Fonio (*Digitaria exilis*) as a staple food in Mali: an approach to upgrade nutritional value

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Thesis

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To my beloved

husband Guillaume,

young daughter Sika,

and little son Sènami

A mes chers parents Dorothée et Louis

In memory of Romain A.M. Dossa and Lidwien van der Heijden
who played key roles from the beginning of this thesis but did not see its end

Abstract

Background

With the increasing nutritional and health problems related to the global food crisis, the potential contribution of traditional foods to alleviation of poverty, nutritional deficiencies and health issues has been emphasized. Fonio *(Digitaria exilis)* is the most ancient West African cereal representing a key crop in food supply during crop shortfall periods. Less is known about the potential of fonio to contribute to nutrition and health in West Africa. The value chain approach for nutritional goals is a set of strategies through which values are added to products for improvement of nutrition among vulnerable groups, while creating benefits for stakeholders. Strategies in a value chain approach comprise agricultural strategies, processing, and consumer-oriented actions to enhance acceptability.

Objectives

In this thesis we explored value chain strategies as possible solutions to existing nutritional problems among West African women, using fonio as product. To achieve this objective, specific research questions were investigated through the following cross-sectional studies: *i)* baseline assessment of nutrition (iron) status, iron intake and adequacy to define the nutritional context for operating the value chain approach; *ii*) assessment of socio-cultural acceptability of fonio as strategic entry point for consumer-oriented activities; *iii*) investigating processing (dephytinisation and fortification) as strategy for adding nutritional value to fonio.

Methods

Subjects involved were women of reproductive age randomly selected in Bamako, the capital city of Mali (108 women aged 15-49 y-old from 3-stage cluster sampling procedure), and Cotonou, the largest city of Benin (16 women aged 18-30 y-old from simple random sampling for an iron absorption study). Data collection included anthropometric and blood indicators measurement; dietary assessment based on a duplicate 24-h dietary recall, indirect and rapid assessment techniques; food ethnography focused on fonio, including an availability survey on market, a food consumption survey on the uses of fonio, and an acceptability survey based on a behavioral model; and an iron absorption study using stable isotopes.

Results

Investigating the nutritional context for operating value chain strategies indicated that overweight/obesity prevalence was 19%, and 25% of women were iron-deficient based on low serum ferritin concentration. Forty-six percent and 53% women were at risk of inadequate iron and overall micronutrient intake respectively. Intakes of grains, vegetables and legumes/nuts were significantly associated with iron intake (*rho* = 0.3; $P < 0.05$) but not with the probability of adequacy of iron intake. Assessing the socio-cultural acceptability of fonio as entry point for consumer-based strategies revealed that average daily frequency

consumption (68% consuming 1 to 3 times/month) and daily portion size of fonio (152 g) was relatively low as compared to other staples like rice and millet. Fonio consumption was strongly predicted by intention to consume ($r = 0.78$, $P < 0.001$), which was influenced by positive beliefs and attributes ($\beta = 0.32$, $P < 0.05$). Subjective norms, namely the opinion of the husband, the family and the neighborhood motivated intention to consume fonio ($r = 0.26$, $P < 0.001$). Perceived barriers such as time-consuming processing and lack of skills in cooking fonio had a significant interaction between intention to consume and fonio consumption ($\beta = -0.72$, $P < 0.05$). Exploring processing as strategy for adding nutritional value to fonio showed that dephytinisation with intrinsic wheat phytase reduced phytate-to-iron molar ratio from 23.7:1 to 2.7:1 and iron fortification decreased the molar ratio to 0.3:1. Dephytinisation with wheat phytase and fortification significantly increased iron absorption from 2.6% to 8.2% in fonio porridges.

Conclusions and recommendations

Dephytinisation with native wheat phytase and iron fortification appeared relevant for adding nutritional value to fonio. Nonetheless, the achievement in iron absorption might not be sufficient to consider fonio as an appropriate food for improving iron status through iron fortification. However, as staple food contributing to food security, consumer-oriented activities for enhancing fonio consumption should emphasize positive attitudes and opinions of men, family and neighbors, while strengthening skills of women in cooking good quality fonio meals. For the value chain approach to be relevant, the impact of value-added fonio products on smallholders' income should be assessed, as well as the effect of the improved income on the nutritional outcome of vulnerable communities.

Content

Chapter 1

General Introduction

BACKGROUND

Fonio, a traditional cereal with potential in Sub-Saharan Africa

For decades, food security in developing communities has been dependent on traditional food products locally produced and consumed in identifiable geographical areas (1). In sub-Saharan Africa, traditional grains encompass white maize (*Zea mays*), sorghum (*Sorghum bicolor*), millets (*Pennisetum sp*), fonio (*Digitaria sp)*, teff (*Eragrostis tef*), and also amaranth seeds (*Amaranthus caudatus*) (2).

Fonio is the most ancient traditional cereal cultivated across West African dry savannah regions along the Sudanese zone (3). Particularly white fonio *(Digitaria exilis)* is primarily grown in Guinea, Mali, Burkina Faso, Ivory Coast, Nigeria and Benin, in marginal, mountainous and hilly zones with sandy, poor and degraded soils, without fertilizers and pesticides (4). In 2009, about 460 685 tons have been produced on 519 846 hectares in western Africa (5). In Mali, Burkina Faso and Guinea semiarid and subhumid areas, fonio contributes to 17-21 % of staple cereals requirement (6)

Due to its large ecological adaptability and the relatively short crop cycle of some varieties, fonio is believed to have a high potential as key-crop in agriculture and food supply in its traditional cultivation zones, particularly when annual harvests fail or during local crop shortfall periods (2, 3). In West African semi-arid areas, fonio is the major part of the diet during harvest periods, and in sub-humid zones it is stored during the post-harvest period, for use as food supply during the food shortage periods (6).

Although accounting for only about 10% of the global aggregate cereal output, the contribution of fonio and other traditional grains to food requirement is significant in many developing countries, for they are subsistence-oriented food staples (2). They may have high nutritional value, with some potential for combating the 'hidden hunger' caused by micronutrient deficiencies. Beside their importance to food supplies and health, traditional grains as fonio can also contribute to improved income among the most food-insecure population groups (2).

Challenges facing fonio as a traditional food crop in Sub-Saharan Africa

Major constraints reported in fonio cultivation and harvesting are related to the small size of the grain, showing tiny kernels of 700 µm size (7). This leads to tedious and time-consuming post-harvest and cooking processes (3, 8, 9). As most traditional cereals, fonio is produced in traditional systems with little or no external inputs (4). Most activities are manually performed, making post-harvest activities more laborious and time-consuming (8). Meanwhile, urbanization and related changes leading to an increased demand of industrially produced and sophisticated foods induced a rapid shift of coarse grains to non-traditional grains (10, 11). This resulted in a decrease in consumption of traditional foods, particularly in urban areas. In addition, there has been an increasing inadequacy in research investment between the need to follow-up these substantial lifestyles changes occurring in developing areas and the need to improve the production and processing chains of the traditional grains (12). This led to a turn of farmers to the cultivation of exportable cash crops (13). Therefore, the productivity, output and harvested areas of traditional food crops such as fonio appeared to be reducing over the past years in several West African countries (12, 13).

Subsequently, fonio has been undervalued by decision makers, receiving decreased attention. Hence there has been a lack of improved agronomic practices, little efforts for agricultural research, low post-harvest promotion including losses, inadequate marketing channels and processing technologies, poor product quality, and no up-to-date information to upgrade the quality of the grain (12, 13). As a consequence, fonio has been classified as “lost crop of Africa”, “minor” or “neglected” crop without economic potential (3).

Re-considering the role of fonio in strategies against the global food crisis

With the global food crisis, trends in malnutrition, household food security and poverty have been worse, especially in Sub-Saharan Africa, and the need for an accelerated reduction of malnutrition and poverty has been emphasized (14). Evidence of the potential micronutrient-value of some underutilized species has been reported along with information on ways to consider those available biological food resources in strategies to reduce malnutrition (15, 16). Therefore, national and international research and development institutions have come to appreciate the roles that traditional grains like fonio can play for nutrition and food security. Subsequently, many neglected species and varieties have been reconsidered to become potential vehicles for improved nutrition, as well as convenient sources of increased food supply and income (15, 16). Hence, a renewed interest in fonio is growing, especially in West African urban areas (17).

During the past decade, national agricultural research centers of the main producing West-African countries have collected and conserved important germplasm of fonio genetic resources (18). Moreover, in the perspective of defining appropriate strategies for conservation of fonio on farm, information has been produced on ethnobotanical and indigenous knowledge related to fonio production, diversity, use and conservation, genome content and size of the crop as well as its genetic diversity (18-20). To improve fonio processing and cooking techniques, modern and new equipments have been developed at small companies and women's groups in West Africa (8). Also, modern small-scale processing industries for ready-to-cook products have been developed in urban areas and modern fonio-based recipes have been experienced (8). However, less effort has been made to document and improve the potential of fonio to contribute to nutritional and health-related issues in West Africa.

Fonio is the most ancient West African traditional cereal, with a large ecological adaptability. As staple grain for the food shortage periods, there is a need to reconsider the potential of fonio to contribute to nutrition and food security among vulnerable groups of West African communities.

Fonio IP6 project: upgrading quality and competitiveness of fonio for improved livelihoods in West Africa

In order to explore properties of fonio as a potential healthy and nutritive food for urban areas in Sub-Saharan Africa and European countries, while generating income for local producers, the interdisciplinary project FONIO "*Upgrading quality and competitiveness of fonio for improved livelihoods in West Africa*" was launched in 2006 for 3 years. FONIO is a collaborative research project funded by the EU Specific International Scientific Cooperation Activities (INCO). It involved research institutions from 3 European countries (France, Netherlands and Belgium) and 4 West African countries (Mali, Guinea, Burkina Faso and Senegal). The main goal of the project was to develop research on production, post-harvest and food technology for fonio products of high nutritional value, good sensory quality and commercial value (21). The FONIO project focused on 3 strategies: *i*) development of farming and cropping systems to increase fonio production as well as the value of various uses -grain, straw and by-products as animal feed, and improving soil fertility-, for creating opportunities to improve the livelihoods of producers; *ii*) investigating the nutritional quality and improving the nutritional value of fonio products for adding substantial health/nutrition value to fonio products to contribute to better nutrition and health; *iii*) investigating processing and marketing systems (women's association, small and medium enterprises) in order to facilitate economic access to local communities, and promote the development of export markets for improved/new fonio products (21). The main expected outputs of the FONIO project were to alleviate (hidden) hunger and poverty burden among women who are involved in the fonio production chain as producers, processors or traders; and to improve local living standards through a sustainable development of the fonio commodity chain from rural zones to urban or export markets. A secondary expected output was to maintain fonio cultivation and so contributing to the conservation of natural local resources and preservation of the local biodiversity (21).

The FONIO project has been initiated with the main goal to develop research on production, post-harvest and food technology in order to arrive at fonio products of high nutritional value, good sensory quality and commercial value.

IMPROVING FONIO VALUE FOR BETTER NUTRITION IN WEST AFRICA

The value chain approach for nutrition goals

The value chain approach is a supply chain along which values are added to a product, from production of the raw food to the final product. Series of activities and actors are involved in this process. The objective of the value-chain approach is to obtain a product with improved affordability, availability, acceptability and quality, in order to increase food security and diet quality (22). Value chain approaches for nutrition goals in resource poor settings mainly aim to address the lack of access to, and the low intake of high-quality foods and balanced diets, being the key underlying determinants of nutritional problems in developing countries (22). Therefore, a set of nutrition-oriented strategies are initiated to improve the nutrition value of the foods that do or potentially can contribute consistently to the daily intake of a wide range of nutrients. In general, these strategies involve different sectors. Their perspectives are to create long-term, sustainable benefits for food security and livelihoods, while improving the nutrient quality and intake of the upgraded products. As such, nutritional outcomes can be enhanced among vulnerable groups (22).

For the present research, implementing the value chain approach for nutritional purposes would target the need to set fonio as a key crop in development policy, by adding substantial and consistent (nutrition/health) value to fonio products for West African vulnerable groups for whom fonio is part of the diet (13). The value chain approach should primarily focus on women as vulnerable group, for they contribute to more than 50% of production, processing and marketing activities of fonio, as well as cooking in the households (18, 23). Thus, targeting women for nutrient- improved and healthy fonio products would be a way to guarantee a healthy consumption pattern for the households consuming fonio products. Improving the value of fonio products for the consumers, would bring forward the benefits of fonio as a valuable food that can help with their health problems, hence resulting in a renewed interest and increased demand of fonio. This added value would also lead to a better market value for fonio (13, 23). Furthermore, the increased market value and demand for fonio products will encourage farmers to allow larger cropping areas for fonio and boost fonio production, resulting in an improvement of income and living conditions of local producers and processers (13, 23).

