (<0.001)
DDS, Adequate diversity (%)[3] (%≥5 food groups)	Children	**37 (28-45)[a]**	14 (6-23)[b]	12 (4–21)[b]	20 (1–31)[a,b]	23 (27–19)	16 (<0.001)
	Adult females	52 (42-62)[c]	19 (12–26)	**6 (1–12)[a]**	**1 (1-3)[a]**	20 (24–17)	16 (<0.001)
Calcium inadequacy (%)[3]	Children	**90 (83–97)**	95 (85–100)	99 (97–100)	97 (91–100)	93 (91–95)	
	Adult females	**81 (64–95)**	94 (89–97)	95 (90–98)	99 (98–100)	91 (89–93)	
Riboflavin inadequacy (%)[3]	Children	50 (38–80)	66 (56–76)	71.5 (57–86)	**47 (29–64)**	59 (53–65)	
	Adult females	**75 (45–100)**	90 (72–100)	87 (69–100)	99 (94–100)	87 (85–88)	
Folate inadequacy (%)[3]	Children	**29 (20–39)**	34 (23–44)	47 (33–72)	43 (22–61)	36 (33–39)	
	Adult females	47 (31–65)	57 (41–71)	**44 (33–63)**	94 (88–100)	58 (55–61)	
Zinc inadequacy (%)[3]	Children	48 (41–56)	56 (45–71)	74 (66–86)	**36 (24–51)**	54 (51–57)	
	Adult females	**44 (29–58)**	51 (35–61)	54 (412–79)	90 (85–97)	56 (53–59)	
Vitamin A inadequacy (%)[3]	Children	**46 (36–57)**	55 (42–70)	64 (51–74)	61 (43–79)	54 (50–57)	
	Adult females	**42 (32–55)**	50 (37–60)	66 (49–82)	78 (59–97)	56 (52–60)	
Vitamin C inadequacy (%)[3]	Children	44 (29–100)	**41 (32–54)**	51 (37–69)	43 (30–60)	43 (38–54)	
	Adult females	**38 (14–56)**	41 (27–53)	40 (27–56)	86 (73–100)	48 (44–51)	
Thiamin inadequacy (%)[3]	Children	**9.9 (3.9–17)**	18 (8.5–28)	25 (14–37)	17 (9.5–28)	18 (14–33)	
	Adult females	34 (18–49)	37 (27–45)	**19 (0–34)**	96 (93–100)	44 (41–47)	
Vitamin B6 inadequacy (%)[3]	Children	**26 (18–37)**	41 (22–58)	42 (32–55)	30 (15–42)	34 (30–37)	
	Adult females	**16 (0.7–27)**	22 (15–31)	25 (16–36)	83 (71–94)	30 (20–35)	
Iron inadequacy (%)[3]	Children	**17 (9.8–25)**	28 (16–39)	39 (24–60)	21 (4.9–55)	25 (22–29)	
	Adult females	35 (22-47)	39 (29-48)	29 (25-35)	**93 (84-97)**	45 (38-52)	

Table 2 – Continued

		"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4[4]	Total	Wald (p-value)
Niacin inadequacy (%)[3]	Children	**5.3 (2.3–9.3)**	11 (4–18)	23 (10–35)	8.2 (2.9–14)	12 (7.9–20)	
	Adult females	17 (7.9–26)	**12 (4.1–21)**	16 (7.3–25)	71 (54–89)	24 (22–27)	
Vitamin B12 inadequacy (%)[3]	Children	**2.1 (0.1–5.6)**	6.5 (1.4–12)	15 (4.5–29)	18 (1.7–36)	8 (5.9–13)	
	Adult females	**8 (0.3–20)**	8.7 (4.7–13)	13.8 (2.9–96)	52 (40–65)	16 (14–18)	

* Healthy and unhealthy food group intake were classified according to the Global Diet Quality Score (GDQS) food group definitions (22).[1] Values are mean g/day ± sd [2] Food groups considered unhealthy when consumed in excessive amounts according to the GDQS. [3] Values are % (95%, CI) [4] Pattern 4 for children = "Refined, red meat and vegetable" and adult females = "Low energy intake". **Bolded values** indicate food groups that contributed most to the characterisation of each dietary pattern.

[a,b,c] If the mean of a variable has the same superscript (e.g. a and a) for two dietary patterns, the pairwise comparison between the two dietary patterns had $p \geq 0.05$;

if the means have a different superscript (e.g. a and b), the pairwise comparison between the two dietary patterns had a $p < 0.05$.

Abbreviations: GDQS; Global Diet Quality Score, DDS; Diet Diversity Score

Associations between with dietary patterns and socio-demographics

Dietary patterns were associated with city of residence and maternal education among children, and with city of residence and education among adult females. In adult females, additional associations were observed with ethnicity, occupation, housing type, and number of dependent children (Wald test ≤0.05) (**Table 3**).

In both groups, participants in the "Fish, vegetables and oil" and "Dairy and sweet" patterns were more often living in Bouaké and Daloa and more likely to live in a "common commune or shack". The "Fish, vegetables and oil" pattern was associated with lower education of both the children's primary caregivers and adult females. Specifically, 28% of children's primary caregivers in this pattern had no education, compared to 8-13% in the other three patterns. Similarly, 33% of adult females in this pattern had no education, compared to 15-28% in the other patterns. Children's primary caregiver in this pattern were also more frequently engaged in informal work (62% vs. 41-48% in the other patterns).

The "Poultry and fruit" pattern in children and adult females showed several contrasts with the other patterns. Participants were predominately from Abidjan (53% of children's primary caregiver and 54% of adult females) and had higher education levels. Specifically, 37% of children's primary caregivers and 44% of adult females in this pattern had completed secondary education, compared with 16-31% and 32-39%, respectively, in the other patterns. Among children, their primary caregivers were most often engaged in informal work (45% vs 31-35% in other patterns), whereas among adult female in this pattern were younger (mean 27.8 ± 9.3), included a higher proportion of unemployed or students (57% vs. 32-43%) and had fewer dependent children (mean 1.9 ±2.1).

Among adult females, the "Low energy intake" pattern was more common in Abidjan and Bouaké and was associated with a higher proportion living in an apartment (45% vs. 29-40% in the other patterns). Among children, the "Refined, red meat and vegetable" pattern was distributed across all the four cities and showed no significant socio-demographic differences compared to the other patterns.

Associations between dietary patterns and nutritional status

No significant differences in nutritional status were observed across dietary patterns in either children or adult females (**Table 3**). The only exception was mean height among adult females, which was higher in the "Low energy intake" pattern (160 ± 6 cm) compared to the other three patterns (128-155 cm).

Table 3. General characteristics of the total population by dietary patterns

				"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4	Total	Wald (p-value)
Participant		n (%)	Children	156 (36)	108 (25)	100 (23)	71 (16)	434	
			Adult females	104 (24)	139 (32)	112 (26)	79 (18)	434	
		kcal/day ± sd	Children	2325 ± 74	2156 ± 93	**1615 ± 81**	2410 ± 129	2134 ± 38	**50 (<0.001)**
			Adult females	3170 ± 1920	3219 ± 1834	2727 ± 1176	**1242 ± 485**	2720 ± 1312	**50 (<0.001)**
	Age (y)		Children	9.1 ± 2.1[a]	9 ± 2.2[a]	9.1 ± 2.2[a]	9.2 ± 2.2[a]	9.1 ± 1.9	0 (0.94)
			Adult females	28.5 ± 8.9[a,b]	30.6 ± 9.9[a]	27.8 ± 9.3[b]	31.1 ± 10.4[a]	29.4 ± 8.8	**9 (0.04)**
	Mother's age (y)		Children	35.8 ± 9.8[a]	36.3 ± 10.7[a]	35.5 ± 8.6[a]	36.9 ± 10.2[a]	36 ± 8.6	1 (0.82)
	Sex (%)	Female	Children	55 (46–64)	51 (40–63)	63 (52–74)	50 (37–64)	55 (51–60)	3 (0.440)
	City (%)	Abengourou	Children	6 (2–11)[a]	10 (3–16)[a]	10 (4–15)[b]	11 (3–20)[a,b]	9 (6–11)	**20 (0.017)**
			Adult females	11 (5–17)[a]	10 (4–15)[a]	3 (-1–6)[b]	15 (6–23)[c]	9 (6–12)	**36 (<0.001)**
		Abidjan	Children	33 (24–41)	19 (10–29)	**53** (42–64)	40 (26–53)	35 (31–40)	
		(Youpougon)	Adult females	19 (11–27)	26 (18–34)	**54** (44–64)	41 (29–53)	34 (30–39)	
		Bouke	Children	**31** (23–39)	**42** (31–53)	20 (10–29)	20 (9–31)	29 (25–34)	
			Adult females	**35** (25–44)	**27** (19–35)	23 (15–32)	**38** (26–50)	30 (26–34)	
		Daloa	Children	**30** (22–38)	29 (19–39)	18 (9–27)	29 (17–41)	27 (23–31)	
			Adult females	**36 (26–45)**	**37 (28–46)**	20 (12–29)	6 (-1.1–13)	27 (23–31)	
	Ethnicity (%)	Akan	Children	37 (28–46)[a]	46 (35–57)[b]	50 (39–62)[a]	34 (21–47)[a]	42 (37–46)	22 (0.250)
			Adult females	30 (21–40)[a]	34 (26–43)[a]	**63** (53–73)[b]	**65** (53–77)[b]	46 (42–51)	**40 (0.00)**
		Gour	Children	8 (3–13)	**9 (2–15)**	5 (-0.2–10)	3 (-2.5–8)	7 (4–9)	
			Adult females	8 (3–14)	**11** (6–17)	1 (-1.6–4)	5 (-0.8–11)	7 (5–9)	
		Krou	Children	18 (11–25)	0.4 (-0.8–2)	**24 (15–34)**	31 (19–43)	17 (14–20)	
			Adult females	11 (4–17)	**19** (12–26)	8 (2–13)	13 (5–21)	13 (10–16)	

Table 3. Continued

			"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4	Total	Wald (p-value)
	Mande du Nord	Children	21 (14–28)	29 (19–38)	11 (3–18)	**16 (7–26**)	20 (16–23)	
		Adult females	**23** (15–32)	17 (10–24)	**18** (10–26)	4 (-1.6–9)	16 (13–20)	
	Mande du Sud	Children	7 (3–12)	**6 (0.8–10)**	5 (0–9)	9 (2–16)	6 (4–9)	
		Adult females	8 (3–14)	**9** (4–14)	4 (-0.2–8)	5 (-0.5–10)	7 (4–9)	
	Non Ivorian	Children	**5 (1–9)**	4 (-0.1–9)	1 (-1.8–4)	6 (-0.5–13)	4 (2–6)	
		Adult females	**11** (5–17)	5 (1–10)	3 (-1.1–6)	8 (0.8–15)	6 (4–9)	
	Other	Children	**4 (0.3–8)**	6 (0.9–12)	4 (-0.2–8)	1 (-1.9–4)	4 (2–6)	
		Adult females	**8** (3–14)	4 (0.8–8)	4 (-0.3–7)	0.7 (-2.2–4)	4 (2–6)	
Weight (kg)		Children	27.6 ± 11.8[a]	30.2 ± 32.2[a]	30.7 ± 24.6[a]	29.8 ± 14.8[a]	29.3 ± 22.2	3 (0.46)
		Adult females	**8** (3–14)	4 (0.8–8)	4 (-0.3–7)	0.7 (-2.2–4)	4 (2–6)	1 (0.86)
Height (cm)		Children	110 ± 64.6[a]	112 ± 59.2[a]	**123 ± 41.4[b]**	116 ± 52.8[a]	114 ± 50.9	4 (0.22)
		Adult females	128 ± 62.8[a]	128 ± 83.2[a]	**155** ± 49.9[b]	**160 ± [6]**b	141 ± 56	**59 (<0.001)**
BMI class (%)[1]	Thin	Children	14 (8–20)	9 (3–16)	11 (3–18)	6 (-0.7–12)	11 (8–14)	4 (0.88)
		Adult females	**4** (0–8)[a]	2 (-0.5–5)[a]	2 (-1.2–5)[a]	1 (-1.9–5)[a]	2 (0.9–4)	12 (0.24)
	Normal	Children	81 (74–88)	83 (75–92)	80 (71–89)	85 (75–95)	82 (78–86)	
		Adult females	41 (31–51)	**48 (**39–58)	**53** (43–64)	39 (26–51)	46 (41–51)	
	Overweight	Children	5 (0.7–8)	5 (0.2–10)	8 (2–15)	6 (-0.8–12)	6 (4–8)	
		Adult females	40 (30–50)	26 (18–34)	30 (20–39)	**45 (32–57)**	33 (29–38)	
	Obese	Children	0.4 (-1.3–2)	2 (-1.5–6)	1 (-1.7–4)	3 (-1.7–8)	1 (0.3–3)	
		Adult females	15 (8–23)	**23** (16–31)	15 (7–23)	15 (6–25)	18 (14–22)	

Table 3. Continued

				"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4	Total	Wald (p-value)
Mother/adult females	Education (%)	None	Children	13 (8–18)[a]	**28 (20–35)[b]**	8 (4–13)[a]	12 (6–18)[a]	16 (12–19)	**18 (<0.001)**
			Adult females	23 (16–30)[a]	**33 (25–41)[a]**	**15 (9–20)[b]**	28 (19–37)[a]	25 (21–29)	**15 (0.00)**
		Primary	Children	53 (48–58)	56 (50–61)	47 (40–54)	52 (46–59)	52 (47–57)	
			Adult females	24 (19–28)	26 (21–30)	20 (16–25)	25 (21–30)	24 (20–28)	
		Secondary	Children	29 (23–36)	16 (10–21)	**37 (29–45)**	31 (22–39)	28 (24–32)	
			Adult females	39 (33–45)	32 (26–38)	44 (38–50)	36 (28–43)	38 (33–42)	
		Tertiary	Children	4 (2–7)	1 (0.2–2)	**8 (3–12)**	5 (2–8)	4 (2–6)	
			Adult females	14 (9–20)	9 (5–13)	**21 (15–28)**	11 (6–16)	14 (11–17)	
	Occupation (%)	Student/ unemployed	Children	43 (34–52)[a]	**57 (46–68)[a]**	32 (22–43)[b]	40 (27–54)[a]	44 (39–48)	9 (0.160)
			Adult females	39 (29–49)[a]	28 (20–37)[a]	**45 (35–55)[b]**	30 (19–41)[a]	35 (31–40)	**37 (<0.001)**
		Informal	Children	35 (27–44)	31 (20–41)	**45 (34–56)**	35 (22–48)	36 (32–41)	
			Adult females	43 (33–53)	**62 (53–71)**	41 (32–51)	48 (36–61)	50 (45–54)	
		Salary/ Manager	Children	22 (15–29)	12 (5–20)	22 (13–32)	24 (13–36)	20 (16–24)	
			Adult females	18 (10–26)	10 (4–16)	14 (7–21)	22 (12–32)	15 (12–18)	
	Marital status (%)	Single	Children	46 (37–55)	50 (39–61)	48 (37–59)	41 (28–54)	47 (42–51)	12 (0.200)
			Adult females	48 (38–58)	43 (34–52)	**62** (52–72)	37 (25–49)	48 (43–53)	6 (0.43)
		Partnership	Children	31 (23–39)	37 (27–48)	37 (26–48)	49 (36–63)	37 (33–42)	
			Adult females	33 (24–43)	36 (28–45)	**28** (19–37)	31 (20–42)	32 (28–37)	
		Married	Children	22 (15–29)	9 (2–15)	13 (5–21)	9 (1–17)	14 (11–18)	
			Adult females	18 (10–25)	18 (11–25)	**6** (1–12)	29 (18–40)	17 (13–20)	
		Divorced	Children	-	-	-	-	-	
			Adult females	0.8 (-1.1–3)	0.8 (-0.8–2)	3 (-0.5–6)	0 (0–0)	1 (0.2–2)	

Table 3. Continued

				"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4	Total	Wald (p-value)
		Widow	Children	1 (-0.9–3)	4 (-0.2–8)	2 (-1.1–5)	0.4 (-1.3–2)	2 (0.6–3)	
			Adult females	0.9 (-1.1–3)	2 (-0.6–5)	0.7 (-1.4–3)	3 (-1.5–7)	2 (0.4–3)	
Household	Housing type (%)	Shack	Children	**52 (43–61)[a]**	59 (48–70)[a]	34 (23–45)[b]	44 (31–57)[a,b]	48 (44–53)	11 (0.100)
			Adult females	48 (38–58)[a,b]	**50 (41–59)[a]**	42 (32–52)[a,b]	34 (22–46)[b]	44 (40–49)	**113 (<0.001)**
		Apartment	Children	31 (23–39)	32 (21–43)	**47 (35–58)**	34 (22–47)	35 (31–40)	
			Adult females	32 (23–41)	29 (21–37)	**40 (30–50)**	**45 (32–57)**	35 (31–40)	
		Villa	Children	**17 (10–24)**	9 (2–16)	19 (11–28)	22 (11–33)	16 (13–20)	
			Adult females	20 (12–28)	21 (13–28)	18 (10–26)	22 (12–32)	20 (16–24)	
	Household type (%),	Other	Children	10 (5–15)	15 (7–22)	7 (1–12)	10 (2–17)	10 (7–13)	9 (0.150)
			Adult females	22 (13–30)	21 (14–29)	26 (17–35)	19 (9–29)	22 (18–26)	4 (0.67)
		Monogamy	Children	86 (80–92)	75 (66–85)	92 (86–98)	88 (80–97)	85 (82–88)	
			Adult females	70 (61–79)	68 (60–77)	74 (65–83)	78 (67–88)	72 (68–76)	
		Polygamy	Children	4 (0.9–8)	**10 (4–16)**	1 (-1.4–4)	2 (-2–6)	5 (3–7)	
			Adult females	9 (3–14)	11 (5–16)	**0 (0–0)**	3 (-1.7–8)	6 (4–8)	
	Expenditure (%),	<2000Fr	Children	20 (13–27)[a]	32 (22–42)[a]	34 (24–45)[a]	20 (9–31)[a]	26 (22–30)	7 (0.280)
			Adult females	25 (16–34)[a]	17 (10–23)[a]	20 (11–28)[a]	24 (13–34)[a]	21 (17–25)	7 (0.28)
		2000-4000Fr	Children	58 (50–67)	48 (37–59)	51 (39–62)	61 (48–74)	54 (50–59)	
			Adult females	61 (51–71)	66 (57–74)	60 (50–70)	55 (43–68)	61 (57–66)	
		>4000Fr	Children	21 (14–29)	20 (11–29)	15 (7–24)	19 (9–29)	19 (16–23)	
			Adult females	14 (7–21)	18 (11–25)	20 (12–28)	21 (11–31)	18 (14–22)	
	Num of dependent children		Children	4.2 ± 3[a]	4.4 ± 2.9[a,b]	3.9 ± 2.3[b]	3.9 ± 2.7[b]	4.1 ± 2.4	3 (0.38)
			Adult females	2.6 ± 2.7[a]	3.1 ± 3.5[a]	**1.9 ± 2.1[b]**	3.1 ± 3.1[a]	2.7 ± 2.7	**16 (0.00)**

Table 3. Continued

		"Dairy and sweet"	"Fish, vegetable and oil"	"Poultry and fruit"	Pattern 4	Total	Wald (p-value)
Num of people living in household	Children	7.5 ± 4.4[a]	7.8 ± 4.4[a]	7 ± 3.6[b]	6.9 ± 3.6[b]	7.4 ± 3.6	3 (0.43)
	Adult females	6.6 ± 3.9[a]	7.1 ± 5.3[a]	6.2 ± 3.9[a]	6.5 ± 5.1[a]	6.6 ± 4.3	2 (0.52)

Data are presented as mean ± standard deviations (SD) or percentage (95%CI) [1] For children z-scores cut-offs for BMI/age <-2SD (thinness), between -2SD and +1 SD (normal weight), BMI-for-age > +1 SD (overweight), BMI-for-age > +2 SD (obesity), and height-for-age < −2 SD (stunting). For adults, nutritional status was assessed using the Body Mass Index (BMI) (kg/m2) cut-offs. **Bolded values** indicate variables that contributed most to the characterisation of each dietary pattern.

[a,b,c] Indicate differences between any two dietary patterns by pairwise comparison, with similar superscripts indicating no difference.

Abbreviations: Not Assessed (NA)= not applicable, FR, West African Franc, conversion rate in 2021 was 0.0015 euro.

DISCUSSION

This study identified four dietary patterns in both children and adult females in urban Côte d'Ivoire. Two patterns, the "Fish, vegetable and oil" (characterised by a higher consumption of fish, dark green leafy vegetables, deep orange fruits, and oil) and the "Dairy and sweet" (characterized by a higher consumption of dairy, sweet foods and juice), were common to both population groups, suggesting shared dietary behaviours. These patterns, together with the "Refined, red-meat and vegetable" for children, were associated with healthier diets in terms of lower risk of micronutrient inadequacies and NCDs (GDQS), higher diet diversity (DDS) and nutrient adequacy, compared to the "Poultry and fruit" pattern in children and the "Low energy intake" pattern in adult females. Healthier patterns were more common in Bouaké and Daloa (outside of Abidjan) and among participants with lower education, whereas the less healthy 'Poultry and fruit' pattern was more common among children and adult females in Abidjan, and adult females with higher education level, occupation and housing status and fewer dependent children.

Our findings align with Popkin's model of nutrition transition (14), in which cereal-based diets shift toward animal sourced foods, cheaper (vegetable) oils, added sugars and energy-dense nutrient-poor processed foods [14] as urbanization increases (38–40) and income rises (41). The inclusion of both staple and animal-sourced foods across all identified patterns suggests that Ivorian urban diets already reflect this transition, differing from earlier reports in urban populations in Nigeria, Ghana and Burkina Faso, that described predominantly starchy diets with limited animal products (42–44). These findings suggest that Ivorian urban diets are already reflecting key features of the region's ongoing nutrition transition.

The "Dairy and sweet" pattern identified in our study resembles those previously reported for adult populations in urban Nigeria ("Dairy and sugar") and Ghana ("Sweet tooth") (42,43). Other patterns encountered in Nigeria ("High intakes and vegetables" and "Low (food and energy) intakes") were comparable to our "Fish, liquid oil, vegetable" and "Low energy intake" patterns in adult females. However unlike the Nigerian "Dairy and sugar" pattern, which had the lowest GDQS total (due to higher GDQS -), our "Dairy and sweet" maintained a moderate total GDQS and DDS due to the concurrent consumption of foods considered healthy (higher GDQS+) and unhealthy (higher GDQS -). In all three studies, sweets, primarily derived from baked goods, sugar-sweetened beverages, and added sugars in drinks, consistently formed the main unhealthy dietary component (42,43). These similarities suggest emerging convergence in urban dietary

patterns across West Africa as unhealthy and sweetened foods become more available.

Diet quality, assessed using the Global Diet Quality Score (GDQS), the Dietary Diversity Score (DDS), and estimated nutrient adequacy, varied across dietary patterns but remained suboptimal overall. Most participants were classified as being at high risk of nutrient inadequacy and NCDs by the GDQS, and few met the DDS criterion of consuming ≥5 food groups, indicating a generally poor diet quality despite some relative differences between patterns. Across indicators, higher GDQS and DDS values were generally in accordance with greater nutrient adequacy, except in the "Fish, oil and vegetable" pattern among children, which showed a high GDQS but low DDS and intermediate nutrient adequacy compared to other patterns. This discrepancy reflects key differences between the indicators. DDS captures only the number of healthy food groups consumed, whereas GDQS accounts for both healthy and unhealthy foods and incorporates quantities consumed. In the "Fish, oil and vegetable" pattern, children consumed a limited range of food groups (yielding a low DDS) but relatively high amounts of foods defined as healthy by GDQS, alongside low intakes of unhealthy foods, resulting in a higher GDQS. This illustrates how diets with low diversity but dominated by a few nutrient-dense foods can score well on GDQS while remaining limited in diversity and overall nutrient adequacy.

Although overall diet quality was poor in both population groups, the relatively healthier patterns, especially the "Dairy and sweet", suggest some dietary diversification within the urban settings studied. This observation is consistent with the broader regional evidence that urban food environments in West Africa (Nigeria, Senegal and Mali) are becoming more diverse, as reported by the OECD/Sahel and West Africa Club based on national household data from 2014–15 to 2018–19 (12). Our results therefore reflect within-urban variation, suggesting that diversification in urban food environments may also extend beyond major metropolitan centres.

Sub-Saharan Africa continues to report relatively low consumption of foods high in sugar, salt and fat compared with regions further along the nutrition transition, such as Latin America (45). Recent household survey and market data across West African cities indicate a continued reliance on traditional staples in urban and rural cities, alongside a gradual increase in packaged and sweetened products, primarily in urban settings (12). Popkin & Ng (2022) reported that daily intakes of sugar-sweetened beverages in Latin America routinely exceed 500–800 g per person, levels far above those currently recorded in African urban cohorts, which remain around 200–300 g/day for most populations (e.g.,

in our study 194 and 327 g/day for children and adult females, respectively) (38). These findings suggest that urban West Africa remains in the early phase of nutrition transition, offering a window for preventive policy action.

The higher prevalence of overweight and obesity in adult females compared to children observed in our study could not be explained by dietary patterns. Despite differences in energy intake (**Supplement Table 3 and 4**), diet types and quality were similar across children and adult females, except for adult female's "Low energy intake" pattern, where under-reporting is likely (as discussed below). In a diet modelling study assessing dietary quality in 185 countries between 1990 and 2018 using nationally representative data, comparable overall diet quality was similarly observed between adult females and children across most regions (46). Together, these findings suggest that body weight differences may be more strongly influenced by total energy intake, physical activity, or sociocultural norms rather than diet quality alone. In sub-Saharan Africa, higher body weight is often culturally valued among adult females (47). At the same time, urbanisation and occupational changes have reduced physical activity. These factors, combined with reproductive and physiological changes after adolescence and during reproductive years (e.g. pregnancy related weight gain and metabolic shifts), may further contribute to the higher prevalence of obesity in adult females (14). Given the consistently higher burden of overweight and obesity, research should continue to prioritise adult females, focusing on biological, behavioural, and sociocultural pathways that heighten vulnerability to obesity in changing food environments (7,48).

The "Low energy intake" pattern in adult females warrants careful interpretation, as it showed the lowest mean energy intake (approximately 1242 kcal/day), poorest diet quality, and the highest prevalence of overweight/obesity (~60%), suggesting underreporting, a common bias in dietary recalls especially among overweight individuals (49). Adult females in this pattern were also taller on average, implying higher energy needs and reinforcing the likelihood of underestimation. The impact of under-reporting depends on its form: if it occurs at the intake level, relative proportions of food groups remain valid, though nutrient adequacy is underestimated; if it occurs at the food group level, entire food groups, particularly unhealthy ones, may be omitted. The latter would compromise the validity of this pattern's interpretation.

Dietary patterns were strongly shaped by urbanicity and socio-economic context, reflecting an early but heterogeneous stage of the nutrition transition. The healthier, "Fish, vegetables and oil" and "Dairy and sweet" patterns, were more common outside of Abidjan and among participants with lower education. In

contrast, the less healthy "Poultry and fruit" pattern, was more common among children and adult females in Abidjan, and among adult females with higher education, occupational status, and housing conditions, and fewer dependent children. This suggests that increasing urbanicity, in this case living in Abidjan, may contribute to a shift away from traditional or common dietary practices (such as the fish, green leafy vegetable and oil), even when these are healthier and locally accessible. These findings are broadly consistent with observations from other LMICs, where higher socio-economic status is often associated with less healthy diets, unlike high-income countries where the reverse is observed (14,46,50). However, the specific pattern observed here does not fully align with classic descriptions of the nutrition transition, which emphasise increased consumption of energy-dense, ultra-processed foods among wealthier urban groups (14). Notably, consumption of overtly unhealthy foods was not highest in the most urbanised settings, suggesting a more nuanced transition.

This complexity aligns with global evidence showing that traditional socio-demographic indicators such as education and urbanicity do not consistently predict diet quality across contexts (46). Instead, food environment characteristics, such as food access, affordability, and retail infrastructure, may be more sensitive predictors of dietary patterns in LMICs (51,52). For example, a Word Bank report found through a case study using household survey interviews that many urban Ivorian consumers prioritised retailer's proximity over price or quality (53).

The main strength of this study is that it is the first to characterise dietary patterns among children and adult females in Côte d'Ivoire using a data-driven approach. Including both population groups within the same analytical framework allowed identification of shared and distinct dietary patterns, providing insight into common household-level behaviours and strengthening the relevance of findings for interventions that target families rather than individuals alone. Dietary patterns were identified using Latent Class Analysis, a probabilistic, model-based approach that accounts for uncertainty in class membership and reduces misclassification. In addition, the use of a bias-adjusted three-step method allowed for correction of classification error, resulting in more accurate estimations of associations between dietary pattern and other variables. Food group categorisation was guided by the GDQS to minimise subjectivity enhancing comparability with other studies, and was validated by multiple experts.

This study has several limitations. First, data were collected in four urban cities of Côte d'Ivoire and may not represent national diets. Second, potential under-reporting particularly among adult females, in whom overweight and obesity

are common, as mentioned above may have affected our results. Third, dietary patterns and diet quality were characterised using a single 24-hour recall in the LCA method, which does not capture usual intake. Nutrient adequacy was estimated using the two-day intake data, however bootstrapping was restricted to 50 iterations due to poor model convergence, likely related to small numbers within some patterns.

In summary, four dietary patterns were identified in urban Ivorian children and adult females. Patterns rich in fish, vegetables, and dairy were associated with higher diet quality and nutrient adequacy, whereas poultry- and low-intake patterns showed poorer quality. These findings highlight opportunities to strengthen culturally familiar, nutrient-dense dietary behaviours in urban populations.