Strategies to add nutritional value to foods through the value chain approach include *i)* agricultural strategies to improve the nutrient content of foods, *ii*) food processing to improve the level and uptake of nutrient from foods, and *iii*) improving the acceptability of the food with consumer-oriented strategies (**Figure 1**) (22). In the next sections, these strategies are explained in the global context of the value chain approach for nutritional goals, together with some propositions for their operationalization with fonio as staple food. Prior to this description, the nutritional context (defined by existing food consumption patterns and nutritional problems) in which these strategies will be applied needs to be considered, since the primary goal in improving the nutritional value of fonio is to achieve enhanced nutritional outcomes.

Adding nutritional value to fonio through a value chain approach can be a relevant nutrition research goal for improving household food security and nutritional/health status of West African women.

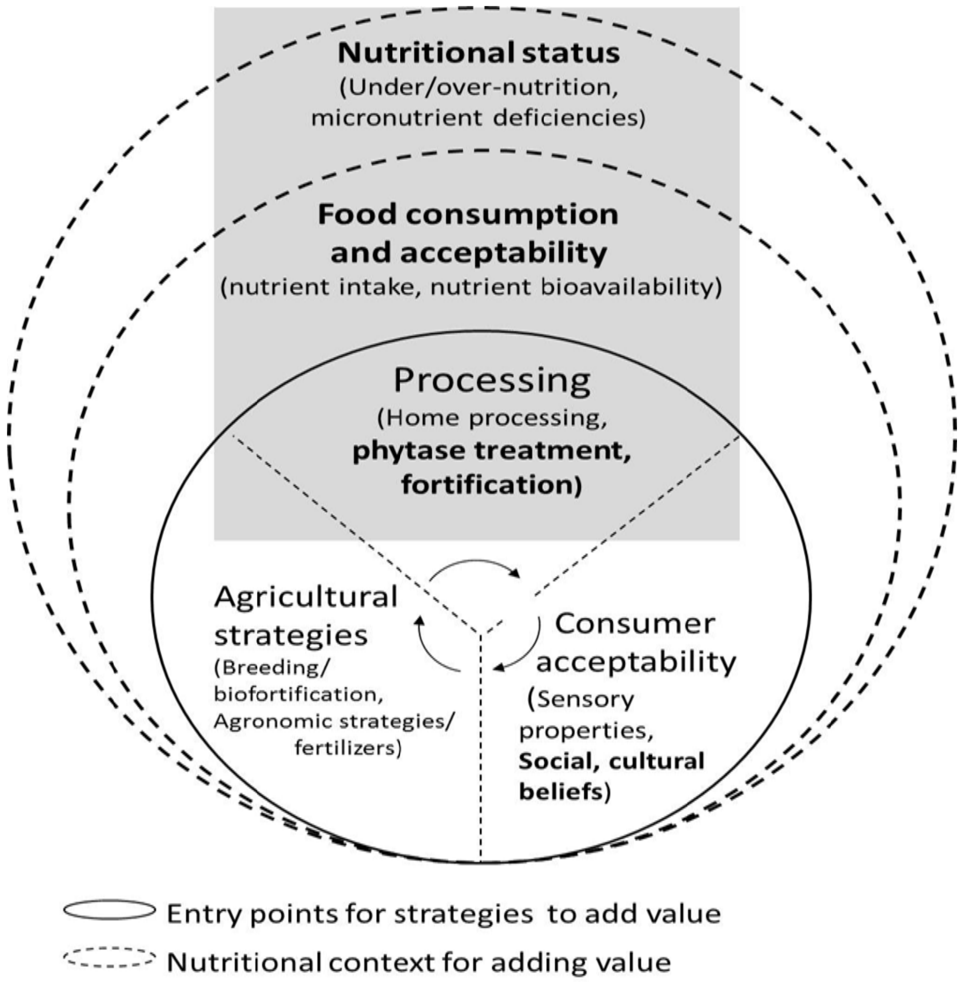

Figure 1: Conceptual framework for adding value to a product: the value chain approach for nutritional goals. Adapted from Hawkes and Ruel (22)

Nutritional disorders among women of reproductive age in West Africa

Anemia and nutritional iron deficiency

Anemia defined as low haemoglobin (Hb) concentration (24) is the most common physiological disorder in developing countries (25). A decrease of Hb concentration below the cut-off of <12 g/dL indicates overall anemia among women of reproductive age (26). A prevalence of anemia > 5% among vulnerable groups defines anemia as a public health problem (27). Anemia is of public health importance among women of reproductive age because of high requirements and losses during youth, pregnancy and lactation (25). In Africa, the prevalence of anemia among non-pregnant women is 44% (27). In West Africa, 54% of the women aged 15-49y living in urban areas in Mali are anemic (28).

Iron is the major component of Hb. As iron cannot be excreted by the body, its concentration is regulated by the iron absorbed from the diet (29). Nutritional iron deficiency (ID) occurs in case of failures to meet physiological requirements from dietary iron (30). Three stages of nutritional ID are usually detected: depleted iron stores, iron deficient erythropoiesis and iron deficiency anemia (IDA) (26). The first stage of ID is defined by a progressive decrease of the iron stored in the liver as ferritin and haemosiderin. At this stage, the supply of iron to the body is not compromised and the concentration of circulating iron is normal. However the progressive depletion of iron stores is reflected by a fall in serum ferritin (SF) concentration (26, 29). Referred to as iron deficiency without anemia, the iron-deficient erythropoiesis stage is characterized by an exhaustion of iron stores. The iron supply to erythropoietic cells progressively decreases, as does the erythrocyte protoporphyrin concentration, with an increase in serum transferrin receptors (sTfR) concentration. Hb may decline slightly at this stage, but the concentration usually remains within the normal ranges (26, 29). The third stage of ID is characterized by a total exhaustion of iron stores and declining levels of circulating iron. The main feature is anemia defined as a reduction in Hb concentration in the red blood cells, arising from the restriction of iron supply to the bone narrow (26, 29).

Related to the 3 stages of ID development, useful indicators for differentiating nutritional IDA from overall anemia are SF and sTfR concentrations (27, 31, 32). SF is the only measure of iron that can reflect a deficient, excess or normal iron status. A decline in SF below the cut-off of 12 µg/L reflects iron depletion for women of reproductive age. Combined with low Hb levels, decreased SF concentration indicates IDA (26). However, SF is an acute phase protein that may be elevated in inflammatory conditions, resulting in misleading high ferritin concentrations (33). To control for the effect of inflammatory conditions, SF is usually associated with the measurement of C-reactive protein (CRP) and alpha-1 glycoprotein (AGP) (26, 34). CRP is the commonly used parameter for acute infections, while AGP is used to detect chronic infections (26). In situations where infections highly influence SF levels, sTfR may be used to detect true IDA. sTfR are iron-related proteins that regulate the uptake of tissue iron to body cells (31, 32). They are much less influenced by ongoing inflammatory processes. The expression of sTfR at body cells surface is proportional to the requirement of iron (26, 29). Cutoff values of > 8.5 mg/L (depending on the analysis method used) associated with low SF and Hb concentration indicate true IDA among women of reproductive age (26).

Nutritional ID continues to be the most common single micronutrient deficiency of public health importance in West Africa (30). It has been estimated that half of the prevalence of overall anemia among women of reproductive is related to nutritional ID (25, 27). As in most developing countries, Hb has been the only one indicator used to estimate ID in West Africa (28) because of the low-cost and simplicity of the measurement (35). However its specificity is low (36) especially in developing countries where infections rates are still high (37, 38). SF and sTFR are more specific and accurate in detecting nutritional IDA (31, 32, 35, 39) but they are scarcely used because of their high costs and the lack of accurate measurement devices in developing countries (30). This results in a failure to differentiate other causes of anemia from nutritional ID, the prevalence of the latter being an indicator of the effectiveness of interventions for reducing ID in developing countries (29, 40). For instance, iron fortification has been recommended for groups at risk of ID in developing countries (40-42) but to date, it has been difficult to assess the effectiveness of iron fortification programs in these countries (30). Therefore, an adequate assessment of iron status is essential for a suitable evaluation of the effectiveness of iron fortification (29). Particularly for women of reproductive age in West Africa, there is a need to document their iron status using accurate biochemical indicators in order to design and monitor appropriate programs for alleviating the burden of ID among them.

ID and weight status

Beside chronic energy deficiency and micronutrient deficiencies, overweight and obesity have become an emerging public health concern in developing countries during the past decade (43-48). Urban communities are the most at risk of overweight and obesity because of the transition in the habitual diet, associated with a sedentary lifestyle defined by decreased physical activity due to the sedentary nature of work activities, changing modes of transportation, and increasing urbanization (10, 45, 48). Prevalences of overweight ranging from 10 to 70% have been reported for women living in urban areas in developing countries (49). In Bamako, the prevalence of overweight and obesity increased from 10% in 2001 to 31% in 2006 (30, 48). Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health (48, 50). Increased BMI is a key determinant of cardiovascular diseases, diabetes, musculoskeletal disorders and some cancers (48).

Recently, significant correlations between iron biomarkers, fat mass and BMI have been reported, and it has been suggested that excess adiposity may negatively affect iron status (51, 52). The mechanisms explaining reduced iron status in obese individuals have not been clearly determined. Assumptions about this correlation include a possible lower iron intake from poor-quality diets (53). However, it has been demonstrated that dietary iron intakes of normal-weight individuals was not significantly higher than iron intakes of overweight adults (51). Another hypothesis is related to the function of hepcidin, an hormone secreted by the liver (54). Hepcidin expression is induced by inflammation (54, 55). In obese individuals, the chronic adipose tissue inflammation associated with obesity (56, 57), increases the secretion of hepcidin (58, 59). In the presence of hepcidin, iron absorption from the gastrointestinal route is blocked whereas its uptake by macrophages increased (54, 55). This could reduce dietary iron absorption (60, 61). Higher circulating hepcidin concentrations have been associated with lower iron status in overweight children in industrialized countries (60). Adiposity have been reported to predict lower iron absorption among young women in emerging countries undergoing nutrition transition (62).

Despite this evidence, the association between ID and weight status has not been investigated in developing countries. Both ID and obesity result in overall poor health conditions and are associated with increased mortality risk (48, 50). Particularly in urban areas of developing countries where lifestyles are changing at a rapid pace (48), there is a need to document ID in association with the increasing weight status among women of reproductive who are the most at risk of these nutritional comorbidities.

West African women of reproductive age living in urban areas are at double risk of iron deficiency and overweight/obesity. Estimates of prevalence of iron deficiency using specific iron biomarkers and their association with weight status are insufficiently documented.

Nutrient adequacy among women of reproductive age in West Africa

Nutrient intake and adequacy

Like in many developing countries, low intake of micronutrients from poor diets is the major cause of micronutrient deficiencies in West Africa. Habitual traditional West African diets are monotonous, mainly based on grains, roots and tubers. Main protein sources are plant-based. Animal source foods are consumed in insufficient amounts (41, 63, 64). Iron bioavailability and hence uptake from these diets is generally low (65-68). Low iron bioavailability from habitual plant-based diets has been associated with ID in rural African children (69).

In recent years, traditional diets have progressively turned to transitional diets in urban areas, as a consequence of the increasing urbanization process observed in most of West African cities. These diets are often described as rich in saturated fats and based on highly processed energy-dense foods with low levels of micronutrients (45, 70). Thus, beside the increasing exposure to chronic diseases, communities living in West African urban areas are also at risk of low iron and other micronutrients intake from the diet (45, 48).

Despite this evidence, micronutrient intake among women of reproductive age living in such settings, as well as its association with the diet has been documented in very few West African countries (71-74). Particularly among women living in urban areas facing the double risk of overweight and ID, there is a need to document the extent of the risk of nutrient inadequacy from their diet in comparison to the dietary recommendations. Diet diversification/modification has been emphasized as an approach to ensure adequate iron intake from diets in developing countries (75-79). However, as in a number of West African countries, there is a lack of systematic data reporting on diet and food group intake of women in Mali. Especially in urban areas where traditional and transitional diets are consumed simultaneously, foods and food groups contributing substantially to nutrient intakes and adequacy need to be explored as potential for increasing the variety and the diversity of the diets.

Assessing dietary intake among women of reproductive age in West Africa

The 24-h dietary recall (24hDR) has been developed to fill the need of an accurate cost-effective, and non-invasive dietary assessment tool, with the ability to quantify daily amounts, types of food, and intakes of micronutrient (77, 80). The 24hDR in national or international surveys allow comparison of data on the dietary pattern of heterogeneous populations (77, 80, 81). A number of studies have adjusted or computerized this method for assessing dietary intake among different groups in industrialized countries (82-87). In developing countries, the 24hDR has been adapted to the context of low literacy rate by training interviewers to probe respondents and help them recall foods and amounts consumed (88). However, as most self-reported dietary assessment methods, the 24hDR is marked by the bias of food intake misreporting (80, 88) which along with the day-to-day variation in individual diets may lead to under or over estimation of the intake, limiting the precision of prevalence of inadequate intake estimates (89, 90). Especially in West Africa human development indexes are the lowest compared to other African regions, and illiteracy rate is still high among women (91). In addition traditional dietary practices such as shared plate-eating (many household members sharing meals in a same plate) are still common (74), even in urban areas, challenging the precision of diet assessment. There is a need to report updated information on the day-to-day variation from the diet in such settings. This will contribute to an accurate assessment of their usual intake, thus decreasing the risk of misleading estimation of prevalence of high or low intakes (92, 93).

Low intake and absorption of iron from the diet is a major cause of the nutritional iron deficiency among women of reproductive age in West African urban areas. However, information on nutrient intake from urban diet patterns and its association with the diet diversity still need to be documented, along with the ability of the diet assessment method to report accurate information on the dietary intake.

Strategies to add nutritional value to products in a value chain approach

Strategies to be implemented for upgrading the nutritional value of fonio within the above described nutritional context comprise agricultural strategies, processing and improvement of acceptability. In the following, the feasibility of these strategies as well as factors to be influenced to increase acceptability of fonio are described.

Agricultural strategies

Agricultural strategies are applied at production level of the value chain. They contribute to increasing the micronutrients content in staple foods through agronomic strategies, breeding or genetic engineering (94, 95). For instance, biofortification can be explored as a breeding technique to produce new varieties with higher concentrations in iron and zinc and a higher grain yield than the traditional landraces of traditional crop such as fonio. This aspect is not further addressed as it goes beyond the scope of the present research.

Processing

Moving forward in the value chain, upgrading nutritional quality of foods can be achieved through processing, including home processing and fortification. Home processing is mainly used to improve retention of micronutrients or reduce the concentration of phytate (96), present in most plant-based foods and binding minerals into non bioavailable complexes (66, 68). Home processing encompasses practices like soaking, malting, cooking, germination, fermentation (79, 97-108); or parboiling, particularly for cereals, to enhance nutrient content yield at milling (109-111). Another, relatively new, approach is the use of natural phytase of plant-based foods or microbial phytase treatment to reduce phytate level (112-114).

Adding nutritional value through processing can also be achieved by fortification, which is the addition of minerals and/or vitamins to a food vehicle with the goal of increasing their concentration in the fortified product (41). Selected food vehicles for fortification are either staple foods consumed on a regular basis and in relatively consistent amounts by a large share of vulnerable population, or products with specific value for specific groups of the population, like indigenous foods (41).

Consumer acceptability

Activities aiming to upgrade nutritional value of a food can be effective only if the latter is accepted by the targeted vulnerable group of the community. Consumer-oriented strategies within value-chain approaches are meant to satisfy this condition. They comprise a set of community-based activities intended to stimulate triggers influencing the consumers' acceptability for a value-added product (115). They usually include nutrition education activities at consumers' level, and policies influencing activities targeting decision makers (22).

Processing and investigating fonio acceptability may be key entry points for adding value to fonio products in a value chain approach with nutrition goals targeting women in West Africa.

Strategic entry points for adding nutritional value to fonio

Acceptability of fonio

The acceptability and utilisation of foods in societies depend upon four main internal and external factors regulated by cognitive processes (115). These factors are related to: - information obtained from observing, handling, and consuming the food, - information acquired from the surrounding social and cultural context; - information gained from the physiological effects (pleasure, satiety, dislike, discomfort) experienced when eating and after eating a certain food; and - comparison with information stored in the memory of past experiences (115). The Theory of Planned Behavior (116) is one of the most predictive persuasion theories applied to various fields interested in the relationships between beliefs, attitudes, behavioural intentions and behaviour. For nutritional goals, the TBP has been used to measure the social acceptability of foods as the effect of internal and external factors on the intention to consume these foods (117-119). For health related purposes in nutrition, the TBP has been combined with the Health Belief Model (120, 121). This combined model integrates individual beliefs, social beliefs and perceived barriers related to the use of the product, as well as health and nutritional outcomes related to the decision to use or not to use the product (120, 121).

Previous investigations on the dietary role of fonio in West African societies reported that the grain was essentially cultivated for home consumption in rural areas (3, 4, 6, 122). The average individual amount consumed was 4.4 kg/year in Mali (123) with a range of 650 to 840 g/year in urban areas (124). Fonio is consumed by every age group, as couscous or as porridges made from grain or flour (122), but the consumption of fonio porridge as infant food has not been reported.

Reported beliefs and factors related to the uses of fonio in West African communities showed that the dietary role of the grain is interrelated with religious and sociocultural beliefs and uses (3, 4, 8, 123, 124). It is often reported to be the tastiest, nutritious and easy to digest cereal. For long time, fonio has been reserved particularly for chiefs, royalty, and special occasions like Ramadan for Muslims (3, 4, 123). For some rural communities, fonio is the most important food ingredient in traditional and religious events (4). In some communities, fonio is believed to help prevent blood clotting after women give birth (4, 123), and fonio porridge is recommended for breastfeeding women to stimulate milk production (4, 123). The difficult post-harvest processing, the time-consuming cooking process, and the high-cost of fonio products compared to other traditional cereals are often reported as limitation factors regarding the consumption of fonio as daily cereal (3, 4, 8, 123, 124).

However, this evidence is mostly based on anecdotal information reported in descriptive studies and so far, no systematic approach has been used to show their actual effect on the consumption of fonio. Particularly in urban areas facing rapid shifts to transitional diets (10, 45), other non-identified important socio-cultural and cognitive determinants, as well as factors related to the nutritional quality of the grain may influence its consumption. To assess the acceptability of fonio in West African urban areas, those factors, as well as their interrelationships with existing determinants and their influence on the consumption of fonio, need to be appropriately documented using behavioral models. Documenting the acceptability of fonio in West urban areas is an essential step for bringing forward the necessity to add nutritional value to fonio products.

Processing: Phytate degradation and fortification

The food composition table of Mali (TACAM) reported an iron content of 8.5 mg/100g dry weight for the husked raw grain of fonio, being higher than other cereals, except for sorghum (125). However it has been recently shown that after home processing, the iron concentration of fonio is rather low, being 0.8 to 1.8 mg/100g dry weight (126). The grains are also reported to have high brewing and malting potentials (127, 128) and the potential of fonio for traditional and technological processing have been reported (129). However, as most plant-based foods, fonio grains contain phytic acid that forms complexes with iron, and thus reducing its bioavailability (66, 68). A recent investigation on phytate content in fonio reported a concentration of 123 mg/100g dry weight after cooking (126), indicating that the phytate-to-iron molar ratio from fonio products is much greater than the optimal level of <0.4:1 required to achieve a significant increase in iron absorption (130, 131). Adding value to fonio could therefore only be achieved by increasing both the iron content and bioavailability in fonio products, hence a better contribution of fonio-based diets to the daily intake of iron can be expected.

Iron fortification of staple foods has been recommended as a strategic processing option to increase the content of available iron in foods (30, 41). Water soluble ferrous sulfate, is the suitable iron fortificant in developing countries as it is cheaper and shows similar bioavailability as native iron, compared to poorly water soluble and insoluble compounds. Also, at a low fortification level, sensory properties alteration of the fortified foods led by ferrous sulfate can be reduced (30, 41, 42). Recently, Zimmermann et al (132) reported that iron absorption from ferrous sulfate was well up-regulated in iron deficiency, and emphasized its use for low-level fortification in iron-deficient populations (132). However, iron absorption from highly bioavailable iron compounds can be inhibited by phytic acid in cereals-based foods (65, 133, 134). Therefore, reducing phytate level is essential to improve iron absorption from iron-fortified foods (133).

Phytic acid can be degraded by adding microbial phytase to foods (112-114, 135-138), or by enhancing the activity of the intrinsic phytase present in a large range of plant-based foods (112, 114, 137, 139-145). Using home processing practices, Egli et al. (146) showed that adding wheat to cereal-based foods mixtures completely degraded phytate at optimal incubation conditions. The efficacy of this technique in improving iron absorption from staple cereals needs to be demonstrated in humans but up till now, evidence has not been reported.

Although iron fortification has been shown to be potentially cost-effective for West African countries, very few efficacy trials are being implemented to bring forward evidence of the positive effects of iron fortification. In Mali, fonio is a traditional staple cereal for some communities in rural as well as in urban areas. Increasing the content of iron using ferrous sulfate as a cheap iron compound after reducing the concentration of phytate in fonio products is a strategy to be investigated as entry point for adding value to fonio products through a value chain approach. An iron absorption study with iron-fortified fonio products is an essential exploratory step towards enhancing the contribution of fonio to iron intake of women living in urban areas.

A number of outstanding research questions need to be addressed in order to achieve nutritional value-added fonio products using acceptability and home processing/fortification as entry points in a value chain approach

RATIONALE AND OBJECTIVES

Fonio is the most ancient West African traditional cereal having a large ecological adaptability. In some communities, fonio is a staple grain particularly during the food shortage periods. As such, its potential for contributing to food security in West African communities, while alleviating nutritional deficiencies among vulnerable groups has been reconsidered. The FONIO project was initiated with the vision to develop fonio products of high nutritional value, good sensory quality and commercial value. The value chain approach for nutritional goals is a set of strategies that can be explored for upgrading the nutritional value of fonio products. Particularly for West African women living in urban areas facing the double risk of iron deficiency and overweight/obesity, adding nutritional value to fonio products can be relevant for improving their nutritional/health status while enhancing household food security. Processing and investigating fonio acceptability may be key entry points of a value chain approach for adding nutritional value to fonio products. However, a number of outstanding research questions need to be addressed before achieving this.
As part of the FONIO project, the main objective of the present research was to upgrade the nutritional value of fonio through home processing and fortification. To achieve this objective, some specific research questions are addressed through the chapters of the thesis.

Chapter 2 addresses the need of defining the nutritional context for operating the value chain approach by providing updated and specific data on nutritional problems faced by West African women. Results of a baseline assessment of nutrition (iron) status among a sample of Malian women of reproductive age are presented, with as scientific objective to investigate the association between iron deficiency and weight status. In **chapter 3**, the intake and adequacy of iron and other micronutrients are examined among the same sample of women, in association with the intake of food groups from their diets. Given that a 24-h dietary recall was designed for the purposes of the research, the ability of the method to provide estimates of day-to-day variation in intake was discussed, with implications for assessing usual intake from Malian diets. This study is described in **chapter 4.**

Chapter 5 focussed on the importance of fonio in urban areas, by describing the role of the grain in urban diet patterns, as well as the beliefs, attributes and perceived barriers related to its consumption. The influence of these factors on the intention to consume fonio was investigated in **chapter 6** to document the acceptability of fonio in urban areas.

Chapter 7 presents an iron absorption study investigating the effect of degrading phytate in fonio with intrinsic wheat phytase on iron absorption from iron-fortified fonio products in a sample of Beninese young women.

Main findings, as well as methodological strengths and limitations are discussed in **chapter 8** along with implication for future research and practice.

The women involved in our research were randomly selected in Bamako and Cotonou, the largest cities of Mali and Benin (West Africa) respectively (**Figure 2**). The population size of the two cities were 1 690 471 (Bamako) and 862 445 inhabitants (Cotonou) of which more than 50% are female (147).