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SUPPLEMENTARY MATERIAL

Supplement Table 1. Profile of each food group[1] stratified by dietary pattern cluster for children

	Cluster1	Cluster2	Cluster3	Cluster4	Overall	Wald (p-value)
Cluster Size	0.36	0.25	0.23	0.16	435	
Citrus fruits						
Non-consumers	92 (87–97)	100 (100–100)	97 (94–101)	92 (84–99)	95 (93–97)	120 (<0.001)
Consumers	8 (3–13)	0 (0–0)	3 (-1–6)	8 (1–16)	5 (3–7)	
Deep orange fruits						
Non-consumers	100 (100–100)	89 (83–96)	98 (95–102)	93 (87–99)	96 (94–98)	180 (<0.001)
Consumers	0 (0–0)	11 (4–17)	2 (-1.8–5)	7 (0.5–13)	4 (2–6)	
Other fruits						
Non-consumers	89 (84–94)	100 (100–100)	83 (75–91)	97 (92–102)	92 (89–94)	209 (<0.001)
Consumers	11 (6–16)	0 (0–0)	17 (9–25)	3 (-2.1–8)	8 (6–11)	
Dark green leafy vegetables						
Non-consumers	95 (91–99)	73 (64–83)	100 (100–100)	92 (84–100)	90 (88–93)	250 (<0.001)
Consumers	5 (0.5–9)	27 (17–36)	0 (0–0)	8 (0.2–16)	10 (7–12)	
Other vegetables						
Below Median	52 (43–61)	33 (23–44)	78 (68–88)	56 (43–70)	54 (49–59)	23 (<0.001)
Above Median	48 (39–57)	67 (56–77)	22 (12–32)	44 (30–57)	46 (41–51)	
Legumes						
Non-consumers	100 (100–100)	93 (87–98)	82 (74–90)	85 (75–94)	91 (89–94)	35 (<0.001)
Consumers	0 (0–0.1)	7 (2–13)	18 (10–26)	15 (6–25)	9 (6–11)	
Nuts and seeds						
Non-consumers	77 (69–84)	63 (52–74)	96 (90–102)	74 (62–86)	77 (73–81)	11 (0.012)
Consumers	23 (16–31)	37 (26–48)	4 (-2–10)	26 (14–38)	23 (19–27)	
Liquid oils						
Non-consumers	5 (0.1–11)	18 (9–27)	38 (27–49)	48 (35–62)	23 (19–27)	31 (<0.001)
Below	44 (35–53)	46 (35–57)	35 (25–46)	26 (13–38)	39 (35–44)	
Above	51 (42–60)	36 (26–47)	27 (16–37)	26 (14–38)	38 (33–42)	
Fish and shellfish						
Non-consumers	7 (2–13)	0.1 (0–0.1)	45 (34–56)	44 (31–57)	20 (17–24)	547 (<0.001)
below	39 (31–48)	55 (44–65)	31 (20–41)	38 (25–51)	41 (36–45)	
above	53 (45–62)	45 (35–56)	24 (14–34)	18 (6–29)	39 (34–43)	

Supplement Table 1. Continued

	Cluster1	Cluster2	Cluster3	Cluster4	Overall	Wald (p-value)
Poultry and game meat						
Non-consumers	88 (82–94)	94 (88–100)	78 (69–87)	99 (94–103)	89 (86–92)	10 (0.018)
Consumers	12 (6–18)	6 (0.1–12)	22 (13–31)	1 (-3–6)	11 (8–14)	
Eggs						
Non-consumers	77 (69–84)	100 (100–100)	52 (41–63)	75 (64–86)	76 (73–80)	222 (<0.001)
Consumers	23 (16–31)	0.1 (0–0.2)	48 (37–59)	25 (14–36)	24 (20–27)	
High fat dairy						
Non-consumers	27 (18–35)	94 (88–101)	99 (95–102)	80 (69–91)	69 (65–72)	60 (<0.001)
Consumers	73 (65–82)	6 (-1–12)	1 (-2.1–5)	20 (9–31)	31 (28–35)	
Red meat						
Non-consumers	39 (31–48)	51 (40–62)	49 (38–60)	45 (32–58)	45 (41–50)	25 (<0.001)
Below	51 (42–59)	44 (33–55)	25 (15–36)	21 (9–32)	38 (34–43)	
Above	10 (4–16)	5 (-1–12)	26 (16–35)	35 (22–47)	17 (13–20)	
Refined grains and baked goods						
Below Median	58 (49–67)	49 (38–60)	99 (97–101)	0.2 (0.1–0.3)	56 (52–60)	780 (<0.001)
Above Median	42 (33–51)	51 (40–62)	0.9 (-0.9–3)	100 (100–100)	44 (40–48)	
Sweets and ice cream						
Non-consumers	0.1 (0.1–0.1)	84 (76–92)	72 (61–82)	30 (18–43)	42 (39–46)	1393 (<0.001)
Below	52 (44–60)	16 (8–24)	17 (8–26)	26 (14–38)	31 (27–35)	
Above	48 (40–56)	0.3 (-0.1–0.7)	11 (4–19)	43 (30–57)	27 (23–31)	
Sugar sweetened beverages						
Non-consumers	94 (90–98)	100 (100–100)	90 (83–96)	96 (90–102)	95 (93–97)	11 (0.014)
Consumers	6 (2–10)	0 (-0.1–0.2)	10 (4–17)	4 (-1.8–10)	5 (3–7)	
Juice						
Non-consumers	50 (41–59)	100 (99–100)	57 (46–68)	69 (56–81)	67 (63–71)	64 (<0.001)
Consumers	50 (41–59)	0.4 (-0.1–0.8)	43 (32–54)	31 (19–44)	33 (29–37)	
White roots and tubers						
Non-consumers	7 (2–12)	3 (-2.3–8)	12 (5–19)	69 (58–81)	17 (14–20)	508 (<0.001)
Below	47 (38–55)	62 (51–73)	65 (54–76)	31 (19–42)	52 (47–57)	
Above	46 (37–55)	35 (25–46)	23 (13–33)	0.1 (0.1–0.2)	31 (26–35)	

Supplement Table 1. Continued

	Cluster1	Cluster2	Cluster3	Cluster4	Overall	Wald (p-value)
Cruciferous vegetables						
Non-consumers	98 (96–100)	99 (97–101)	100 (100–100)	100 (100–100)	99 (98–100)	27 (<0.001)
Consumers	2 (-0.3–4)	1 (-1–3)	0 (0–0)	0 (0–0)	0.9 (0–2)	
Deep orange vegetables						
Non-consumers	98 (96–100)	100 (100–100)	99 (97–101)	100 (100–100)	99 (98–100)	24 (<0.001)
Consumers	2 (-0.4–4)	0 (0–0)	1 (-1.4–3)	0 (0–0)	0.9 (0–2)	
Deep orange tubers						
Non-consumers	100	100	100	100	100	
Whole grains						
Non-consumers	97 (94–100)	99 (98–101)	100 (98–101)	98 (95–101)	98 (97–99)	5 (0.180)
Consumers	3 (0.5–6)	0.8 (-0.6–2)	0.5 (-0.9–2)	2 (-1.4–5)	2 (0.6–3)	
Low fat dairy						
Non-consumers	100	100	100	100	100	
Processed meat						
Non-consumers	99 (98–101)	100 (99–100)	100 (100–100)	100 (100–100)	100 (99–100)	24 (<0.001)
Consumers	0.5 (-0.5–2)	0.2 (-0.2–0.6)	0 (0–0)	0 (0–0)	0.2 (-0.2–0.7)	
Purchased deep fried foods						
Non-consumers	100	100	100	100	100	

[1] Food items were grouped into the food groups of the Global Diet Quality Score
All values reported as % (95% CI) unless specified

Supplement Table 2. Profile of each food group[1] stratified by dietary pattern cluster for adult females

	Cluster1	Cluster2	Cluster3	Cluster 4	Overall	Wald (p-value)
Cluster Size	0.3	0.26	0.24	0.18	434	
Citrus fruits						
Non-consumers	98 (96–101)	94 (90–99)	97 (93–100)	100 (100–100)	97 (96–99)	50 (<0.001)
Consumers	2 (-0.8–4)	6 (1–10)	3 (-0.2–7)	0 (0–0)	3 (1–4)	
Deep orange fruits						
Non-consumers	96 (92–99)	96 (92–100)	96 (92–100)	100 (100–100)	97 (95–98)	4 (0.2800)
Consumers	4 (0.8–8)	4 (0.1–8)	4 (-0.1–8)	0 (-0.2–0.3)	3 (2–5)	
Other fruits						
Non-consumers	95 (92–99)	96 (92–100)	90 (85–96)	96 (91–101)	94 (92–97)	3 (0.3200)
Consumers	5 (0.8–8)	4 (0.2–8)	10 (4–15)	4 (-1–9)	6 (3–8)	
Dark green leafy vegetables						
Non-consumers	84 (78–91)	94 (88–99)	84 (77–91)	86 (78–95)	87 (84–90)	4 (0.2500)
Consumers	16 (9–22)	6 (1–12)	16 (9–23)	14 (5–22)	13 (10–16)	
Other vegetables						
Non-consumers	1 (-2.1–4)	12 (6–19)	6 (1–11)	29 (18–40)	10 (8–13)	46 (<0.001)
Consumers	24 (16–32)	55 (44–65)	44 (34–54)	70 (59–80)	45 (40–49)	
Legumes						
Non-consumers	97 (94–100)	95 (91–99)	94 (90–99)	100 (100–100)	96 (95–98)	65 (<0.001)
Consumers	3 (0.1–6)	5 (0.7–9)	6 (1–10)	0 (0–0)	4 (2–5)	
Nuts and seeds						
Non-consumers	74 (67–82)	85 (77–92)	76 (68–85)	92 (84–99)	81 (77–84)	8 (0.0500)
Consumers	26 (18–33)	15 (8–23)	24 (15–32)	8 (1–16)	19 (16–23)	
Liquid oils						
non-consumers	16 (9–23)	13 (6–20)	22 (13–30)	38 (26–50)	20 (17–24)	30 (<0.001)
below	31 (22–39)	55 (44–65)	33 (24–43)	44 (32–56)	40 (35–44)	
above	53 (44–62)	32 (23–42)	45 (35–55)	18 (8–29)	40 (35–44)	
Fish and shellfish						
Non-consumers	6 (1–11)	18 (10–26)	18 (10–26)	49 (38–60)	20 (16–23)	278 (<0.001)
Below	33 (24–41)	48 (38–58)	34 (25–44)	51 (39–62)	40 (36–45)	
Above	61 (52–70)	34 (25–44)	48 (38–58)	0.2 (0.1–0.4)	40 (36–44)	
Poultry and game meat						
Non-consumers	90 (85–96)	76 (67–84)	86 (79–93)	100 (98–101)	87 (84–90)	12 (0.0086)
Consumers	10 (4–15)	24 (16–33)	14 (7–21)	0.3 (-0.9–2)	13 (10–16)	

Supplement Table 2. Continued

	Cluster1	Cluster2	Cluster3	Cluster 4	Overall	Wald (p-value)
Eggs						
Non-consumers	72 (64–80)	81 (73–89)	56 (46–66)	98 (93–103)	75 (71–79)	19 (<0.001)
Consumers	28 (20–36)	19 (11–27)	44 (34–54)	2 (-3.1–7)	25 (21–29)	
High fat dairy						
Non-consumers	88 (82–94)	93 (87–99)	25 (16–34)	89 (80–97)	74 (71–78)	105 (<0.001)
Consumers	12 (6–18)	7 (1–13)	75 (66–84)	11 (3–20)	26 (22–29)	
Red meat						
Non-consumers	50 (41–59)	50 (39–60)	46 (36–56)	30 (18–41)	45 (41–50)	18 (0.0053)
Below	27 (18–35)	19 (10–27)	33 (23–42)	51 (39–63)	30 (26–35)	
Above	24 (16–31)	32 (22–41)	21 (13–30)	19 (9–29)	24 (20–28)	
Refined grains and baked goods						
Non-consumers	0 (0–0)	36 (27–44)	0 (0–0)	5 (0–10)	10 (8–13)	2165 (<0.001)
Below	17 (10–23)	64 (56–73)	35 (26–45)	76 (65–87)	44 (40–49)	
Above	83 (77–90)	0.1 (0.1–0.2)	65 (55–74)	18 (8–29)	45 (42–49)	
Sweets and ice cream						
Non-consumers	100 (100–100)	78 (70–87)	0.2 (0.1–0.2)	91 (83–98)	69 (66–71)	3033 (<0.001)
Consumers	0.1 (0–0.1)	22 (13–30)	100 (100–100)	9 (2–17)	31 (29–34)	
Sugar sweetened beverages						
Non-consumers	94 (90–98)	86 (79–93)	96 (92–100)	88 (80–96)	91 (89–94)	6 (0.1200)
Consumers	6 (2–10)	14 (7–21)	4 (-0.3–8)	12 (4–20)	9 (6–11)	
Juice						
Non-consumers	85 (79–92)	79 (71–87)	78 (70–86)	88 (80–96)	82 (79–86)	4 (0.2700)
Consumers	15 (8–21)	21 (13–29)	22 (14–30)	12 (4–20)	18 (14–21)	
White roots and tubers						
Non-consumers	20 (12–27)	0.1 (0–0.1)	33 (24–42)	58 (47–69)	25 (21–28)	1511 (<0.001)
below	52 (43–61)	25 (17–34)	28 (19–37)	42 (31–53)	38 (33–42)	
above	28 (20–36)	74 (66–83)	39 (29–49)	0.1 (0.1–0.2)	38 (34–42)	
Cruciferous vegetables						
Non-consumers	99 (97–101)	100 (100–100)	99 (97–101)	99 (96–102)	99 (98–100)	2 (0.4800)
Consumers	1 (-0.7–3)	0 (-0.1–0.1)	1 (-1–3)	1 (-1.6–4)	0.9 (0–2)	
Deep orange vegetables						
Non-consumers	100 (100–100)	99 (97–101)	100 (100–100)	99 (96–101)	100 (99–100)	17 (<0.001)
Consumers	0 (0–0.1)	0.8 (-0.9–3)	0 (0–0)	1 (-1.3–4)	0.5 (-0.2–1)	

Supplement Table 2. Continued

	Cluster1	Cluster2	Cluster3	Cluster 4	Overall	Wald (p-value)
Deep orange tubers						
Non-consumers	100	100	100	100	100	
Whole grains						
Non-consumers	100 (100–100)	98 (96–101)	100 (100–100)	100 (100–100)	100 (99–100)	33 (<0.001)
Consumers	0 (0–0)	2 (-0.7–4)	0 (0–0)	0 (0–0)	0.5 (-0.2–1)	
Low fat dairy						
Non-consumers	100	100	100	100	100	
Processed meat						
Non-consumers	100 (100–100)	99 (97–101)	99 (97–101)	100 (100–100)	100 (99–100)	18 (<0.001)
Consumers	0 (0–0)	0.9 (-0.9–3)	0.9 (-1–3)	0 (0–0)	0.5 (-0.2–1)	
Purchased deep fried foods						
Non-consumers	100	100	100	100	100 (296–-96)	

[1] The Global Diet Quality Score was used to categorise foods into food group

All values reported as % (95% CI) unless specified

Supplement Table 3. Mean (SD) usual nutrient intake according to dietary pattern for school-aged children (N=434)

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total	Wald
Proportion/n	0.36 (156)	0.25 (108)	0.23 (100)	0.16 (71)	435	
Energy, kcal	2325 ± 922	2156 ± 965	1615 ± 808	2410 ± 1087	2134 ± 786	50 (<0.001)
Calcium, mg	485 ± 392	376 ± 605	207 ± 134	343 ± 263	371 ± 344	88 (<0.001)
Iron, mg	12.6 ± 5.8	14.7 ± 12.4	9.8 ± 6.2	16.2 ± 12.2	13.1 ± 7.4	26 (<0.001)
Zinc, mg	7.2 ± 2.9	6.7 ± 3.5	5.2 ± 2.6	8.4 ± 4.3	6.8 ± 2.7	51 (<0.001)
Vitamin A, µg RAE[1]	673 ± 1058	627 ± 1596	444 ± 1005	441 ± 692	571 ± 935	5 (0.2000)
Thiamin, mg	1 ± 0.49	1.1 ± 0.65	0.74 ± 0.49	1 ± 0.76	0.97 ± 0.48	24 (<0.001)
Riboflavin, mg	0.98 ± 0.68	0.77 ± 0.88	0.72 ± 0.92	1.1 ± 1.3	0.89 ± 0.72	10 (0.0160)
VitB6, mg	1.5 ± 0.91	1.3 ± 1.4	1.3 ± 1.1	1.3 ± 0.77	1.4 ± 0.88	6 (0.0940)
VitB12, µg	5.3 ± 5.3	5.4 ± 6.5	6.1 ± 11.4	5.9 ± 12.8	5.6 ± 7	0 (0.9300)
Vitamin D, µg	3.7 ± 2.9	3.7 ± 4.1	2.6 ± 2.5	2.6 ± 2.7	3.2 ± 2.6	12 (0.0059)
Vitamin C, mg	74.4 ± 78.2	93.4 ± 171	59 ± 82.1	65 ± 68.7	74.1 ± 86.9	4 (0.2500)
Folate, µg DFE[2]	248 ± 151	258 ± 214	192 ± 160	207 ± 164	231 ± 139	10 (0.0230)
Niacin, mg	16 ± 10.2	18 ± 14.4	12.6 ± 11.5	16.8 ± 12.9	15.8 ± 10.1	10 (0.0210)

Abbreviations: RAE, Retinol Activity Equivalent; DFE, Dietary Folate Equivalent

Supplement Table 4. Mean (SD) usual nutrient intake according to dietary pattern for adult females (N=434)

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total	Wald
Proportion/n	139 (32)	112 (26)	104 924)	79 (18)	434	
Energy, kcal	3219 ± 1834	2727 ± 1176	3170 ± 1920	1242 ± 485	2720 ± 1312	50 (<0.001)
Calcium, mg	450 ± 292	377 ± 205	744 ± 449	182 ± 130	453 ± 286	88 (<0.001)
Iron, mg	21 ± 13.6	16.2 ± 7.1	19.6 ± 11.9	7 ± 3.2	16.9 ± 9.5	26 (<0.001)
Zinc, mg	11.2 ± 7.1	8.6 ± 5	11.6 ± 8.8	4.9 ± 2.3	9.5 ± 5.4	51 (<0.001)
Vitamin A, µg RAE[1]	1486 ± 1685	185 ± 147	823 ± 826	355 ± 577	784 ± 1053	5 (0.20)
Thiamin, mg	1.3 ± 1.1	1.5 ± 1.3	1.3 ± 0.98	0.46 ± 0.26	1.2 ± 0.68	24 (<0.001)
Riboflavin, mg	1.2 ± 1.7	0.84 ± 0.36	1.7 ± 1.5	0.39 ± 0.2	1.1 ± 0.79	10 (0.02)
VitB6, mg	2 ± 1.3	1.9 ± 1.3	2.3 ± 1.3	0.75 ± 0.45	1.8 ± 1	6 (0.09)
VitB12, µg	6.8 ± 5.2	14.6 ± 13.7	5.8 ± 4.4	1.9 ± 1.4	7.6 ± 7.9	0 (0.93)
Vitamin D, µg	7.3 ± 6.9	3.9 ± 3.3	5.4 ± 4	1.4 ± 1.2	4.9 ± 4.5	12 (0.01)
Vitamin C, mg	81.6 ± 72.8	182 ± 141	92.3 ± 114	30.3 ± 46.8	101 ± 92.1	4 (0.25)
Folate, µg DFE[2]	270 ± 153	463 ± 298	286 ± 136	98.1 ± 67.7	292 ± 187	10 (0.02)
Niacin, mg	27.2 ± 19.4	22.6 ± 19.2	24.2 ± 19.1	9.5 ± 4.2	22.1 ± 13.8	10 (0.02)

Abbreviations: RAE, Retinol Activity Equivalent; DFE, Dietary Folate Equivalent

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Chapter 5

Statistical analysis plan: Effectiveness of a multi-nutrient fortified milk-based porridge in Nigerian school-aged children: a randomised, double-blind trial embedded in a school feeding programme

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Abstract

Background: Anaemia and multiple micronutrient deficiencies remain widespread among Nigerian children. This has been attributed to poor dietary diversity, limited intake of animal-source foods, and persistent gaps in the coverage and effectiveness of nutrition interventions. Evidence on food-based strategies to improve iron status in school-aged children is limited, despite the potential of school-feeding programmes as a delivery platform. A fortified milk-based maize porridge, developed by FrieslandCampina, provides iron, iodine, zinc, vitamin A, and vitamin B12 at levels contributing approximately one-third of recommended daily intakes for school-aged children per serving. Therefore, we developed a clinical trial that aims to evaluate whether daily consumption of this fortified porridge improves iron status and other micronutrient biomarkers in Nigerian primary school-aged children (5-8 years old).

Methods: This double-blinded, three-arm, randomised controlled trial will enrol 951 children aged 5–8 years from 17 primary schools in Ibadan, Nigeria. Participants will be randomised in a 1:1:1 ratio to receive: (1) multi-nutrient fortified milk-based maize porridge with 3.3 mg iron (low-dose); (2) multi-nutrient fortified milk-based maize porridge with 6.6 mg iron (high-dose); or (3) non-fortified milk-based maize porridge (control). One 300g serving will be provided per child each school day for five months. Co-primary outcomes are haemoglobin concentration (g/L) and serum ferritin (µg/L) at endpoint. Secondary outcomes include the prevalence of anaemia, iron deficiency, iron-deficiency anaemia, and status of iodine, vitamin A, vitamin B12 and vitamin D. Tertiary outcome is cognitive performance scores for attention and short term memory. A hierarchical testing strategy will be used to account for multiplicity, beginning with the high-dose versus control comparison. Multilevel models will account for clustering by school and will adjust for baseline covariates and inflammation where appropriate. To be registered at ClinicalTrials.gov prior to enrolment.

Discussion: This trial will provide evidence on the effectiveness of a multiple-micronutrient fortified milk-based maize porridge delivered through a school-based programme in improving iron status and related micronutrient outcomes in school-aged children. The findings may inform the design of fortified foods within school-feeding programmes, and guide future product development for commercial food companies and nutrition policies in Nigeria.

BACKGROUND AND RATIONALE

One in 3 Nigerian children under the age of 5 years old experience anaemia (58%) and/or stunting (40%), indicating substantial nutritional inadequacies in the diet (1). Multiple micronutrient deficiencies persist, including iron (21%), vitamin A (31%), and zinc (35%) (2). Although approximately two-thirds of anaemia is attributed to iron deficiency (3), anaemia in Nigeria is multifactorial and reflects additional deficiencies, infections, and inflammatory conditions (2). These nutritional deficits are linked to limited dietary diversity and low intake of animal-source foods. Only 6% of children aged 6–23 months meet the minimum acceptable diet, highlighting restricted access to diverse and nutrient-rich foods (1).

Nigeria's nutrition response includes targeted interventions for young children (6–59 months), adolescent girls (10–14 years), and women of reproductive age and pregnant women (15–49 years), as detailed in the National Micronutrient Deficiency Control (MNDC) guidelines (4). Untargeted strategies involve mandatory fortification of salt with iodine (since 1993), sugar and oils with vitamin A, and various flours with vitamin A, iron, and zinc (since 2002). Recent national assessments found compliance rates of 74% for vitamin A in sugar, 100% for iodine in salt and iron/zinc in wheat and semolina flour, but lower compliance for vitamin A in vegetable oil (31%) and wheat flour (26%). Voluntary fortification (such as in bouillon) is rising and biofortification remains low (3-13%). Despite multiple layers of targeted and untargeted programmes, persistent high prevalence of vitamin A, iron and zinc deficiencies suggest large unmet coverage and implementation gaps remain (1).

National data for school-aged children (>5 years) are limited. Available subnational studies highlight concern for iron, vitamin A, zinc, and iodine deficiencies. A systematic review estimated that 86% of Nigerian children (0–19 years) are iodine deficient, 19% are vitamin A deficient, 12% are iron deficient, and 32% are zinc deficient, while vitamin D and vitamin B12 status remain largely unmeasured (5). Evidence on dietary inadequacies in this age group are also scarce. A cross-sectional study in Ibadan reported that more than 60% of children aged 4–8 years had inadequate intakes of calcium, copper, iron, folate, and vitamins A, D, and E, although protein intake was within the acceptable macronutrient range (6). A broader review of dietary intake among Nigerian infants, children, adolescents, and pregnant and lactating women found that total protein intake appeared adequate, but protein quality may be insufficient (7).

School-feeding programmes represent an established platform for improving child (>5 years) nutrition in Nigeria. These programmes aim to increase dietary adequacy by providing a regular meal during the school day and are widely used to support learning, attendance, and overall child welfare (8,9). In Nigeria, the Renewed Hope National Home-Grown School Feeding Programme (RH-NHGS-FP) aims to provide meals to primary school-aged children, with the intention of supplying a meaningful proportion of daily nutrient requirements (10). Although school feeding can improve dietary intake (11,12), evidence for its impact on micronutrient status, particularly iron status, remains limited (13–15). Food-based iron interventions have shown variable effectiveness in school-aged children, while supplementation programmes, though more efficacious, face operational challenges in routine implementation (16–18). Given that anaemia in this age group is multifactorial and the specific nutrient and non-nutrient drivers remain poorly characterised, school-feeding programmes offer a relevant delivery platform for evaluating the effect of fortified foods on micronutrient status (16,18).

FrieslandCampina Sub-Saharan Africa business group developed a multi-nutrient fortified milk-based maize porridge as a potential complement to school feeding programmes. The product combines fermented maize with milk to provide high-quality protein and is fortified with iron, iodine, zinc, vitamin A, and vitamin B12 at levels supplying roughly one-third of recommended daily intakes per serving. Increasing intake of these micronutrients through this multiple fortified food product may improve nutrient status among school-aged children and influence outcomes such as anaemia. Therefore, we aim to evaluate the effect of daily consumption of this fortified porridge on iron status and other micronutrient outcomes in Nigerian primary school-aged children (5-8 years old).

Objectives

The co-primary objectives of this randomised controlled trial are to evaluate the effect of daily consumption of a milk-based maize porridge fortified with multiple micronutrient (MMN) at different iron doses (hierarchical testing), compared with a control porridge, on haemoglobin (g/L) and serum ferritin concentrations (ug/L) at the end of the 5-month intervention in Nigerian primary school-aged children aged 5-8 years.

The secondary objectives are to evaluate the effects of the intervention, compared with the control group, on the prevalence of iron-deficiency (ID), iron-deficiency anaemia (IDA), and anaemia, and on biomarker concentrations of iodine, soluble transferrin receptor (sTfR), vitamin A, vitamin B12, and vitamin D, at end point.

The tertiary objectives are to evaluate the effects of the intervention, compared with the control group, on cognitive performance including, attention (digit span backward) and short-term memory (digit-span forward), at end point.

METHOD

Trial Design

This study concerns a double-blinded, randomised controlled trial with three parallel arms and an intervention period of five months (five days per week). A schematic overview of the study design is shown in **Figure 1**. Eligible children will be individually randomised in a 1:1:1 allocation ratio to one of three study arms using block randomisation.

- Arm 1: multi-fortified maize-milk product containing 3.3 mg/serve of ferrous fumarate per serving
- Arm 2: multi-fortified maize-milk product containing 6.6 mg/serve of ferrous fumarate per serving
- Arm 3: control group (maize-milk product without multiple micronutrient fortification)

Participants will consume one serving of 300 g per school day for the total intervention period of five months. Recruitment, screening, and enrolment will be conducted at the school level using a staggered approach. All eligible children within a given school will be recruited and screened consecutively. Once the predefined target number of enrolled participants for a school is reached and baseline assessments are completed, the intervention will commence at that school. Baseline and endline assessments will therefore be conducted on a staggered schedule across schools, with the timing of endline assessments corresponding to completion of the five-month intervention period at each school.

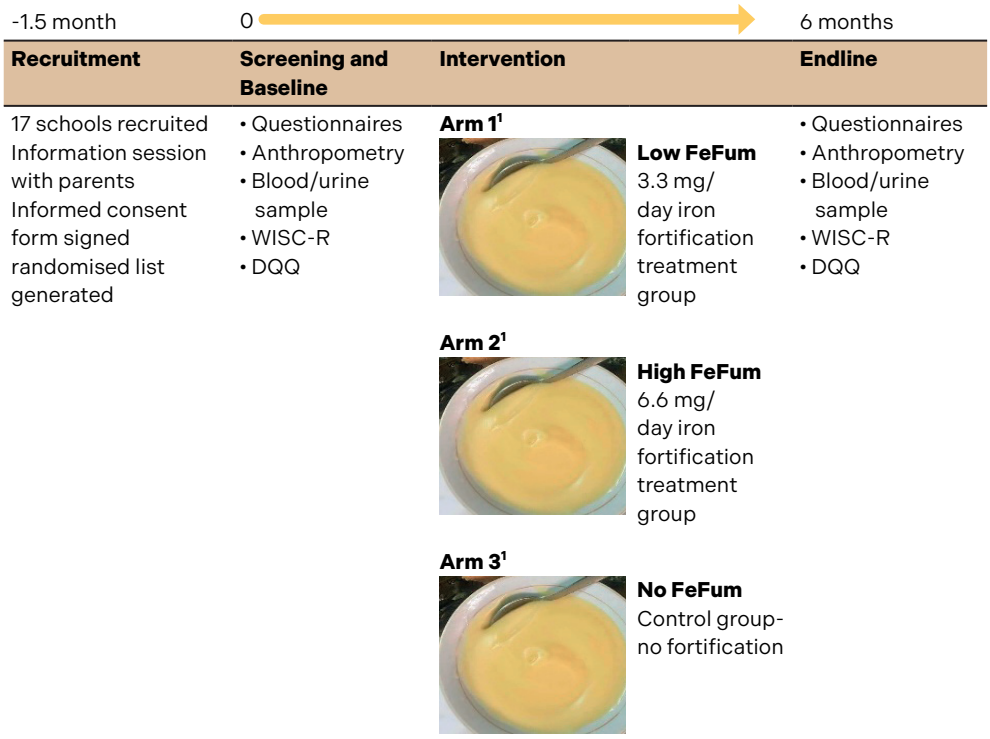


Figure 1: Study design

[1]Study products (multiple micronutrient fortified maize-milk porridge) will be blinded and given a randomly assigned code "A", "B" or "C"

Abbreviations: FeFum, Ferrous Fumarate; DQQ, Diet Quality Questionnaire; WISC-R, Wechsler Intelligence Scale for Children- Revised

Participants

Study population

Participants are school-aged children aged 5-8 years, that are not severely malnourished. The sample size will be 951 children for a sufficiently powered study (see section 3.1 for sample size calculation), to be recruited from approximately 17 primary schools, as it is expected that around 180 children will be within this age group per school, and 1/3rd (~60 children per school) will be eligible and consent to participate in the study.

Geographical location

Primary schools located in Ibadan, Nigeria that are located within the catchment area of the Institute of Child Health (ICH) of the University of Ibadan. The ICH, located within the premises of the University College Hospital, runs routine Child Welfare Clinics, which provides immunization, routine medical consultations,

growth and child development monitoring, and nutrition clinics including food demonstrations. Only schools without a school feeding programme will be included.

Eligibility criteria

Inclusion criteria

1) Nigerian children in primary schools, aged 5-8 years (age will be verified by school or ICH register).
2) Willing and able to consume the supplied servings of the treatment product or the provided control product.
3) Having written and oral informed consent from the parent/legal guardian and the participants gave oral and/or written assent.

Exclusion criteria

1) Children suffering from severe malnutrition and/or micronutrient deficiencies defined as (such children will be referred to a nearby health centre for appropriate treatment):
    a. Children with WHZ, WAZ < - 3 SD;
    b. Children showing clinical signs of severe (chronic) anemia i.e. visibly pale, jaundice and lethargic.
2) Children with a history of or who at the time of recruitment suffer from chronic illness, including (but not limited to) renal diseases, thyroid disease, (metabolic) bone disease, genetic and/or congenital disorders e.g. Down syndrome and sickle cell anaemia, hepatic dysfunction, thalassemia or chronic diarrhea (e.g. irritable bowel syndrome). Based on medical history examination.
3) Children with a physical disability or handicap that prevents participation in the study.
4) Children with food allergies or intolerances.
5) Children participating in any other clinical research (or having participated in any such study in the previous half year).
6) Children or a child's family who are intending to withdraw from school or move out of the study region within the study period.
7) Children consuming prescribed 'iron/folic acid, and (multi-)vitamin, and mineral supplements' during the course of the study within the last 2 months prior to the study as a minimum.

All subjects that have not received deworming in the past 3 months prior to baseline will be given deworming treatment before the trial begins (after screening and before baseline).

Intervention

During the school day, children will receive the assigned study product at lunch-time in designated distribution lines corresponding to groups A, B, and C. The three intervention arms will be: (1) Maize-milk product fortified with 3.3 mg Fe as ferrous fumarate, (2) Maize-milk product fortified with 6.6 mg Fe as ferrous fumarate, and (3) non-fortified maize-milk product (control). All products will be identical in appearance and preparation, and administration procedures will be standardised across schools to minimise performance bias.

Investigational product

The study product is a fermented maize porridge, with soy, milk, and multiple micronutrient fortification. The composition of 1 serve of the multi-fortified maize-milk product is provided in **Table 1**. For preparation, 800 g of the powdered product is first mixed with 400 mL of water to form a paste, after which, 2.8 L of boiling water is added and stirred until smooth. The prepared product is then portioned into individual cups (approximately 16) using 300-g measuring spoons, which corresponds to approximately 50 g powder reconstituted with 250 mL water.

One serving of multi-fortified maize-milk product (300 g) with 3.3 mg iron contains 33% of the daily recommended amount for a 5–8-year-old child for iron, 35% for iodine, 49% for zinc, 77% for vitamin A, 42% for vitamin B12 and 26% for vitamin D. The second study arm contains 6.6 mg iron as the premix quantity is doubled, which equals to 66% for iron, 70% for iodine, 98% for zinc, 154% for vitamin A, 83% for vitamin B12 and 53% for vitamin D. None of these nutrients will be consumed over or close to the upper level (UL) with one serving of product. The National Homegrown School Feeding Programme (NHGSFP) has defined nutrition standards for school feeding programmes in Nigeria (10). The investigational product was developed based on these standards **(Table 1).**

The amount of iron in the study product (3.3 mg/serve) is based on an existing commercial product available on the Nigerian market. This formulation uses ferrous fumarate as the iron compound and was designed to meet the regulatory definition of a "good source" of iron, providing more than 30% of the adult nutrient reference value (NRV: 14 mg) in accordance with the local, National Agency for Food and Drug Administration and Control (NAFDAC) regulations (19).

Using this existing study product as the starting point, we subsequently estimated the amount of iron required through fortification to improve iron intake in the target population such that the prevalence of inadequate intake would be reduced to approximately ≤5%, in line with FAO/WHO food fortification

guidelines (20). Tassy *et al* (2021) reported the usual intake of iron to be 9.4 ± 2.2 mg per day among children in a similar age group (4-8 years old) and in the same geographical area (Ibadan, Nigerian) as the present study population (21). Iron requirements of children (4–8 years) are described by WHO/FAO as being skewed, based on the analysis of NHANES data indicating a coefficient of variation of approximately 75% (20). Therefore, we applied the WHO/FAO full probability approach to define the target requirement distribution for our population.

Table 1. Nutritional composition of the intervention product per study group per day

Nutritional information[1]	Control[3] (units, (%) NRV[4])	Low-dose[3] (units, % NRV[4])	High-dose[3] (units, % NRV[4])	NHGSFP Standards (%)[5]
Energy (kcal)	205 (12)	205 (12)	205 (12)	-
Protein (g)	6.7 (35)	6.7 (35)	6.7 (35)	46
Carbohyrdates (g)	33.1	33.1	33.1	-
Sugar (g)	19	19	19	-
Added sugar (g)	8.3	8.3	8.3	-
Total fat (g)	4.6	4.6	4.6	-
SAFA (g)	21	2.1	2.1	-
Sodium (mg)	37.5	37.5	37.5	-
Calcium (mg)[2,]	131 (13)	140 (14)	154 (15)	-
Iron (mg)[2]	0.6 (6)	3.3 (33)	6.5 (66)	33
Zinc (mg)[2]	0.7 (14)	2.5 (49)	4.9 (98)	40
Iodine (ug)[2]	12.5 (14)	31.5 (35)	63 (70)	-
Vitamin A (ug-RE)[2]	168 (42)	308 (77)	616 (154)	42
Vitamin B12 (ug)[2]	0.3 (25)	0.5 (42)	1 (83)	-
Vitamin D (ug)[2]	0.2 (1)	3.95 (26)	7.9 (53)	-
Vitamin C (mg)[2]	0	25.7 (103)	51.4 (206)	-
Vitamin B1 (mg)[2]	0	0.285 (48)	0.57 (95)	-
Vitamin B2 (mg)[2]	0	0.25 (42)	0.5 (83)	-
Vitamin B3 (mg)[2]	0	3.5 (44)	7 (88)	-
Vitamin B6 (mg)[2]	0	0.33 (55)	0.66 (110)	-
Folic acid (ug)[2]	0	106 (53)	212 (106)	-

[1] Calculated from the formulation specifications provided by the manufacturer. [2] Nutrients in the multiple micronutrient fortification premix. [3] Serving is 50 g powder (300 g ready-to-consume) milk-based maize porridge per child per day. [4] NRVs derived from the Institute of Medicine (22). [5] NHGSFP= National Home Grown School Feeding Programme nutrient requirements for foods to be included in the Nigerian school feeding programme (10).

Abbreviations: - Not available; SAFA= saturated fatty acids; RE= Retinol Equivalent; NRV= Nutrient Reference Values.

For Nigerian children aged 5–8 years, a diet with moderate iron bioavailability (10%) was assumed, consistent with cereal-based diets that include vitamin C but limited animal-source foods. Using WHO/FAO requirement distributions for children aged 4–8 years, the usual iron intake associated with an inadequacy probability of approximately 5% was estimated at **16.2 mg/day.** Given a baseline usual intake distribution with a mean of 9.4 mg/day and a standard deviation of 2.2 mg/day, this corresponds to an additional **6.8 mg of iron** per serving per day required to reduce the prevalence of inadequate iron intake to ≤5%. This approach is consistent with WHO recommendations for population-level fortification planning in settings characterized by skewed iron requirements and moderate dietary iron bioavailability **(Supplement 1).**

Outcome measures

The co-primary outcomes are mean concentrations of haemoglobin (g/L) and serum ferritin (µg/L) assessed at the end of the 5-month intervention period. Serum ferritin concentrations will be adjusted for inflammation using the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia (BRINDA) regression correction approach with continuous C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) (23).

The secondary outcomes are soluble transferrin receptor concentration (sTfR, mg/L; adjusted for inflammation using the BRINDA method); the prevalence of anaemia (haemoglobin <115 g/L); iron deficiency (serum ferritin <15 µg/L or sTfR >8.3 mg/L); iron-deficiency anaemia (concurrent anaemia and iron deficiency). Additional secondary outcomes include endpoint concentrations of retinol-binding protein (RBP, adjusted for inflammation using the BRINDA method) (24), vitamin D, vitamin B12, iodine, and zinc, as well as the prevalence of deficiencies in these micronutrients. Vitamin A deficiency is defined as RBP <0.70 µmol/L (25); vitamin D deficiency as serum 25(OH)D <50 nmol/L and insufficiency as 50–75 nmol/L (26); vitamin B12 deficiency as serum cobalamin <148 pmol/L (27); and iodine deficiency as median urinary iodine concentration <100 µg/L (28). Body iron will be calculated using Cook's formula (29).

The tertiary outcomes are cognitive performance measures assessed at end point, including attention (digit span backward), short-term memory (digit span forward), and total cognitive performance score.

Additional measurements, to know dietary diversity, illness, and growth indicators, will be assessed to characterise the dietary and health context of participants during follow-up rather than to evaluate intervention effects. Information on illness episodes will be collected repeatedly throughout the intervention

period (monthly), and dietary intake and anthropometric measurements will be obtained at baseline and end point. These variables will be summarised descriptively by study group over time to assess background differences during the trial and to support interpretation of the primary and secondary outcomes. No formal hypothesis testing of intervention effects will be performed for these measures.

Study procedure

School recruitment and consent

Primary schools without a school feeding programme and located near the ICH centre will be invited to participate after approvals from the ethics committee, Oyo State Ministry of Education, and school boards. The PI and study team will meet head teachers and staff to explain the trial, then provide children and parents with written information and hold parent information sessions. Parents/guardians will receive an information sheet and consent form (translated into Yoruba where needed); consent will be obtained by signature or thumbprint after the information has been read and explained, with explicit statements on voluntariness, the right to withdraw, and optional future use of data and samples (**Supplement 2 and 3**).

Screening

Following parental consent, children will be screened for eligibility, including assessment of general health, anthropometry (height and weight), vitamin/mineral supplement use, clinical signs of micronutrient deficiency, and relevant medical history. Children meeting inclusion and exclusion criteria will be enrolled; those who are ineligible (e.g. with severe deficiency or other contraindications) will not receive study products or be followed further.

Randomization and blinding

Within each participating school, eligible children will be individually randomised in blocks of varying size (e.g. 3, 6, 12) to one of three groups, stratified by school to maintain balance between arms. An independent statistician will generate the allocation sequence, and study products will be packaged identically and labelled A, B, or C; participants, field staff, and outcome assessors will remain blinded to allocation.

Data collection

Data will be collected at screening, baseline (before the intervention), end point (after 5 months of intervention), and throughout follow-up to monitor compliance, illness and safety. All information will be captured in an electronic

case report form (CRF) using the Viedoc electronic data capture system (BCplatforms Ltd, Turku, Finland), with a study-specific template in English. The internet connectivity at schools may be unreliable, and therefore data collection will first be completed on paper CRFs and subsequently entered into the electronic database by trained research assistants. Equipment and utensils used for data collection measurements are shown in **Supplement 4**.

At baseline, demographic characteristics (sex, age), socio-economic status, medical history, and medication use will be recorded (**Supplement 5**). Socio-economic status will be assessed using a Wealth Index derived by principal component analysis of household assets, housing conditions, access to basic services, and occupation classified according to the Nigerian Standard Classification of Occupations; households will be ranked and grouped into tertiles from poorest to wealthiest, and individual wealth indicators will be used if PCA assumptions (eigenvalues >1) are not met.

At baseline and end point, standing height will be measured to the nearest 0.1 cm with a portable stadiometer, and weight to the nearest 0.05–0.10 kg using a calibrated digital scale, with duplicate readings taken according to standard procedures. If the difference between the two measurements exceeds 0.5 cm for height or 0.1 kg for weight, a third measurement will be obtained. The mean of the two closest measurements will be used for analysis. WHO growth standards will be used to derive height-for-age, weight-for-age, and BMI-for-age z-scores.

In addition, at baseline and end point, dietary intake and cognitive performance will be assessed. Dietary intake will be evaluated using the Diet Quality Questionnaire (DQQ), adapted for the Nigerian population and covers 29 food groups (30) (see **Supplement 6**). Cognitive performance will be assessed using the Wechsler Intelligence Scale for Children–Revised (WISC-R) digit span subsets (31), which measure attention (digit span backward) and short-term memory (digit-span forward) (see **Supplement 7**).

At baseline and end of the intervention, biological samples will be collected for assessment of micronutrient status, inflammation, and infection. Venous blood samples (approximately 13 mL per time point) will be collected by trained phlebotomists under aseptic conditions, with topical anaesthesia (Emla® 2.5% cream). Blood will be drawn into K□EDTA tubes (3mL EDTA vacutainer) for haematology analyses and plain serum tubes (10-mL clot activated vacutainer) for biochemical analyses.

Haemoglobin concentration and complete blood count will be measured on the day of collection using an automated haematology analyser (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) at University College Hospital, Ibadan. EDTA whole blood samples will be maintained at 2–8 °C prior to analysis or aliquoting (into 0.5 mL aliquots), according to assay requirements. If the whole blood is not analysed immediately, it can be stored for 2-3 days in the refrigerator. After this, before measurement, the tubes will be put on a roller bank for 10 minutes to homogenize the blood. The whole blood will be apportioned into 0.5 mL aliquots, stored, and transported at −20°C or lower.

For serum preparation, whole blood collected in plain tubes will be allowed to clot at room temperature for 30–40 minutes and subsequently centrifuged at 1,000–2,000 × g for 10 minutes in a refrigerated centrifuge. Serum will be aliquoted into trace-element-free polypropylene cryovials in predefined volumes (ranging from 0.5-2.0 mL) to avoid repeated freeze–thaw cycles. The samples should be maintained at 2–8°C while handling. If the serum is not analysed immediately, the serum aliquots should be stored and transported at −20°C or lower.

Serum ferritin, soluble transferrin receptor, C-reactive protein, alpha-1-acid glycoprotein and retinol-binding protein will be analsyed at the VitMin Lab (Germany) using their validated multiplex ELISA method, in duplicate and with repeat testing when plate coefficients of variation are ≥10% (32). Serum vitamin D (25-hydroxyvitamin D) and vitamin B12 will be determined using validated LC-MS/MS and immunoassay methods, respectively (Masdiag, Poland and Dicoon/CWZ Nijmegen, the Netherlands) (33,34).

For analyses at the VitMin Lab, serum samples will be provided in 0.5 mL tubes micronic tubes supplied by the laboratory (VitMin Lab). The target serum volume per tube will be 50–100 µL, with approximately equal volumes across tubes. Volumes below 25 µL may preclude analysis, while volumes above 100 µL may increase handling difficulties and analytical error. Accurate documentation of the unique 10-digit identification code located on the base of each tube will be ensured prior to shipment.

All serum, plasma and whole blood aliquots will be stored at −80 °C or lower in a study-dedicated, temperature-monitored freezer at the Institute of Child Health, in accordance with Good Clinical Practice and biosafety guidelines, with a back-up generator. Samples will be shipped in batches frozen using validated temperature-controlled packaging with continuous temperature monitoring to the designated analytical laboratories.

Malaria infection will be assessed at baseline by light microscopy following WHO procedures, with thick and thin blood films prepared. Thick and thin blood smears will be prepared, Giemsa-stained, and independently read by two trained microscopists blinded to treatment allocation and clinical data. Discordant readings will be resolved by a third reader (35).

Spot urine samples (approximately 10 mL) will be collected at baseline and endpoint for assessment of iodine status in a 100-mL plastic bottle. Urine samples will be stored at −20 °C and shipped frozen to Wageningen University, the Netherlands, for determination of urinary iodine concentration using the modified Sandell–Kolthoff method (36).

All biological samples will be labelled using unique participant identification codes to ensure traceability while maintaining participant confidentiality. Sample collection, processing, storage, transport, and analysis will follow standardized operating procedures, and detailed records will be maintained to ensure sample integrity and data quality throughout the study.

Compliance with study product consumption will be monitored daily throughout the intervention period. Study cooks and designated research assistants at each school will record whether each child received one serving and whether at least 50% of the serving was consumed (**Supplement 8**). One cook will be responsible for approximately 20 children per lunch meal, and each school will have three cooks recording intake for the respective study groups. Compliance records will be collected and reviewed regularly by a research assistant and cross-checked against school attendance registers. Any discrepancies will be addressed promptly.

A nurse or nutritionist will visit each school once per week at unscheduled times. During these visits, compliance records will be reviewed, and school-level product use will be assessed by verifying delivery and consumption of the required quantities of study product. Leftover study product will be collected to prevent distribution outside the study.

Morbidity data will be collected monthly using a structured illness questionnaire (**Supplement 9**) administered by telephone to parents or caregivers over the 5-month intervention period. Information on the number, type, and frequency of illnesses, as well as healthcare visits, will be recorded. In addition, daily school attendance will be monitored by research assistants, and reasons for absence will be documented where possible.

Follow-up, withdrawal and retention of study participants

Participants will be followed throughout the intervention period, with endpoint assessments mirroring those collected at baseline. To support participant retention and optimize compliance, detailed information on each child's place of residence will be collected at enrolment. When necessary, a trained research assistant will accompany the caregiver and child after enrolment to verify and document clear directions to the residence in the case report form. This information will be used to facilitate follow-up in cases of non-attendance or non-compliance with study product consumption.

School attendance will be monitored continuously by the research assistants. If non-compliance is identified, the issue will be escalated to the principal investigator, and follow-up will be conducted with the caregiver by telephone or home visit as appropriate. Caregivers will receive reminder telephone calls prior to scheduled study visits. To support retention, participants will receive free treatment for common illnesses during the study period and a modest financial incentive (USD 5) at each study visit.

Participation in the study is voluntary, and subjects may withdraw at any time without consequence. In addition, the investigator may withdraw a subject for medical or safety reasons. Suspected infections, including COVID-19, will prompt referral to local health services; confirmed infections will be documented as adverse events, and the child may continue participation where clinically appropriate.

Withdrawal requests may be communicated either directly by the child through the school-based research assistant, with confirmation from the caregiver, or by the caregiver contacting the research team. All withdrawals will be documented in the electronic case report form, including the reason for withdrawal where available. Following withdrawal, the child will discontinue receipt of the study product and will not undergo further study-related assessments.

Participants who withdraw will not routinely be replaced. The sample size calculation incorporated an anticipated dropout rate of 20%. Replacement may be considered only if withdrawal occurs within the first two weeks of the intervention or if overall attrition exceeds 20%. No follow-up assessments will be conducted after withdrawal, although the timing and reason for withdrawal will be recorded for reporting purposes.

Adverse event assessment and reporting

Adverse events (AEs) will be monitored throughout the trial and managed according to regulatory and ethics-committee requirements (**Supplement 10**). Any undesirable medical occurrence in a participant, will be documented on a dedicated AE form and in the electronic CRF, with an assessment of seriousness and relatedness to study participation. Serious adverse events (SAEs), including events that are fatal, life-threatening, require or prolong hospitalisation, cause persistent disability, or represent other important medical events, will be reported by the investigator to the sponsor and principal investigator within 24 hours of first awareness, and subsequently to the ethics committee within 7 days of first knowledge. All AEs and SAEs will be followed until resolution or stabilisation, with additional tests, treatment, or referral arranged as needed. The sponsor may temporarily halt the trial if participant safety is at risk, and all participants (and their guardians) will be informed of any safety-related changes. Parents can report (S)AEs at any time, and symptoms suggestive of intolerance (e.g. indigestion or lactose intolerance) will be actively queried at screening and recorded during the duration of the study.

Data management

Data will be captured using unique study identification numbers; the link between identification numbers and personal identifiers will be stored separately under restricted access and destroyed within six months after study completion. Pseudonymised datasets will be stored, and, where necessary, variables will be aggregated or recoded to minimise re-identification risk; trial master and investigator files, including the final dataset, will be archived for at least 25 years. An independent monitor will conduct risk-based monitoring of adherence to the protocol and accuracy of eCRF data against source documents, according to a predefined monitoring plan. Before unblinding, a blinded data review meeting will be held to define the intention-to-treat and per-protocol analysis sets, review and classify protocol deviations (e.g. ineligibility, missing primary outcomes, safety-related events, or inadequate product compliance), and document any changes.

Ethics and oversight

This study will be conducted according to the principles of the Declaration of Helsinki (2013), Good Clinical Practice (GCP), and in accordance with the local Medical Research Involving Human Participants Act (National Code of Health Research Ethics, 2003, Federal Ministry of Health) and ethical requirements. Local requirements include receiving the ethical clearance release letter from the Ethical Committee for Human Studies of the University of Ibadan. Informed

consent letter has to be signed by one parent or guardian and witnessed by the school authority, after receiving information from the research team.

The sponsor will arrange a subject insurance, prior to study start, to compensate subjects for any trial-related injury. This is as per The Federal Republic of Nigeria Official Gazette, Clinical Trial Regulations, 2021 (37). All children in the 3 study arms will be reimbursed for their participation in the study. All subjects will receive an incentive worth 2,000 naira (approximately 5 US Dollars) at each study visit during which outcome measurements are collected, specifically, at baseline and at end point. A school incentive will also be considered for example a book allowance and extra study product.

The study protocol will be registered at a public database (www.clinicaltrials.gov) after approval from the accredited ethical commission is received and before the first subject is screened. The results of this study will be made publicly available by means of a scientific publication in a peer-reviewed journal (in collaboration between investigator and sponsor), regardless of the outcome of the study. All data will be reported in aggregated form showing group results.

STATISTICAL PLAN

Statistical analyses will be conducted using R (version 2024.09.1). Anthropometric z scores will be calculated in R (version 2024.09.1) using the WHO 2007 growth reference for children and adolescents aged 5–19 years, implemented through the appropriate R package (e.g. *anthroplus*), which applies World Health Organization methodology for the calculation and reporting of height-for-age, weight-for-age, and BMI-for-age z scores in children aged 5 years and older (38).

Sample size determination

A total of 951 participants (317 per group) will be randomised to obtain at least 759 evaluable participants assuming a 20% drop out rate. This sample size provides 80% power to detect a two-sided difference in haemoglobin concentrations between the low-dose treatment group and the placebo group. The calculation assumed a mean difference of 2 g/L and a standard deviation of 8 g/L. These assumptions were informed by findings from a comparable study population and intervention design reported by Andang'o et al. 2007 (39).

For serum ferritin, the co-primary outcome, the required sample size was lower, at least 179 participants per group (537 in total), needed to achieve 80% power,

based on an assumed mean difference of 5 ug/L and a standard deviation of 15 ug/L. Therefore, the sample size derived from the hemoglobin calculation was selected to ensure adequate power for both primary outcomes.

The high-dose treatment group (6.6 mg iron) is expected to produce a larger effect on the primary outcomes; therefore, the sample size calculation was conservatively based on the estimated effect of the low-dose treatment group (3.3 mg iron).

Multiplicity was considered because two co-primary outcomes (hemoglobin & serum ferritin) will be analysed. However, given these two co-primary outcomes are related and highly correlated, no adjustment for multiple comparisons will be applied (40). Instead, a hierarchical testing procedure with a fixed-sequence approach will be used to address multiplicity between the two comparisons (high-dose and control; followed by low-dose and control). The high-dose comparison will be conducted first, and the low-dose comparison will be formally tested only if the high-dose comparison rules out a null effect; otherwise, the low-dose results will be reported as exploratory.

Statistical analysis

Definition of analysis populations

The following populations will be defined with the dataset of this study:

Per Protocol (PP): The PP population consists of subjects who:
1. Meet all eligibility criteria;
2. Have primary outcome data (hemoglobin and ferritin) available for both baseline and endpoint;
3. Have no adverse events or take medication that could interfere with primary outcomes;
4. Are compliant to product intake for at least 80% of the school-day servings over the intervention period (minimum 84 days) and consume more than 50% of each serving.

Modified-Per Protocol (mod-PP)[1]: The mod-PP population will include subjects who:
1. Meet all eligibility criteria;

[1] *Amendment to protocol 11/2024*. During the blind data review meeting (DRM), it was determined that no participants met the compliance definition for the PP population particularly due to treatment compliance definition. Therefore, a mod-PP population is included to replace the PP analysis set.

2. Have primary outcome data available (hemoglobin and ferritin) for both baseline and endpoint;
3. Have no adverse events or take medication that could interfere with primary outcomes;
4. Are compliant with product intake for at least 80% of the school days over 3 months (minimum 50 days) and consume more than 50% of each serving.

The choice of a minimum of 50 compliant days was based on observed adherence patterns during the study, as no evidence from comparable studies was available regarding the minimum number of consumption days required to influence outcomes.

Intention to Treat (ITT) population: The ITT population will include all randomised subjects who consumed the study product at least once.

Efficacy analyses will be performed using both ITT and PP populations, with the PP analysis considered primary[2]. Unless stated otherwise, a two-sided statistical significance level of $\alpha = 0.05$ will be used where applicable[3]. Normality will be assessed using Q–Q plots and/or the Shapiro–Wilk test. If variables are non-normally distributed, data transformation may be applied or non-parametric alternatives may be used.

Presentation of analysis outcomes

For categorical variables, number and percentage of subjects within each category will be presented. Percentages will be calculated using the number of subjects with non-missing data and reported with one decimal place, except for values of 100%, which will be presented without decimals.

For continuous variables, the number of participants, number of missing observations, mean, median, standard deviation, Q1, Q3, interquartile range, minimum, and maximum will be presented.

Demographic variables

Demographic and baseline characteristics will be summarized for the ITT population set by treatment groups. Variables recorded at or shortly before randomisation include sex (male, female), age (years), weight (kg), length (cm),

2 *Amendment to protocol, 11/2024* The mod-PP population will be considered the primary analysis set.
3 *Amendment to protocol 04/*2025 The interpretation of treatment effects has been updated as follows. Primary interpretation will be based on the effect size and 95% confidence intervals. P-values will be reported as supporting information and not used as the sole basis for statistical inference.

height-for-age Z score, weight-for-age Z score, body mass index (kg/m^2), BMI-for-age Z-score, and wealth index.

Primary analysis

The primary outcome will be analyzed in both the ITT and PP populations, with the PP[2] analysis considered primary. Treatment effects will be estimated by comparing groups at the end of intervention relative to the control group.

The primary objectives will be evaluated using hierarchical testing: the comparison between the high-dose group (6.6 mg iron) and the control group will be tested first. Only if this comparison demonstrates evidence against the null hypothesis[4] will the comparison between the low-dose group (3.3mg iron) and the control be formally tested.

Multilevel models will be used to analyse the primary outcomes, with participants nested within schools to account for clustering and non-independence of observations. Baseline concentrations of haemoglobin and serum ferritin will be included as covariates to account for potential baseline imbalances in prognostic factors. Model fit statistics and intraclass correlation coefficients will be examined to assess and optimise model performance.

Secondary analysis

Secondary outcomes related to iron status will be analysed using the ITT population. Binary outcomes, including iron deficiency, iron-deficiency anaemia, anaemia, inflammation, will be analysed using multilevel mixed-effects Poisson models with robust error variance to address potential overestimation of associations for common outcomes. Baseline haemoglobin and ferritin concentrations will be included as covariates. C-reactive protein and alpha-1-acid glycoprotein will be included as covariates only for analyses of biomarkers that are not already adjusted for inflammation (e.g. serum ferritin, soluble transferrin receptor, and retinol-binding protein).

All other micronutrient status and deficiency outcomes will be analysed using the ITT population. Continuous outcomes, including inflammation-adjusted retinol-binding protein, vitamin D, vitamin B12, iodine, and zinc concentrations, will be analysed using multilevel mixed-effects models with participants nested within schools. Models will adjust for relevant baseline covariates, including

[4] *Amendment to protocol, 11/2024* The mod-PP population will be considered the primary analysis set. *Amendment to protocol 04/*2025 The interpretation of treatment effects has been updated as follows. Primary interpretation will be based on the effect size and 95% confidence intervals. P-values will be reported as supporting information and not used as the sole basis for statistical inference.

baseline biomarker concentrations. Model fit statistics and intraclass correlation coefficients will be assessed to optimise model specification.

Binary outcomes for micronutrient deficiencies (vitamin A deficiency, vitamin D deficiency, and vitamin B12 deficiency) will be analysed using multilevel mixed-effects Poisson regression models with robust variance estimation.

Tertiary analysis

Tertiary outcomes will be analysed using the intention-to-treat (ITT) population. Cognitive performance will be assessed using the Wechsler Intelligence Scale for Children–Third Edition (WISC-III). Three scores will be derived per participant: digit span forward (0–16 points), digit span backward (0–14 points), and the maximum subtest score (0–30 points). Differences in these scores at end point between each intervention group and the control group will be analysed using multilevel mixed-effects models, adjusting for baseline cognitive scores and accounting for clustering within schools. Model fit statistics and intraclass correlation coefficients will be examined to optimise model performance.

Additional measurements

Dietary intake data collected using the Diet Quality Questionnaire (DQQ) will be used to assess dietary diversity. Consumption of the 29 food groups will be summarised descriptively at baseline and end point by study group and overall. In addition, the Dietary Diversity Score (DDS), a population-level indicator based on consumption of ten distinct food groups during the previous day, will be calculated. The DDS is a semi-continuous score ranging from 0 to 10 and will be reported as means and standard deviations for each study group and overall at baseline and end point (41)[5].

Methods in analysis to handle protocol nonadherence and other statistical considerations

Missing data will be imputed when more than >10% of values are missing. Outcome variables will not be imputed.

Outliers

Plausibility of outliers will be inspected on a case-by-case basis.

Values below limit of detection

Analytical values that are below the limit of detection (LOD) will be replaced by LOD divided by 2: LOD/2. Alternative approaches may be considered depending on the proportion of values below LOD.

[5] Deviation from SAP; this was originally reported as a tertiary outcome

Covariates
Covariates will be considered when appropriate. For the secondary outcomes (anaemia, iron-deficiency, and iron-deficiency anaemia), biomarkers will be adjusted for inflammation (via the BRINDA methodology) before classification. Additional potential covariates include age, sex, wealth index, parental education, and anthropometric indicators. Covariates will be retained if they change the effect estimate by at least 10%. Baseline measures will be included when available.

Methods for additional analyses (e.g. subgroup analyses)
Potential effect modification by age, sex, and baseline nutritional status will be assessed by including an interaction term between the potential modifier and the treatment group. Interaction terms with $p < 0.20$ will be further explored through stratified analyses.

Interim analyses
No interim analyses will be conducted.

Safety Analysis
Adverse events and illnesses reported on the illness form will be tabulated by treatment group, event type, severity, and possible relationship to study participation. Events will be coded using MedDRA version 25.1. Serious adverse events will be tabulated or listed and described in the study report, including assessment of their relationship to study participation (42).

Dissemination plans
Findings will be disseminated through a peer-reviewed scientific manuscript and presentations to local collaborators at the College of Child Medicine (University of Ibadan), FrieslandCampina Wageningen and Nigeria, and at scientific conferences.

DISCUSSION

This trial will evaluate whether fortification of a milk-based maize porridge improves iron status and related micronutrient status compared with an otherwise similar unfortified porridge in 5-8-year-old school-aged children in Nigeria. The study addresses an evidence gap on food-based iron interventions in school-aged children, and will test a commercially relevant, multi-nutrient fortified product delivered through a school-feeding programme. If effective, the findings could support incorporation of fortified porridge or similar products

into national school-feeding programmes to reduce anaemia and micronutrient deficiencies.

The design builds directly on the Kenyan trial by Andang'o *et al.* in comparable age groups and school settings, while differing in the iron compound and dose (39). Whereas the Kenyan study used NaFeEDTA at relatively high-doses, this trial uses ferrous fumarate at doses selected to be technologically feasible and cost-compatible with commercial production (Appendix of this thesis) and National Agency for Food and Drug Administration and Control communication (approximately 30% of the nutrient reference value), with an additional higher-dose arm to explore a potential dose–response. This will provide important information on whether a more affordable iron compound and commercially realistic fortification levels can still meaningfully improve iron status.

Anaemia and iron status are influenced by infections, inflammation, and haemoglobinopathies (16). The protocol therefore incorporates systematic assessment and management of key co-factors, including malaria diagnosis and treatment, de-worming, and screening for haemoglobinopathies. Biomarker adjustment for inflammation will further support accurate attribution of effects to the intervention. The school-based delivery model is expected to support good adherence, as the porridge is locally adapted, taste-tested, and offered five days per week within an existing school routine.

Implementing a large randomised trial in public schools is operationally complex. The protocol therefore includes independent monitoring, a pilot phase to refine procedures and assess acceptability, a structured site initiation and staff training to support protocol fidelity. The results will inform both policy decisions on fortification within school-feeding schemes and product-development decisions for the industry partner, including whether higher fortification levels or further reformulation are warranted. By including multiple micronutrients in addition to iron, the study may also shed light on the extent to which broader micronutrient improvements translate into reductions in anaemia risk.

Trial status

The trial commenced with enrolling the first subject in January 2024 and the last subject out in July 2024. The analysis has been conducted and the primary and secondary outcomes have been analysed and written into the first version of a manuscript, which was submitted to a journal in January 2026. The tertiary outcomes, namely cognitive performance are yet to be analysed or reported.

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SUPPLEMENTARY INFORMATION

Supplement Table 1.a Predicting the effect of iron fortification on usual iron intake distributions in children, (WHO, 10% bioavailability, normal requirement distribution)

Percentile	Distribution of usual food intake (servings/ day)	Iron intake before fortification (mg/day)	After fortification (+3.3 mg/day)	After fortification (+6.6 mg/day)	Intake in relation to requirement (6.3 mg/day)
Group at risk of inadequacy					
5	1	5.8	9.1	12.4	Below / Above / Above
10	1	6.6	9.9	13.2	Meets / Above / Above
25	1	7.9	11.2	14.5	Above / Above / Above
50	1	9.4	12.7	16.0	Above / Above / Above
Group at potential risk of excess					
75	1	10.9	14.2	17.5	Above / Above / Above
90	1	12.2	15.5	18.8	Above / Above / Above
95	1	13.0	16.3	19.6	Above / Above / Above

b. Predicting the effect of iron fortification on usual iron intake distributions in children (WHO, 5% bioavailability, normal requirement distribution)

Percentile	Distribution of usual food intake (servings/ day)	Iron intake before fortification (mg/day)	After fortification (+3.3 mg/day)	After fortification (+6.6 mg/day)	Intake in relation to requirement (12.6 mg/day)
Group at risk of inadequacy					
5	1	5.8	9.1	12.4	Below / Below / Meets
10	1	6.6	9.9	13.2	Below / Below / Above
25	1	7.9	11.2	14.5	Below / Below / Above
50	1	9.4	12.7	16.0	Below / Below / Above
Group at potential risk of excess					
75	1	10.9	14.2	17.5	Below / Above / Above
90	1	12.2	15.5	18.8	Below / Above / Above
95	1	13.0	16.3	19.6	Above / Above / Above

c. Predicting the effect of iron fortification on usual iron intake distributions in children (WHO, 5% bioavailability, skewed requirement distribution)

Percentile	Distribution of usual food intake (servings/day)	Iron intake before fortification (mg/day)	After fortification (+3.3 mg/day)	After fortification (+6.6 mg/day)	Intake in relation to requirement (≈31.5 mg/day)
Group at risk of inadequacy					
5	1	5.8	9.1	12.4	Below / Below / Below
10	1	6.6	9.9	13.2	Below / Below / Below
25	1	7.9	11.2	14.5	Below / Below / Below
50	1	9.4	12.7	16.0	Below / Below / Below
Group at potential risk of excess					
75	1	10.9	14.2	17.5	Below / Below / Below
90	1	12.2	15.5	18.8	Below / Below / Below
95	1	13.0	16.3	19.6	Below / Below / Below

d. Predicting the effect of iron fortification on usual iron intake distributions in children (WHO, 10% bioavailability, skewed requirement distribution)

Percentile	Distribution of usual food intake (servings/day)	Iron intake before fortification (mg/day)	After fortification (+3.3 mg/day)	After fortification (+6.6 mg/day)	Intake in relation to requirement (≈16.2 mg/day)
Group at risk of inadequacy					
5	1	5.8	9.1	12.4	Below / Below / Below
10	1	6.6	9.9	13.2	Below / Below / Below
25	1	7.9	11.2	14.5	Below / Below / Below
50	1	9.4	12.7	16.0	Below / Below / Meets
Group at potential risk of excess					
75	1	10.9	13.9	17.7	Below / Below / Above
90	1	12.2	15.2	19.0	Below / Below / Above
95	1	13.0	16.0	19.8	Below / Below / Above

Supplement 2. Participant information sheet

Study Name: Assessing the effectiveness of a multiple-micronutrient fortified maize pap on the nutrient status of Nigerian school-aged children: Randomised Controlled Trial

Principal Investigator & Contact Details:

Name	Department/ Address	Email	Phone

Purpose of the Research

You are invited to participate in a research study. It is important to us that you first take time to read through and understand the information provided in this brochure. Nevertheless, before you take part in this research study, the study will be explained to you and you will be given the chance to ask questions. After you are properly satisfied that you understand this study, and that you wishes to take part in the study, you must sign the informed consent form. You will be given a copy of this consent form to take home with you.

You are invited because you're a child between the age of 5-8 years old attending a school in Ibadan within a 5km area of the Ibadan Institute of Child Health Centre.

The purpose of this study is to test the effectiveness of a maize pap product that has been fortified with a number of micronutrients such as iron on the nutrient status of the school-aged children. FrieslandCampina is paying for this research. We expect to recruit about 60 subjects from this site over a period of 6 months. In total, about 951 will participate in this study.

What procedures will be followed in this study

If you agree to take part in this study, we will have to ask you questionnaires to see if you are eligible for the study, after this with your consent we will collect a blood sample, a urine sample, measure height and weight and ask a number of questions relating the demographic information, illnesses, dietary quality and

cognition. This will be done at two points, at the beginning and the end of the study. The study procedures will take approximately 2-3 hours' time.

Your responsibilities in this study

- If you agree to participate in this study, you will be asked to consume 300g of pap product at lunchtime in school on each school day (5 days a week) for 5 months.
- For measurements as explained above you will also be expected to take part in these procedures at school. This will happen on two days; one at the beginning of the study and one at the end of the study. It is expected the measurements will take 2-3 hours.
- Your participation in the study will last 6 months in total, as there is holidays included in this time period where you will not receive pap product.

Possible risks and side effects

The possible risks and side effects of:

- Consuming this study product is low. Side effects include; allergic reaction. At excessive consumption levels side effects may include; diarrhea, fever and vomiting and increased risk of malaria.
- Having blood withdrawn are also low. Side effects may include pain or discomfort, bruising, dizziness or fainting, infection and an allergic reaction.

Possible benefits from participating in the study

- If you participate in this study, you may reasonably expect to benefit from the trial product in the following way; improvement to nutrient status (Hemoglobin, ferritin, zinc, vitamin A and iodine) and be at a reduced risk of anaemia and iron deficiency. *[OR]*
- There is no assurance you will benefit/ no known benefit from participation in this study. However, your participation in this study may add to the (medical) knowledge about the use of this product.

Payment/ incentive if participating in the study

- Your participation will be incentivized by receiving a payment of naira's to the equivalence of 5USD at each measurement; at the beginning and end of the study. You will also be receiving the product as mentioned for a period of 5 months.

Voluntary participation

Your participation in this study is voluntary. You may stop participating in this study at any time. Your decision not to take part in this study or to stop your

participation will not affect your medical care or any benefits to which you are entitled. If you decide to stop taking part in this study, you should tell the Principal Investigator or your doctor. You retain the rights to ask the investigators to discard or destroy any unused samples. Specimens and their derived products obtained in this study will be used for the purposes of this study. You need to indicate on the consent form if you agree to storage of additional derived products and use in future studies.

Compensation for injury

If you are physically injured due to the study procedures in this study, [details of clinical trial insurance] will pay the medical expenses for the treatment of that injury.

Confidentiality

Information collected for this study will be kept strictly confidential. Your records, to the extent of the applicable laws and regulations, will not be made publicly available. In the event of any publication regarding this study, your identity will remain confidential.

How will my information be used?

The study team will use your information to conduct this study. Once the study is done using your information, we may share study data/ specimens with other researchers so they can use it for other studies in the future. We will not share your name or any other personal information that would let the researchers know who you are. We will not ask you for additional permission to share this de-identified information for future studies.

Who to contact if you have questions

Should you have further questions about the study or need to contact someone in the event of a research-related adverse event or injuries related to the study, please contact the Principal Investigator: Professor Adebola E. Orimadegun.

The study has been reviewed by the Institutional Review Board" (IRB) for ethics approval.