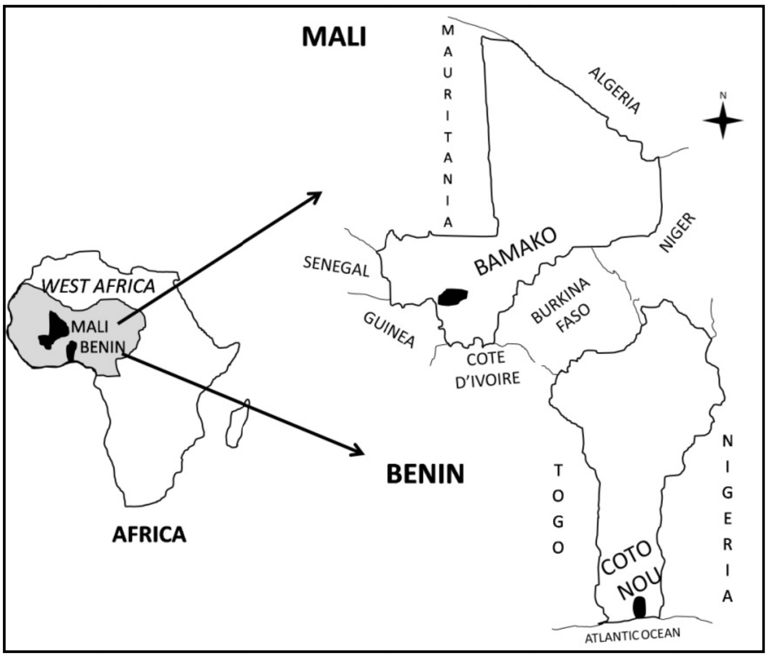

Figure 2. Maps of Benin and Mali in West Africa showing study sites

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Chapter 2

Weight status and iron deficiency among urban Malian women of reproductive age

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Abstract

The present study investigated the association between weight status and Fe deficiency (ID) among urban Malian women of reproductive age. Height, weight, serum ferritin (SF), soluble transferrin receptor (sTfR) and C-reactive protein (CRP) concentrations were measured in sixty apparently healthy women aged 15–49 years old in Bamako, Mali. Prevalences of overweight and obese were 19 and 9%, respectively. SF was non-significantly different between overweight (84 µg/l) and normal-weight women (52 µg/l). The prevalence of ID (SF < 12 µg/l) was 9% in the overweight group and no true ID (sTfR > 8.3 mg/l) cases were recorded in the overweight and obese groups. The prevalence OR of ID (SF < 12 µg/l) in the overweight group was NS (OR = 0.3; *P* = 0.363). Conversely, the chronic energy deficiency group was at a significantly higher risk of ID than the normal-weight group, adjusting or not for CRP (OR = 7.7; 95%CI 1.49, 39.96; *P* = 0.015). The lack of association between overweight and ID in the present study could be due to the fact that the excess of body fat of the women might not be critical to induce chronic inflammation related to reduced Fe absorption. Future research based on a larger convenience sample should be designed to further investigate associations between overweight, obesity and ID in developing countries.

Introduction

Fe deficiency (ID) continues to be the most prevalent single micronutrient deficiency among women of reproductive age in Mali, West Africa (1). In the meantime, obesity has become an emerging public health concern in developing countries, especially in urban communities (2, 3). In Bamako, 10 % of women were overweight in 2001 (4). Recently, significant correlations between serum Fe, soluble transferrin receptors (sTfR), fat mass and BMI have been reported, and it was suggested that excess adiposity may negatively affect Fe (5-8) status. Both ID and obesity increase the risk of overall poor health and are associated with increased risk of mortality (1, 9) but definitive mechanisms explaining low Fe status in the obese have not been clearly determined (10). The association between Fe status and obesity should be explored further, as obesity and ID continue to evolve worldwide, and the combined impact of these nutritional comorbidities is unknown (11). Because very few studies have investigated the association between ID and weight status in a developing context, exploratory research is a necessary step to bring forward consistent conclusions about relationships between ID and overweight/obesity. The present study examined the association between weight status and ID among women of reproductive age in urban Mali, using part of the data originally collected for a larger research project named FONIO (EU/INCO no. 0015403). (Fonio is the name of a West African traditional cereal). Although the research was not powered to study the relationship between weight status and ID, results were found to be interesting to publish in a research communication – as data on developing countries are still lacking – and to generate points of discussion about the interaction between obesity and Fe status in developing countries for further research. We hypothesised that overweight women have a higher risk of ID than normal-weight women, irrespective of Fe intake.

Subjects and methods

For the purpose of the FONIO project, 108 Malian women aged 15–49 years old were randomly selected from 108 households of Bamako, using a three-stage cluster sampling (12) and the random walk method (13). The sampling procedure has been fully described elsewhere (14). For the present study, a sub-sample of sixty women was selected from the main sample of the FONIO project, based on their willingness to participate in anthropometry and blood sample collection. Due to the small sample size, statistical power of the results is limited. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethical committee of the Malian National Institute of Research in Public Health (Mali). Verbal consent was witnessed and a written informed consent was obtained from all the participants before data collection. All the data were collected over a period of 3 months.

Anthropometry

Body weight and height were measured early in the morning, from fasting subjects according to WHO standardised procedures (15). Weight was measured with a SECA Model 761 platform spring balance scale with a 150 kg maximum range and 0.5 kg graduation (Seca 761; Seca, Hamburg, Germany). Height was measured with a body measuring tape with 2200 mm maximum range and 1 mm graduation. BMI (kg/m^2) was calculated from anthropometric data and subjects were divided into four groups: chronic energy deficiency (CED) defined as BMI < 18.5 kg/m^2, normal weight defined as 18.5 ≤ BMI ≤ 24.9 kg/m^2, overweight defined as 25 ≤ BMI<30 kg/m^2 and obesity defined as BMI ≥30 kg/m^2 (16).

Blood samples and biochemical measurements

Whole blood was collected by venepuncture from overnight fasting subjects in K2 EDTA tubes (2 ml) for Hb, and in normal tubes (6 ml) for sTfR, serum ferritin (SF) and C-reactive protein (CRP). Hb was measured using an automated counter (Horiba ABX Micros 60-OT16, Montpellier, France). Anaemia was defined as Hb < 120 g/l (17). SF was analysed by chemiluminescent immunoassay using the Beckman Access Immunoassay DXL System (Immulite/Ferritin, Beckman Coulter, Inc., Fullerton, CA, USA). sTfR was analysed by ELISA (Ramco, Stafford, TX, USA), using the quality-control materials supplied by the company. ID was defined as SF <12 µg/l. Because SF can be influenced by a high CRP level (18), true ID was also defined using sTfR > 8.3 mg/l (17). High sensitive CRP was measured using latex nephelometry (BNProSpec System; Dade Behring Limited, Tokyo, Japan), normal reference value: CRP >7.5 mg/l (19).

Dietary and nutrient adequacy assessment

Energy and Fe intake was assessed by two non-consecutive 24 h dietary recalls as described in Gibson & Ferguson (13). All days of the week were included in the 24 h recalls except weekends and special event days to reduce bias due to occasional consumption of foods with exceptionally high Fe or other (micronutrients) content. Foods were weighed with digital dietary scales with a 10 kg maximum range and 2 g graduation. Procedures for dietary assessment and food intake data processing have been fully described elsewhere (14). Probability of Fe adequacy was derived from the intake of the 2 recall days, assuming a bioavailability factor of 5% regarding the diet of the women. As Fe requirement distribution is not normally distributed (20), probability of Fe adequacy was determined by the intake of absorbed Fe from the Institute of Medicine table for adult women (21).

Statistical analysis

Statistics were performed using SPSS 15 for windows (SPSS, Inc., release 15.0.1.1, Chicago, IL, USA, 2007). A P-value < 0.05 was considered significant. Normality of variable distribution in BMI groups was assessed using the Shapiro-Wilk test. Mean Fe intake, SF and sTfR distributions were (log) transformed for normality. One-way ANOVA test was used for means comparison; when significant, the Tukey’s minimum significant difference was used as a post hoc test, assuming homogeneity of variances. Pearson’s χ^2 test was used for comparison of proportions. Logistic regression was performed to determine the prevalence OR of ID in overweight and CED groups using the normal-weight group as reference. The model was adjusted for CRP by excluding three cases (each from the CED, normal-weight and obese groups) with simultaneously high CRP (> 20 mg/l) and ferritin levels (> 65 mg/l), and by including CRP as a covariate in the model.

Results

The mean age of the women was 32.5 (SD 10) years. BMI ranged between 16.6 and 38.7 with a mean of 23.4 kg/m^2 (**Table 1**). Prevalences of overweight and obesity were 19 and 9%, respectively. About 16% of the women suffered from CED. Mean daily Fe intake appeared to be the lowest in the obese group (13 mg) as compared to the normal-weight (19 mg) and CED groups (22 mg) but this was NS. Of the women, 20% had an adequate intake of Fe (probability of Fe adequacy = 0.2); 30 and 20% of the women in the CED and overweight groups had an adequate intake respectively, while none of the obese women had an adequate Fe intake. However, there was no significant difference between the groups. Mean concentration of SF was 39 mg/l, being significantly higher in the obese group (84 μg/l) compared to the CED group (15 μg/l). However, there was no significant difference between SF of the overweight group (52 μg/l) and SF of the normal-weight group (36 μg/l). Using SF as an indicator, the prevalence of ID was 25%, with the lowest prevalence recorded in the overweight group (9%). No ID cases were recorded among obese women. Overall sTfR levels and means among groups were not higher than the normal reference value for detecting true ID (> 8.3 mg/l). Subsequently, overall prevalence of true ID among women was low (9%). Also, no true ID cases were recorded in overweight and obese groups. Mean CRP level was lower (2.4 mg/l) than the reference value for detecting present inflammation (> 7.5 mg/l). Overweight and obese groups showed higher CRP values as compared to normal and CED groups but this was NS. Except for the three cases which were deleted because of simultaneously very high CRP (> 20 mg/l) and ferritin levels (SF > 65 μg/l), five out of the fifty-seven women (9%) showed an elevated CRP level (> 7.5 mg/l), of which one was in the overweight group (9%), one was in the CED group (11%) and three were in the normal-weight group (9%). The proportion of women with elevated CRP level (> 7.5 mg/l) was non-significantly different between groups. Because none of the obese women was Fe deficient, only overweight women were included in the regression model. Also, because sTfR indicated zero prevalence among the overweight and obese women, only SF was used as an Fe biomarker in the model. The prevalence OR of ID (SF < 12 μg/l) in the overweight group was < 1 and NS (OR = 0.3; 95%CI 0.04, 3.24; P = 0·363). Adjusting for CRP level did not influence the relationship (OR = 0.3; 95% CI 0.03, 2.99; P = 0.314). Conversely, the CED group was at a significantly higher risk of ID than the normal-weight group (OR = 7.1; 95%CI 1.41, 36.08; P = 0.017). The relationship remained significant after adjusting for CRP level (OR = 7.7; 95%CI 1.49, 39.96; P = 0.015).

Discussion and conclusion

Based on the studies suggesting that obesity may be a causal factor in the development of ID in adults, one might expect that indications of poor Fe status would be observed in overweight and obese adults, especially in settings where the double burden of malnutrition and micronutrient deficiencies exists simultaneously (8). In the present study, we investigated the association between weight status and ID among urban Malian women of reproductive age. There was a lack of association between ID and BMI in overweight women; the prevalence OR of ID was low and NS for overweight women, using normal weight as reference; mean SF was not significantly different between overweight and normal-weight women; overweight women showed the lowest ID prevalence.

Study limitation

Limitations of the present study included the use of SF as a unique biomarker in the regression model. SF may be elevated in inflammatory conditions even in the presence of true ID, and therefore resulted in a misleadingly high ferritin concentration (18). Indeed, the present results showed a trend of higher CRP and ferritin levels in the overweight and obese groups than in the normal-weight and CED groups, although this was not statistically significant. sTfR concentration has been suggested as the best indicator for detecting true ID because it is not influenced by infection or by inflammatory processes (22). Also, as indicated by Looker et al. (23, 24), an individual must show an abnormal value for two or more indicators to be considered Fe deficient. This did not apply to the present study because sTfR concentration indicated zero prevalence of ID among overweight and obese groups and thus was not included in the model.