Supplement 3: Informed consent Form (ICF)

Title: Assessing the effectiveness of a multiple-micronutrient fortified maize pap on the nutrient status of Nigerian school-aged children: Randomized Controlled Trial

Principal Investigator & Contact details

Name	Department/ Address	Email	Phone

I voluntarily consent to take part in this research study. I have fully discussed and understood the purpose and procedures of this study. This study has been explained to me in a language that I understand. I have been given enough time to ask any questions that I have about the study, and all my questions have been answered to the best of my doctor's ability.

Please tick one (if applicable)

□ I agree to the storage of my biological samples of blood and urine and their products for future use. I understand that this will allow the possibility of conducting future studies.

□ I disagree to the storage of my biological samples of blood and urine and their products for any future research. All biological samples of blood and urine specimens and their derived products obtained will be used solely for the purpose of this study and will be destroyed after its completion.

Name of Subject ID number Signature Date & Time

Witness Statement (if applicable)

I, the undersigned, certify to the best of my knowledge that the participant signing this informed consent form had the study fully explained in a language understood by him/her and clearly understands the nature, risks and benefits of his/ her participation in the study.

Name of Wtness Signature Date & Time

Investigator Statement

I, the undersigned, certify that I explained the study to the participant and to the best of my knowledge the participant signing the informed consent form clearly understands the nature, risks and benefits of his/her participation in this study.

Name of person administering consent Signature Date & Time

Supplement 4: Equipment and utensils

For measurements



Wristband



Electronic scales



Stadiometer



Tape measure

For collecting biological samples



A Vacutainer safety lok set



3mL EDTA (anticoagulant) vacutainer

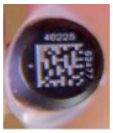


10mL Plain vacutainer (clot activated tube)



100mL plastic bottle

For storing and transporting biological samples





0.5 mL tubes from Micronic provided by VitMin Lab



1.5/2mL Eppendorf tubes



10mL tube



Solar powered Freezer - thermocool



Neopor shipment box

Supplement 5: Demographic survey

What is the sex of the child? Male ☐ Female ☐ Other ☐	Group assignment: A ☐ B ☐ C ☐
Age (as at last birthday in years):	Date of birth: ___/___/_____
Home address:	
Parent's/caregiver's mobile phone number:	
What is the age of the mother (year)? ☐	What is the age of the father (year)? ☐
Who does the child live with? Mother ☐ Father ☐ Carer ☐ Others ☐ (specify).....	
Who is the main care provider in the household? Mother ☐ Father ☐ Carer ☐ Others ☐ (specify).....	
How many are there in the same household, both adults and children? ☐	
How many brothers or sisters? (write number or zero if none): ☐	
What is the child's position among mother's children? (write number 1 for first, 2 for second etc.) ☐	
Ethnicity of the child? Yoruba ☐ Igbo ☐ Hausa ☐ Others ☐ (specify).....	
Family religion: Christianity ☐ Islam ☐ African traditional religion ☐ Others ☐ (specify).....	
What is the marital status of the mother (carer) of the child? 1. Married or living together 2. Divorced / separated 3. Widowed 4. Never married and never lived together ☐	
What is the highest level of education the mother has completed? 1. No education 2. Primary 3. Secondary 4. Tertiary ☐	

What is the highest level of education the father has completed?
1. No education
2. Primary
3. Secondary

Tertiary

What class is the child in now?

What is the occupation of the mother?
1. Manager
2. Professional
3. Technician's and associate professionals
4. Clerical support workers
5. Service and sales workers
6. Skilled agricultural, forestry and fisher workers
7. Craft and related trade workers
8. Plant and machine operators and assemblers
9. Elementary occupations
10. Other (specify).....

What is the occupation of the father?
1. Manager
2. Professional
3. Technician's and associate professionals
4. Clerical support workers
5. Service and sales workers
6. Skilled agricultural, forestry and fisher workers
7. Craft and related trade workers
8. Plant and machine operators and assemblers
9. Elementary occupations
10. Other (specify).....

Do you own a television?	Yes ☐	No ☐
Do you own a radio?	Yes ☐	No ☐
Do you own a mobile phone?	Yes ☐	No ☐
Do you own a bicycle?	Yes ☐	No ☐
Do you own a car or motorcycle?	Yes ☐	No ☐
Do you own a refrigerator?	Yes ☐	No ☐
Do you have access to the internet?	Yes ☐	No ☐

What type of material is used for the roof of your house?
1. Thatch
2. Iron sheet
3. Tiles
4. Other (specify)

What type of material is used for the floor of your house?
1. Earth
2. Cement
3. Wood
4. Other (specify)

Other (specify)

How many rooms does your family have?

Does your house have access to electricity?
Does your house have access to clean drinking water?
What type of toilet facilities does your household use? 1. Flush toilet 2. Pit latrine 3. Open defecation 4. Other (specify).....
What is the main source of cooking fuel used in your household? 1. Electricity 2. Gas 3. Charcoal 4. Wood 5. Other (specify).....

Supplement 6: Nigerian Diet Quality Questionnaire (DQQ)

DIET QUALITY QUESTIONNAIRE (DQQ)



NIGERIA

Read: Now I'd like to ask you some yes-or-no questions about foods and drinks that you consumed yesterday during the day or night, whether you had it at home or somewhere else.

First, I would like you to think about yesterday, from the time you woke up through the night. Think to yourself about the first thing you ate or drank after you woke up in the morning ... Think about where you were when you had any food or drink in the middle of the day ... Think about where you were when you had any evening meal ... and any food or drink you may have had in the evening or late-night... and any other snacks or drinks you may have had between meals throughout the day or night.

I am interested in whether you had the food items I will mention even if they were combined with other foods.

Please listen to the list of foods and drinks, and if you ate or drank ANY ONE OF THEM, say yes.

	Yesterday, did you eat any of the following foods:	(circle answer)
1	Rice, couscous, bread, spaghetti, masa, pap, semo, or other swallow made from grains?	YES or NO
2	Porridge, egbo / dambu, corn, or Ofada rice?	YES or NO
3	Sweet potatoes that are white inside, fufu, gari, abacha, yam, lafun, black amala, or plantain?	YES or NO
4	Beans, awara from soy, moin moin, bambara groundnuts, pigeon peas, African yam beans, or other local beans?	YES or NO
	Yesterday, did you eat any of the following vegetables:	
5	Carrots, pumpkin, squash that is orange inside, or sweet potatoes that are orange inside?	YES or NO
6.1	Ewedu / ayoyo, pumpkin leaves, water leaves, bitter leaves, green, soko leaves, or baobab leaves?	YES or NO
6.2	Zobo leaves, afang / okazi, karkashi, garden egg leaves, sweet potato leaves, or cowpea leaves?	YES or NO
7.1	Tomatoes, garden eggs, okro, cucumber, green pepper, cabbage, or green beans?	YES or NO
	Yesterday, did you eat any of the following fruits:	
8	Mango, pawpaw, golden melon, or locust bean fruit?	YES or NO
9	Orange, tangerine, or grapefruit?	YES or NO
10.1	Banana, guava, soursop, watermelon, cashew fruit, apple, or pineapple?	YES or NO
10.2	Avocado pear, coconut flesh, dates, African bush pear, African elemi, or African cherry?	YES or NO
	Yesterday, did you eat any of the following sweets:	
11	Cakes, cookies, sweet biscuits, wafers, chin chin, or puff puff?	YES or NO
12	Sweets, chocolates, ice cream, groundnut brittle or sesame brittle?	YES or NO
	Yesterday, did you eat any of the following foods of animal origin:	
13	Eggs or egg rolls?	YES or NO
14	Wara from milk, or dairy cheese?	YES or NO
15	Yogurt, nono, or fura de nono?	YES or NO
16	Sausages, hot dogs, corned beef, or kilishi?	YES or NO
17	Cow meat, ram meat, goat meat, or any organs from these animals?	YES or NO
18	Pig meat, rabbit, dog meat, or bush meat?	YES or NO
19	Chicken, turkey, guinea fowl, quail, duck, or pigeon?	YES or NO
20	Fish, canned fish, crab, prawns, or shrimp?	YES or NO
	Yesterday, did you eat any of the following other foods:	
21	Groundnuts, melon seeds, ogbono, breadfruit seeds, walnuts, or cashews?	YES or NO
22	Packaged potato chips such as Pringles, or packaged plantain chips?	YES or NO
23	Instant noodles such as Indomie or Chikki?	YES or NO
24	Fried plantain, fried sweet potato, fried yam, akara, egg roll, samosa or spring rolls, fried fish, or fried meat?	YES or NO
	Yesterday, did you have any of the following beverages:	
25	Milk including powdered milk?	YES or NO
26	Tea with sugar, coffee with sugar, 3-in-1, choco drink, Milo or Bournvita?	YES or NO
27	Fruit juice, fruit flavoured drinks, zobo, or kunu?	YES or NO
28	Soft drinks such as Coca-Cola, Fanta, Sprite, or chapman, malt drinks, or energy drinks such as Red Bull?	YES or NO
	Yesterday, did you get food from any place like...	
29	Mr. Biggs, Big Treat, Domino's, KFC, Tantalizer, or other places that serve meat pie, chicken pie, pizza or burgers?	YES or NO

Adapted by the Global Diet Quality Project, www.dietquality.org, 2021.
Supported by the EU and BMZ (through GIZ), USAID, The Rockefeller Foundation, and SDC.





INSTRUCTIONS:

1) Read the DQQ exactly as written. Do not include additional dialogue or probing questions. Do not add or remove food items. It is important for the integrity of the questionnaire and comparability of results to not modify the DQQ. Further instructions on how to use the DQQ can be found at our website, www.dietquality.org

2) If you desire to collect information on additional food items, or to add supplementary questions, these additional questions can be placed at the end of the questionnaire.

3) The Global Diet Quality Project has undertaken a systematic process to identify food items for each food group. If you would like to suggest changes to the DQQ, please contact the team using the "Contact Us" button on our website.

Supplement 7: WISC-R: Digit Span (Supplementary Verbal Subtest)

WISC-III – Memory for Digit Span

Ages: 7 years and older

Examiner: _____

Date: _____

Child ID: _____

Step 1: Eligibility

Is child's age 7 years or older?

☐ Yes ☐ No

Step 2: Instructions to Examiner

- This section assesses the child's memory for number order.
- Each sequence has more digits than the last.
- It is normal for the child not to remember all sequences.

Step 4: Instructions for Digits Forward

- Read each digit span **only once** at 1 digit per second.
- Pause for response after each part, then score.
- Do **not** encourage if child does not respond.
- Stop when child misses **both parts** of any item.
- To score: No omissions or reversals allowed.

Instruction to Child:

"I am going to say some numbers. Listen carefully, and when I am through, say them right after me."

Digits Forward Measurement

Digits forward mearurment

1. A. 3 - 8 - 6 #2	Correct	1. B. 6 - 1 - 2 #3	Correct
2. A. 3 - 4 - 1 - 7 #4	Correct	2. B. 6 - 1 - 5 - 8 #5	Correct
3. A. 8 - 4 - 2 - 3 - 9 #6	Correct	3. B. 5 - 2 - 1 - 8 - 6 #7	Correct
4. A. 3 - 8 - 9 - 1 - 7 - 4 #8	Correct	4. B. 7 - 9 - 6 - 4 - 8 - 3 #9	Correct
5. A. 5 - 1 - 7 - 4 - 2 - 3 - 8 #10	Correct	5. B. 9 - 8 - 5 - 2 - 1 - 6 - 3 #11	Correct
6. A. 1 - 6 - 4 - 5 - 9 - 7 - 6 - 3 #12	Correct	6. B. 2 - 9 - 7 - 6 - 3 - 1 - 5 - 4 #13	Correct
7. A. 5 - 3 - 8 - 7 - 1 - 2 - 4 - 6 - 9 #14 Correct		7. B. 4 - 2 - 6 - 9 - 1 - 7 - 8 - 3 - 5 #15 Correct	

Total Correct (Step 5): _____

Step 6: Instructions for Digits Backward

Instruction to Child:

"Now I am going to say some more numbers, but this time when I stop, I want you to say them backward. For example, if I say 9–2–7, what would you say?"

Digits Backward Measurement

8. A. 2 - 5 (ANS = 5 - 2) #16	Correct	8. B. 6 - 3 (ANS = 3 - 6) #17	Correct
9. A. 5 - 7 - 4 (ANS = 4 - 7 - 5) #18	Correct	9. B. 2 - 5 - 9 (ANS = 9 - 5 - 2) #19	Correct
10. A. 7 - 2 - 9 - 6 (ANS = 6 - 9 - 2 - 7) #20	Correct	10. B. 8 - 4 - 9 - 3 (ANS = 3 - 9 - 4 - 8) #21	Correct
11. A. 4 - 1 - 3 - 5 - 7 (ANS = 7 - 5 - 3 - 1 - 4) #22	Correct	11. B. 9 - 7 - 8 - 5 - 2 (ANS = 2 - 5 - 8 - 7 - 9) #23	Correct
12. A. 1 - 6 - 5 - 2 - 9 - 8 (ANS = 8 - 9 - 2 - 5 - 6 - 1) #24	Correct	12. B. 3 - 6 - 7 - 1 - 9 - 4 (ANS = 4 - 9 - 1 - 7 - 6 - 3) #25	Correct
13. A. 8 - 5 - 9 - 2 - 3 - 4 - 2 (ANS = 2 - 4 - 3 - 2 - 9 - 5 - 8) #26	Correct	13. B. 4 - 5 - 7 - 9 - 2 - 8 - 1 (ANS = 1 - 8 - 2 - 9 - 7 - 5 - 4) #27	Correct
14. A. 6 - 9 - 1 - 6 - 3 - 2 - 5 - 8 (ANS = 8 - 5 - 2 - 3 - 6 - 1 - 9 - 6) #28	Correct	14. B. 3 - 1 - 7 - 9 - 5 - 4 - 8 - 2 (ANS = 2 - 8 - 4 - 5 - 9 - 7 - 1 - 3) #29	Correct

Total Correct (Step 6): _____

Presence of Adults

For each category, mark presence and effect:

- **Mother:** ☐ Present ☐ Not present | Effect: ☐ Harmful ☐ None ☐ Improved
- **Father:** ☐ Present ☐ Not present | Effect: ☐ Harmful ☐ None ☐ Improved
- **Guardian:** ☐ Present ☐ Not present | Effect: ☐ Harmful ☐ None ☐ Improved
- **Relative:** ☐ Present ☐ Not present | Effect: ☐ Harmful ☐ None ☐ Improved
- **Family/Friend:** ☐ Present ☐ Not present | Effect: ☐ Harmful ☐ None ☐ Improved
- **Other (specify):** _____ | Effect: ☐ Harmful ☐ None ☐ Improved

Children Present

- **Sibling:** ☐ Present ☐ Not present | Effect: ☐ Harmful ☐ None ☐ Improved
- **Friend:** ☐ Present ☐ Not present | Effect: ☐ Harmful ☐ None ☐ Improved
- **Other (specify):** _____ | Effect: ☐ Harmful ☐ None ☐ Improved

Child's Energy Level

☐ Low (tired) ☐ Medium ☐ High

Was section terminated prematurely?

☐ Yes ☐ No

Reason (check all that apply):

☐ Parent/Guardian terminated/refused

☐ Child refused

☐ Major interruption

☐ Child could not understand task

☐ Language problem

☐ Emotional condition

☐ Physical condition

☐ Other (specify): _____

Supplement 8: Compliance dairy

Compliance Diary

Date for given portion: _____

Study week: _____

Calendar week: _____

Research Assistant (RA): _____

Feeding Record

Did the child eat more than half of the pap?

☐ Yes ☐ No ☐ Not present

Did you have breakfast?

☐ Yes ☐ No

What did you have for breakfast?

☐ Milk

☐ Pap

☐ Bread

☐ Rice

☐ Other (specify): _____

Remarks for this day:

Supplement 9: Illness questionnaire

Monthly Illness Questionnaire at baseline (enrolment day 0)

This section of the instrument should be administered at baseline and once every month on a Monday. Thus, each participant should have only SIX of this section completed.

In the past 1 month has the child experienced the following illness or health problem?	YES ☐	NO ☐
If yes, how many times did the child experience the illness in the past 1 month?	☐	
In the past 1 month, was the child hospitalized?	YES ☐	NO ☐
If yes, how many times was the child hospitalized in the past 1 month?	☐	
In the past 1 month, did the child have a cough or difficulty in breathing?	YES ☐	NO ☐
In the past 1 month did the child have a diarrhea or loose stools?	YES ☐	NO ☐
In the past 1 month did the child have vomit or complain of nausea?	YES ☐	NO ☐
In the past 1 month did the child have skin problems or rashes?	YES ☐	NO ☐
In the past 1 month did the child have an earache or ear problems?	YES ☐	NO ☐
In the past 1 month did the child have a constipation?	YES ☐	NO ☐
In the past 1 month did the child have an infection?	YES ☐	NO ☐
Were any drugs or medication taken in the past 1 month?	YES ☐	NO ☐

If Yes, what drugs or medications?.....

Supplement 10: Adverse Events

AE #	Type of Adverse Event	Date AE started	Severity	Is it related? Yes/No	SAE? Yes/No	Date AE ended	Outcome	Briefly describe the event (symptoms, signs, evolution, relevant examinations, biological data, residual effect):	Was study intervention discontinued due to event?
1.	Fever								
2.	Coughing or difficulty breathing								
3.	Diarrhea or loose stools								
4.	Constipation								
5.	Vomiting or nausea								
6.	Skin problems or rashes								
7.	Earache or ear problem								
8.	Accident (e.g. Broken bone, burn)								
9.	Infection								
10.	Other (please specify)								

Severity:
1= Mild - Transient or mild discomfort (<48 hours); no intervention required.
2=Moderate - Mild to moderate limitation of activity-some assistance may be needed; no or minimal medical intervention/therapy required.
3=Severe - Marked limitation in activity, some assistance usually required; medical intervention/therapy required; hospitalization possible.
4= Life threatening
Intervention Related: Y= May be related to the study Pap with Milk; N=May not be related to the study medication
Outcome: 1=Resolved, 2= Resolved with sequelae, 3=Ongoing, 4=Deceased 9=Unknown
SAE* If yes, complete SAE form and submit it to principal investigator and study IRBs

5



Chapter 6

Effectiveness of a multi-nutrient fortified milk-based maize porridge in Nigerian school-aged children: a randomised, double-blind trial embedded in a school feeding programme

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Abstract

Background: Anaemia affects over 1.9 billion people globally, with iron deficiency anaemia responsible for nearly two-thirds of cases. In Nigeria, school-aged children experience a high burden of anaemia and micronutrient deficiencies. Fortified foods in school feeding programmes may help address these deficiencies. This study assessed the effectiveness of a multi-nutrient fortified milk-based maize porridge on iron status as the primary outcome and on other micronutrient status outcomes, including vitamin A, vitamin B12, vitamin D and iodine, as secondary outcomes among Nigerian school-aged children aged 5–8 years.

Methods: We conducted a double-blind, randomised, controlled superiority trial in 17 public primary schools in Ibadan, Nigeria (ClinicalTrials.gov, NCT06251427). Healthy children received a low-dose fortified (3.3 mg iron/day), high-dose fortified (6.6 mg iron/day), or non-fortified control porridge for five months. Randomisation was computer-generated, stratified by school and concealed from participants, school staff and researchers. Venous blood was collected at baseline and endpoint. Multilevel linear and Poisson regression models were used to estimate intervention effects, accounting for school clustering.

Findings: Between January and July 2024, 945 children were randomised, 319 to control, 312 to low-dose and 315 to high-dose groups. At baseline, anaemia prevalence was 74% and iron deficiency (inflammation-adjusted) was 9.5%. At endpoint, the high-dose group did not differ from control in haemoglobin (−0.5%, 95% CI −3.4 to 2.5) and serum ferritin (1.9%, 95% CI -6 to 10.5) in intention-to-treat (ITT) analysis (n=945). Vitamin D concentration, a secondary outcome, improved modestly in the high-dose group (3.8%, 95% CI 0.6 to 7).

Interpretation: Consumption of multi-nutrient fortified milk-based maize porridge for 5 months through a Nigerian school feeding programme did not improve iron status but modestly increased vitamin D concentration.

INTRODUCTION

Anaemia remains a major global health concern, affecting over 1.9 billion people, or approximately 24% of the world's population. Iron deficiency is the leading cause, accounting for nearly two-thirds of anaemia cases worldwide (1). In children, iron deficiency is associated with impaired psychomotor, cognitive, and socioemotional development, with effects persisting into adolescence (2). Notably, the prevalence of iron deficiency anaemia in sub-Saharan Africa is the highest globally, and iron interventions in the region have often shown limited effectiveness, likely due to differences in dietary patterns, infection burden, and contextual factors compared to other regions (3).

Public health strategies to reduce iron deficiency anaemia have largely focused on children under five and pregnant women, while school-aged children (5–12 years) remain largely overlooked (3–5). In Nigeria, anaemia prevalence is high across all age groups: National data from 2021 show that 31% of children under five are anaemic, while sub-national estimates indicate a 39% prevalence among school-aged children (6,7). Iron deficiency and iron deficiency anaemia affect approximately 12% (range between 10-25%) and 17% of school-aged children from sub-national estimates, respectively (7). Other micronutrient deficiencies are also common in this age group, including vitamin A (16%), and iodine (59%) (7), alongside dietary inadequacies in calcium, iron, folate, and vitamins A, D, and E (8). However, national data on nutrient intake and status among school-aged children remains sparse.

School feeding programmes present an opportunity to address micronutrient deficiencies at scale, while supporting local food systems and educational output. This potential has been highlighted by the United Nations School Meals Coalition, including at the UN Food Systems Summit +4 in July 2025, and is reflected in Nigeria's Renewed Hope National Home-Grown School Feeding Programme, which aims for each school meal to provide at least 33% of children with recommended dietary allowances (9). However, despite evidence that school feeding improves overall nutrient adequacy, few randomised controlled trials have shown improvements in iron status with food-based iron fortification in school-aged children (10–13). While supplementation is often more effective, its large-scale implementation poses challenges in terms of feasibility, cost, and safety (14). Anaemia in this age group is often poorly characterized and likely multifactorial, with contributions from infections and other micronutrients deficiencies (5).

Given the multiple nutrient inadequacies found in Nigerian school-aged children (5-8 years old), a multi-nutrient fortified fermented maize, soy and milk-based porridge containing ferrous fumarate was developed. This study assessed the effectiveness of this product to increase haemoglobin and serum ferritin concentration (primary outcome) over five months when delivered through a school feeding programme compared with an unfortified porridge. Secondary outcomes included soluble transferrin receptor, anaemia, iron deficiency, iron deficiency anaemia, and status of vitamin A, vitamin B12, vitamin D and iodine.

METHOD

Study design

This was a double-blind, randomised controlled superiority effectiveness trial embedded within a school feeding programme. The trial had three parallel arms (i.e. high iron, low iron, control) and a five-month intervention period. It was conducted from 7th January to 30th July 2024 in 17 public primary schools in Ibadan North Local Government Area, Nigeria. Schools were eligible if they were located within a 5-km radius of the Institute of Child Health (ICH), University of Ibadan, and had no existing school feeding programme. A pilot study was conducted prior to implementation to optimise product preparation and delivery. Input from community stakeholders, including teachers, community leaders, and representatives from the local Ministry of Education, was collected and used to inform the study design and operational procedures. The study protocol was approved by Oyo State Ministry of Health Research Ethics Committee (HREC) (approval number: AD 13/479/612A) and registered at ClinicalTrials.gov (NCT06251427).

Participants

Eligible participants were apparently healthy children aged 5–8 years attending the selected schools. Recruitment was conducted at the school level, all children within the eligible age range (5–8 years) were screened for inclusion at each participating school. Information sessions were conducted at schools with parents or legal guardians prior to enrolment, after which written informed consent was obtained.

Exclusion criteria included severe malnutrition (weight-for-age or height-for-age z-score <−3 SD), clinical signs of severe anaemia, chronic illness (e.g. renal, thyroid, hepatic, or genetic disorders), physical disability, food allergy, recent participation in other clinical studies, planned relocation, or recent use of iron/multivitamin supplements.

Randomisation and masking

Participants were randomly assigned in a 1:1:1 ratio to one of three study arms (A, B, or C) using block randomisation with randomly varying block sizes of 3, 6, and 12, stratified by school. The randomisation list was generated in advance by an independent statistician for each school, and participants received the next sequential assignment upon enrolment. A class list with child's name and group allocation was made per school, to ensure correct allocation during meal distribution. Intervention products were labelled "A," "B," or "C" by an independent staff member not involved in the study, with the label-to-treatment code concealed in a sealed envelope until database lock or in case of a serious adverse event. All products were indistinguishable in packaging and appearance when prepared. Participants, caregivers, field staff, cooks, and investigators remained blinded to group allocation throughout the trial.

Procedures

Following screening and baseline assessments, participants received a daily serving of one of the three products: (1) fortified milk-based maize porridge containing 3.3 mg iron (low-dose group); (2) fortified milk-based maize porridge containing 6.6 mg iron (high-dose group); and (3) unfortified milk-based maize porridge (control group).

Intervention food "pap" is a traditional fermented maize-based porridge commonly consumed by children in Nigeria. The porridge was prepared daily by trained on-site cooks (three per school) and served at lunch time on the school days for five months. Preparation followed a standardised protocol, with the powdered product reconstituted with water and portioned into individual servings equivalent to approximately 50 g dry powder. The nutritional composition of the product is shown in **Table 1** and was calculated from manufacturer-provided formulation specifications.

Baseline and endpoint assessments included demographic information, anthropometry, dietary quality, venous blood collection, urine sampling, and malaria testing. Deworming was provided at baseline for children not treated in the preceding three months and malaria was treated when diagnosed at baseline. All fieldworkers, phlebotomists, and laboratory staff were trained and blinded to group allocation.

Individual compliance (porridge consumption) was visually assessed, with at least half of the portion confirmed as consumed, and recorded daily by field staff. Monthly, illness questionnaires were administered to caregivers via phone interviews, and an on-site monitoring visit was conducted by an independent

study monitor. Caregivers could also report events directly to school staff or fieldworkers. All AEs and SAEs were coded, graded for severity and relatedness using the Medical Dictionary for Regulatory Activities (MedDRA) by a medical practitioner and reviewed by the principal investigator.

Table 1. Nutritional composition of intervention product per daily serving[1] by treatment group

Nutritional information[2]	Control (units, % NRV[4])	Low dose (units, % NRV[4])	High dose (units, % NRV[4])	National school Standards (%)[5]
Energy (kcal)	205 (12%)	205 (12%)	205 (12%)	-
Protein (g)	6.7 (35%)	6.7 (35%)	6.7 (35%)	46
Carbohyrdates (g)	33.1	33.1	33.1	-
Sugar (g)	19	19	19	-
Added sugar (g)	8.3	8.3	8.3	-
Total fat (g)	4.6	4.6	4.6	-
SAFA (g)	21	2.1	2.1	-
Sodium (mg)	37.5	37.5	37.5	-
Calcium (mg)[3,]	131 (13%)	140 (14%)	154 (15%)	-
Iron (mg)[3]	0.6 (6%)	3.3 (33%)	6.5 (66%)	33
Zinc (mg)[3]	0.7 (14%)	2.5 (49%)	4.9 (98%)	40
Iodine (ug)[3]	12.5 (14%)	31.5 (35%)	63 (70%)	-
Vitamin A (ug-RE)[3]	168 (42%)	308 (77%)	616 (154%)	42
Vitamin B12 (ug)[3]	0.3 (25%)	0.5 (42%)	1 (83%)	-
Vitamin D (ug)[3]	0.2 (1%)	3.95 (26%)	7.9 (53%)	-
Vitamin C (mg)[3]	0	25.7 (103%)	51.4 (206%)	-
Vitamin B1 (mg)[3]	0	0.285 (48%)	0.57 (95%)	-
Vitamin B2 (mg)[3]	0	0.25 (42%)	0.5 (83%)	-
Vitamin B3 (mg)[3]	0	3.5 (44%)	7 (88%)	-
Vitamin B6 (mg)[3]	0	0.33 (55%)	0.66 (110%)	-
Folic acid (ug)[3]	0	106 (53%)	212 (106%)	-

[1]One serving is 50 g powder (300 g ready-to-consume) fortified milk-based maize porridge per child per day. [2] Calculated from the formulation specifications provided by the manufacturer. [3] Nutrients in the micronutrient fortification premix. [4] Nutrient Reference Value as recommended by the Institute of Medicine, 2006. [5] NHGSFP= National Home Grown School Feeding Programme nutrient requirements for foods to be included in the Nigerian school feeding programme (Federal Ministry of Humanitarian Affairs and Poverty Reduction, 2024).

Abbreviations: - Not available; SAFA= saturated fatty acids; RE= Retinol Equivalent; NRV= Nutrient Reference Value.

Outcomes

Co-primary outcomes were mean haemoglobin concentration (g/L) and serum ferritin (µg/L) at endpoint (5 months). Serum ferritin was adjusted for inflammation using the BRINDA regression-correction approach with CRP and AGP as continuous variables (15). Secondary outcomes included differences in mean soluble transferrin receptor (sTfR) concentration (mg/L) adjusted for inflammation, prevalence of anaemia (Hb <115 g/L), iron deficiency (inflammation-adjusted serum ferritin <15 µg/L or sTfR >8.3 mg/L), iron deficiency anaemia (IDA, defined as concurrent anaemia and iron deficiency), mean retinol-binding protein (RBP) adjusted for inflammation, vitamin D, vitamin B12, and iodine and their deficiencies at endpoint (16). Vitamin A deficiency was defined as inflammation-adjusted RBP <0.70 µmol/L (17). Vitamin D deficiency was defined as serum 25(OH)D <50 nmol/L and insufficiency as 50-75 nmol/L (18). Vitamin B12 deficiency was defined as serum cobalamin <148 pmol/L (19). Iodine deficiency was defined as median urinary iodine concentration <100 µg/L (20). Body iron was calculated using Cook's formula (21).

Measurements

Wealth indicator information was collected at baseline using a structured questionnaire that included information on household assets, housing conditions, access to basic services and occupation, classified according to the Nigerian Standard Classification of Occupations (NSCO) system. A Wealth Index was constructed using principal component analysis (PCA), which generates a household wealth score from multiple indicators. Households were ranked by their wealth score and categorized into tertiles, representing socio-economic status groups from poorest to wealthiest. When eigenvalues >1 were not met, individual wealth variables were used in the analysis instead.

Standing height was measured to the nearest 0.1 cm using a portable stadiometer (DETECTO, Model (PHR, Free-Standing Portable Height Rod), Cardinal Scale Manufacturing Co., Webb City, MO, USA). Children were measured barefoot in the Frankfort plane, with two readings recorded and averaged. If measures differed by >10%, a third measure was taken. Weight was measured to the nearest 0.05–0.10 kg using a portable digital flat scale (seca 877, seca gmbh & co. kg, Hamburg, Germany). The scale was placed on a firm, level surface, zeroed before each measurement, and calibrated daily with a standard test mass; children were weighed without shoes and in light clothing. Anthropometric indices, including height-for-age z-score (HAZ), weight-for-age z-score (WAZ), and BMI-for-age z-score (BAZ) were calculated using WHO standards (22).

Venous blood samples (5 mL) were obtained from each participant by trained phlebotomists under standard aseptic conditions into sterile 5 mL K×EDTA vacutainer tubes. An additional 5 mL of blood was drawn at baseline and endpoint into serum clot-activator tubes (without gel). All haematological analyses were performed using a Mindray BC-10 Automated Hematology Analyser (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Haemoglobin was measured on the same day as blood was drawn at the University College Hospital, Ibadan. Whole blood samples were centrifuged using an Eppendorf 5702 bench-top centrifuge (Eppendorf AG, Hamburg, Germany), operated at the manufacturer-recommended settings for serum/plasma separation (3,000 rpm for 10 minutes at room temperature), aliquoted, and stored at −80°C at the Institute of Child Health, University of Ibadan, in accordance with Good Clinical Practice (GCP). Samples were later shipped frozen with Phase Change Material to NutriLab, Justus Liebig University, Giessen, Germany.

Serum concentrations of ferritin (SF), soluble transferrin receptor (sTfR), C-reactive protein (CRP), alpha-1-acid glycoprotein (AGP), and retinol-binding protein (RBP) were measured at the VitMin Lab (Germany) using their validated multiplex sandwich ELISA method, as previously described (23). The inter-assay CVs for SF, sTfR, RBP, CRP, and AGP were 2.3%, 3.6%, 3.6%, 5.8%, and 8.1%, respectively, indicating high analytical precision. Vitamin D (25-hydroxyvitamin D; Masdiag, Poland) and vitamin B12 (Dicoon, CWZ Nijmegen, Netherlands) were measured using High-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and competitive electrochemiluminescence immunoassay (ECLIA), respectively. Malaria diagnosis was performed by light microscopy following the World Health Organization (WHO) standard protocol for malaria parasite detection and quantification (24) using thick and thin blood smears parasite detection and density estimation, while the thin film was used for species identification. Stained slides were examined by two independent trained microscopists blinded to the participant data. All assays were run in duplicate. Spot urine samples were collected to assess iodine status and stored at −80°C. Samples were sent to Wageningen University, The Netherlands, for urinary iodine concentration (UIC) analysis using the modified Sandell–Kolthoff method (25). All measurements were performed in duplicate, and any plates with a coefficient of variation (CV) ≥10% had measurements repeated to ensure accuracy.

Dietary intake was assessed using the Diet Quality Questionnaire (DQQ), adapted for the Nigerian population and covering 29 food groups (26). We compared the proportion of children in each group consuming each of the food groups at

baseline and at endpoint. To evaluate overall dietary diversity, we calculated the Dietary Diversity Score (DDS), a population-level indicator based on the consumption of ten distinct food groups over the previous day. The DDS is a semi-continuous score (0-10), reported as the population mean and standard deviation, and was compared between study groups at baseline and endpoint.