Table 1. Characteristics of the women by weight category and mean comparison between CED, normal, overweight and obese groups*
(Means values and standard deviations)

	All groups (n=57)		CED (n=9)		Normal weight (n=32)		Overweight (n=11)		Obese (n=5)		Mean comparison
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
BMI (kg/m^2)	23.4	4.9	17.6	0.7	21.9	1.98	27.2	1.2	34.9	3.4	
Energy intake (kj/d)	8946	2432.7	9496	1814.4	9088	2297.4	8085	3536.4	8959	1096.2	P = 0.596
Iron intake (mg/d)	18.4	7.9	22.3	8.8	18.5	7.4	17.4	9.1	13.0	2.7	P = 0.209
PAiron	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.3	0.0	0.0	P = 0.107
Hb (g/l)	123	15	113	22	123	12	128	16	128	6	P = 0.123
SF (µg /l)	39.3	51.0	14.8	14.6	33.8	31.5	52.2	72.3	83.5	104.3	P = 0.004†
sTfR (mg/l)	4.6	3.6	7.6	7.3	4.2	2.5	3.7	0.8	3.7	1.2	P = 0.094
CRP (mg/l)	2.4	3.6	1.3	2.5	2.0	3.9	3.8	3.4	3.7	2.4	P = 0.315
Iron status	All groups		CED		Normal weight		Overweight		Obese		
Anemia (%)											
Hb<12g/dl	35		44		38		36		0		P = 0.371
Iron deficiency (%)											
SF<12µg/l	25		67		22		9		0		P = 0.008†
sTfR>8.5mg/l	9		22		9		0		0		P = 0.310
Elevated CRP (%)											
CRP >7.5 mg/l	9		11		9		9		0		P = 0.906

PAiron probability of iron adequacy; SF serum ferritin; sTfR serum transferrin receptors; CRP C-reactive protein;

*Oneway ANOVA for means comparison and Pearson' χ^2 for comparison of proportions

†P < 0.05

Nonetheless, SF has been indicated as the most specific biochemical test that correlates with relative total body Fe stores. Looker et al. (24) reported that models including ferritin are more likely to detect an early stage of ID. Indeed, low SF levels reflect depleted Fe stores and hence are a precondition for ID in the absence of infection (17). In our model, a correction has been made to reduce bias related to the use of ferritin by excluding three cases with simultaneously high CRP and ferritin levels (> 65 mg/l), and by including CRP as a covariate in the model.

The second limitation of the present study was the probability that under-reporting of dietary intake could have occurred, as indicated by the lowest energy intake of the overweight group as compared to the normal-weight and CED groups (non-significantly different though). Also, the prevalence of inadequate intake of Fe was almost 100% in the obese group for a zero prevalence of ID. Dietary under-reporting has been shown to be particularly prevalent in obese subjects (25). In the present study, the interviewers were carefully trained and supervised in order to minimise reporting errors, and it was not possible to detect under-reporting during food intake data collection.

Iron deficiency in overweight/obese

The results of the present study confirmed those of Karl et al. (10) who reported that decreased Fe status was not associated with increasing adiposity among overweight and overfat non-obese American women. One hypothesis developed to explain the association between high rates of ID among overweight individuals is that overweight may be associated with lower Fe intake from a poor-quality diet (26). The present study did not support this hypothesis; mean Fe intake was not-significantly different between weight categories. This confirmed the results of previous studies which showed that dietary Fe intakes in overweight adults were not lower than in normal-weight individuals (5, 7).

A second hypothesis is that chronic inflammation and increased leptin production in obesity increase hepcidin secretion from the liver (27) which, along with hepcidin produced by adipose tissue (28), could reduce dietary Fe absorption (29). Recently, higher circulating hepcidin concentrations have been associated with lower Fe status in overweight children (30). Owing to the fact that obesity is an excess accumulation of body fat resulting in a chronic inflammation condition (31), Karl et al. (10)suggested that a certain critical level of relative body fat mass may be required to support an association between body composition and poor Fe status in obese adults. This might be applicable in the present study. In our sample, none of the obese women was Fe deficient and although body fat was not assessed, the mean BMI of overweight women (27 kg/m^2) suggested that the level of body fat may not be enough to induce the critical chronic inflammation level associated with obesity. Although hepcidin level was not measured in the present study, the rate of chronic inflammation observed among overweight women was 9% and the NS difference between BMI categories showed that CRP was not associated with weight status. All this together supported the assumption that the lack of association between ID and overweight in the present study might be due to the fact that the fat mass in overweight women did not meet the critical level needed to induce an inflammatory process, and thus the production of hepcidin leading to the reduction of absorbed Fe. In addition, an inverse relationship has been found between physical activity and weight gain, and it has been suggested that physical inactivity could be another factor associated with ID in obesity (5), but this assumption has not yet been tested in human subjects. McClung et al. (32) investigated the effects of physical activity on moderate Fe-deficient rats' body composition. They reported that moderate ID may cause increased body fat accretion, while physical activity attenuates that effect. Physical activity was not measured in the present study but 90% of the women in our sample are housewives, with activities such as cleaning the house, shopping at the market, cooking and getting water (from the well). This indicates that the potential increase of ID due to increased fat mass in overweight women could be attenuated by the physical activity level of the women, but this hypothesis could not be verified with our data.

Iron deficiency in chronic energy deficiency

The present study showed that individuals in the CED group are significantly at risk of ID using SF as the biomarker, although they apparently have the highest Fe intake as compared to the other groups. However, the probability of Fe adequacy of the CED group (0.3) indicated that the prevalence of inadequate intake is 70%, which is close to the observed prevalence of ID in the group (67%). This confirms the recommendation that the estimate of Fe inadequacy prevalence should agree with the prevalence of ID (20).

Conclusion and public health significance

The present study was the first exploratory research about association between ID and weight status in an urban West African context. From a public health perspective, the study revealed that ID, CED and obesity are all occurring at the public health level among women of reproductive age in urban Mali. However, based on our sample, there were no ID cases among obese women and no association between ID and weight status among overweight women. This could be due to the fact that in our sample the excess of body fat might not be critical to induce chronic inflammation related to reduced Fe absorption. Future research based on larger convenience samples should be designed to further investigate associations between overweight, obesity and ID in developing countries along with causal factors related to this association.

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Chapter 3

Food groups associated with a composite measure of probability of adequate intake of 11 micronutrients in the diets of women in urban Mali

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Abstract

The prevalence of micronutrient deficiency is high among women of reproductive age living in urban Mali. Despite this, there are little data on the dietary intake of micronutrients among women of reproductive age in Mali. This research tested the relationship between the quantity of intake of 21 possible food groups and estimated usual micronutrient (folate, vitamin B-12, calcium, riboflavin, niacin, vitamin A, iron, thiamin, vitamin B-6, vitamin C, and zinc) intakes and a composite measure of adequacy of 11 micronutrients [mean probability of adequacy (MPA)] based on the individual probability of adequacy (PA) for the 11 micronutrients. Food group and micronutrient intakes were calculated from 24-h recall data in an urban sample of Malian women. PA was lowest for folate, vitamin B-12, calcium, and riboflavin. The overall MPA for the composite measure of 11 micronutrients was 0.47 ± 0.18. Grams of intake from the nuts/seeds, milk/yogurt, vitamin A rich dark green leafy vegetables (DGLV), and vitamin C-rich vegetables food groups were correlated (Spearman's *rho* = 0.20–0.36; $P < 0.05$) with MPA. Women in the highest consumption groups of nuts/seeds and DGLV had 5- and 6-fold greater odds of an MPA > 0.5, respectively. These findings can be used to further the development of indicators of dietary diversity and to improve micronutrient intakes of women of reproductive age.

Introduction

Micronutrient malnutrition is a global public health problem; it undermines the health, development, and economic potential of millions of people worldwide. Women of reproductive age are among the most vulnerable to micronutrient deficiencies due to physiologically higher micronutrient requirements during the reproductive life stage (1). The impact of poor maternal micronutrient status is transmitted intergenerationally from mother to child, resulting in less optimal fetal growth and development (2). In Bamako, Mali, it is estimated that 54% of women 15–49 y with a child under 5 y suffer from anemia (3) and that the Mali national gross domestic product is decreased by 2.7% due to vitamin and mineral deficiencies (4). Food-based approaches have been cited as one of the primary means to improve micronutrient status and control other problems related to poor diet quality (5). However, women's dietary intake receives insufficient attention in many developing countries. In Mali, there are no nationally representative data on women's food intake. Available dietary intake information is often fragmented and, due to diverse data collection methodologies, cannot be compared internationally or over time within the same country. This is partly due to the fact that collecting information on dietary intake is costly and time consuming. The challenges of collecting accurate quantitative information on dietary intake can be even greater in developing countries, where literacy levels are low, restricting the types of survey instruments that can be used (6). In addition, shared-plate eating requires adaptation of survey instruments and additional time for data collection (7). Because of the challenges associated with quantitative dietary data collection, simpler methods yielding information on dietary intake are needed. Simple counts of the number of food groups consumed have been shown in developing country settings to be adequate proxy indicators of micronutrient intake among children (8-12) and also among adults in diverse settings (13-15). Although the use of food group diversity indicators (FGI)[1] has shown promise in different settings, methodologies for indicator construction have varied (16). For infants and children aged 6–23 mo, the WHO (17) has defined a FGI that sums 7 food groups. For older children and adults, there is currently no standard indicator and no consensus on the level of aggregation of foods or number of food groups to sum when creating FGI. This

[1] Abbreviations used: AI, adequate intake; DGLV, vitamin A-rich dark, green, leafy vegetables; EAR, estimated average requirement; FGI, food group diversity,indicator; MPA, mean probability of adequacy; PA, probability

study used 2 non consecutive days of 24-h dietary recall to examine the correlation between a set of 6 and 21 food groups, micronutrient intakes, and mean probability of adequacy (MPA), a summary measure of probability of adequacy (PA) across 11 micronutrients, among a sample of women of reproductive age in Bamako, Mali. The results highlight specific food groups that may have potential to improve the micro-nutrient adequacy of the diet of women of reproductive age in Mali and help to advance knowledge about FGI construction.

Materials and Methods

The data used in this study are from a cross-sectional food consumption survey undertaken as part of the FONIO project funded by the Sixth European Union Framework Program. Some results presented in this paper were part of a multi-site study, the Women's Dietary Diversity Project (WDDP), funded by the Food and Nutrition Technical Assistance II Project and its predecessor project, Food and Nutrition Technical Assistance. Full results of the Mali site report are available elsewhere (18). The main objective of the WDDP was to analyze the relationship between simple FGI and the micronutrient adequacy of the diet of women in resource-poor settings. A standardized protocol used by all collaborators specified the micronutrients of focus (folate, vitamin B-12, calcium, riboflavin, niacin, vitamin A, iron, thiamin, vitaminB-6, vitamin C, and zinc) and provided standardized methods for indicator construction and calculation of PA. A description of the research protocol can be found in the summary paper in this supplement (14).

Study site and sampling methodology. Study participants were women 15–49y living in Bamako, Mali. Ethical clearance for the study was obtained from National Institute of Public Health Research in Mali. All women included in the study were informed of the study purpose and provided verbal consent.

A total sample size of 108 women was calculated based on the original FONIO objective to provide an estimate of iron intake. Five women dropped out of the survey sample, leaving 103 women with dietary intake data available from the first recall. Of the 103 women with dietary intake from the first recall, 6 did not participate in the second recall due to unavailability. During analysis, 1 woman was considered a severe outlier and was deleted

of adequacy; RE, retinol equivalent; TACAM, Table de Composition des aliments du Mali; WDDP, Women's from analysis, leaving a total sample of 102 women with 1d of intake data and a subsample of 96 women with 2 non consecutive days of recall.

Women were selected using a 3-stage cluster sampling method (19). The initial sampling frame consisted of Bamako's 72 quarters; 12 were selected for sampling using probability proportional to size. In each selected quarter, 9 households were randomly selected using the random walk method. All women aged 15–49y living in the selected households were listed, and1apparently healthy nonpregnant, nonlactating woman belonging to a Malian sociolinguistic group was randomly selected from eligible women in each household. If the selected woman was not responsible for food preparation on the recall day, amounts of ingredients used in recipes were obtained from the person responsible for meal preparation on the recall day.

Dietary intake data collection. Two 24-h dietary recalls were performed from February to April 2007, which corresponds to the postharvest season. The 2 recalls were from non consecutive days with 2–11 d separating the recall periods. Weekends and special event days were excluded. The participants' daily food intake was assessed by a quantitative 24-h recall method adapted to the context of shared-plate eating, which involved asking the respondent to use known-weight utensils on the recall day to help them visualize the amount of food consumed (7). Participants were asked to name all the food and drinks consumed during the preceding day, including anything consumed outside the home. Then they were asked to describe ingredients and cooking methods of any mixed dishes and the place and time of consumption. Finally, the amounts of all foods, beverages, and ingredients of mixed dishes were estimated either in household units or monetary value[2]. For meals consumed within the household, the total amount of the cooked food and the amount consumed by the respondent were estimated in household units to derive the proportion consumed by the respondent from the total volume of the dish. Food weights were measured using digital dietary scales (Soehnle, Plateau Art, model 65086, 10 kg maximum) and recorded to the nearest 2g. Information on consumption of fortified food products was not collected. However, most of the fortified foods commonly available in the study area are

Dietary Diversity Project; YORV, vitamin A-rich deep yellow, orange, and red vegetables.

[2] When a monetary unit was used to describe a portion, these foods or ingredients were converted from monetary value to weight equivalent by using the mean weight per fixed monetary value obtained from several vendors in the main market areas

designed for infants and preschool children. These foods were not consumed by any of the women in our study.

Food composition data. The food composition values used in this study are based on a food composition table developed for a larger project (a detailed description of the food composition table's development is provided elsewhere (20)) and relied primarily on the Table de Composition des aliments du Mali (TACAM) (21). The nutrients for all staple foods in TACAM are expressed as cooked. To account for nutrient losses during cooking in other foods where only raw values are provided in TACAM, such as fish, meat, and vegetables, USDA Retention Factors release 6 (22) were applied to the nutrient values of the raw foods. Twelve percent of the nutrient values were not in TACAM[3], including 10 foods for which all values were taken from other sources. For other foods, missing values were replaced most commonly for vitamin B-6, folate, and zinc. Missing values were first obtained from USDA release 20 (23) and, if necessary, from the International Mini List (24). Retinol equivalents (RE) were calculated as the sum of retinol and β-carotene, using the following conversions: 1 mg retinol = 1 mg RE and 1 mg beta-carotene = 0.167 mg RE, as recommended by the FAO/WHO (1). Nutrient values from sources other than the TACAM were adjusted to account for differences in moisture content[4].

Food groups and food group intakes. Food group combinations based on 6 and 21 food groups were used in this analysis (**Table 1**). For simplicity of presentation, results from only the most disaggregated set of food groups (21 food groups) are presented in most tables and figures. Notable results based upon the least disaggregated set of food groups (6 food groups) are described in the text. Construction of FGI was based on food group intakes (grams) reported on only the first day of recall to coincide with the overall aim of the WDDP to test 1-d FGI that could be used in large-scale surveys.

[3] Vitamin B-6 values reported for cooked staples in the Table de Composition des aliments du Mali were very low, so vitamin B-6 values for cooked staples from the USDA and International Mini List were used.
[4] International Mini List tables do not report moisture content. The moisture content for foods taken from the International Mini List was estimated based on the closest food match from the USDA.

Table 1. Food groups

Food groups (6)	Food groups (21)
All starchy staples	Grains and grain products All other starchy staples
All legumes and nuts	Cooked dry beans and peas Soybeans and soy products Nuts and seeds
All dairy	Milk/yogurt Cheese
Other animal-source foods	Organ meat Eggs Small fish eaten whole with bones Large whole fish/dried fish/shellfish and other seafood Beef, pork, veal, lamb, goat, game meat Chicken, duck, turkey, pigeon, guinea hen, game birds Insects, grubs, snakes, rodents and other small animals
Vitamin A-rich fruits and vegetables	Vitamin A-rich dark green leafy vegetables Vitamin A-rich deep yellow/orange/red vegetables Vitamin A-rich fruits
Other fruits and vegetables	Vitamin C-rich vegetables Vitamin C-rich fruits All other vegetables All other fruits

***Assessing usual micronutrient intakes, PA, and MPA*.** The probability approach (25) was used to estimate adequacy for all micronutrients except calcium, where the PA was calculated following the recommendations of Foote (26). Nutrient intake distributions and intraindividual SD distributions based on both days of intake were skewed for most micronutrients and were Box-Cox transformed prior to analysis. Estimated usual micronutrient intakes were calculated as best linear unbiased predictors (27). Estimated average requirements (2) were used to assess the PA of thiamin, riboflavin, niacin, vitamin B-6, vitamin B-12, vitamin C, vitamin A, zinc, folate, and iron. An adequate intake (8) was used for calcium. Because iron requirements are skewed, the PA for iron was estimated using the Institute of Medicine tables for adult women (28). Iron and zinc requirements were based on a bioavailability assumption of 10% for iron and 34% for zinc, determined on the basis of the dietary pattern of the sample (1). The PA associated with estimated usual intake for each micronutrient was calculated taking into account nutrient requirement distributions and inter-and intra-individual variation in intake. MPA was constructed as a summary indicator, taking the mean of all PA.

Statistical methods. The relationship between food group intake (grams) and estimated usual micronutrient intake and food group intake and MPA was assessed by Spearman correlation due to the fact that food group intake (grams) was not normally distributed and was untransformed. For food groups observed to have a significant correlation with MPA, a categorical variable of food group intake was created. Categories of intake were defined as follows: 1) for those food groups for which all women consumed at least 1 g, a low, middle, and high consumer category was created based on tertiles of food group intake; 2) for other food groups, the low intake category was defined as an intake of 0 g and the middle and high intake categories defined by using the median intake among consumers as the cutpoint. Binary logistic regression models were used to assess these categorical food group intake variables as predictors of MPA > 0.50. Because energy intake from individual food groups, most notably nuts/seeds, is endogenous to the model, logistic regression models controlled for total energy intake from other food groups and age. However, results were not influenced by age in any model. Therefore, only results unadjusted for age are presented. STATA/IC version 10 was used for all analyses (29). $P < 0.05$ was considered significant. Values in the text are means ± SD or medians (25th and 75th interquartile range).

Results

Sample characteristics. The mean age in the sample was 31 ± 10.5y, with 16% adolescents (15–18y) (**Table 2**). The literacy rate, defined as having attended primary school or Islamic school, was 65%. Median energy intake was 8474 kJ (2024kcal) (6753 kJ, 10,521 kJ), with 11% of dietary energy from protein, 57% from carbohydrates, and 32% from fat.

Table 2: Descriptive statistics of Malian women [1,2]

Variable	
Age, *y*	31.5±10.5
Education, *% literate*[3]	65
Energy, *kJ/d*	8600 ± 3000
Total carbohydrate, *g/d*	310 ± 112
Total fat, *g/d*	73 ± 35
Total protein, *g/d*	57.7±25.6

[1] Adapted with permission from (18)
[2] Values are mean ± sd, or %, n = 102
[3] Percentage of women who reached at least primary school level or went to Islamic school

Diet patterns and food group intake. The diet of this urban sample is based on a starchy staple, mainly refined white rice, refined wheat flour or millet, accompanied by a sauce typically made from vegetables and beef or fish. The most commonly consumed food groups did not differ substantially between recall days. Of the 21 possible food groups, those most commonly consumed on both recall days (based on the subsample of women with data for 2 recall days) included grains and grain products (grains); vitamin-C rich vegetables; vitamin A-rich deep yellow, orange, and red vegetables (YORV); and nuts and seeds (nuts/seeds) (**Figure 1**).

The food groups beef, pork, veal, lamb, goat, and game meat (red meat); large whole fish/dried fish/shellfish and other seafood (large fish); all other vegetables (other vegetables); milk and yogurt (milk/yogurt); vitamin-A rich dark, green, leafy vegetables (DGLV); and all other starchy staples (other staples) were consumed by the majority of women on at least 1 recall day.

The food groups soybeans and soy products; cheese; organ meat; chicken, duck, turkey, pigeon, guinea hen, and game birds; insects, grubs, snakes, rodents, and other small animals; and other fruits were not consumed by any women on either day of recall. Those food groups not consumed by any woman are not presented in the remainder of the analysis

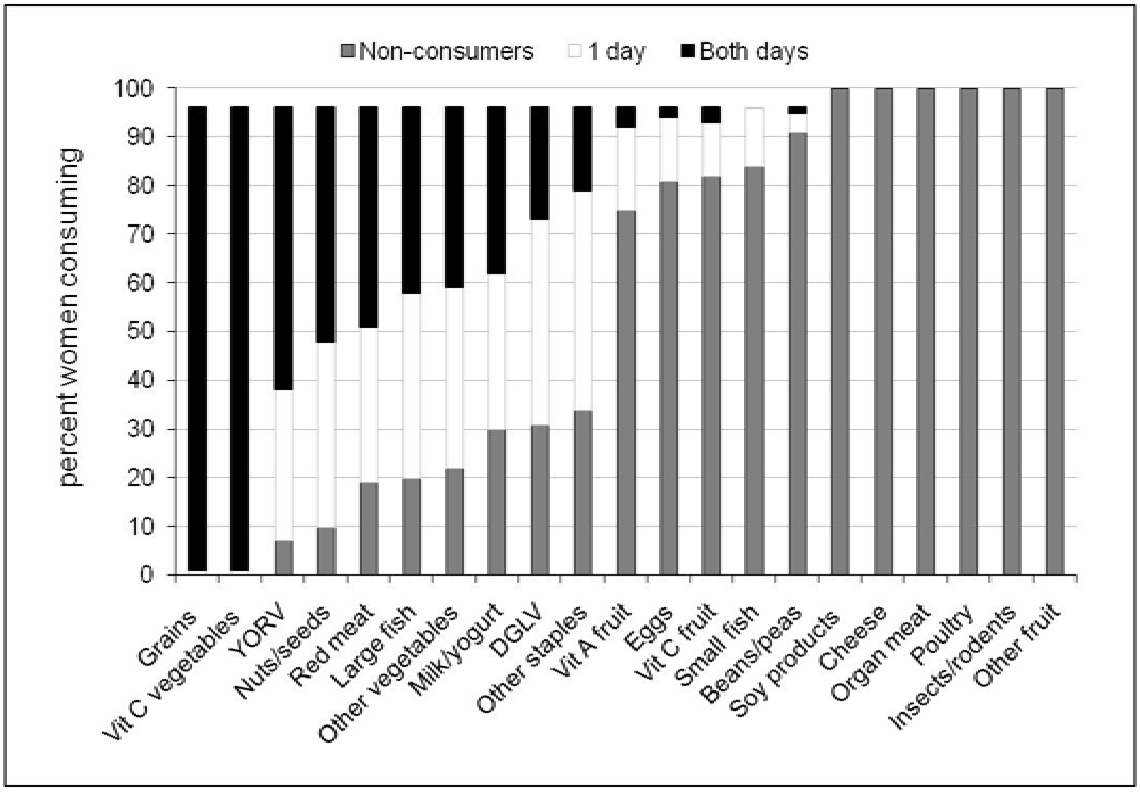

Figure 1. Percent of Malian women consuming individual food groups on both, one, or no days of recall (n = 96). Figure is reproduced with permission from (18).

Micronutrient intakes, PA, and MPA. Median micronutrient intakes were below the EAR for folate, vitamin B-12, riboflavin, niacin, vitamin A, and the AI for calcium (**Table 3**). Median intakes were above the EAR for vitamin B-6, vitamin C, and zinc and equal to the EAR for thiamin. The PA was < 0.25 for vitamin B-12 and folate; ranged between 0.25 and 0.49 for riboflavin, calcium, and niacin; and was > 0.50 for iron, vitamin A, vitamin B-6, thiamin, zinc, and vitamin C. The MPA for the 11 micronutrients was 0.47 ± 0.18.

Contribution of food groups to nutrient intakes. The contribution of each food group (of the 21 groups) to nutrient intake depended on both the quantity consumed (grams) and the nutrient density of the food items consumed within that group (**Tables 4 and 5**). Median intake of grains was 775 g, which accounted for 43% of the total energy intake of the sample, over 50% of the total thiamin intake, and over one-third of the total vitamin B-6, iron, and zinc intakes. Nuts/seeds provided the next largest contribution to energy, although median intake was only 2g. Nuts/seeds also supplied over one-third of the niacin intake and 10% or more of the thiamin, folate, and iron intakes.

Table 3. Micronutrient intakes, EAR and PA for Malian women[1-3]

Nutrient	Mean	Median	EAR	SD[4]	PA (Mean)
Folate (μg/d)	131±82.5	119.1	320[5,9]	32.0	0.00
Vitamin B-12 (μg/d)	1.5±1.0	1.3	2.0[5]	0.2	0.17
Calcium (mg/d)	444±318	375	1,000[7,9]	--	0.27
Riboflavin (mg/d)	0.8±0.4	0.7	0.9[6,9]	0.09	0.28
Niacin (mg/d)	10.6±6.5	8.3	11[6,9]	1.65	0.31
Vitamin A (RE/d)	358±295	245	270[5,9]	54.0	0.50
Iron (mg/d)	16.1±8.8	14.2	--[8]	--	0.54
Thiamin (mg/d)	1.0±0.5	0.9	0.9[6]	0.09	0.59
Vitamin B-6 (mg/d)	1.2±0.5	1.2	1.1[6,9]	0.11	0.67
Vitamin C (mg/d)	62.6±34.5	58.4	38[6]	3.8	0.88
Zinc (mg/d)	10.2±5.8	8.8	6.0[9,10]	0.75	0.96
MPA	0.47±0.18	0.48			

[1] Adapted with permission from (18)
[2] Mean and median nutrient intakes are for the first observation day. PA are based on estimated usual intake, calculated using repeat observations for a subset of the sample, incorporating information from both rounds of data collection.
[3] Values are mean ± sd
[4]All SD were calculated based on EAR and CV, which was assumed to be 10% for all micronutrients except 15% for niacin (30), 20% for vitamin A (27) and 25% for zinc (31).
[5] Values are taken directly from the WHO/FAO 2004 requirements (32).
[6] EAR were back-calculated from recommended nutrient intake (RNI) values of WHO/FAO 2004 requirements (32).
[7] Not an EAR, but rather AI (33). Following Foote et al (26), PA are calculated to be: 0% when intake ≤ ¼ of the AI; 25% for intakes > ¼ and ≤ ½ of the AI; 50% for intakes > ½ and ≤ ¾ of the AI; 75% for intakes > ¾ and ≤ AI; and 100% for intakes above the AI.
[8] PA for iron intake were estimated using the IOM tables for adult women (27). A bioavailability assumption of 10% was used for this study (32).
[9] For the adolescents group (15-18 y, n=16), a value of 0.8±0.08 mg was used for riboflavin, 12±1.2 mg for niacin, 7.0±0.88 mg for zinc (30% bioavailability), 365±73 RE for vitamin A, 330±33 μg for folate, 1.0±0.1 mg for vitamin B-6 and 1,300 mg AI for calcium.
[10] This value is the estimated median requirement of zinc to be used for diets with a higher bioavailability (mixed of refined vegetarian diets), as suggested by IZiNCG (31).