Statistical Analysis

Data was analysed in modified per protocol (mPP) and intention-to-treat (ITT) analysis sets. The mPP was defined by the trial steering committee prior to outcome analysis as a reduced minimal exposure threshold of 50 days (80% of 3 months with more than half of each serving consumed), because no children met the original threshold of 84 days (80% of 5 months with more than half of each serving consumed) due to operational constraints. Sample size was calculated to detect a mean difference of 2 g/L in haemoglobin (SD 8 g/L) between low-dose and control groups with 80% power and ⊠ = 0.05, accounting for 20% attrition, based on previous trial outcomes (12). A total of 951 children (317 per arm) were required. This also provided sufficient power for serum ferritin (mean difference of 5 µg/L; SD 15 µg/L) and comparisons between high-dose and control groups.

Data were entered using Viedoc (BioPlatforms, version 4.87.9347.29005) and analysed in RStudio (version 2024.02.0). Model assumptions were checked using Q–Q plots and Shapiro–Wilk tests; residual distributions were further assessed visually (Q–Q plots, histograms) and with the Kolmogorov–Smirnov test. Non-normally distributed variables were log-transformed before analysis. Covariates were included based on a change-in-estimate criterion (≥10% change in effect size).

A hierarchical testing approach was used for the co-primary outcomes: comparisons began with the high-dose versus control group, and only if a null effect was ruled out was the low-dose versus control comparison conducted. Treatment effects were evaluated using multilevel linear models for continuous outcomes, with random intercepts for school, adjusted for baseline values. For untransformed outcomes, results are reported as mean differences with 95% confidence intervals; for log-transformed outcomes, beta estimates were exponentiated and expressed as percentage differences relative to control.

Binary outcomes were analysed using Poisson regression with a log link and robust standard errors (via cluster-robust sandwich estimators) to estimate relative risks and 95% confidence intervals, assuming constant time at risk. Models were adjusted for baseline covariates and clustered by site. Robust standard

errors were computed using the vcovCL: Clustered Covariance Matrix Estimation function from the sandwich package in R.

Model assumptions were checked for residual uniformity, dispersion and multicollinearity. No imputation was performed for missing values. To assess potential effect modification, interaction terms between the exposure and prespecified covariates (sex, age, baseline nutritional status, inflammation, and malaria) were added to the models individually. Subgroup analyses were subsequently performed for interactions with p-values <0.20.

Sensitivity analyses were performed to investigate the within-group effects (baseline vs. end of study) for retinol-binding protein, vitamin D, iodine, and vitamin B12 and were estimated using a linear mixed-effects model with a time × group interaction term and a random intercept for subject ID to account for repeated measures. This analysis was conducted because the control product was fortified with these nutrients (**Table 1**).

RESULTS

Of the 1,138 children who were screened for eligibility, 946 were randomised across 17 schools into three groups, and 945 commenced the intervention (see **Figure 1** for participant flow). This cohort formed the intention-to-treat population, comprising 319 in the control, 312 in the low-dose iron group, and 315 children in the high-dose iron group. A total of 819 (86.5%) children completed the trial. Reasons for dropout were similar across groups and included school withdrawal, relocation, and refusal of blood sampling.

Children received the study product on an average of 56.4 ± 15.9 days during the five-month intervention period, out of a mean of 65.6 ± 15.7 possible feeding days, which was lower than the originally intended 105 days. The proportion of children meeting the modified per protocol criteria was 86% in the control group, 91% in the low-dose group and 89% in the high-dose group. During the intervention, children were absent from school for an average of 8.8 ± 5.6 days.

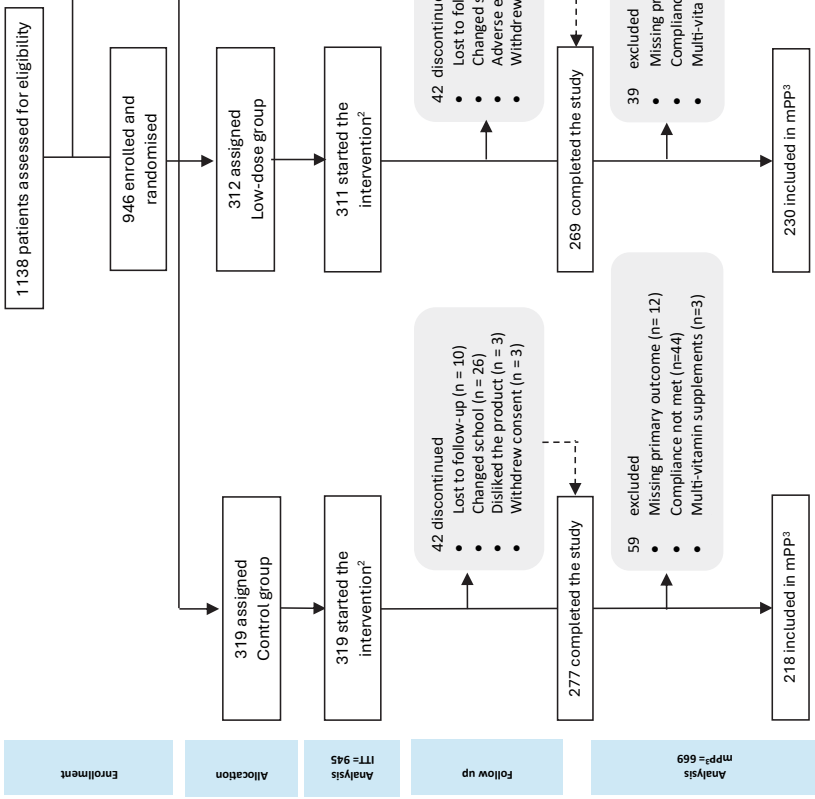


Figure 1: Flow diagram of recruitment, screening, enrolment, allocation, follow-up and analysis of study participants.

mPP= modified per protocol, ITT= intention-to-treat, [1]n=1, participant never consumed the study product and is therefore not defined as ITT; [2] ITT defined as starting the intervention, [3]mPP defined as an intervention threshold of 50 days (80% of 3 months), as no children met the original criteria of 84 days (80% of 5 months with more than half of the serving consumed.

Baseline characteristics were comparable across groups (**Table 2**). Most children (74%) were anaemic (median Hb concentration 108 g/L), 9.7% had iron deficiency, and 8.0% had iron deficiency anaemia (IDA). Most cases (93%) of iron deficiency were attributed to elevated soluble transferrin receptor concentrations (median 5.7 mg/L) and the remaining cases (1.7%) were attributed to low inflammation-adjusted serum ferritin (median 54.5 ug/L). Acute Inflammation (CRP>5 mg/L) was present in 15% and chronic inflammation (AGP >1g/L) in 34% of children. Malaria parasitaemia was detected in 12% of children at baseline and treated appropriately. Prevalence of vitamin A deficiency was 30%, vitamin D deficiency was 12% and median urinary iodine concentration was 81 ug/L, indicating mild iodine deficiency. No cases of vitamin B12 deficiency were observed. More detailed information on biochemical indicators is shown in **Supplement Figure 1**. Diet quality across groups were similar with an average diet diversity score of 5.04 (1.76), more information on diet quality is shown in **Supplement Table 1**.

Table 2: Baseline characteristics by intervention group (intention-to-treat analysis)

	Total n=945	Low-dose n=311	High-dose n=315	Control n=319
Demographic characteristics				
Age (years)	6.7 ± 1.0	6.7 ± 1.0	6.6 ± 1.1	6.7 ± 1.1
Female (n, %)	445 (47)	145 (47)	155 (49)	145 (45)
Wealth Index (n, %)[1]				
Low	295 (33)	96 (33)	88 (30)	111 (37)
Medium	295 (33)	102 (35)	103 (35)	90 (30)
High	295 (33)	93 (32)	104 (35)	98 (33)
Anthropometric characteristics				
Height-for-age Z score ± SD	-0.1 ± 1.1	-0.2 ± 1.1	-0.0 ± 1.2	-0.2 ± 1.2
BMI-for-age Z score ± SD	-0.9 ± 0.8	-0.9 ± 0.8	-0.9 ± 0.8	-0.9 ± 0.8
Stunted[2] , (n,%)	34 (3.8)	7 (2.3)	9 (2.9)	18 (5.6)
Thin[3] (n,%)	91 (9.7)	27 (8.7)	35 (11)	29 (9.1)
Overweight[4] (n,%)	9 (1)	3 (1)	2 (0.6)	4 (1.3)
Diet Diversity Score[5]	5.04 (1.76)	5.18 (1.68)	4.89 (1.79)	5.04 (1.81)
Haematological indices				
Haemoglobin (g/L)	108 (101, 115)	108 (101, 114)	109 (101, 114)	108 (101, 116)
Anaemia[6], (n,%)				
Total	691 (74)	234 (75)	230 (75)	227 (72)
Mild	170 (18)	49 (16)	70 (22)	51 (16)
Moderate	487 (51)	173 (56)	151 (48)	163 (51)
Severe	34 (4)	12 (4)	9 (3)	13 (4)
Normocytic[7]	527 (76)	178 (76)	169 (74)	180 (79)

Table 2: Continued

	Total n=945	Low-dose n=311	High-dose n=315	Control n=319
Microcytic[8]	118 (17)	41 (18)	43 (19)	34 (15)
Macrocytic [9]	46 (7)	15 (6)	18 (8)	13 (6)
Low MCV + MCH[10]	126 (18)	45 (19)	44 (19)	37 (16)
Malaria[11] (n,%)	111 (12)	34 (11)	40 (13)	37 (12)
Iron & nutrient status				
Serum Ferritin[12] (µg/L)	54.5 (39.2,73.6)	55.3 (40.6,77.2)	54.2 (39, 71.6)	53.9 (38.9,73.1)
ID from serum ferritin[13] (n,%)	16 (1.7)	2 (0.6)	7 (2.2)	7 (2.2)
Soluble transferrin receptor [12] (mg/L)	5.7 (4.9, 6.7)	5.8 (5, 6.8)	5.7 (5, 6.6)	5.7 (4.9, 6.7)
ID from Soluble transferrin receptor[14] (n,%)	82 (9.0)	35 (11.6)	23 (7.7)	24 (7.8)
ID[15] (n,%)	88 (9.7)	36 (12)	25 (8.4)	27 (8.8)
IDA[16] (n,%)	73 (8.0)	29 (9.5)	21 (7.0)	23 (7.4)
Body iron Stores[17] (mg/kg BW)	6.6 (5.3, 7.8)	6.7 (5.6, 7.9)	6.6 (5.3, 7.7)	6.6 (5.1, 7.7)
Inadequate BIS[18] (n,%)	2 (0.2)	0	0	2 (0.7)
CRP (mg/L)	0.7 (0.2, 2.4)	0.6 (0.2, 2.7)	0.7 (0.2, 2.4)	0.7 (0.2, 2.3)
Elevated CRP[19] (n,%)	140 (14.8)	48 (15.4)	49 (15.6)	43 (13.5)
AGP (g/L)	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)
Elevated AGP[20] (n,%)	323 (34.2)	102 (32.8)	110 (34.9)	111 (34.8)
Retinol-binding protein[12] (µmol/L)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 0.9)	0.8 (0.7, 1.0)
Vitamin A deficiency[21] (n,%)	273 (30)	92 (30)	96 (32)	85 (28)
Vitamin B12 (pmol/L)	589 (452, 768)	580 (457, 788)	600 (449, 755)	583 (454, 775)
Vitamin B12 deficiency[22] (n,%)	0	0	0	0
Vitamin D (ng/mL)	26.4 (22.6,29.9)	26.4 (22.9,29.4)	26.3 (22.6,30.7)	26.4 (22.3,29.8)
Vitamin D deficiency[23] (n, %)	112 (12)	36 (12)	36 (12)	40 (13)
Vitamin D insufficency[24] (n, %)	683 (75)	237 (78)	213 (71)	233 (76)
Iodine[25] (ug/L)	81.3 (51.6, 122)	82.3 (51.9, 122)	81 (47.8, 124)	80.6 (53.4, 119)

Data are mean ± SD, median (p25, p75) or frequency (%) unless indicated otherwise. [1] Wealth Index categories (Low, Medium, High) were derived using Principal Component Analysis (PCA) based on ownership variables. [2] Stunted: Height-for-age Z-score < −2 SD. [3] Thin: BMI-for-age Z-score < −2 SD. [4] Overweight: BMI-for-age Z-score > +1 SD. [5] Diet Diversity Score (DDS) represents the number out of 10 unique food groups consumed in the previous 24 hours (range 0–10), based on the Global Diet Quality Questionnaire (DQQ).[6] Anaemia was defined as haemoglobin concentration < 115 g/L. Severity was classified as mild (110-115 g/L-), moderate (80-109 g/L), or severe (0- 80 g/L). [7] Normocytic anaemia: haemoglobin < 115 g/L with MCV 73–87 fL. [8] Microcytic anaemia: haemoglobin < 115 g/L with MCV < 73 fL. [9] Macrocytic anaemia: haemoglobin < 115 g/L with MCV > 87 fL. [10] Low MCV and MCH were defined as MCV < 73 fL and MCH < 24 pg. [11] Presence of malaria parasitaemia detected by light microscopy using thick and thin blood smears. [12] Adjusted for inflammation using the BRINDA methodology [13]Iron deficiency was defined as inflammation-adjusted serum ferritin <15 ug/L .[14] Iron

deficiency was defined as inflammation-adjusted soluble transferrin receptor >8.3mg/L. [15] Iron deficiency was defined as inflammation-adjusted serum ferritin <15 ug/L or inflammation-adjusted soluble transferrin receptor >8.3mg/L. [16] Iron deficiency anaemia (IDA) was defined as iron deficiency combined with haemoglobin concentration < 115 g/L. [17] Body iron stores were calculated as −(log10 (sTfR/ferritin) − 2.8229) / 0.1207, where sTfR is in mg/L and ferritin in µg/L and inflammation-adjusted. [18] Body iron stores < 0 mg/kg were considered indicative of iron deficiency. [19] Elevated CRP was defined as > 5 mg/L. [20] Elevated AGP was defined as >1 g/L. [21] Vitamin A deficiency was defined as inflammation adjusted RBP < 0.7 µmol/L. [22] Vitamin B12 deficiency was defined as serum vitamin B12 < 148 pmol/L. [23] Vitamin D deficiency was defined as 25OHD < 20 ng/mL (50 nmol/L), based on IOM and AAP recommendations. [24] Vitamin D insufficiency was defined as 25OHD < 30 ng/mL (75 nmol/L), according to Endocrine Society and Paediatric Endocrinology guidelines, especially for populations with darker skin or limited sun exposure. [25] Iodine concentration was measured from spot urine samples. The median was compared to the WHO threshold of < 100 µg/L; prevalence of iodine deficiency was not calculated.

Abbreviations: ITT= Intention-to-treat; MCV= Mean Corpuscular Volume; MCH=Mean Corpuscular Haemoglobin; ID= iron deficiency; IDA= iron deficiency anaemia; CRP= C-Reactive Protein; AGP=Alpha-1-Acid Glycoprotein.

No group differences were observed for haemoglobin, or serum ferritin at endpoint in ITT analysis. The adjusted log-transformed percent difference in the high-dose group compared to control was -0.5 % for haemoglobin (95% CI −3.4 to 2.5) and 1.9% for serum ferritin (95% CI −6 to 10.5) (**Table 3**). Results from the ITT analyses were similar to those from the mPP, with no evidence of an intervention effect (Total n=669; **Supplement Table 2**).

No group differences were observed for soluble transferrin receptor or body iron stores at endpoint in ITT analysis. The adjusted log-transformed percent difference in the high-dose group compared to control was −0.4% for soluble transferrin receptor (95% CI −4.7 to 4.1) and 1.7% (95% CI −4.8 to 8.6) for body iron stores. Similarly, no group differences were found for the prevalence of anaemia, iron deficiency or iron deficiency anaemia. The adjusted percent difference in the high-dose group compared to control was 1% for anaemia (95% CI −17 to 23), 5% for iron deficiency (95% CI −32 to 61), and −12% for iron deficiency anaemia (95% CI −46 to 42) (**Table 3**).

Irrespective of treatment, iron status (haemoglobin, serum ferritin, soluble transferrin receptor and body iron stores) deteriorated over the intervention period. At endpoint, the total prevalence of anaemia was 75%, iron deficiency 15% and iron deficiency anaemia 12%. Regardless of treatment, vitamin A and vitamin D status improved over the intervention period, while vitamin B12 and iodine decreased. At the endpoint, no group differences between high-dose and control group were observed for serum retinol-binding protein (vitamin A), vitamin B12, or median urinary iodine concentrations (**Table 3**). The adjusted log-transformed percent difference in the high-dose group compared to the

control was −0.2% for retinol-binding protein (95% CI −4.7 to 4.5; p=0.935), -0.6% for vitamin B12 (95% CI −6.8 to 6.1; p=0.860) and −5% for iodine (95% CI −15 to 6; p=0.358). No group differences were observed for vitamin A deficiency (adjusted effect −10%, 95% CI −37 to 31; p=0.593). No cases of vitamin B12 deficiency were found.

Vitamin D status, measured by serum 25(OH)D, was higher in the high-dose group compared to control at endpoint with an adjusted log-transformed percent difference of 3.8% (95% CI 0.6 to 7; p=0.021). The prevalence of vitamin D deficiency (25OHD <20 ng/mL) decreased from 12% at baseline to 3% in the high-dose group and 1.5% in the low-dose group, compared to 5.4% in the control group. The adjusted percent difference for vitamin D deficiency in the high-dose group compared to control was −46% (95% CI −78 to 26; p=0.166). The prevalence of vitamin D insufficiency (25OHD < 30 ng/mL) decreased from 75% at baseline in the high-dose group to 52% in the high-dose group and 58% in the low-dose group, compare to 60% in the control group. The adjusted percent difference for vitamin D insufficiency in the high-dose group compared to control was −33% (95% CI −31 to 9; p=0.217).

No evidence of effect modification was detected for any assessed variables; notably, baseline iron deficiency did not alter the intervention effects. In the within-group analyses, retinol-binding protein concentrations increased in all groups: 8.0% (95% CI 4.6, 11.6) in the high-dose group, 7.6% (95% CI 4.2, 11.1) in the low-dose group and 8.7% (95% CI: 5.2, 12.3) in the control group. Vitamin D concentrations also increased in all groups: 14.3% (95% CI 11.6, 17.1) in the high-dose group, 11% (95% CI 8.8, 14.1) in the low-dose group and 12.4% (95% CI: 9.6, 15) in the control group. In contrast, serum ferritin declined, with greater reductions in the control (-4.4%, 95%CI -9.3 to 0.8), and the low-dose group (-4.9%, 95%CI -9.6, 0.1), compared to the high-dose group (-1.6%, 95% CI -6.6 to 3.6). Soluble transferrin receptor concentrations also increased only in the control group, with an increase of 4.2% (1.3, 7.2) (**Supplement Table 3**).

Of the 29 serious adverse events recorded (28 hospitalisations and 1 death), none were attributed to study participation following independent causality assessment. A detailed list of SAEs is provided in **Supplement Table 4**. The frequency of mild symptoms (diarrhoea, respiratory infections) was low and balanced across groups.

Table 3: Effects of the multi-micronutrient fortified milk-based porridge product on primary and secondary outcomes in school-aged children, comparing the Low-dose and High-dose groups with the control group (intention-to-treat analysis, n=945)

Outcome variable at endline	**Control n=319**	**Low-dose n=311**	**High-dose n=315**	**p value [1]**
Haemoglobin (g/L), *n=818*	108 (99, 115)	106 (99, 114)	107 (98, 114)	0.727
Treatment effect	reference	-0.9% (-3.8 to 2) [2]	-0.5% (-3.4 to 2.5) [2]	
Serum Ferritin[3] (µg/L), *n=809*	52.1 (36.7, 74.2)	51.7 (40.2, 73)	54.1 (38.6, 69.4)	0.648
Treatment effect	reference	3.9% (-4.3 to 12.7) [2]	1.9% (-6 to 10.5) [2]	
Soluble transferrin receptor[3] (mg/L), *n=809*	6.1 (5.3, 7.3)	6.2 (5.2, 7.3)	6.2 (5.2, 7.4)	0.865
Treatment effect	reference	-0.1% (-4.4 to 4.4) [2]	-0.4% (-4.7 to 4.1) [2]	
Body iron Stores[4] (mg/kg BW), *n=809*	6.2 (4.8, 7.4)	6.2 (5.1, 7.4)	6.2 (4.9, 7.5)	0.626
Treatment effect	reference	4.3% (-2.4 to 11.5) [2]	1.7% (-4.8 to 8.6) [2]	
Anaemia (n(%))[5]	207 (75)	204 (76)	206 (75)	0.921
Treatment effect	reference	2% (-16 to 24) [6]	1% (-17 to 23) [6]	
Iron Deficiency (n(%))[7]	41 (15)	40 (15)	42 (16)	0.833
Treatment effect	reference	1% (-35 to 57) [6]	5% (-32 to 61) [6]	
Iron Deficiency Anaemia (n(%))[8]	36 (13)	29 (11)	31 (11)	0.603
Treatment effect	reference	-16% (-49 to 37) [6]	-12% (-46 to 42) [6]	
Retinol-binding protein[3] (µmol/L), *n=809*	0.9 (0.7, 1)	0.9 (0.7, 1)	0.9 (0.7, 1.1)	0.935
Treatment effect,	reference	-0.7% (-5.2 to 4) [2]	-0.2% (-4.7 to 4.5) [2]	
Vitamin A deficiency (n(%))[9]	61 (22)	51 (19)	54 (20)	0.593
Treatment effect	reference	-13% (-40 to 26) [6]	-10% (-37 to 31) [6]	
Vitamin B12 (pmol/L), *n=815*	555 (420, 717)	552 (402.5, 717)	553 (431.5, 694)	0.860
Treatment effect	reference	-1.8% (-8 to 4.8) [2]	-0.6% (-6.8 to 6.1) [2]	
Vitamin D (ng/mL), *n=815*	28.5 (25.4, 32.5)	28.9 (25.9, 31.9)	29.4 (25.6, 33.9)	0.021/0.323
Treatment effect	reference	1.6% (-1.5 to 4.8) [2]	3.8% (0.6 to 7) [2]	

Table 3: Continued

Outcome variable at endline	Control n=319	Low-dose n=311	High-dose n=315	p value [1]
Vitamin D deficiency (n(%))[10]	15 (5.4)	4 (1.5)	8 (3.0)	0.166
Treatment effect	reference	-72% (-92 to -24) [6]	-46% (-78 to 26) [6]	
Vitamin D insufficiency (n(%))[11]	166 (60)	156 (58)	141 (52)	0.217
Treatment effect	reference	-3% (-22 to 21) [6]	-33% (-31 to 9) [6]	
Iodine[12] (ug/L), *n=807*	60.6 (39.9, 101.1)	58.3 (41.3, 103.5)	60 (36.9, 97.9)	0.358
Treatment effect	reference	1.5% (-9.1 to 13.5) [2]	-5% (-15 to 6) [2]	

Data are median (IQR) or n(%) unless otherwise stated. Treatment effects are measured as group differences (continuous outcomes) or relative risks (categorical outcomes) compared with the control group. Although 945 children were randomised (intention-to-treat population), the analysis sample varied by outcome due to missing baseline or endline measurements; n indicates the number of participants with available data included in each analysis[1] *p* value corresponds to the comparison of the high-dose group vs. control and if null-effect was ruled out the low-dose group vs control is also given. [2] Treatment effect (%, 95% CI) values indicate the difference between groups, expressed as a percentage relative to the control group, obtained by exponentiating effect estimate from log-transformed data. [3] Adjusted for inflammation using the BRINDA methodology. [4] Calculated using the formula: body iron = −(log10(sTfR/ ferritin) − 2.8229) / 0.1207, where sTfR is measured in mg/L and ferritin in µg/L. [5] Anaemia was defined as haemoglobin concentration < 115 g/L. [6] RR (%, 95% CI) values indicate the percent difference in relative risk (RR) compared to the control group, calculated as (RR − 1) × 100%, with 95% confidence intervals. Relative risks were estimated using modified Poisson regression with robust standard errors. [7] Iron deficiency was defined as inflammation-adjusted serum ferritin <15 ug/L or soluble transferrin receptor >8.3 mg/L. [8] Iron deficiency anaemia (IDA) was defined as iron deficiency[7] combined with haemoglobin concentration < 115 g/L.[9] Vitamin A deficiency was defined as inflammation-adjusted RBP < 0.7 µmol/L.[10] Vitamin D deficiency was defined as 25OHD < 20 ng/mL (50 nmol/L) according to IOM and AAP recommendations for bone health and prevention of rickets. [11]Vitamin D insufficiency was defined as <30 ng/ mL (equivalent to 75 nmol/L) according to Endocrine Society and Paediatric Endocrinology recommendations, especially for populations with darker skin and limited sun exposure. [12] Iodine concentration was measured from spot urine samples. The median can be compared to a threshold of <20 µg/L is severe, 20-50 µg/L moderate and 50-100 µg/L mild deficiency.

Abbreviations: AGP = α_1-acid glycoprotein; BRINDA = Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia; BW = body weight; CI = confidence interval; CRP = C-reactive protein; IQR = interquartile range; RBP = retinol-binding protein; RR = relative risk; sTfR = soluble transferrin receptor; AGP = α_1-acid glycoprotein

DISCUSSION

This randomised-controlled trial found that providing a high-dose multi-micronutrient fortified milk-based maize porridge (delivering ~6.6 mg elemental iron per day) through a school feeding programme did not improve iron status, measured by concentrations of haemoglobin, serum ferritin, soluble transferrin receptor or by estimated iron stores, nor did it reduce the prevalence of anaemia, iron deficiency or iron deficiency anaemia among Nigerian school-aged children aged 5–8 years over a five month period. In contrast, vitamin D status did improve in the high-dose group compared to the control. However, no differences between groups were observed for vitamin A, B12, or iodine status. Notably, vitamin A and D status improved in all treatment groups.

The absence of an observed effect of iron intervention on haemoglobin and ferritin should be interpreted in the context of adequate baseline iron stores and a high burden of infection and inflammation. Anaemia prevalence was higher than expected (74% compared with estimations of 25%) whereas iron deficiency anaemia was lower than expected (9 versus 13-15%). Thus, anaemia was frequently present in the absence of biochemical iron deficiency, and most children had adequate iron stores even after adjustment for inflammation. When iron deficiency was identified, it was predominantly functional, characterized by normal or elevated ferritin alongside increased soluble transferrin receptor concentrations, indicating iron sequestration rather than depletion. This pattern is consistent with inflammation-associated anaemia, in which iron absorption and erythropoiesis are blunted despite iron availability, likely mediated by hepcidin-induced blockade of ferroportin (27). Although hepcidin was not measured, this biological profile provides a plausible explanation for the lack of response to iron fortification in this setting.

The high baseline burden of subclinical inflammation, indicated by elevated AGP (34%) and elevated CRP (12%), alongside malaria (12%), and vitamin A deficiency (30%), likely sustained this functional block described above. In this context, increasing iron stores, the usual goal of fortification, offers little benefit, as stores are already sufficient. Instead, only the small subgroup with iron-responsive functional deficiency might benefit from iron provided "at the right time" for direct use in erythropoiesis**,** as was seen in an Indian school feeding trial using mungbean dahl with vitamin C-rich guava (28). However, given that only approximately 8% of children had iron deficiency anaemia, the potential impact of iron-based interventions was inherently limited. These findings align with evidence that iron interventions have limited impact in high-infection-

burden settings unless integrated with infection control, malaria prevention, deworming, and broader nutritional strategies (3,5).

Iron status declined in all groups, and the prevalence of iron deficiency and iron deficiency anaemia increased over the trial period, despite stable anaemia prevalence. This likely reflects seasonal and economic pressures: food prices rose by ~30% the currency devaluated by 75% between June 2023 and June 2024, and endline data were collected during the rainy season, when dietary diversity typically declines, consistent with the lower diet diversity scores observed in this study (**Supplement Table 1**). In this deteriorating context, the high-dose group showed smaller declines in ferritin than the control group, suggesting some absorption and storage of iron from the intervention, but insufficient to improve functional iron availability or red cell production. Similar findings have been reported in Ghanaian school-aged children, where iron-fortified cowpea meals, with vitamin C rich drinks, modestly increased ferritin without changing soluble transferrin receptor or anaemia prevalence in a setting with a high burden of inflammation (13).

Vitamin A and D status improved across all study arms, reflecting fortification of the control and intervention products. However, these improvements in vitamin A status were not associated with any observable effect on anaemia. Although vitamin A fortification alone or in combination with iron and multiple micronutrients has reduced anaemia in other settings, including Tanzanian school-aged children (29), correction of vitamin A deficiency in the present study was likely insufficient to overcome inflammation-related constraints on iron mobilisation and erythropoiesis. Vitamin D status improved modestly, with the largest gain in the high-dose arm, reducing both deficiency and insufficiency. This finding is relevant given the high prevalence of vitamin D deficiency among African school-aged children, which has been linked to low vitamin D and calcium status and inadequate dietary intake (30). While vitamin D deficiency was relatively uncommon in this cohort compared with estimates suggesting that up to one-third of the African population is affected, the continued occurrence of nutritional rickets in the region indicates the need to consider combined vitamin D and calcium interventions (31).

Strengths of this study include its large sample size, real-world school programme setting, high acceptability and practical application of the fortified food in schools. The product formulation followed WHO-recommended levels and included multiple micronutrients due to anaemia known multi-factorial causes (32). Additionally, deworming and malaria treatments were monitored and given, when necessary, before the intervention began.

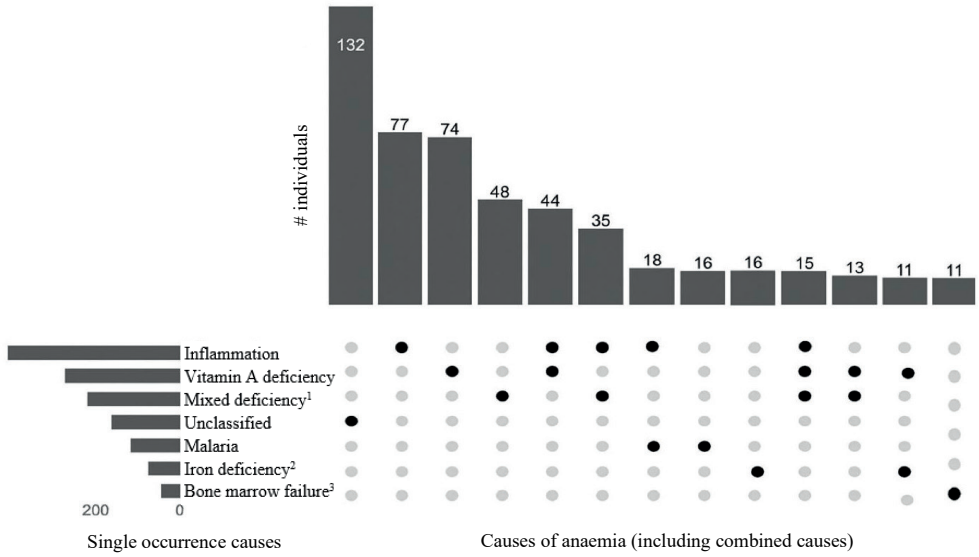
Limitations include lower-than-expected prevalence of iron-responsive anaemia, reduced treatment exposure duration, and potential lower bioavailability of the iron source in the age group and food matrix. Fortification relies on sustained intake of modest iron doses over sufficient duration, in contrast to high-dose supplementation. In this trial, intervention exposure was shorter than planned: although treatment adherence on school days was high, children consumed the product on only approximately 56 of the 67 intervention days over the 105-day (5 month) trial period, largely due to unforeseen logistical constraints. This reduced exposure may have been limited by the biological impact of fortification, particularly in (replete) young children, for whom higher iron intakes from ferrous fumarate may be required to achieve a response (33).

Clear guidance on optimal fortification doses for this compound in this age group is limited, and the achieved exposure and fortification level may therefore have been insufficient within the study timeframe. Most iron-fortification studies with positive effects on iron status have been 5 months at minimum (12) and mostly longer (10), approximately 7 months (11). Theoretical rather than analytical nutrient composition was used, and the control contained some micronutrients (vitamin A, vitamin D and iodine) from the milk, which may have reduced between-group differences. Future studies aimed at reducing anaemia and/or iron deficiency anaemia should first understand the specific causes of anaemia in the population and tailor interventions accordingly (5). Further research is also needed to identify effective strategies to reduce anaemia of inflammation beyond those implemented in this study, such as malaria and parasite monitoring and treatment.

CONCLUSION

In conclusion, in this high-inflammation, high-anaemia population, multimicronutrient fortification of a dairy-based porridge did not improve iron status or anaemia prevalence among Nigerian school-aged children in a school feeding programme. In settings where iron deficiency anaemia prevalence is low and iron stores are adequate but functionally unavailable, fortification alone is unlikely to address the multifactorial causes of anaemia. Policy should prioritise integrated strategies combing infection control, deworming, improved school attendance, and targeted use of highly bioavailable iron compounds for children with iron-responsive anaemia, while addressing other prevalent deficiencies, notably vitamin A, vitamin D, and calcium, to achieve meaningful public health gain.

SUPPLEMENTARY MATERIAL



Supplement Figure 1: Occurrence of single and combined causes of anaemia in the intention-to-treat population (n=691) at baseline. Horizontal bars on the left show the number of individuals with a single cause (e.g. inflammation or iron deficiency). Vertical bars represent the number of individuals with a combination of causes. The dot matrix below indicates which causes are present in each combination. Causes assessed included nutritional deficiencies, inflammation, infection, bone marrow–related disorders, and haemoglobinopathies (sickle cell disease, *n=6*; haemoglobin C disease, *n=2*; and SC disease, *n=1*), which were identified using genotype data and accounted for a small proportion of anaemia cases. [1]Mixed deficiency defined as combined iron deficiency and vitamin A deficiency; [2] Iron deficiency defined as inflammation-adjusted ferritin < 12 µg/L or inflammation-adjusted sTfR > 8.3 mg/L; [3] Bone Marrow Failure defined as white blood cell < 4.5 $\times 10^9$/L and platelets < 175 $\times 10^9$/L.