Table 4. Intake of food groups by Malian women [1,2]

Food group	All women (n=102)		Consumers	Consumers only	
	g/d		*%*	*g/d*	
Grains (g/d)	743±333	775.5 (497,958)	100	743±333	775.5 (497,958)
Other staples (g/d)	61±93	0 (0,92)	42	144±103	124 (81,176)
Beans/peas (g/d)	5±5	0 (0,0)	4	134±76	121.5 (67,212)
Nuts/seeds (g/d)	37±37	2 (0,80)	70	53±56	25 (2,99)
Milk/yogurt (g/d)	75±136	0 (0,86)	48	155±161	94 (35,287.5)
Red meat[3] (g/d)	42±38	41.5 (0,67)	70	61±31	52 (40,81)
Large fish[4] (g/d)	25±38	12 (0,38)	56	45±41	35 (19,56.5)
Small fish[5] (g/d)	0.3±2	0 (0,0)	5	6±4	5 (3,10)
Eggs (g/d)	4±15	0 (0, 0)	8	52±16	57.5 (55,60)
DGLV[6] (g/d)	30±56	0 (0,25)	41	73±67	87.5 (7,125)
YORV[7] (g/d)	8±9	5 (3,8)	82	9±9	5 (4,9)
Vitamin C-rich vegetables (g/d)	212±115	209.5 (121,283)	100	212±115	209.5 (121,283)
All other vegetables (g/d)	16±22	11 (0,26)	60	27±22	24 (14.5,35)
Vitamin A-rich fruits (g/d)	4±13	0 (0,0)	12	32±24	20 (13,50.5)
Vitamin C-rich fruits (g/d)	15±68	0 (0,0)	9	167±174	42 (12.5,357)

[1]Values are mean±sd or median (interquatile range)
[2]Adapted with permission from (18)
[3] Beef, pork, veal, lamb, goat, game meat
[4] Large whole fish/dried fish, shellfish and other seafood
[5] Small fish eaten whole with bones
[6] Vitamin A-rich DGLV
[7] Vitamin A-rich YORV

Table 5. Contribution of food groups (21) to energy and micronutrient intakes[1-3]

Food groups	Energy	Folate	Vitamin B-12	Calcium	Riboflavin	Niacin	Vitamin A	Iron	Thiamin	Vitamin B-6	Vitamin C	Zinc
						(%)						
Grains	43.0	8.8	0.2[4]	10.5	24.8	14.7	3.3	40.3	53.0	35.1	0.8	39.4
Other staples	3.1	6.1		1.5	2.0	5.9	1.3	2.2	5.9	12.9	15.0	1.2
Beans/peas	0.2	8.3		0.3	0.4	0.2		0.8	1.1	0.4		0.7
Nuts/seeds	10.3	13.0		5.6	6.2	37.5	0.1	17.6	10.6	8.3		9.0
Milk/yogurt	4.6	5.0	30.4	43.4	29.4	4.4	20.2	0.3	5.0	3.6	0.9	19.5
Red meat	4.8	1.6	30.6	1.0	8.4	14.4		9.4	6.9	4.3		17.0
Large fish	2.0	2.3	31.1	5.7	3.7	6.9	0.4	4.5	1.7	5.3	0.4	3.8
Small fish		0.1	0.5	0.7		0.2		0.1		0.2		0.1
Eggs	0.3	0.6	4.1	0.4	1.5	0.1	1.7	0.5	0.4	0.5		0.6
DGLV	0.4	12.2		10.6	7.3	2.1	35.7	5.8	2.3	3.3	6.4	1.2
YORV	0.2	1.8		0.5	0.8	1.5	11.6	1.0	1.1	2.1	0.6	0.4
Vitamin C-rich vegetables	3.9	33.0		17.1	11.7	8.8	12.3	14.1	8.8	17.0	66.5	6.2
All other vegetables	0.1	1.9		0.5	1.0	0.5	3.5	0.6	0.9	1.3	1.8	0.4
Vitamin A-rich fruits	0.5	0.1		0.1	0.1	0.1	7.5	0.1	0.1	0.1	2.1	
Vitamin C-rich fruits	0.6	2.2		0.3	0.6	0.8	0.6	0.1	0.5	3.9	2.7	0.3

[1] Reproduced with permission from (18).
[2] Percentages might not total 100 due to nutrient contributions from foods excluded from the groups comprising the FGI (e.g., fats, sweets, alcohol).
[3] Empty cells mean no contribution.
[4] This value comes from butter croissant (0.16 µg/100 g).

The median intake of vitamin C-rich vegetables, which included cabbage, tomatoes, and onions, was 209 g. This food group supplied two-thirds of the vitamin C intake, one-third of the folate intake, and over 10% of the riboflavin, vitamin B-6, vitamin A, and iron intakes. Milk/yogurt supplied only 5% of the energy intake but .20% of the riboflavin, vitamin B-12, vitamin A, and calcium intakes. Eggs did not supply > 5% of the intake for any micronutrient. Red meat supplied < 5% of the total energy intake but one-third of the vitamin B-12 intake and > 10% of the niacin and zinc intakes. DGLV supplied < 1% of the total energy intake but 36% of the vitamin A intake and 10% or more of the folate and calcium intakes.

Correlations between grams of intake from individual food groups, micronutrient intakes, and MPA. When using the highest level of food group aggregation (6 food groups), intake (grams) for each food group was positively correlated with estimated usual intakes (mg, µg, or RE) of between 4 and 7 individual micronutrients ($P < 0.001$–0.01) and with MPA ($P < 0.001$–0.01) (**Table 6**). With further disaggregation into 21 food groups, associations became more specific to certain subgroups. Grams of intake from the food groups nuts/seeds, milk/yogurt, DGLV, and vitamin C-rich vegetables were significantly correlated with intakes (mg, µg, or RE) of 5 or more individual micronutrients (**Table 7**) and with MPA ($P < 0.001$ - 0.01). The 3 micronutrients with the lowest PA were folate, vitamin B-12, and calcium. Beans/peas, nuts/seeds, DGLV, and vitamin C-rich vegetables were significantly correlated with folate intakes (micrograms); milk/yogurt, eggs, and red meat were significantly correlated with vitamin B-12 intakes (micrograms); and milk/yogurt was significantly correlated with calcium intakes (milligrams).

Table 6. Correlations between intakes of 6 food groups (g/d) and estimated usual micronutrient intakes or MPA[1]

Food group	Folate	Vitamin B-12	Calcium	Riboflavin	Niacin	Vitamin A	Iron	Thiamin	Vitamin B-6	Vitamin C	Zinc	MPA
	µg			*mg*		*RE*			*mg*			
All starchy staples	0.13	-0.08	0.12	0.11	0.17	-0.04	0.34*	0.47*	0.50*	0.22*	0.40*	0.25*
Legumes and nuts	0.42*	-0.03	0.13	0.15	0.56*	0.13	0.37*	0.36*	0.21*	-0.01	0.27*	0.35*
All dairy	0.10	0.50*	0.54*	0.49*	-0.01	0.36*	-0.09	0.02	0.01	-0.02	0.28*	0.25*
Other animal source foods	0.22*	0.55*	0.26*	0.32*	0.18	0.15	0.11	0.22*	0.15	0.28*	0.22*	0.27*
Vitamin A rich fruits and vegetables	0.43*	0.05	0.13	0.19	0.19	0.55*	0.17	0.09	0.21*	0.29*	0.09	0.28*
Other fruits and vegetables	0.26*	0.05	0.08	0.14	0.16	0.02	0.19	0.29*	0.35*	0.36*	0.20*	0.22*

[1]Estimated usual micronutrient intakes and MPA incorporate information from both rounds of data collection * $P < 0.05$

Table 7. Correlations between intakes of 21 (g/d) food groups and estimated usual micronutrient intakes and MPA[1]

Food group (g)	Folate	Vitamin B-12	Calcium	Riboflavin	Niacin	Vitamin A	Iron	Thiamin	Vitamin B-6	Vitamin C	Zinc	MPA
		µg		*mg*		*RE*			*mg*			
Grains	0.09	-0.11	0.09	0.07	0.15	-0.11	0.33*	0.44*	0.44*	0.17	0.39*	0.19
Other staples	0.14	0.09	0.09	0.11	0.01	0.24	0.00	0.06	0.19	0.21*	0.03	0.14
Beans/peas	0.30*	-0.06	-0.09	-0.11	-0.03	0.02	0.04	0.02	-0.08	-0.17	0.05	-0.05
Nuts/seeds	0.29*	-0.02	0.16	0.18	0.57*	0.12	0.35*	0.37*	0.24*	0.05	0.24*	0.36*
Milk/yogurt	0.10	0.50*	0.54*	0.49*	-0.01	0.36*	-0.09	0.24	0.01	-0.02	0.28*	0.25*
Eggs	0.10	0.25*	0.10	0.14	-0.09	0.17	-0.12	-0.07	-0.00	0.05	-0.15	0.09
Small fish	0.10	0.00	0.02	0.02	0.19	0.07	0.18	0.00	0.17	0.07	0.13	0.08
Large fish	-0.01	0.14	0.04	-0.03	0.17	-0.15	0.11	0.10	0.19	0.05	0.06	0.09
Red meat	0.16	0.28*	0.14	0.23*	0.07	0.16	0.05	0.15	-0.02	0.18	0.21*	0.14
DGLV	0.42*	0.03	0.18	0.22*	0.27*	0.45*	0.34*	0.11	0.09	0.12	0.22*	0.27*
YORV	0.01	0.03	-0.04	-0.07	0.03	-0.02	-0.04	0.09	0.18	0.16	0.07	0.03
Vitamin C-rich vegetables	0.25*	0.04	0.07	0.12	0.16	-0.03	0.18	0.27*	0.30*	0.33*	0.20*	0.20*
Vitamin A-rich fruits	0.02	0.01	-0.03	-0.01	-0.06	0.24*	-0.13	-0.03	0.03	0.16	-0.16	0.04
Vitamin C-rich fruits	0.06	0.08	0.01	0.04	-0.12	0.15	-0.21*	-0.15	0.02	0.14	-0.26*	-0.01
All other vegetables	0.06	0.06	0.13	0.11	0.03	0.14	0.14	0.16	0.19	0.18	0.15	0.15

[1] Estimated usual micronutrient intakes and MPA incorporate information from both rounds of data collection.

Logistic regression of MPA > 0.50 and grams of intake from significant food groups. Binary logistic regression results for the 4 food groups (of the 21) that were significantly correlated with MPA did not differ when controlling for age but did change slightly when adjusted for energy intake from other food groups (**Table 8**). The MPA was higher for the highest consumers within each food group compared with the lowest consumers with a linear trend for consumers of nuts/seeds (*P*-trend < 0.01) and DGLV groups (*P*-trend < 0.01).