Supplement Table 1: Comparison of diet diversity between treatment groups at baseline and endpoint in the ITT Population (n = 944)

Indicator	Baseline [1]	End of study [1]	Effect [2]	p-value [3]
Diet Diversity Score [4]				
Control [5]	5.04 (1.81)	4.56 (2.09)	Δ -0.54 (-0.84–-0.23)	0.001
Low-dose [6]	5.18 (1.68)	4.66 (2.10)	Δ -0.54 (-0.84–-0.25)	0.000
High-dose [7]	4.89 (1.79)	4.67 (2.16)	Δ -0.12 (-0.43–0.19)	0.454
Grains, white roots and tubers, and plantains [8]				
Control [5]	315/319 (98.7%)	272/277 (98.2%)	OR 0.80 (0.21–3.00)	0.737
Low-dose [6]	309/311 (99.4%)	265/269 (98.5%)	OR 0.50 (0.09–2.73)	0.421
High-dose [7]	310/314 (98.7%)	267/272 (98.2%)	OR 0.80 (0.21–3.00)	0.737

Supplement Table 1: Continued

Indicator	Baseline[1]	End of study[1]	Effect[2]	p-value[3]
Pulses (beans, peas and lentils)[9]				
Control[5]	194/319 (60.8%)	101/277 (36.5%)	OR 0.36 (0.26–0.51)	0.000
Low-dose[6]	203/311 (65.3%)	94/269 (34.9%)	OR 0.27 (0.18–0.40)	0.000
High-dose[7]	180/314 (57.3%)	104/272 (38.2%)	OR 0.48 (0.34–0.68)	0.000
Nuts and Seeds[10]				
Control[5]	108/319 (33.9%)	108/277 (39.0%)	OR 1.18 (0.83–1.69)	0.361
Low-dose[6]	126/311 (40.5%)	114/269 (42.4%)	OR 1.07 (0.75–1.54)	0.712
High-dose[7]	112/314 (35.7%)	102/272 (37.5%)	OR 1.11 (0.77–1.59)	0.582
Dairy[11]				
Control[5]	81/319 (25.4%)	111/277 (40.1%)	OR 2.02 (1.39–2.94)	0.000
Low-dose[6]	74/311 (23.8%)	111/269 (41.3%)	OR 2.15 (1.46–3.15)	0.000
High-dose[7]	72/314 (22.9%)	106/272 (39.0%)	OR 2.31 (1.58–3.36)	0.000
Meat, poultry and fish[12]				
Control[5]	224/319 (70.2%)	188/277 (67.9%)	OR 0.80 (0.54–1.19)	0.275
Low-dose[6]	214/311 (68.8%)	199/269 (74.0%)	OR 1.26 (0.86–1.86)	0.237
High-dose[7]	212/314 (67.5%)	198/272 (72.8%)	OR 1.37 (0.94–2.00)	0.103
Eggs[13]				
Control[5]	62/319 (19.4%)	64/277 (23.1%)	OR 1.17 (0.77–1.76)	0.464
Low-dose[6]	64/311 (20.6%)	59/269 (21.9%)	OR 1.08 (0.70–1.65)	0.742
High-dose[7]	60/314 (19.1%)	65/272 (23.9%)	OR 1.45 (0.93–2.25)	0.098
Dark green leafy vegetables[14]				
Control[5]	195/319 (61.1%)	153/277 (55.2%)	OR 0.77 (0.54–1.09)	0.134
Low-dose[6]	203/311 (65.3%)	161/269 (59.9%)	OR 0.80 (0.56–1.14)	0.209
High-dose[7]	190/314 (60.5%)	159/272 (58.5%)	OR 1.00 (0.69–1.44)	1.000
Other vitamin A-rich fruits and vegetables[15]				
Control[5]	215/319 (67.4%)	149/277 (53.8%)	OR 0.40 (0.25–0.64)	0.000
Low-dose[6]	121/311 (38.9%)	33/269 (12.3%)	OR 0.22 (0.14–0.36)	0.000
High-dose[7]	102/314 (32.5%)	41/272 (15.1%)	OR 0.36 (0.23–0.57)	0.000
Other vegetables[16]				
Control[5]	215/319 (67.4%)	149/277 (53.8%)	OR 0.40 (0.25–0.64)	0.000
Low-dose[6]	221/311 (71.1%)	137/269 (50.9%)	OR 0.26 (0.16–0.42)	0.000
High-dose[7]	220/314 (70.1%)	139/272 (51.1%)	OR 0.40 (0.26–0.61)	0.000
Other fruits[17]				
Control[5]	98/319 (30.7%)	80/277 (28.9%)	OR 0.85 (0.58–1.23)[18]	0.389
Low-dose[6]	76/311 (24.4%)	80/269 (29.7%)	OR 1.30 (0.88–1.92)[18]	0.194
High-dose[7]	78/314 (24.8%)	89/272 (32.7%)	OR 1.59 (1.06–2.36)[18]	0.023

[1] *Baseline and Endpoint*: for continuous variables, values are presented as mean (standard deviation); for categorical variables, values are presented as n (%) of participants consuming the item. [2] *Effect (95% CI)*: for continuous variables, results are expressed as the mean difference (Δ) between Endpoint and Baseline with 95% confidence intervals; for categorical variables, results are expressed as odds

ratios (OR) with 95% confidence intervals, derived from logistic mixed-effects models. [3] *p-value*: for continuous variables, obtained from paired *t*-tests; for categorical variables, obtained from generalized mixed-effects logistic regression models with time (Baseline vs Endpoint) as a fixed effect and subject as a random intercept. [4] *Diet Diversity Score (DDS)* represents the number out of 10 unique food groups consumed in the previous 24 hours (range 0–10), based on the Global Diet Quality Questionnaire (DQQ). [5] control group, n= 319

[6] low-dose group, n= 311. [7] high-dose group, n = 314. [8] *Grains, white roots, tubers, and plantains* – DQQ Questions 1, 2, 3.

[9] *Pulses (beans, peas, and lentils)* – DQQ Question 4. [10] *Nuts and seeds* – DQQ Question 21. [11] *Dairy* – DQQ Questions 14, 15, 25. [12] *Meat, poultry, and fish* – DQQ Questions 16, 17, 18, 19, 20. [13] *Eggs* – DQQ Question 13. [14] *Dark green leafy vegetables* – DQQ Question 6 (and sub-questions 6.1, 6.2 if applicable). [15] *Other vitamin A–rich fruits and vegetables* – DQQ Questions 5 and 8. [16] *Other vegetables* – DQQ Question 7 (and sub-questions 7.1, 7.2 if applicable). [17] *Other fruits* – DQQ Questions 9 and 10 (and sub-questions 9.1, 10.1, 10.2 if applicable). [18] The mixed-effects logistic model for *other fruits* showed a singular fit; therefore, a conditional logistic regression (equivalent to McNemar's test) was used to estimate the OR (95 % CI) and p-value. Food-group definitions and scoring the Global Diet Quality Project (*Global Diet Quality Project*, n.d.)

Supplement Table 2: Effects of the multi-micronutrient fortified milk-based porridge product on the primary outcomes in school-aged children, comparing the Low-dose and High-dose groups with the control group (per protocol population, n=669)

Outcome Variable	Total	Control n=218	Low-dose n=232	High-dose n=226	p-value
Haemoglobin (g/L)					
Baseline	10.8 (10.2, 11.5)	10.9 (10.1, 11.6)	10.8 (10.2, 11.4)	10.9 (10.2, 11.4)	
End of study	10.7 (9.9, 11.4)	10.8 (9.9, 11.4)	10.6 (9.9, 11.5)	10.8 (9.9, 11.5)	
Effect (log-transformed)[1]		Reference		0.2% (-3, 4)	0.908
Serum Ferritin (µg/L)					
Baseline[2]	54.2 (39.5, 72)	53.9 (38.9, 71.1)	55.1 (42, 74.2)	54 (39.5, 69.3)	
End of study[2]	52.1 (38.4, 71.4)	51.6 (35.3, 74.2)	50.6 (40.1, 72.3)	54.1 (38.4, 68.2)	
Effect (log-transformed)[1]		Reference		4% (-5, 14)	0.423

Data are median (IQR) or n(%) unless otherwise stated. Treatment effects are measured as group differences (continuous outcomes) or relative risks (categorical outcomes) compared with the control group. [1] Effect (%, 95% CI) values indicate the difference between groups, expressed as a percentage relative to the control group, obtained by exponentiating effect estimate from log-transformed data. [2] Adjusted for inflammation using the BRINDA methodology

Supplement Table 3: Within group (pre and post analysis) changes[1] in biomarkers over the study period in the ITT population (n=945)

Biomarker	Control	p-value	Low-dose	p-value	High-dose	p-value
Haemoglobin	-0.9% (-3.7%, 1.9%)	0.509	1.8% (-1%, 4.6%)	0.211	0.3% (-2.5%, 3.1%)	0.848
Serum ferritin	-4.4% (-9.3%, 0.8%)	0.094	-4.9% (-9.6%, 0.1%)	0.056	-1.6% (-6.6%, 3.6%)	0.536
Soluble Transferrin Receptor	4.2% (1.3%, 7.2%)	0.004	0.6% (-2.2%, 3.4%)	0.691	1% (-1.8%, 3.9%)	0.480
Retinol binding protein	8.7% (5.2%, 12.3%)	0.000	7.6% (4.2%, 11.1%)	0.000	8% (4.6%, 11.6%)	<0.001
Vitamin B12	-8.1% (-11%, -5.2%)	0.000	-7.1% (-9.8%, -4.2%)	0.000	-7.3% (-10.2%, -4.4%)	<0.001
Vitamin D	12.4% (9.6%, 15.1%)	0.000	11% (8.8%, 14.1%)	0.000	14.3% (11.6%, 17.1%)	<0.001
Iodine	-19% (-27.3%, -9.2%)	0.000	-22% (-29.7%, -12.6%)	0.000	-24.6% (-32.5%, -15.8%)	<0.001

[1] Within-group changes were assessed using linear mixed-effects models with random intercepts for participants and school, comparing post- vs pre-intervention biomarker values within each treatment group. All biomarkers were analysed on the log scale, and results are expressed as percentage changes derived from exponentiated model coefficients.

Supplement Table 4: Safety Analysis (Adverse Events)[1]

		Adverse Event				SAE
System Organ Class	**Preferred Term**	**Low-dose n (%)**	**High-dose n (%)**	**Control n (%)**	**Total n (%)**	**n (%)[2]**
	n	311	315	319	945	
General Disorders	Pyrexia	12 (3.9%)	16 (5.1%)	11 (3.4%)	39 (4.1%)	15
Respiratory Disorders	Cough/Dyspnoea	4 (1.3%)	2 (0.6%)	7 (2.2%)	13 (1.4%)	12
Gastrointestinal Disorders	Diarrhea	2 (0.6%)	2 (0.6%)	6 (1.9%)	10 (1.1%)	8
	Vomiting/Nausea	4 (1.3%)	1 (0.3%)	3 (0.9%)	8 (0.8%)	4
	Constipation	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
Skin Disorders	Rash/Dermatitis	0 (0.0%)	0 (0.0%)	3 (0.9%)	3 (0.3%)	2
Ear Disorders	Otitis Media	2 (0.6%)	4 (1.3%)	0 (0.0%)	6 (0.6%)	4
Injury	Injury/Bone fracture/Burn	3 (1.0%)	4 (1.3%)	0 (0.0%)	7 (0.7%)	1
Other	Other	2 (0.6%)	2 (0.6%)	3 (0.9%)	7 (0.7%)	2
Total		29	31	33	93	48

AE= adverse events, (s)AE= serious adverse events, [1] Based on Medical Dictionary for Regulatory Activities (MedDRA) (1) [2] A total of 48 sAE symptoms were reported, this included 28 hospitalisations and 1 death.

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Chapter 7

GENERAL DISCUSSION

This thesis aimed to understand the nutritional needs of Ivorian and Nigerian populations, to reformulate fortified food products based on this information, and to evaluate their effectiveness on nutrition and health outcomes in a real-world setting through a school feeding programme. These objectives were addressed through studies in three research themes: (1) understanding the nutritional needs of the target population by identifying nutrient inadequacies and dietary patterns among school-aged children and women of reproductive age in Côte d'Ivoire; (2) assessing the technological and sensory feasibility of a product reformulated through fortification; and (3) evaluating the effectiveness of a multi-nutrient fortified food product delivered through a school-feeding program in Nigeria. Together, these research themes operationalised the practical framework (see Introduction Figure 1) developed to align food-industry innovation with public-health nutrition goals.

SUMMARY OF MAIN FINDINGS

Table 1 provides an overview of the main findings across the three research themes.

Theme 1 focused on understanding the nutritional needs of the target populations. In Côte d'Ivoire, where population-level data were limited, we identified highly prevalent inadequacies in calcium, riboflavin, and zinc among urban school-aged children and women of reproductive age. Women also showed a high prevalence of overweight and obesity, suggesting an early stage of the nutrition transition. Dietary patterns were city-specific but largely similar between children and women. Nutrient modelling using *NutriR* produced similar inadequacy estimates across gamma, log-normal and observed intake distributions; however, the approach still depends on high-quality primary intake data highlighting the continued resource intensity of dietary assessment in low-and-middle income countries (LMICs).

Theme 2 evaluated the technological and sensory feasibility of reformulating flavoured dairy powder products by substituting the current iron compound (ferric pyrophosphate) with more bioavailable alternatives. Encapsulated ferric pyrophosphate was the only compound with acceptable technological and sensory properties but was five times more expensive, making replacement economically infeasible.

Theme 3 assessed the effectiveness of a multi-nutrient fortified dairy-based maize porridge in Nigerian school-aged children. Despite the high baseline

prevalence of anaemia (74%), nearly all children had adequate iron stores, and the intervention did not improve iron status or haemoglobin concentrations. However, modest improvements were observed for vitamin D (high-dose group) and vitamin A (across all groups).

Overall, these studies demonstrate that effective market-driven fortification depends on the availability of population-specific nutrient and health data to identify priority issues (such as anaemia and its underlying causes), the technological feasibility of potential solutions, and their evaluation (Table 1). This thesis applied a practical framework to align food-industry innovation with public-health nutrition goals in Côte d'Ivoire and Nigeria. Although tailoring market-driven multi-micronutrient fortification to population-specific nutritional needs did not improve iron status or reduce anaemia among Nigerian school-aged children, the findings provide important insights that structure the discussion that follows. Specifically, four key topics are addressed: (1) the complexity of assessing and interpreting nutritional needs; (2) the multifactorial aetiology of anaemia, which in this context was likely driven by inflammation, vitamin A deficiency, and other non-iron factors rather than iron deficiency; (3) the need for stronger evidence-based guidance and evaluation to ensure market-driven fortification strategies are biologically effective and contextually relevant; and (4) the resource and contextual constraints in low-income settings that limit both evidence generation and the application of available knowledge.

SUMMARY OF FINDINGS BY FRAMEWORK THEMES

Table 1: Summary of the three research themes and their key outcomes.

Research theme	Description	Research question	Activity	Outcomes
1:Understanding population nutritional needs	Effective product design requires a clear understanding of the nutrient status, dietary patterns and usual nutrient intake of the target population. This ensures that fortification addresses the most relevant micronutrient deficiencies, as outlined in the WHO/FAO framework [1].	Identify nutrient inadequacies and dietary patterns among SAC and WRA in Côte d'Ivoire (**chapter 2, 3 and 4**) and nutrient status among SAC in Nigeria (**chapter 6**)	Cross-sectional dietary intake survey in Côte d'Ivoire (n= 443 children and n=441 women). Analysis: nutrient inadequacies and dietary pattern analysis	*Côte d'Ivoire*: Intake of most nutrients was adequate for both SAC and WRA, except for calcium (99% and 96% inadequacy, respectively), riboflavin (53 and 79% inadequacy) and zinc (51 and 53% inadequacy). More than 50% of urban women were overweight or obese, while most children were within a healthy weight range (2). Four dietary patterns were identified among SAC and WRA. Two patterns were shared across both groups: (1) "Fish, oil and vegetables" and (2) "Dairy and sweet". A third pattern "Poultry and citrus fruit", was also observed in both groups, although in WRA this pattern additionally included red meat and white tubers, whereas the remaining pattern in SAC was "Refined, red meat and vegetables" and in WRA "Low energy intake". Dietary patterns were associated with city of residence. The fish and dairy patterns, more common in Bouaké and Daloa, were associated with lower or moderate NCD risk, higher diet quality, and greater nutrient adequacy. In contrast, poultry (children) and low-intake (women) patterns were characterised by poorer diet quality. Nutritional status indicators, including overweight and obesity (49% in women vs. 7% in children), were not clearly explained by diet patterns.

Table 1: Continued

Research theme	Description	Research question	Activity	Outcomes
			Cross-sectional nutrient status baseline data from the RCT among SAC in Nigeria (n=945 SAC 5-8 years old).	*Nigeria SAC:* Prevalence of anaemia was 74%, iron deficiency 9.5% (primarily elevated sTfR), and 0.2% had inadequate body iron stores. High prevalences of vitamin A (30%) deficiency, vitamin D (12%) deficiency, and chronic inflammation (AGP=34%) were observed. No vitamin B_{12} deficiency was detected. Median urinary iodine was 81 µg/L, which falls in the WHO classification for mild iodine deficiency (3).
			Comparison of methodologies for estimating nutrient inadequacy: modelling method NutriR vs. EAR cut point and probability of adequacy, using 1-day and usual nutrient intake (12% repeated 24HR).	The prevalence of inadequate nutrient intake was similar across methods, with an average difference of ±5 percentage points (range 0.2–7). The smallest differences (0.4–0.3 percentage points) were between NutriR and EAR cut point (methods 6 vs. 4) and NutriR and probability of adequacy (methods 6 vs. 5). Larger differences (~12 percentage points) occurred when using single 24HR data (methods 1–3) instead of usual intake (methods 4–6)(4).

Table 1: Continued

Research theme	Description	Research question	Activity	Outcomes
2: Product (re) formulation via fortification	The design of voluntary fortification strategies must balance public-health relevance with consumer uptake. Key technical and regulatory considerations include nutrient selection, bioavailability, product safety, affordability, and sensory acceptability (1).	To assess the physical, sensory, and chemical stability of replacing ferric pyrophosphate with higher bioavailability iron compounds (ferrous sulfate, ferrous fumarate, ferrous bisglycinate, encapsulated ferric pyrophosphate, and ferrous ammonium phosphate) in flavoured dairy matrices (**appendix**)	Four bench trials were conducted: (1) a trial using chocolate-flavored milk powder with cereal flakes, currently on the Ivorian market, stored at 20 °C, 30 °C, and 40 °C for 1 month; (2) a trial using chocolate- and vanilla-flavored milk powders sold in the Philippines, stored for 12 months under ambient conditions; (3) the flavored milk powder products were further verified with 2AFC sensory tests stored for 12 months under ambient conditions; (4) and, by accelerated shelf-life test, stored at 40 °C for twelve weeks.	Trial 2-4 showed that encapsulated ferric pyrophosphate was the only iron compound suitable to replace ferric pyrophosphate in the flavoured milk powders; however, it was approximately five times more expensive. Ferrous fumarate was suitable only in the cereal-containing chocolate product (Trial 1), with stability up to four months in ambient conditions.

Table 1: Continued

Research theme	Description	Research question	Activity	Outcomes
3: Evaluate product/ programme effectiveness on health and nutrition	This theme assesses whether fortification efforts translate into measurable improvements in nutrition and health outcomes. Evaluation aligns with the monitoring and evaluation step of the WHO/FAO framework, linking product performance to population-level impact (1).	Assess effectiveness of a multi-nutrient fortified food product in a school feeding programme (**chapter 5 and 6**)	Randomised controlled trial in SAC (n=945, 5–8 years) in Ibadan, Nigeria, with three study arms (control, LD, HD) over five months. Primary outcomes: haemoglobin and serum ferritin.	No differences in iron status were observed between the high-dose group and the control group. No differences were found in anaemia, iron deficiency, or iron-deficiency anaemia, likely due to the relatively adequate baseline iron stores in this population. Vitamin D concentrations were higher in the high-dose group compared to control at endpoint (adjusted difference 4%, 95% CI 0.6 to 7). Vitamin A status improved by approximately 8% (4.6, 11.5) across all groups after 5 months. No changes were observed for iodine and vitamin B12 status.

Abbreviations: SAC, school-aged children; WRA, women of reproductive age; LD, low-dose; HD, high dose; IDA, iron-deficiency anaemia; sTfR, soluble transferrin receptor; 24HR, 24 hours recalls; RCT, randomised controlled trial; NCD, non-communicable disease; EAR, Estimate Average Requirement; 2AFC, Two-Alternative Forced Choice.

Understanding nutritional needs is complex in LMIC (Côte d'Ivoire and Nigeria)

Understanding nutritional needs in LMIC settings such as Côte d'Ivoire and Nigeria remains complex. WHO/FAO guidance highlights the importance of three complementary types of information to inform food fortification strategies: biochemical indicators of deficiency, dietary intake-based estimates of nutrient adequacy, and dietary pattern data (1). In practice, these data are rarely available simultaneously or show good alignment. This challenge was evident in both country contexts examined in this thesis, albeit in different ways. In Nigeria, decisions to evaluate iron fortification were informed by national and sub-national dietary and biomarker evidence from other population groups, as nutrient status data were unavailable for the specific intervention target group (school-aged children) at baseline. As a result, the intervention was implemented in a population that was later shown to have largely adequate iron stores. In Côte d'Ivoire, dietary intake data could not be validated against biochemical indicators within the same population, and available sub-national and quasi-national biomarker data showed inconsistent with dietary intake data. In both settings, intake-based estimates of nutrient inadequacy did not consistently correspond with biochemical indicators of deficiency, complicating their interpretation for characterising population nutrition needs. These discrepancies likely reflect limited generalisability of sub-national studies, geographical variation in dietary patterns and disease burden, biological regulation and body stores of nutrients, and methodological challenges in both dietary intake and biomarker assessment.

The decision to evaluate iron fortification in the randomised controlled trial was based on the available evidence to characterise the nutritional needs of Nigerian school-aged children: national survey findings (NFCMS 2021) in young children and women (5), the long-standing global assumption that iron deficiency underlies one-half to two-thirds of anaemia cases (6), and limited pre-existing information on school-aged children (7). A dietary intake survey among school-aged children (4–8-years) in Ibadan indicated high dietary iron inadequacy (62%) (7). In contrast, baseline biomarker data from the randomised controlled trial in Ibadan showed that iron deficiency in school-aged children was approximately 10%, and body iron stores were adequate for nearly all of the participants. These findings demonstrate that nutrient inadequacy and biochemical deficiency can diverge substantially across age groups and contexts, highlighting the need to investigate the aetiology of nutrient-related conditions directly within the population of interest, with priority given to biomarker assessment.

Existing biomarker data from school-aged children in Taabo, south-east Côte d'Ivoire, (n=252) collected in 2025 (8), conflicted with our dietary intake findings (2). In this study, iron deficiency based on ferritin and transferrin, was reported in 100% of children, whereas our intake data suggested that fewer than 15% of children had inadequate iron intake. This lack of concordance was likewise evident among Ivorian women using quasi-national data (2,9) and in the Nigerian NFCMS 2021 (5).

These discrepancies are further amplified by population and contextual differences. Despite comparable age groups, the study in Taabo was conducted in a different setting from the urban populations included in our dietary intake survey, limiting direct comparability (8). Geographical variation in dietary patterns may further help explain the differences between sub-national nutrition data. In Côte d'Ivoire, dietary patterns differed substantially across cities, influencing overall diet quality, including nutrient inadequacy (Chapter 4). Similar heterogeneity is evident at the global level, where modelling studies demonstrate that nutrient inadequacies vary by region, sex, and age group (10). Notably, nutrients with the highest estimated prevalence of inadequate intake often do not correspond to those with the highest prevalence of biochemical deficiency in preschool children and women of reproductive age (11). These discrepancies, likely reflect differences in dietary patterns, environment exposures, infection burden and inflammation. Together, these findings emphasise the importance of interpreting dietary and biochemical data together and where possible, collecting both types of data concurrently within the same population.

Interpretation of iron inadequacy in literature is currently limited by the use of differing requirement frameworks and assessment methods. Many low-and-middle income countries, including Nigeria and Côte d'Ivoire lack national dietary reference values, and therefore rely on FAO/WHO estimated average requirements (EARs) or the more recently recommended harmonized-average requirement by Allen *et al* 2000 (12). In practice, studies variably apply the EAR, the Recommended Nutrient Intake, or alternative agency requirements (e.g. EFSA, IOM) (12–14). These requirements depend heavily on assumed dietary iron bioavailability (5%, 10%, or 15%), yet guidance on selecting the most appropriate level for a given population is limited. In practice studies either; do not specify the method used, apply data-intensive models such as the Hallberg and Hulthén approach, which requires detailed information on iron status and dietary composition (e.g. meat and phytate intake) (15), or rely on proxy indicators such as national Human Development Index classifications (10) as we did here. In addition, iron requirements are skewed in key population groups, particularly women and some agencies assuming this also for children (1,13), meaning that the full

probability approach should be applied using the full iron requirement distribution rather than the EAR cut-point method; however, this is rarely applied in practice (16). Together, these factors lead to wide variation in iron requirements applied and, consequently, in reported iron inadequacy estimates, complicating both their interpretation and the design of iron fortification strategies. Clearer guidance and greater caution are therefore needed when interpreting iron inadequacy estimates in LMIC settings.

Anaemia is multi-factorial, and its aetiology needs to be understood better

Global and national strategies typically prioritise iron deficiency as the primary cause of anaemia. This view is based on widely cited yet methodologically limited, estimates, most notably the longstanding assumption that, in the absence of biomarker data, approximately 50% of anaemia is attributable to iron deficiency (17). Although this rule-of-thumb has been applied for decades, it is based on limited evidence, varies substantially across settings and population groups, and does not account for confounding factors such as inflammation or infection. It also conflates association with impact, as the proportion of anaemic individuals with iron deficiency is not equivalent to the proportion of anaemia that would respond to iron interventions (18).

A more recent estimate by Gardner *et al.* (2023) stated that approximately two-thirds of global anaemia is attributable to iron deficiency; however, this estimate is derived from Global Burden of Disease (GBD) modelling in which a substantial proportion of unexplained ("residual") anaemia is statistically assigned to iron deficiency. As emphasised by the *Lancet Haematology Commission*, such attribution reflects modelling assumptions rather than direct biological evidence and does not establish iron deficiency as the causal mechanism, particularly in settings with a high burden of inflammation and infection (6). National analyses, such as those from the Nigerian National Food Consumption and Micronutrient Survey (NFCMS), also identify iron deficiency as a major correlate for anaemia. While informative for describing population patterns, cross-sectional data cannot distinguish between iron deficiency as a cause, consequence, or correlate of anaemia in the presence of inflammation and infection (19). As a result, current evidence has reinforced an iron-centred paradigm that underpins global recommendations, such as population-wide iron interventions in high-anaemia settings, despite limited aetiological resolution regarding the dominant biological drivers of anaemia in specific contexts (20,21).

Iron fortification has been shown to improve iron status, anaemia and iron-deficiency anaemia in several meta-analyses, especially where populations are

iron deficient (22–24). Anaemia interventions that focus exclusively on iron are however unlikely to be effective where other causes predominate, as seen in the intervention trial with Nigerian school-aged children in this thesis. Despite previous reports of high dietary iron inadequacy (62% among 4–8-year-olds in Ibadan) (7), our study found that school-aged children in the same region had adequate iron stores at baseline, yet more than 70% were anaemic. This indicates that anaemia in this group was largely driven by non-iron causes, likely inflammation, vitamin A deficiency and unknown causes. Similar inconsistencies might be expected in other populations based on national data and findings from this thesis. In Ivorian children and women high anaemia prevalences are reported from national DHS data, and dietary iron intake in our study was largely adequate. Similarly, anaemia is high among Nigerian women, yet iron deficiency was mild (10%) and inadequacy was approximately 45% (19).

Moreover, intervention evidence shows that even where iron deficiency is prevalent its reduction does not necessarily translate into meaningful reductions in total anaemia. In Côte d'Ivoire, a trial reported large reductions in iron deficiency and iron-deficiency anaemia after consuming iron-fortified complementary foods, yet anaemia prevalence decreased by only a few percentage points, suggesting that most anaemia was driven by non-iron causes (25). Similar patterns have been observed in school-feeding trials using food-based iron delivery. In India, Rani *et al.* 2024 reported improvements in haemoglobin without commensurate changes in iron stores, suggesting benefit limited to a small subgroup with iron-responsive functional deficiency (26), while in Ghana, Egbi *et al.* 2015 observed modest increases in ferritin without reductions in soluble transferrin receptor or anaemia prevalence in a high-inflammation setting (27). Together, these findings demonstrate that while iron fortification can effectively reduce iron-deficiency anaemia when it is limiting, its impact on total anaemia is often modest and context-specific, highlighting the need for interventions to address the broader aetiology of anaemia, including inflammation and other nutritional constraints (28) .

Stronger evidence-based guidance and evaluation are needed for voluntary fortification

Although this trial did not demonstrate improvements in iron status or anaemia following multi-micronutrient voluntary fortification, it reinforces the need for rigorous evaluation of such initiatives (1). Evaluation is essential not only to assess effectiveness, but also to refine strategies toward more effective solutions. In this study, anaemia was associated with inflammation and vitamin A status; however, correction of vitamin A deficiency did not reduce anaemia prevalence, and despite malaria and parasitic infections being treated at baseline, inflam-

mation was still high. These findings indicate that addressing identified correlates alone may be insufficient and that a substantial proportion of anaemia remains unexplained by commonly measured nutritional and infectious factors. This underscores the need for deeper investigation into non-identified drivers of anaemia and for strategies that reduce inflammation beyond malaria and helminth control. Although a few large-scale industry-led fortification initiatives, such as fortified bouillon and condiments, have generated substantial empirical evidence, rigorous evaluation remains the exception rather than the rule for market-driven fortification more broadly (29–32). Without systematic monitoring and evaluation, programmes risk being inefficient or potentially harmful. For example, findings from Nigerian women indicate that iodine status is already adequate or even excessive, suggesting that additional voluntary iodine fortification could increase the risk of hyperthyroidism (19). Effective monitoring systems are therefore essential to ensure that fortification aligns with population health needs and safety standards.

In many settings, fortification regulations emphasise compliance with nutrient content and labelling standards rather than evidence of biological effectiveness. For example, regulations of the National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria specify permissible fortification levels and nutrient claims but do not require assessment of health impact. Nutrient claims are permitted at 5%, 15%, and 30% of the Nutrient Reference Value (NRV), corresponding to "contains", "good source", and "high source", respectively (33). While compliant with regulatory standards, these levels are considerably lower than those shown in efficacy trials to improve iron status and do not account for differences in iron bioavailability (34). In our study, intervention products contained 33% (low dose) and 66% (high dose) of the NRV, yet did not improve iron status, indicating regulatory compliance alone may be insufficient to ensure biological impact. Incorporating considerations of bioavailability and evidence of efficacy in target priority populations into fortification policies could better align fortification efforts with biological meaningful outcomes.

Lack of resources remains a barrier to translating knowledge into practice

Limited resources in low- and middle-income countries constrains both the generation of high quality nutrition evidence needed to accurately characterise population nutritional needs, and the implementation of evidence-based nutrition interventions. For the work presented in this thesis, resource constraints manifested in two distinct ways. First, limited financial and infrastructural capacity restricted the collection of high-quality dietary intake, biomarker, and intervention-effectiveness data needed to accurately define

nutritional problems and design appropriate solutions. Second, even when robust data and technically feasible solutions were available, economic and logistical constraints limited their translation into affordable and scalable interventions.

The first challenge relates to constraints on evidence generation, specifically the scarcity of reliable data on dietary intake and nutrient status. In Côte d'Ivoire, the absence of recent national nutrition data necessitated the dietary survey in this thesis (2). More broadly, global funding cuts threaten the sustainability of large-scale surveillance systems such as the Demographic and Health Surveys, which provide essential information on anaemia and other population health indicators (35,36). While modelling tools such as *NutriR* can support assessment of nutrient adequacy, their application still depends on high-quality primary intake data, including estimates of usual intake distributions (multi-day dietary intake assessment), which are resource-intensive to collect (37). Similarly, digital data-collection tools such as INDEXX, used in the Nigerian National Food Consumption and Micronutrient Survey, may reduce some operational costs but remain dependent on repeated dietary recalls, trained personnel and stable internet access, which are not consistently available in many low-resource settings (5).

The second challenge concerns the translation of technical solutions into affordable and scalable interventions. Iron is a particularly complex nutrient to fortify as maintaining bioavailability requires careful control of oxidation and product stability, thereby limiting the suitability of many iron compounds across different food vehicles (38). The bench-scale trials conducted in this thesis showed that, although encapsulated ferric pyrophosphate could replace the current iron compound (ferric pyrophosphate) to enhance bioavailability, it was five times more expensive. Other iron compounds with higher bioavailability, such as NaFeEDTA, were also excluded during the bench trials (appendix) due to prohibitive costs (23,34). These findings illustrate that even when scientific and technological solutions exist, financial constraints often prevent their application. This gap between evidence-based solutions and economic feasibility helps explain why iron fortification has progressed more slowly than other fortification programmes, such as those targeting iodine or folic acid.

Strengths

This thesis contributes novel nutrition and health evidence from studies conducted in Côte d'Ivoire and Nigeria. The dietary intake survey provides one of the first comprehensive assessments of nutrient inadequacies and dietary patterns among women and children in Côte d'Ivoire using individual-level dietary

intake data, addressing a major evidence gap in this context. In addition, the randomised controlled trial is the first to evaluate the effectiveness of a multi-micronutrient food product among Nigerian school-aged children. Together, these studies provide new insights for the design and evaluation of nutrition interventions in settings where anaemia and micronutrient deficiencies are prevalent.

Studies within this thesis used validated and context-appropriate data collection and analytical methods. Usual intake was assessed using a standard multiple pass 24-hour recall as previously described and validated for other low- and middle-income countries (39) and was repeated for 12% of participants rather than reliance on a single day 24-hour recall. Although repeat recalls were conducted in a small subsample (12% of participants), this design allowed estimation of within-person variability and correction using the Statistical Program to Assess Dietary Exposure (SPADE), thereby improving the accuracy of population-level usual intake distributions. This approach strengthens the interpretation of nutrient inadequacy estimates compared with single-day intake assessments (shown in chapter 3).

This study adopts a food-based, pattern-oriented approach to dietary assessment that moves beyond single nutrients, food groups, or a priori diet scores, aligning with contemporary food-systems thinking about diet quality and population nutrition. Dietary patterns were identified using Latent Class Analysis (LCA), a probabilistic, data-driven method that captures heterogeneity in habitual food consumption without imposing predefined assumptions about diet quality. The use of a bias-adjusted three-step approach accounted for uncertainty in class membership and reduced misclassification, strengthening estimation of associations between dietary patterns and socio-demographic and nutritional factors (40). Food group categorisation was guided by the Global Diet Quality Score (GDQS), minimising subjectivity, enhancing comparability with other studies, and supported by expert validation (41–43).

Finally, the randomised controlled trial was designed to balance scientific rigour with real-world applicability. Including all eligible children rather than restricting participation to those who were iron deficient at baseline reflects how school-feeding interventions are implemented at scale and acknowledges that baseline iron status modifies biological response (44). This design provides a realistic estimate of population level effectiveness within a low- and middle-income school feeding context, where individual screening is rarely feasible. In addition, the intervention used a commercially available product formulated with technologically feasible ingredients, and adhered to WHO food-fortification guidance

while operating within real-world manufacturing constraints. Together, these features enhance the external validity and policy relevance of the findings.

At the same time, the trial incorporated rigorous methodological procedures to minimise bias and strengthen internal validity, including computer-generated randomisation, allocation concealment, blinding of product, participants, and fieldworkers, and an independent external monitoring. Current best practices in anaemia research were applied, including venous haemoglobin measurement using an automated haematology analyser, as opposed to capillary sampling and point-of-care devices such as HemoCue; inflammation adjustment using the BRINDA method addressing a major source of bias in the interpretation of iron and vitamin A biomarkers in infection-prone settings (45), and multiple micronutrient status indicators were assessed allowing evaluation of non-iron-related contributors to anaemia. Baseline malaria and parasitic infections were identified and treated, and haemoglobinopathies were assessed, thereby reducing confounding and strengthening causal inference regarding nutritional determinants of anaemia (46).

Limitations

Our results have limited generalisability. First, the dietary intake survey in Côte d'Ivoire included urban populations, and the intervention trial in Nigeria focused on school-aged children in Ibadan, Southwest Nigeria. These groups may not reflect rural or other regional populations. Biochemical data were also unknown for Nigerian school-aged children *a priori*, necessitating assumptions about anaemia aetiology based on other population groups and dietary intake data (7). Consequently, the use of existing dietary intake data from school-aged children in Ibadan, without nutrient status verification, was a major limitation, as the iron-fortification intervention was implemented in a population with adequate iron status. Moreover, the findings cannot be readily extrapolated to the wider target consumer population of market-driven fortified foods beyond children attending public primary schools in Ibadan or similar settings. In Nigeria, where only an estimated 16% of the population can afford a healthy diet (47), such products are likely consumed by middle- and high-income households. This represents a smaller and more socioeconomically homogeneous group than the nutritionally vulnerable populations targeted by public nutrition programmes or mandatory fortification.

Second, the reliability of nutrient intake assessment was constrained by methodological and data availability limitations. Dietary intake was assessed using 24-hour recalls, which are resource intensive and prone to subject recall error (48,49). Portion sizes were estimated using a photographic food atlas (50), and

standard recipes for foods and meals prepared outside the home, including common local dishes such as sauces, were developed by the study team based on information from local food vendors. These methodological issues increase uncertainty in the reported consumption quantities. Data-availability limitations further affected the precision of nutrient-content estimates. The FAO West African Food Composition Table contains few Ivorian foods, fortified foods are not systematically represented (51), and information on nutrient degradation factors, such as vitamin A from cooking, is incomplete (52,53). Together, these constraints increase uncertainty in estimated nutrient intakes.

Third, the selection of iron compounds was constrained by technological feasibility, sensory acceptability and cost. Two commercially available products were assessed in the framework of this thesis: a flavoured milk powder from Côte d'Ivoire and a milk-based porridge from Nigeria. Bench-scale trials of the Ivorian product, which used ferric pyrophosphate as the reference compound, indicated that no alternative iron source with higher-bioavailability could be incorporated without compromising sensory quality or substantially increasing cost (**Appendix 1**). Given the low bioavailability of ferric pyrophosphate and lack of feasible alternatives, this product was deemed unsuitable for evaluating its nutritional effectiveness and was therefore excluded. The Nigerian porridge contained a more suitable iron compound (ferrous fumarate) and thus was selected (23). Ferrous fumarate was used as a pragmatic compromise, offering acceptable sensory and stability characteristics, and bioavailability comparable to ferrous sulfate, but lower than that of NaFeEDTA, which was not used due to its higher cost and unsuitability in dairy matrices (38). However, ferrous fumarate absorption is inhibited by calcium and phytates, and its bioavailability in young children may be lower than expected, potentially due to age-related differences in gastric secretion and iron regulation (54). Although the product contained fermented maize and included vitamin C and iron at or above WHO recommendations (1), these measures may have been insufficient to overcome these inhibitory effects. Overall, while compound-related constraints may have contributed to the modest impact on iron status and exposed limitations in applying the framework to real-world products, adequate baseline iron stores and inflammation-associated functional iron sequestration were likely the primary constraints for effectiveness.

Finally, full compliance with consumption of the study product (five days per week for five months) was not achieved by any participant. The intervention was delivered through a school-feeding programme, reflecting the reality in many low- and middle-income countries, where such programmes are designed to improve access to fortified foods but are frequently affected by operational

constraints. In this study, delayed product availability, prolonged recruitment and staggered intervention start dates, lower-than-anticipated school attendance, and electricity strikes reduced actual exposure. Consequently, the per protocol population was redefined to a three-month consumption period, although consistent with theoretical kinetics of iron repletion (55), longer durations (typically six to twelve months) are often required to improve iron status and iron-deficiency anaemia (56,57).

Implications and future research

This thesis highlights several implications for research, policy, and industry, particularly regarding how market-driven fortification in low-and-middle income countries can align to public health concerns. As identified above, understanding the nutritional needs of the consumers of the market-driven fortified products is important, yet this understanding is often based on incomplete data. Our findings indicate that "filling nutrient gaps" based solely on existing dietary intake data does not necessarily lead to meaningful public health impact, as this approach oversimplifies the relationship between intake and nutrient status. In the intervention trial, for example, providing additional iron based only on estimated intake inadequacy was ineffective because the target population already had adequate iron stores. This illustrates how discrepancies between intake-based inadequacy estimates and biomarker-defined deficiency can undermine intervention effectiveness, particularly in low-resource settings where comprehensive data are rarely available. To ensure that fortification addresses true nutritional needs, biomarker assessment should be prioritised in the intended target population, with dietary intake data used subsequently to inform product design. When data sources conflict, biochemical indicators should take precedence, as they provide a more direct measure of nutrient status. Fortification strategies should therefore be guided by clearly defined public health objectives rather than by intake gaps alone.

Once health objectives are defined, future interventions should be guided by a comprehensive understanding of underlying aetiology, particularly for complex conditions such as anaemia and in regions with high inflammation and infection. As discussed in Section 6.2.2, anaemia in these settings is frequently driven by non-iron causes, including inflammation and other micronutrient deficiencies. Implications for future interventions are therefore clear: programmes should prioritise diagnosis-driven strategies that identify dominant aetiologies within the target population before selecting nutrient interventions (46,58). In this context, non-iron contributors to anaemia warrant greater attention, including neglected tropical infections that drive chronic inflammation (e.g. malaria, hookworm, and schistosomiasis) (58), anaemia of unknown origin,

which was a major contributor in our intervention and inflammatory pathways associated with non-communicable conditions such as overweight and obesity among women of reproductive age (59). Where iron fortification is constrained by food matrix limitations or limited biological response, alternative food-based approaches may be more appropriate. School-feeding trials in India and Ghana reduced anaemia without improving iron status, suggesting contributions from non-iron mechanisms (26,27).

Other important health concerns identified in these populations, but not directly addressed by our intervention, include the double or triple burden of malnutrition among women of reproductive age. For example, among women of reproductive age, where nutrient deficiencies coexist with high rates of overweight and obesity, future research should explore nutritionally sensitive "double-duty actions" that address both ends of the malnutrition spectrum (60). Integrating mothers into school-feeding programmes, as cooks or peer educators, could strengthen sustainability and provide dual benefits for women and children. Calcium also emerged as a consistent nutrient of concern and was the most prevalent inadequacy in both study countries and in modelling analyses at a global level (2,5,37). This warrants further investigation because calcium deficiency has been linked to rickets, a condition that persists in parts of Africa despite being eliminated in other regions of the world (61–64). The public-health relevance of calcium inadequacy may be underestimated, partly because no reliable biomarker exists for assessing calcium status. Continued work is therefore needed to understand the magnitude, causes, and health consequences of calcium inadequacy in these populations.

Advances in affordable analytical and statistical tools could substantially reduce the resource burden of data collection and analysis in low-and-middle-income countries. Tools such as *SAMBA*, which standardise analysis of population-based micronutrient biomarker data and adjusts for inflammation, illustrate the potential for improving data accuracy and comparability (65). Similar tools are needed for anaemia that integrate multiple biomarkers, account for inflammation, and apply consistent diagnostic rules. Improved diagnostic standardisation would also help reconcile inconsistencies in reported anaemia prevalence. This need is highlighted by the Nigerian Food Consumption Micronutrient Survey 2021, where anaemia and iron-deficiency anaemia values in the preliminary report differed substantially from those in the corrected version due to a coding error in haemoglobin adjustments, and values still differ considerably from the Demographic Health Survey estimates (5,19). A similar tool is warranted for dietary intake given issues explained with inconsistency of requirement frame-

works and possible dual data exploration between nutrient intake and status to understand this relationship better.

Private-public alignment opportunities

This thesis operationalised a framework to align market-driven fortification with population nutritional needs. The findings indicate that industry-led formulations are often made in the absence of context-specific biomarker and aetiological data, while public health bodies frequently lack insight into the technological, regulatory and commercial constraints that shape product reformulation. To maximise the public-health contribution of market-driven fortification, public-private collaboration should focus on three tangible functions informed by the empirical findings of this thesis.

First, partnerships should support joint evidence generation and data sharing, particularly in resource-limited settings where capacity to conduct routine biomarker surveys, dietary assessments, and post-market evaluations is limited. Shared investment in baseline aetiological assessments and effectiveness monitoring, embedded within real-world delivery platforms such as school-feeding programmes, could improve both the targeting and accessibility of market-driven fortified foods. Partnerships could also strengthen dietary assessment by addressing gaps in food composition data, which remain incomplete or outdated in many settings, including Côte d'Ivoire. Food manufacturers routinely generate detailed compositional data for their own products for quality control and regulatory compliance; structured mechanisms for sharing such data with public health actors could improve the accuracy of dietary intake estimates and better align fortification strategies with population needs.

Second, partnerships should enable co-design of fortification strategies that account for bioavailability, inflammation, and feasible exposure, rather than relying solely on generic nutrient "claim" targets. Agreement on bioavailability-adjusted fortification benchmarks, informed by WHO/FAO guidance and population-specific evidence, would directly address the limitations observed in the trial described in this thesis.

Third, partnerships should establish shared accountability mechanisms, whereby governments, donors, and industry jointly define measurable health outcomes and share responsibility for evaluation costs, recognising that post-market effectiveness assessment offers limited immediate commercial return but substantial public-health benefit. The Nigerian school-feeding programme illustrates this need: although it has strong national policy support, operational implementation remains constrained and often dependent on external financing

and delivery partners. In such contexts, shared accountability frameworks could enable coordinated co-investment by government, development partners, and industry, aligning incentives for sustained delivery, robust monitoring, and transparent assessment of health impact rather than placing responsibility on a single actor.

Rather than creating new structures, these functions can be operationalised through stronger integration and coordination of existing platforms, such as, GAIN, the Scaling Up Nutrition movement, the Access to Nutrition Index, and national fortification alliances. Aligning these actors around shared priority nutrients, target populations, and evaluation standards would improve coherence across settings. Since economic and public-health objectives do not always align, partnership frameworks should also provide mechanisms to explicitly address trade-offs related to cost, ingredient choice, and programme duration. Structured dialogue supported by qualitative research with industry, government, and academic stakeholders, is needed to identify feasible incentive mechanisms that support sustained engagement while ensuring that market-driven fortified foods deliver demonstrable public-health gains in low- and middle-income country contexts.

CONCLUSIONS

This thesis contributes new evidence on the nutritional challenges faced in Côte d'Ivoire and Nigeria. In Côte d'Ivoire, we found high prevalence of overweight and obesity among women, as well as of inadequacies in calcium, riboflavin and zinc among children and women. Dietary pattern analysis suggests that both these populations are in the early stage of the nutrition transition, highlighting the urgency of addressing obesity prevention alongside potential micronutrient deficiencies. In Nigeria, we found high prevalences of anaemia, mostly caused by inflammation, vitamin A deficiency and unknown causes among school-aged children. Additionally, the multiple micronutrient fortification intervention was not effective in improving iron status, likely limited due to the iron adequate stores of the study population.

Together, these findings demonstrate that intake-based estimates of inadequacy do not necessarily correspond to biochemical deficiency and that iron-centred approaches are unlikely to be effective where anaemia is multifactorial and iron is not limiting. The lack of intervention impact illustrates the risks of designing fortification strategies based primarily on intake data or broad assumptions about anaemia causation. These results underscore the need to

ground market-driven fortification in population-specific understanding of nutritional status and disease aetiology, supported by evaluation of biological effectiveness before scale-up.

However, translating such evidence-driven approaches into practice is constrained by limited resources in low-income settings. Accurately characterising nutritional needs and evaluating intervention effectiveness require prioritisation, beginning with nutrient status assessment, followed by identification of dominant aetiologies and clear definition of target populations. Strengthening evidence-based guidance, evaluation frameworks, and coordination between public health and industry will therefore be essential to ensure that market-driven fortification delivers meaningful and safe public-health impact under real-world constraints.

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7



Appendices

Appendices

Feasibility of using more bioavailable iron forms in fortified dairy powder products: Evidence from four bench-scale trials

Summary

Acknowledgements

About the author

INTRODUCTION

Iron fortification presents technological difficulties: highly soluble iron compounds offer better bioavailability but are more reactive, increasing the risk of colour and flavour changes. Effective fortification therefore requires balancing nutritional efficacy with product stability and sensory acceptability. Dairy was selected as the food vehicle in this study because of FrieslandCampina's commercial focus on and the nutritional value of dairy products. Although dairy components such as calcium and caseins may inhibit iron absorption in single-meal studies, their overall effect on iron status in the diet remains unclear (1). Dairy consumption is relatively low in parts of West and Central Africa (2), and is often combined with tea, coffee, or cereals that contain additional iron-binding compounds.

FrieslandCampina already fortifies selected flavoured milk powders in Côte d'Ivoire and the Philippines with micronised ferric pyrophosphate, a compound widely used due to its stability, low cost, and limited sensory effects. However, even in micronised form, ferric pyrophosphate remains less bioavailable than ferrous salts, and was highly dependent of food matrixes, with no study conducted for dairy, prompting interest in identifying more bioavailable alternatives that are still compatible with dairy matrices (3).

Optimising iron fortification in dairy requires evaluating candidate compounds, to replace lower-bioavailable compounds, such as ferric pyrophosphate, within the specific food system. WHO/FAO guidelines emphasise vehicle-specific testing because interactions with dairy proteins, fats, and carbohydrates may influence both stability or sensory properties. Ferrous bisglycinate is recommended for dairy systems due to its high bioavailability and lower reactivity, but data in real food matrices are limited (4).

In this study, five iron compounds were selected: ferrous sulfate, ferrous fumarate, ferrous bisglycinate, encapsulated ferric pyrophosphate, and ferrous ammonium phosphate—based on expected differences in bioavailability, sensory stability, and cost (**Supplement Table 1**). The selection was guided by an a priori hypothesis that:

1. **bioavailability** would be highest for ferrous salts and chelates (ferrous sulfate, ferrous fumarate, ferrous bisglycinate);

2. **sensory stability** would be more favourable for less reactive or encapsulated compounds (encapsulated ferric pyrophosphate, ferrous ammonium phosphate); and

3. **cost-efficiency** would vary widely, with ferric pyrophosphate and ferrous fumarate representing low-cost options and ferrous bisglycinate and ferrous ammonium phosphate representing higher-cost.

The aim of this study was to assess the technological and sensory feasibility of replacing ferric pyrophosphate with more bioavailable iron compounds in a flavoured milk powder. Selected compounds were evaluated under controlled bench-scale trial conditions for stability, sensory quality, and cost to identify a technically and economically viable formulation. The specific objective was to compare the physical, sensory, and chemical stability of selected iron compounds with ferric pyrophosphate in flavoured dairy matrices.

MATERIALS AND METHODS

Four bench-scale trials were conducted and are described below. A summary of the trial designs can be seen in **Figure 1.**

Trial 1: Chocolate flavoured milk powder with wheat cereal pieces

Product description: The chocolate-flavoured milk powder with wheat cereal pieces was commercially available in Côte d'Ivoire and sold in dry powder format. At consumption, 50 g of powder was reconstituted with 228 mL of water to produce 278 g of ready-to-consume product.

Design

A bench-scale trial *(Trial 1)* was conducted at FrieslandCampina Innovation Centre, Wageningen. This trial involved laboratory-scale preparation and evaluation of fortified flavoured dairy powder with cereal pieces. Four iron compounds were evaluated: 1) ferric pyrophosphate (reference) (DSM), 2) ferrous sulfate (DSM), 3) ferrous fumarate (DSM), and 4) ferrous bisglycinate (Ferrochel), each tested at three fortification levels (4.4, 8.0, and 15 mg Fe per serving). The selection of iron compound and the fortification levels was informed by a structured review of the published literature conducted as part of a student research project, with the findings summarised in **Appendix 1** and detailed in the associated student report (5).

The reference formulation containing ferric pyrophosphate was known to the investigators and served as the control comparator. All test formulations were blinded to assessors and laboratory personnel involved in sensory and stability evaluations. Samples were prepared using identical base ingredients and packaged in identical aluminium-foil sachets, differing only in the iron premix.

Shelf-life test

Products were stored at 20 °C, 30 °C, and 40 °C for four weeks from 16/12/2020 to 16/2/2021. Storage at 40 °C was used to simulate accelerated shelf-life conditions, with one week of storage assumed equivalent to one month of storage at ambient temperature. The four-week period of storage at 40 °C therefore corresponds to approximately four months of ambient storage.

Assessments

Measurements included pH (standard laboratory pH meter), colour and taste by three trained internal assessors. Assessments were conducted at baseline, two weeks, and one month, comparing the reference sample (ferric pyrophosphate) to the three test samples (ferrous sulfate, ferrous fumarate and ferrous bisglycinate).

Assessments were performed for both the dry product and the reconstituted product. Dry-products were assessed visually for colour changes by three trained assessors. Reconstituted-products were prepared by mixing 50 g of product with 228 mL water, followed by pH measurement and sensory assessment of colour and taste.

Colour was assessed by visual comparison against a predefined reference control, containing ferric pyrophosphate. The objective of the assessment was to detect perceptible colour deviations attributable to the iron compound. Trained assessors evaluated each test sample relative to the reference and recorded deviations categorically as "same as reference", "lighter than reference", or "darker than reference". Taste was evaluated in parallel through direct comparison with the reference product, focusing on the presence or absence of detectable differences rather than preference.

Trial 2, 3 and 4: Chocolate and vanilla flavoured milk powder

Product description: The two flavoured milk powder products (chocolate and vanilla) were commercially available in The Philippines at the time of testing. Both are sold in dry format and are to be reconstituted at the point of consumption. The chocolate variant contains 30 g of powder combined with 180 mL of

water, and the vanilla variant has 33 g powder combined with 180 mL of water, yielding approximately 210 g and 213 g of reconstituted product, respectively.

Design

Three subsequent bench-scale trials (Trial 2-4) were conducted at the FrieslandCampina Development Centre (Singapore) using the vanilla and chocolate variants. Iron compounds were selected based on results from trial 1, supplier recommendations (e.g. not to use ferrous fumarate in the vanilla flavour variant) and a screening test to identify iron compounds that were unsuitable for either of the flavour variants. The screening involved hand mixing each iron compound into the flavoured milk powder and observation of any apparent visual or taste change deviations from the reference.

The selected iron compounds for the vanilla product were ferric pyrophosphate (reference) (DSM), ferric pyrophosphate encapsulated (Sudeep), ferrous sulfate (DSM), and ferrous ammonium phosphate (Dr Paul Lohmann), using an iron dose of 3.9 mg Fe/serving. For the chocolate variant iron compounds selected were ferric pyrophosphate (reference), ferric pyrophosphate encapsulated, ferrous sulfate, ferrous fumarate and ferrous ammonium phosphate, using an iron dose of 5.1mg Fe/serving. These iron doses reflect the level of fortification of the products that are currently on the market in The Philippines. The premix included sodium ascorbate at a ratio to iron of 2:1, following WHO guidance, to enhance absorption.

All products were prepared with identical ingredients and aluminium-foil packaging, differing only by the premix. The reference formulation was known to the investigators and served as an unblinded control, whereas all test formulations containing alternative iron compounds were blinded using three-digit codes. Both the dry and the reconstituted products were evaluated. Following reconstitution, samples were left to stand for 30 minutes before analysis.

Trial 2: 12-month shelf-life at ambient temperature

Products were stored under ambient conditions for twelve months (17th July 2023 to 13 June 2024). Assessments were performed at baseline and the end of months 1, 3, 6, 9, and 12.

Trial 3: 12-month shelf life validation at ambient temperature with 2-AFC sensory test

Based on results of Trial 2, the iron compound with the closest sensory performance to the reference (encapsulated ferric pyrophosphate) was selected for a separate shelf-life validation study. Fresh product batches were produced

and stored under ambient conditions for an additional, independent 12-month period (14 August 2024 to 15 July 2025). Assessments were conducted at baseline and at months 1, 3, 6, 9, and 12. The two-alternative forced-choice (2-AFC) sensory test, conducted by the trained panel described above, was included to confirm sensory stability relative to the reference.

Trial 4: 12-week accelerated shelf-life test with 2-AFC sensory test

Trial 4 used the same iron compound (encapsulated ferric pyrophosphate) as Trial 3. Samples were stored at 40 °C for twelve weeks (14 August 2024 to 5th November 2024). Assessments were performed at baseline and at weeks 1, 3, 6, 9, and 12. The 2-AFC sensory validation by the trained panel described above was included to confirm sensory stability.

Assessments

pH was measured with a pH meter (standard laboratory pH meter) and colour with a colourimeter (standard laboratory colourimeter). Visual (colour, flocculation and sedimentation) and taste assessments were conducted by a trained internal assessor (n=1). The assessor was blinded to the identity of the test iron compounds, while the reference reformulation was known and used as a comparator. Samples were rated on a 5-point scale, to record deviations from the reference sample, ranging from 5 points (“conformity with expected sensory profile”) to 1 (“very considerable deviation”).

For *trials 3 and 4*, a blinded two-alternative forced-choice (2-AFC) sensory test was conducted in the FrieslandCampina Sensory Laboratory using an expert trained sensory panel (n=8-11 per test). Each 2-AFC test consisted of a paired comparison between one test product and the reference product of the same flavour, presented simultaneously. Panellists were blinded to the identity of the test sample and were asked to indicate which of the two samples was more intense for each attribute. For chocolate-flavoured samples, attributes included cocoa, milky, sweet and off-flavour. For vanilla-flavoured samples, attributes included milky, sweet, gamey and off-flavour. Responses were coded as (1) same as reference, (2) less than reference, (3) more than reference and (4) not present.

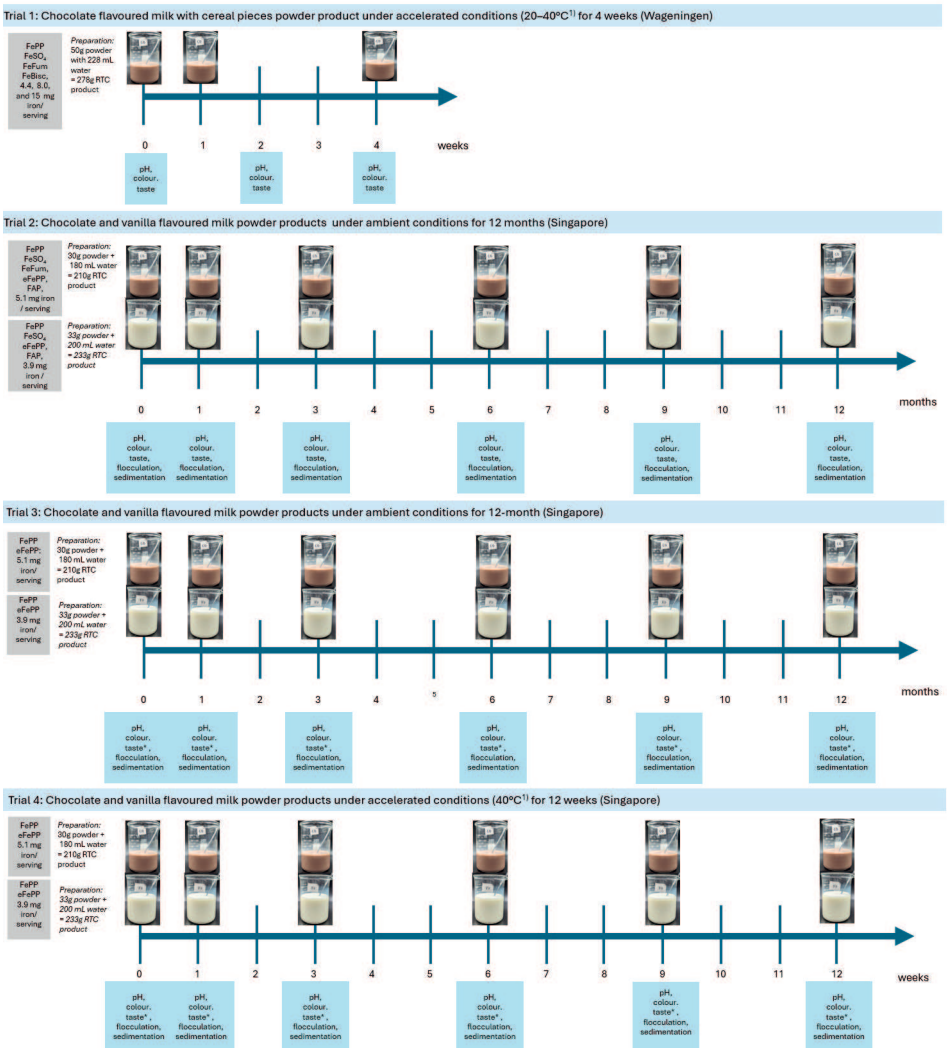


Figure 1. Study design of four bench-scale trials (trial 1-4) assessing the technological feasibility of replacing ferric pyrophosphate (FePP) with better-bioavailability iron compounds in powdered milk products**.** *Trial 1* evaluated chocolate-flavoured milk with cereal pieces over 4 weeks. *Trial 2* expanded on this using chocolate and vanilla powdered milk products over twelve months. *Trial 3* used the highest scoring iron-compound from trial 2 (eFePP) in the chocolate and vanilla powdered milk products over twelve months, and in *Trial 4* in parallel an accelerated shelf life test for twelve weeks was conducted. Technological feasibility outcomes included pH, colour, taste, flocculation, and sedimentation. * two-alternative force choice testing was used. All product formulations were identical except for the iron compound used. FePP= ferric pyrophosphate, $FeSO_4$= ferrous sulfate, FeFum = ferrous fumarate, FeBisc = ferrous bisglycinate, eFePP = ferric pyrophosphate encapsulated, FAP= ferrous ammonium phosphate , [1] Storing the product at 40 °C is an accelerated shelf-life test, where 1 week approximately equates to 1 month under typical ambient temperature conditions. Abbreviations: RTC; ready-to-consume

RESULTS

Trial 1 Result Summary

Colour differences between iron compounds and the reference (ferric pyrophosphate) were observed and became more pronounced during storage. Ferrous fumarate showed the closest colour profile to the reference, with both compounds remaining visually similar across all time points. In contrast, ferrous sulfate and ferrous bisglycinate produced powders and liquid ready to consume (RTC) products that were noticeably darker than the reference, with further darkening during storage.

No differences in pH were observed between any of the iron compounds or over time. Taste assessments of the reconstituted products also revealed no detectable differences compared to the reference.

Based on the four-week accelerated shelf-life study at 40 °C, the Wageningen R&D team (n=3) concluded that ferrous fumarate is the most suitable alternative to ferric pyrophosphate, as it demonstrated comparable colour stability and an unchanged sensory profile. Ferrous sulfate and ferrous bisglycinate were considered unsuitable replacements due to substantial colour changes relative to the reference.

Trial 2 Result Summary

The chocolate product showed colour changes that differed from those observed in Trial 1. Ferrous fumarate displayed the largest colour deviation from the reference (ferric pyrophosphate), progressively turning grey, particularly after the six-month time point. In contrast, ferrous sulfate remained visually similar to the reference throughout storage (**Figure 2**).

All iron compounds showed some degree of flocculation, with the largest deviation from the reference observed for ferrous ammonium phosphate. Both ferrous ammonium phosphate and ferrous fumarate exhibited visible sedimentation from six months onward. Across all variants, samples were perceived as less sweet and less chocolatey than the reference by the internal assessor (n=1). A metallic off-flavour was noted for all samples except encapsulated ferric pyrophosphate, which remained closest to the reference in taste.

For the vanilla product, ferrous sulfate showed the clearest colour deviation from the reference (ferric pyrophosphate). Taste differences emerged after three months, described as an eggy off-flavour by the sensory panel. As with the chocolate variant, all samples exhibited flocculation, with the largest deviation

from the reference recorded for ferrous ammonium phosphate, where black specks were observed as sediment. Additional taste differences for this variant were reported at twelve months, characterised as less milky than the reference by the internal assessor (n=1).

Based on the twelve-month ambient shelf-life study, the Singapore R&D team (n=3) concluded that encapsulated ferric pyrophosphate is the only iron compound that consistently matched the reference across both flavour variants, with no meaningful differences in colour, stability, or taste. All other compounds, ferrous sulfate, ferrous fumarate, and ferrous ammonium phosphate, showed unacceptable deviations and would not be considered further as alternatives for ferric pyrophosphate in chocolate or vanilla powdered milk formats. Encapsulated ferric pyrophosphate, however, is approximately five times more expensive than the current ferric pyrophosphate used in production.

A summary of trial results 1 and 2 is given in **Table 1.**

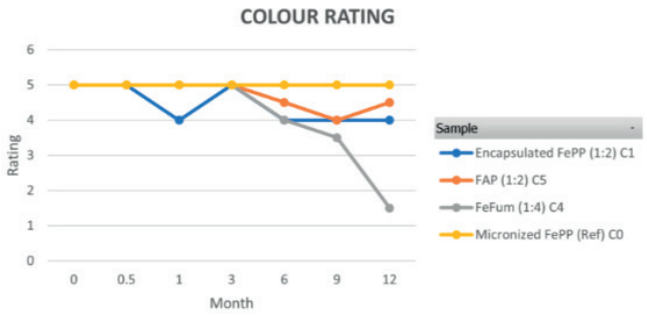


Figure 2 a. Visual assessment and rating 0-5 by internal panel on colour difference form the reference. Rating is based on 0= large deviation and 5= conformity.

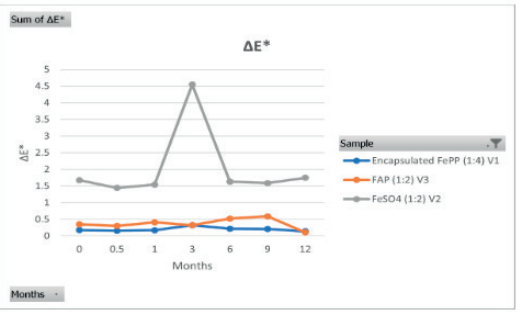
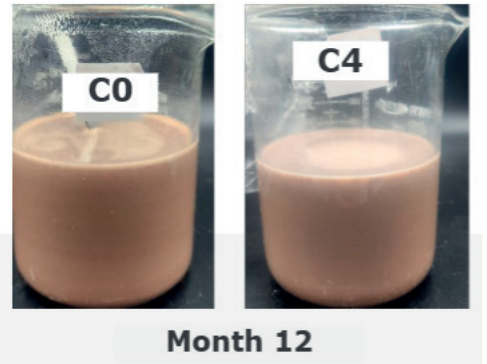


Figure 2b. Colorimeter results showing the difference from reference. ΔE* is the sum of (1) Δ lightness; (2) Δ redness; and (3) Δ yellow.



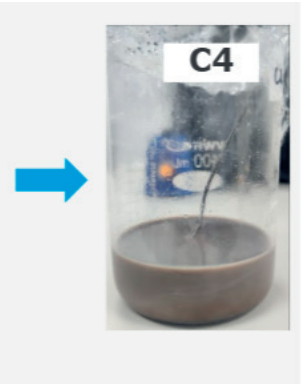


Figure 2c. Photos of the reference (C0) compared to ferrous fumarate (C4) at 12 months. The third image was taken after a prolonged time in the open.

Figure 2. Colour changes in the chocolate flavoured product in trial 2, comparing 3 different iron compounds (FeFum, FAP, eFePP) with the reference (FePP)

Table 1. Outcomes (colour, flocculation, sedimentation, pH, taste) of five test iron compounds compared to the reference in trials 1 and 2.

Outcome	Product[1]	FePP (reference)	eFePP	$FeSO_4$	FeFum	FeBisc	FAP
Colour	1		NA				NA
	2				grey-brown	NA	
	3			greyer	NA	NA	
pH	1		NA				NA
	2						
	3				NA		
Taste	1		NA				NA
	2	M9: metallic/oxidised off-flavour	Less sweet / chocolatey	Less sweet / chocolatey	Less sweet / chocolatey M9: metallic/oxidised off-flavour	NA	Less sweet/chocolatey M9: metallic/oxidised off-flavour
	3			≥M6: eggy-off note	NA	NA	M12: Less milky
Flocculation	1		NA	NA	NA	NA	NA
	2					NA	
	3				NA	NA	Black specs
Sedimentation	1		NA	NA	NA	NA	NA
	2				M6	NA	M6
	3			Black specs	NA	NA	
Overall			1st	Off colour and taste	Turn grey	Off colour	2nd
Cost[2]			1:5	1:1	1:1	1:8	1:3
Bioavailability[3]		+	+	++	++	+++	+++

[1]**Product chocolate** milk with cereal pieces assessed in trial 1; Product 2= chocolate milk assessed in trial 2; and Product 3= vanilla milk assessed in trial 2 [2]Cost ratios are expressed relative to ferric pyrophosphate = 1. For example, a ratio of 1:5 indicates that the alternative compound costs five times more than ferric pyrophosphate[3]Bioavailability key: '+' indicates bioavailability comparable to the reference

(ferric pyrophosphate); '++' indicates approximately twice the bioavailability of the reference; '+++' indicates substantially (three times) higher bioavailability than the reference.
Colour key: blue= reference, grey= not applicable/not tested, green= comparable to the reference , orange= deviates from the reference
Abbreviations: FePP= ferric pyrophosphate, $FeSO_4$= ferrous sulfate, FeFum = ferrous fumarate, FeBisc = ferrous bisglycinate, eFePP = ferric pyrophosphate encapsulated, FAP= ferrous ammonium phosphate, NA, not applicable due to unsuitability based on pre-screening assessment; M, month

Trial Result 3 Summary

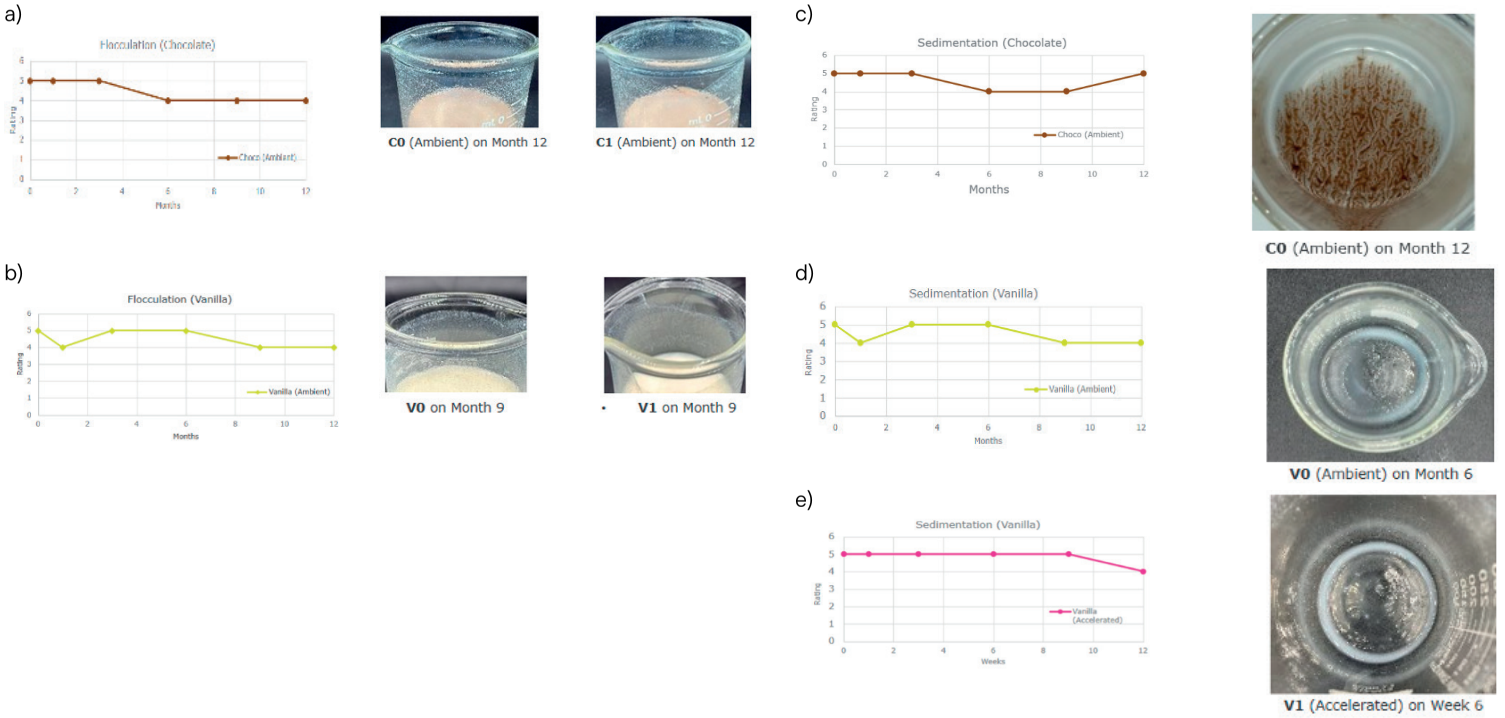
No changes in colour or pH were observed between test and reference samples in dry powder and reconstituted ready-to-consume products for both flavours. Flocculation and sedimentation were observed in all samples, including both reference and test products, for vanilla and chocolate flavours. Sedimentation and flocculation became visible in the chocolate flavoured product from 6 months onwards and after 9 months in the vanilla flavoured product, with similar patterns observed in both test and reference samples (**Figure 3a-d**).

Taste was comparable between reference and test samples based on two-alternative force choice testing conducted under ambient storage conditions. Minor differences relative to the reference were detected only for perceived sweetness, occurring at month 9 for the vanilla-flavoured product and at month 12 in the chocolate flavoured product. No off-flavours were detected in either flavour throughout the study period.

Trial Result 4 Summary

No differences in colour or pH were observed between test and reference samples for either the dry powder or the reconstituted RTC product for both flavours. All samples showed flocculation, both for the reference as well as the test products of both flavours. Sedimentation was visible in the test sample (and not the reference) for the vanilla flavour (**Figure 3c**).

Taste was comparable between reference and test samples based on two-alternative force choice testing after 12 weeks of accelerated shelf life storage. Minor differences relative to the reference were detected only for perceived sweetness for both flavours at week twelve. There was no off-flavour detected for either vanilla or chocolate flavours.



Flocculation occurred in **a)** chocolate and **b)** vanilla under ambient temperature conditions. All assessments were made according to the 5 point scale where 5 is: conformity with expected target sensory profile, 4: minimal deviation from expected target sensory profile, 3: noticeable deviation, 2: considerable deviation, and 1: very considerable deviation.

Sedimentation observed in **c)** chocolate flavoured reconstituted RTC milk product under ambient temperature, showing minimal deviation after 6 months; **d)** vanilla flavoured reconstituted RTC milk product under ambient temperature conditions, also showing minimal deviation after 6 months; and **e)** the accelerated conditions, showing minimal deviations after 10 months.

Figure 3. Results from trials 3 and 4 showing minimal flocculation and sedimentation between FePP (reference) and eFePP (test) samples according to the visual assessment recorded on a 5 point scale.

DISCUSSION

Across bench trials 1 and 2, outcomes of the tested iron compounds relative to the reference (ferric pyrophosphate) differed between products and testing conditions. In Trial 1 (Wageningen), ferrous fumarate was the most suitable replacement, showing no colour, taste, or pH differences relative to the reference after 4 weeks at 40 °C (equivalent to 4 months under ambient temperature conditions), whereas ferrous sulfate and ferrous bisglycinate were unsuitable due to visible colour changes. In Trial 2 (Singapore), results differed from trial 1. Ferrous sulfate again showed unacceptable colour and taste changes, confirming its limited suitability for flavoured milk powders. In contrast, ferrous fumarate showed a grey colour change in the chocolate powder from month six onwards and was excluded from the vanilla product based on supplier guidance during pre-screening. Two additional compounds were evaluated in Trial 2: encapsulated ferric pyrophosphate (eFePP), which performed most consistently and remained closest to the reference, and ferrous ammonium phosphate (FAP), which performed moderately well but formed black specks. In trials 3 and 4 (Singapore), the results reconfirmed the findings from trial 2, where eFePP showed similar performance compared to ferric pyrophosphate for colour, pH and taste outcomes, making it a suitable replacement.

Together, these findings demonstrate that the suitability of iron compounds is highly product-specific and that performance in one matrix does not reliably predict performance in another. This reinforces the need to evaluate iron compounds directly within the intended food vehicle, as even subtle changes in colour, flavour, or texture can influence product quality and consumer acceptance.

The contrasting behaviour of ferrous fumarate between the trials likely reflects differences in both study duration (four months vs. twelve months) and product vehicle. Trial 1 assessed stability after one month at 40 °C (equivalent to four months under ambient conditions), whereas Trial 2 followed products for a full twelve months under ambient conditions. The grey discolouration observed in Trial 2 was only present after month six suggesting that the earlier assessment in Trial 1 may have ended before oxidative changes emerged. Differences in product matrices may also contribute. The cereal pieces in Trial 1 introduce visual complexity that may mask subtle colour changes or may bind to iron in a way that reduces its immediate oxidative reactivity. These findings reinforce the need to evaluate fortificant performance within specific product systems and over realistic shelf-life durations that reflect commercial and regulatory market requirements.

The colour and taste changes observed for the more bioavailable ferrous iron compounds reflect their differing solubilities and oxidative reactivities. Ferrous sulfate is water-soluble, making it highly bioavailable but also highly reactive: once dissolved, Fe^{2+} readily participates in redox cycling that accelerates lipid oxidation, promotes off-flavours, and causes colour fading, such as the brown-to-grey change seen in both trials 1 and 2. Ferrous fumarate, is poorly water-soluble but soluble under gastric conditions, giving it high bioavailability while moderating its reactivity in the dry product. This explains its early stability in Trial 1 and the later onset of discolouration in Trial 2 after prolonged exposure to moisture and oxygen. In contrast, ferric pyrophosphate is water-insoluble and poorly soluble even in dilute acid, greatly limiting iron release and resulting in markedly lower bioavailability (typically 21–74% of ferrous sulfate)(4). It's low solubility reduces its capacity to catalyse oxidation, making it technologically stable but nutritionally less effective. Together, these findings illustrate the trade-off between bioavailability and oxidative stability: compounds that dissolve readily (ferrous sulfate, ferrous fumarate) and deliver more absorbable iron but carry higher sensory risk, whereas compounds that remain insoluble (ferric pyrophosphate) offer stability at the cost of reduced efficacy.

The ferric pyrophosphate used in both trials was a micronised form, selected for its higher bioavailability compared with standard ferric pyrophosphate. According to FAO guidance, reducing ferric pyrophosphate to submicron scale (≈0.5–3 µm, compared with ≈8 µm for conventional FePP) increases its effective surface area and enhances dissolution under gastric conditions, resulting in two- to four-fold higher iron absorption in dairy matrices (4). This corresponds to a relative bioavailability of roughly 40–60% compared with ferrous sulfate. Encapsulated FePP (eFePP) improved stability further in Trial 2. Encapsulation does not change the compound's intrinsic solubility but reduces direct interactions with fats, proteins, and flavour-active components, thereby preventing sensory defects. eFePP showed no detectable changes in colour, pH, or taste over twelve months, and its modest theoretical improvement in bioavailability likely arises from improved dispersion. However, micronisation and encapsulation both increase cost. In this study, eFePP was approximately five times more expensive than micronised FePP, consistent with FAO reports that encapsulation typically increases cost three- to five-fold (4). Although eFePP offered the best overall stability and nutritional profile among the compounds tested, its feasibility depends on whether the gains in performance justify the cost for specific markets and product lines.

The colour and taste challenges associated with the more reactive iron salts have previously been documented in liquid dairy systems (4,6), though such

effects were expected to be less pronounced in powdered products with lower water activity. Our findings, particularly for ferrous bisglycinate, differ from WHO/FAO food fortification guidance that recommends it for dairy products, (4). Oxidation in flavoured milk powders is influenced not only by the iron compound but also by formulation factors, including the citric acid–lactic acid ratio. In this study, pH did not differ from the reference, suggesting that acidifier balance was not the primary driver of changes. Potential mitigation strategies may include the use of encapsulated FePP, adjusting the ascorbic acid-to-iron ratios, or employing reduced-oxygen packaging atmospheres, but these must remain cost-feasible for voluntary fortification.

This study has several strengths. It examined fortificant performance across two laboratories using commercially relevant dairy-based powder products. It adhered to established ascorbic acid–iron ratios (2:1) and integrated cost considerations, which are important for real-world fortification decisions. The inclusion of pH, taste, and colour assessments supported a more complete evaluation of oxidation pathways.

Several limitations should be noted. Trial 1 relied on subjective visual assessment and internal assessors, which may introduce bias. Instrumental colour measurements and trained external sensory panels, as used in Trial 3 and 4, are preferable. Additionally, certain high-bioavailability compounds (e.g., NaFeEDTA) and absorption enhancers (e.g., galacto-oligosaccharides) were not included in this study due to cost. Finally, bench-scale trials cannot fully replicate industrial processing, where heating, shear, spray-drying, and packaging environments influence iron behaviour. Therefore, compounds identified as promising in these bench-scale trials should undergo factory-scale validation to confirm performance under commercial production and packaging conditions before implementation in market-ready products.

CONCLUSIONS

Based on the combined evidence from these four bench trials, encapsulated ferric pyrophosphate is the only alternative iron compound that consistently demonstrated sufficient sensory and technological stability to justify progression to factory-level production trials in chocolate and vanilla flavoured milk powders. A detailed cost–benefit evaluation will be required to determine whether its improved stability and slightly enhanced bioavailability warrant its substantially higher ingredient cost, and to identify the product lines and markets in which such an investment would be feasible.

Ferrous fumarate may merit further evaluation in non-flavoured dairy products or cereal-containing formats, where colour changes are less critical and where its combination of high bioavailability and moderate cost could offer a practical alternative to ferric pyrophosphate. As ingredient technologies evolve and supply chains mature, reassessment of compounds such as ferrous ammonium phosphate is also recommended under storage and sensory conditions relevant to the intended product. Together, these steps will help identify iron fortification strategies that balance nutritional impact, technological feasibility, sensory acceptability, and commercial viability.

SUPPLEMENTARY MATERIAL

Supplement Table 1: Overview of the sensory and chemical properties of different iron compounds focusing on their application in dairy matrices based on published literature, adapted from the literature review presented in the student report (5).

Iron type	Positive results	Negative results	Overall results
Ferrous sulfate (3,4,7)	- Most available and used. Widely studied, predictable absorption. - Effective in a wide range of food vehicles, especially cereals and condiments.	- Highly reactive (Fe^{2+}): causes oxidation, off-flavours, colour changes in foods, especially dairy and beverages. - Causes metallic taste at moderate doses. - Poor sensory stability in dairy and beverage systems.	Useful in low concentrations for iron status improvement.
Ferrous fumarate (3,8–13)	-Similar bioavailability to ferrous sulfate. - Less reactive in dry foods compared to ferrous sulfate. - Better sensory stability early in shelf-life. - Cost is moderate.	- Insoluble in water; can have variable performance in some food systems. - Oxidation can occur under prolonged storage or moisture exposure. - Poor performance in some beverages and light-coloured foods.	Useful alternative to ferrous sulfate for dry, low-moisture foods; typically better sensory stability than ferrous sulfate but still not suitable for all product types.
Encapsulated iron (14–16)	- Encapsulation reduces reactivity with food components, improving colour and flavour stability. - Can maintain high bioavailability if coating dissolves appropriately in gastric conditions. - Useful in food vehicles highly sensitive to oxidation.	Significantly more expensive (3–5×; up to 10× vs ferrous sulfate). - Bioavailability may be reduced if coating is thick or inappropriate. - Requires validation for each coating material and food matrix.	Effective for protecting sensory quality in sensitive foods; appropriate when budget allows and when standard iron compounds cause unacceptable product changes.

Supplement Table 1: Continued

Iron type	Positive results	Negative results	Overall results
Ferrous bisglycinate (4,17–20)	- Very high bioavailability (RBV >100%). - Chelated form reduces some oxidative interactions in certain foods. - Effective at lower doses due to high absorption. - Recommended for dry dairy products.	- High cost (≈17–18× ferrous sulfate per mg iron). - Limited data for large-scale fortification of flavoured products.	Seems to be a good alternative if problems occur with ferrous sulfate/fumarate
Ferric pyrophosphate (3,6)	- Minimal sensory impact due to very low reactivity. - Stable in dairy powders, beverages, and sensitive foods. - Low cost and wide availability. - Micronized version seems to enhance more. 2–4× higher absorption than standard FePP in milk products (FAO).	- Low bioavailability (RBV ≈21–74% of ferrous sulfate). - Requires higher inclusion levels to deliver equivalent absorbed iron. - Poor solubility in water and dilute acid.	Technologically stable but nutritionally modest; widely used when sensory protection outweighs need for high absorption. *Micronised:* Balanced option combining sensory stability with moderate nutritional improvement; used in high-value dairy applications (e.g., Japan).
Sodium iron EDTA (NaFeEDTA) (21,22)	- Has higher bioavailability than ferrous sulfate, especially in high phytate foods - Stable and effective in cereals, sauces, condiments.	- Not suitable for dairy due to off-flavours. - Regulatory limits due to EDTA intake (ADI). - Variable results across products.	Highly effective in cereals and sauces; not appropriate for dairy or flavoured powders.
Electrolytic iron (23,24)	- Low cost. - Minimal sensory impact due to low solubility and reactivity.	- Very low bioavailability (RBV 5–20%). - Ineffective in improving iron status in many intervention studies. - Requires large addition levels, which are impractical.	Not recommended for fortification where effective iron delivery is needed.

Supplement Table 1: Continued

Iron type	Positive results	Negative results	Overall results
Ferrous ammonium phosphate (FAP) (25–27)	- Moderately bioavailable Fe^{2+} compound. - Less reactive than ferrous sulfate due to partial phosphate complexation. - Good stability in several dry food systems. - Lower likelihood of metallic taste.	- Limited global experience and fewer published trials. - Can form visible particles in some matrices. - Higher cost than ferric pyrophosphate. - Supply chain availability still developing.	Emerging alternative with moderate bioavailability and relatively low reactivity; promising for some dry food applications but requires more research and broader validation.

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SUMMARY

This thesis aimed to understand the diet and nutritional needs of populations in Côte d'Ivoire and Nigeria, reformulate and fortify food products, and evaluate their effectiveness on nutritional and health outcomes in a real-world setting through a school feeding programme. These aims were addressed through three research themes: (1) understanding population nutritional needs, by identifying nutrient inadequacies and dietary patterns among school-aged children and women of reproductive age in Côte d'Ivoire; (2) Tailoring product (re) formulation via food fortification and assessing its technological and sensory feasibility; and (3) evaluating the effectiveness of a multi-nutrient fortified food product delivered through a school feeding programme in Nigeria. Together, these studies operationalised a practical framework, using the research themes as programme pillars to align public-health nutrition goals with food-industry innovation. The integration of these pillars provides a model for how industry-led nutrition interventions can support national public health priorities within evolving food systems.

Theme 1 Understanding population nutritional needs

Chapter 2 showed that most urban Ivorian women (>50%) were overweight or obese, whereas school-aged children mostly had a healthy weight. Intake of most nutrients was adequate for both school-aged children and women of reproductive age, with notable exceptions for calcium (99% and 96% inadequacy, respectively), riboflavin (53 and 79% inadequacy) and zinc (51 and 53% inadequacy). Older children (11-12 years) experienced higher prevalence of inadequacies than younger children (6-10 years), and for women of reproductive age, the youngest age group (15-17 years) experienced higher prevalence of inadequacies than older age groups (18-49 years). No differences were found between sexes among children. Notably iron was adequate for approximately >85% of children and women, except for children 11-12 years old where slightly higher inadequacies were found (~30%). Most nutrient inadequacies showed a negative linear relationship with socio-economic status for school-aged children, where the highest inadequacies were seen in the lowest socio-economic status groups. Most school-aged children (69%) and women of reproductive age (56%) met their energy requirements. Protein intake was often below the Acceptable Macronutrient Distribution Range (AMDR) (53% children and 45% women). Ivorian nutrition policies and interventions should generally focus on improving calcium, riboflavin and zinc intake, in addition to vitamin A and folate intake specifically in older children and young women.

Chapter 3 evaluated differences in estimated nutrient inadequacy between a modelling approach (nuriR) that was used previously to identify nutrient

inadequacies globally, and traditional analytical methods (Estimated Average Requirement (EAR) cut point and probability of adequacy methods). When usual intake data were used, differences between methods were minimal (<1%). In contrast, the use of one-day dietary intake data substantially inflated inadequacy estimates. Similarly, failure to apply age-specific nutrient requirements led to larger discrepancies than the choice of analytical method. By modelling usual intake distributions using log-normal or gamma functions, nutriR produced inadequacy estimates comparable to traditional methods when appropriate intake data were available. These findings support the application of nutriR in global modelling efforts, including those used to characterise nutrient gaps across ages and sexes. However, they also highlight its limitations: nutriR does not reduce the need for high-quality usual dietary intake data, and its accuracy remains sensitive to appropriate age categorisation.

Chapter 4, characterised four dietary patterns among school-aged children and women of reproductive age. Two patterns: "Fish, oil and vegetables" and "Dairy and sweet" were highly similar and shared across groups, while a third, labelled "Poultry and citrus fruit" was broadly comparable but in women additionally included red meat and white tubers. In contrast, children additionally displayed a "Refined, red meat and vegetable" pattern whereas women showed a distinct "Low energy intake" pattern, which was least similar to each other. The fish- and dairy-based patterns, more common in Bouaké and Daloa, were associated with lower or moderate socio-demographic indicators but higher diet quality and nutrient adequacy, as well as a lower non-communicable disease risk. In contrast, the poultry pattern among children and low-intake pattern among women were characterised by poorer diet quality. Nutritional status, including overweight and obesity (49% in women and 7% in children), was not clearly explained by dietary patterns. City of residence was the strongest predictor of dietary pattern in both groups. Overall these results suggest that urban Ivorian children and women share dietary patterns consistent with early stages of the nutrition transition. Promoting familiar fish-, vegetable- and dairy based diets, alongside increased dietary diversity, may improve nutrient adequacy although additional work is required to explore causes and to design interventions for the high prevalence of overweight and obesity in women.

Theme 2 Commercial and technical feasibility through (re)formulation of two commercial products

The bench-scale trials presented in the **Appendix** assessed the commercial and technical feasibility of improving iron bioavailability by modifying the iron compound used in fortified dairy products. Encapsulated ferric pyrophosphate was the only iron compound that had similar technological and sensory attributes to

the reference; however its cost was five times higher, rendering it commercially unviable. As a result, reformulation of the flavoured dairy product available in Côte d'Ivoire was not feasible, and this product was excluded from further evaluation in Theme 3. The bench-scale trials indicated that ferrous fumarate represents a viable alternative to ferric pyrophosphate in non-flavoured dairy applications or cereal-containing formats, given its relatively similar cost price and acceptable colour and taste in short-term testing (4 weeks accelerated). However longer bench-scale trials (>4 months under ambient conditions) are needed to confirm these findings. A commercially available milk-based maize porridge containing ferrous fumarate was instead selected as an appropriate multi-micronutrient fortified product developed for Nigerian families and suitable for inclusion in the National School Feeding Programme.

Theme 3 Effectiveness of a tailored food product on nutritional need

Chapter 5 described the study protocol and statistical analysis plan for a randomised controlled trial, while **Chapter 6** presented the trial results. The study evaluated the effectiveness of a multi-nutrient fortified milk- based maize porridge in a school feeding programme in Ibadan, Nigeria. At endpoint, the high-dose group did not differ from control in haemoglobin (−0.5%, 95% CI −3.4 to 2.5) and serum ferritin (1.9%, 95% CI -6 to 10.5) in intention-to-treat analysis (n=945). Secondary outcome vitamin D concentrations were higher in the high-dose group compared to control at endpoint (3.8%, 95% CI 0.6 to 7). Vitamin A status improved by approximately 8% (4.6%, 11.5%) across all groups. Overall, the intervention did not improve iron status or anaemia but produced modest improvements in vitamin D status among Nigerian school-aged children.

The limited impact on iron outcomes of the intervention was most likely attributable to the low prevalence of iron-responsive iron deficiency anaemia in the study population. Although anaemia prevalence was high at baseline (74%), inflammation-adjusted iron deficiency affected only 9.5% of participants. The iron deficiency observed was predominately functional, with body iron stores adequate in 99.8% of participants. This profile was likely driven by a high inflammation burden (elevated AGP in 34% and CRP in 15%), high vitamin A deficiency (30%) and malaria infection (12%). In this context, iron was present, but sequestered through inflammation-mediated hepcidin regulation, limiting its availability for erythropoiesis. These findings highlight the importance of understanding the aetiology of anaemia, including the prevalence of iron-responsive deficiency, before implementing iron-based interventions.

Summary of recommendations

This thesis applied a structured framework to guide alignment between commercial food fortification and public health nutrition goals for school-aged children and women of reproductive ages in Côte d'Ivoire and Nigeria. By integrating population-level dietary data, applied product development, and clinical evaluation, the findings demonstrate that:

- Diagnostic-driven fortification is critical, as nutrient inadequacies alone do not reliably reflect population nutritional needs or predict intervention effectiveness.
- Substantial gaps remain in understanding population-specific causes of anaemia, with a large proportion unexplained in this study.
- Further research is needed on the causes and modifiers of inflammation (beyond malaria and parasites) and their role in anaemia, particularly in LMIC contexts.
- Evidence generation and application in resource-limited settings require innovative approaches to dietary assessment, fortificant bioavailability, and evaluation of market-driven fortification strategies.
- Structured coordination between public health and industry needs is needed to enable market-driven fortification to deliver meaningful public-health impact under real-world constraints.

The results of this thesis underscore the importance of diagnostic-driven fortification, implementation of feasible solutions in resource limited environments, the need to evaluate programme effectiveness, and collaborative models between public health and industry to improve population nutrition outcomes.

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They say you need a village to raise a child, and that is true of my third child, this PhD. I could not have conceived, grown and delivered this PhD without the support of my village, both near and far.

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To my parents, Donna and Trevor, thank you for the endless opportunities you gave me growing up, for instilling a strong work ethic, and for making me feel that the world was always my oyster.

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ABOUT THE AUTHOR



Katie Ayling was born on 6 February 1991 in Camperdown, Sydney, Australia, and grew up in Padstow, a suburb in south-west Sydney.

She completed a Bachelor of Science, majoring in Pharmacology and Physiology, followed by a Master of Nutrition and Dietetics at the University of Sydney. Following her studies, Katie spent over ten years working as a nutrition expert within the food industry, primarily at Nestlé and FrieslandCampina. Through these roles, she gained extensive experience in nutrition research and product development support, alongside opportunities to live and work in the UK, Kenya and Côte d'Ivoire. These experiences sparked a lasting passion for understanding and addressing the nutritional needs of populations in sub-Saharan Africa. It was also in this time, she met her Dutch husband, Steven.

Katie and Steven later moved to the Netherlands with their Ivorian dog, Ouolof. While working at FrieslandCampina, Katie continued to provide nutrition support to the development of food products for the sub-Saharan African region and became increasingly involved in research activities in the region. This included designing and implementing a dietary intake study in Côte d'Ivoire. Seeking to combine her practical experience in food product development with rigorous academic research, she began a PhD as an external candidate at Wageningen University & Research. Her doctoral research focused on the use of multi-micronutrient fortification of dairy products to address nutritional needs in the region and evaluated its potential to improve nutrient status through a randomised controlled school-feeding trial in Nigeria.

In addition to her doctoral research, Katie supervised MSc thesis students and presented her work at national and international scientific conferences. After more than a decade living and working abroad, she relocated back to Australia with her husband and their two young children. She remains deeply committed to making an impact in improving global health equity, with a particular focus on developing practical, evidence-based solutions for disadvantaged and vulnerable populations.

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline specific activities	Organizing institute (s)	Location	Year
Modelling of Habitual Dietary Intake	VLAG	Wageningen, NL	2021
Applied Statistics	VLAG	Wageningen, NL	2023
American Nutrition Society (ANS)	ANS	Online	2021
American Nutrition Society (ANS) workshop clinical trials	ANS	Online	2021
Micronutrient Forum Conference	MNF	The Hague, NL	2023
FANUS	FANUS	Dakar, SN	2023
Nutrition Science Days	NSD	Heeze, NL	2024
Mixed Linear Models	PE&RC	Wageningen, NL	2025
IUNS-INC 2025	IUNS	Paris, FR	2025
Healthy and sustainable diets: synergies and trade-offs	VLAG	Wageningen, NL	2025
Nutrition Science Days	NSD	Heeze, NL	2025

General courses	Organizing institute	City	Year
VLAG PhD week	VLAG	Baarlo, NL	2021
Scientific writing 12	WGS	Wageningen, NL	2022
Introduction to R	VLAG	Wageningen, NL	2023
Presentation skills course for materials scientists (PhD candidates), a joint initiative of 4TU.HTM and dr. Barry Fitzgerald	4TU	Wageningen, NL	2023
Reviewing a Scientific Manuscript	WGS	Wageningen, NL	2024
Philosophy and Ethics of Food Science and Technology	VLAG/WGS	Wageningen, NL	2025
Scientific publications & layout	WGS	Wageningen, NL	2025
Writing Grant Proposals	WGS	Wageningen, NL	2025
Career Assessment (New format)	WGS	Wageningen, NL	2026

Assisting in teaching and supervision activities			Year
Supervising 2 MSc students	Global Nutrition	Wageningen, NL	2023

Other activities	Organizing institute	City	Year
Preparation of research proposal	Global Nutrition	Wageningen, NL	2021
PhD study tour to Australia	Human Nutrition and Health	Melbourne/ Sydney, AU	2024
PhD presentation	Global Nutrition	Wageningen, NL	2021-2025
GCP certificate	GCP Central	Online	2021-2025

AUTHORSHIP STATEMENT

Chapter 1: I proposed the initial outline of thematic areas and refined the concepts through discussions with my supervisors. I drafted the chapter and revised it twice based on feedback from my supervisors (A. Melse-Boonstra and E F. Talsma) and L van Lee.

Chapter 2: I assumed responsibility for an ongoing project that aimed to collate available evidence on nutritional needs in Côte d'Ivoire for the FrieslandCampina Sub-Saharan Africa business. I developed the research question and study design in close collaboration with I. Bovee. Together with Professor Tiahou from the Ivorian Nutrition Society, we refined the study design and implemented the research. I conducted the statistical analyses and wrote the manuscript with feedback and input from I. Bovee, A. Melse-Boonstra and E F. Talsma.

Chapter 3: The research concept originated from a methodological question raised by A. Melse-Boonstra regarding the nutriR approach. I further defined the research question and methodology, conducted the analysis, and wrote the manuscript with input and feedback from my supervisors A. Melse-Boonstra, E F. Talsma and I. Bovee.

Chapter 4: The topic was motivated by observed differences in nutritional status between Ivorian children and women, and by ongoing work on dietary patterns within the chair group Global Nutrition under E.F. Talsma. I defined the research question, developed the methodology, performed the analysis, and wrote the manuscript with input and feedback from A. Melse-Boonstra, E F. Talsma and I. Bovee. K Maasen provided technical support on Latent Class Analysis (LCA), and M. de Jonge assisted with the initial use of LCA in the Ivorian women dataset.

Chapter 5: I wrote the initial version of the statistical analysis plan with support from L. van Lee, I.Bovee, my supervisors, and local research partners A. Orimadegun and Y. Adoulu.

Chapter 6: The concept was developed by the FrieslandCampina Global Nutrition Team and the Sub-Saharan Africa business. I served as project lead and defined the research question, designed the trial, and oversaw implementation, analysis, and manuscript writing. The research question and design were developed in collaboration with I. Bovee, my supervisors, and local research partners A. Orimadegun and Y. Adoulu. The implementation was primarily carried out by the local research team. The statistical analysis plan was developed collabora-

tively with the same partners, I. Bovee and L. van Lee. I drafted the manuscript with input and feedback from all contributors.

Chapter 7: I wrote this chapter and revised it following feedback from A. Melse-Boonstra, E.F. Talsma and L. van Lee.

Appendix: This work originated from discussions within the FrieslandCampina Global Nutrition Team and the Sub-Saharan Africa business regarding potential clinical trial concepts. After initial evaluation of two potential trials, I explored suitable study designs with C. Janssen. It was concluded that the Côte d'Ivoire concept required evaluation of alternative iron compounds, which led to the bench trials in Wageningen and Singapore. In Wageningen, I collaborated with product development (R. Greene) and a technology science student (T. Delemare) to conduct the first bench trial. Subsequent bench trials were conducted in Singapore, with nutrition support from N. Mikulic and product development contributions from S. Winarto, S. Teo, and sensory support from Y. Chen. I consolidated and interpreted all findings.

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