Table 8. Energy-adjusted odds ratios for MPA > 0.5 and selected food groups[1-3]

Food group	Intake *g*	MPA[4]	OR [95%CI]	*P*-value[5]
Nuts/seeds	0	0.41 ±0.18	1.0	
	1-25	0.45±0.18	1.5 (0.5,4.5)	
	> 25	0.55±0.17	4.7 (1.5,14.0)	<0.01
Milk/yogurt	0	0.43±0.17	1.0	
	1-94	0.54 ±0.22	5.8 (1.8,18.9)	
	> 94	0.50 ±0.16	1.4 (0.65,4.2)	0.26
DGLV	0	0.43±0.18	1.0	
	1-88	0.52 ±0.17	1.8 (0.6,5.4)	
	>88	0.54±0.17	6.1 (1.7,21.6)	<0.01
Vitamin C-rich vegetables	< 141	0.44 ±0.20	1.0	
	141-262	0.46 ±0.18	0.8 (0.3,2.3)	
	> 262	0.51 ±0.16	1.0 (0.3,3.1)	0.91

[1] Adjusted for the effect of total energy intake, excluding energy provided from that food group
[2] Cutoff points to form 3 comparison categories were based upon intake distributions for each food group.
[3] Intakes are from first 24-h recall. MPA incorporates information from both rounds of data collection.
[4] Values are mean ± SD or odds ratio (95% CI)
[5] *P* value for linear trend across categories

Discussion

The results indicate that macronutrient distributions, with the exception of fat, were within acceptable population intake ranges of 15–30% fat, 10–15% protein, and 55–75% carbohydrate as defined by the WHO/FAO (34); however, MPA of the sample was low. Grains, nuts/seeds, YORV, vitamin C-rich vegetables, and all other vegetables were the most commonly consumed food groups. This is similar to other studies of dietary patterns in rural Mali, where grains, nuts/seeds, vegetables, and DGLV were consumed by all households in the survey (15). Grams of food group intake from d1 were significantly correlated with MPA for nuts/seeds, milk/yogurt, DGLV, and vitamin C-rich vegetables. Logistic regression results showed the highest consumers of nuts/seeds had 5 times higher odds than non-consumers to have an MPA > 0.50. Consumers in the highest category of DGLV intake had 6 times higher odds of an MPA > 0.50 than non-consumers.

Study limitations. Both the data for individual food groups consumed and the estimated usual micronutrient intakes were calculated from the same 24-h recall data; however, estimated usual micronutrient intakes, A, and MPA were based on 2 recall days, whereas food group intakes represent intakes from only d1. Use of the same round 1 data could lead to artificially higher correlations. Conversely, the random error inherent in all dietary data collection can attenuate correlations (35). The sample size for the original study was designed to measure iron intake. Sample sizes required to measure other micronutrients may be smaller or larger than that needed to estimate iron intake. The relatively small size of the sample may not be adequate to detect significant differences for all micronutrients, particularly vitamin A, vitamin B-12, calcium, folate, and niacin, which had a larger CV than iron in our sample. Additionally, we did not control for some potential confounders of MPA such as educational level and socioeconomic status. PA were low for several micronutrients and MPA was only 0.47. Due to the combination of low MPA and small sample size, only 47 women had an MPA > 0.50, 25 had an MPA > 0.60, and 11 had MPA > 0.70. For these reasons, MPA > 0.50 was used throughout the analysis. There are no clear guidelines on how to define an acceptable MPA level. Ideally, the benchmark chosen to define acceptable MPA would be higher, e.g. 0.80, 0.90. To use a higher and more desirable probability, the sample population studied would need to have micronutrient intakes higher than those observed in this population.

Food group intakes, MPA, and intakes of individual micronutrients. Despite significant correlation with MPA, the logistic regression results for consumer categories and MPA were not significant for the highest consumers of milk/yogurt or any consumer category of vitamin C-rich vegetables. The lack of significant differences for consumers of vitamin C-rich vegetables could relate to the fact that all women consumed some amount of this food group, whereas for the other 3 food groups that were significant predictors of MPA, the reference category was non consumers. Another interesting result was that women who consumed 1–94 g of milk/yogurt had significantly higher odds of better MPA than non-consumers whereas women in the highest consumption (> 94 g) did not. Reasons for this finding were not evident. For example, removing certain outliers did not alter the results. Additionally, there were very few types of milk/yogurt consumed and thus the difference observed is not likely due to consumption of different types of these products.

The nutrients with the lowest estimated prevalence of adequacy were folate, vitamin B-12, calcium, riboflavin, and niacin. These results are similar to those of non-pregnant, non-lactating women in urban Burkina Faso, where the lowest PA were for vitamin B-12, folate, iron, and riboflavin (36). In similar studies in rural Mali, which considered only 7 of our 11 micronutrients, calcium and vitamin A were most limited in the diets of adults (15) and vitamin A, calcium, vitamin C, and riboflavin had the lowest adequacy ratio for children (9). Our Spearman correlation results indicate that increasing intakes of red meat, DGLV, nuts/seeds, and milk/yogurt could increase intakes of some of the key nutrients deficient in our study population. Each of these food groups was consumed on at least 1recallday by a majority of women, indicating that there are no cultural barriers to consumption of foods within these groups. However, for many of the food groups, median portion sizes were small. Qualitative research methods could be used to explore constraints and opportunities related to increasing consumption frequency and quantity of foods within these food groups. These food groups could form the basis of focus for both programmatic efforts and individual dietary choices where the goal is to promote micronutrient adequacy through food-based approaches.

Implications for development of proxy indicators of micronutrient adequacy. Part of the overall goal of the WDDP was to analyze the relationship between simple indicators of dietary diversity and diet quality for women. The analysis in this paper, which had a more in-depth focus on performance of individual food groups, helped to draw attention to the performance and role of certain food groups in achieving better MPA in this particular population from urban Mali. Of the other 4 sites participating in the WDDP, the 3 food groups with the strongest correlation with MPA (controlling for energy intake) were DGLV, vitamin A-rich fruits, and red meat in Burkina Faso (36); vitamin A-rich fruit, DGLV, and other vegetables in Mozambique (37); milk, organ meat, and other vegetables in the Philippines (38); and DGLV, nuts/seeds, and small fish in Bangladesh (39). A similar study among urban adults in Benin found higher intakes of the food groups vegetables and legumes/ nuts were the most strongly associated ($P < 0.001$) with a micronutrient adequacy ratio (40). An analysis of diets of rural schoolchildren in Kenya found that vegetables, fruits, and dairy had the strongest correlations to a diet most likely to be adequate in micronutrients (41). A study of the adequacy of micronutrient density of complementary foods from 9 countries tested how well selected nutrient-dense food groups ("sentinel" food groups) predicted this dimension of diet quality (12). The study also tested FGI. The most consistent sentinel group across all countries was animal source foods, which included dairy and/or eggs and/or meat/fish/poultry. Additionally, in some countries, dairy alone, vitamin-A rich fruits and vegetables, and/or other fruits and vegetables were good indicators of better diet quality of complementary foods. This was related to different diet patterns in the sites; e.g. in Malawi almost no animal source foods were consumed and DGLV were the best sentinel food group to predict micronutrient density. Based on the convergence of evidence from these studies, dairy, eggs fruits and vegetables, particularly DGLV, fish, red meat, and legumes and nuts, or their subgroups are key food groups for developing proxy indicators of micronutrient adequacy based on dietary diversity scores. However, for operational purposes, it is important to note that the food groups contributing most to micronutrient adequacy varied by study site. These findings support using combinations of food groups rather than single food groups in isolation serving as sentinel food groups, because no individual food group seems to perform equally well across diverse contexts.

In conclusion, this analysis showed a strong and positive association between the quantity of intake (grams) of the food groups nut/seeds and DGLV and higher MPA. The food groups milk/yogurt and vitamin C-rich vegetables were also positively and significantly correlated with MPA, although the relationship was not as strong. These results suggest that dietary diversification is a valid strategy to improve micronutrient intake of women of reproductive age, because several food groups contributed substantially to estimated intakes of different individual micronutrients. In Mali, a particular focus on increasing intake of nuts and seeds and DGLV could be used to help improve micronutrient intakes in vulnerable populations.

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Chapter 4

Day-to-day variation in energy, macronutrients and micronutrients intake from a duplicate 24-h dietary recall among urban Malian women of reproductive age and implications for usual intake assessment

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Submitted

Abstract

Day-to-day variation in diet is the most common random error source in dietary assessment hampering accurate estimation of usual intake. The present study reports on the day-to-day variation of urban Malian women diet, with implications for estimating true intake at group and individual level. A duplicate 24-h dietary recall was conducted on independent days with a sample of 108 not pregnant and not lactating women of reproductive age (15-49y), randomly selected in Bamako (Mali), using a three-stage cluster sampling method. One-way ANOVA and general linear model were used to determine intra- and inter-individual variance, unadjusted and adjusted for cluster effects. Adjusted intra-individual variance was used to determine the number of assessment days needed for assessing usual intake. Both intra- and inter-individual variances were used to estimate the number of days required for correctly classifying individuals. The ratio of intra- to inter-individual variance was ≥1 for fat, Zn, Ca, Vitamin A and C, and B6 intake, and <1 for energy, carbohydrates, protein, iron, thiamin and riboflavin intake. Adjusting for cluster effect appeared to increase the variance ratios. The range of days required across nutrients for estimating usual intake was 2-3 (iron, zinc, folate) to 12 (fat) at percentage limit of ±20% for group level, and 1-2 (energy, protein, iron) to 12 (fat) for correctly ranking individual using an attenuation factor of 0.8. For designing future research, these findings should be balanced with research purpose, cost, targeted nutrients, desired precision limit and sampling design to derive the most appropriate required replicates.

Introduction

Day-to-day variation in diet is the most common random error source in dietary assessment hampering accurate estimation of usual intake (1, 2). Usual nutrient intake, defined as the long-run average daily nutrient intake by an individual, is essential for understanding relationships between diet and health status (3). At population level, an accurate estimation of usual intake is required for providing precise estimates of the prevalence of nutrient inadequacy in comparison to dietary recommendations (4). At individual level, accurate estimate of usual intake is also fundamental for ranking correctly individuals for decision making purposes regarding appropriate interventions (5). The extent of the day-to-day variation from diets can be assessed through dietary assessment (3). As daily assessment of diet intake over very long periods is impractical, very costly and often unnecessary, the required number of days for an accurate estimation of the usual nutrient intake can be estimated by quantifying day-to-day variation from a number of replicate dietary assessment over different food seasons (1, 3, 5). The number of days required vary according to the purposes of obtaining reliable estimates of true intake at group level or ranking individuals (1, 5). Some estimates of day-to-day variation in nutrient intake, as well as number of replicates required for assessing usual intake at group or individual level have been previously reported from developed and some developing countries (1, 2, 5-10). Less is known from African countries. Particularly in West Africa, traditional dietary practices such as shared plate-eating (many household members sharing meals in a same plate) are still common, even in urban areas and collecting accurate dietary intake data from such settings is challenging (11). There is a need to report updated information on dietary intakes as well as an estimation of the extent of day-to-day variance in those diets. This will contribute to an accurate assessment of the usual intake, thus decreasing the risk of imprecise estimation of nutrient adequacy prevalence among west African women (3, 12). The present paper reports on day-to-day variation from the diet of urban Malian women of reproductive age assessed by a duplicate 24hDR method, with implications on estimation of true intake at group and individual level.

Subjects and methods

Subjects and sampling

The study was conducted in Bamako, the capital city of Mali. We randomly selected 108 apparently healthy women of reproductive age (15-49y), not pregnant and not lactating from 12 quarters of Bamako, using a three-stage cluster sampling method (13). The sampling procedure has been described elsewhere (14). From the 108 selected, 5 did not complete the 1st day of the 24hDR assessment and 6 were not available for the 2nd day. Thus, a total of 96 women with intake data for the 2 days of 24-hDR assessment were included in variance components analysis.

The ethical committee of the Malian National Institute of Research in Public Health approved the study protocol. Participants were informed in detail, verbally and in written form, about the purposes of the study. Oral agreement was obtained from the participants before dietary assessment and signed informed consent was obtained before anthropometric measurements.

Background information and anthropometry

Baseline information was collected using a short questionnaire, including information about age, marital status, number of children, sociolinguistic group, and occupational activity. Anthropometric measurements (body weight and height) were carried out with a sub-sample of 60 women. Measurements were performed early in the morning, in fasting subjects according to standardized procedures (15). Weight was measured using a SECA platform spring balance model 761, maximum range 150 kg, graduation 0.5 kg. Height was measured using a body measuring tape label MZ10017 (measuring range 2200 mm, graduation 1 mm). Both height and weight were measured twice and the average value was taken.

Dietary assessment

24-h dietary recalls

Trained interviewers conducted two 24-h dietary recalls on non-consecutive days (2d-24hDR) in face-to-face semi-structured interviews at the respondents' home and in their native language. To reduce bias due to occasional consumption of foods with exceptionally high micronutrients content, weekends and special event days were not included in the 24hDR. Participants were interviewed by different enumerators at different sessions to reduce interviewer bias. Food preparers were also probed in case the respondents did not cook on the recall days. A nutritionist supervised the interviews and checked completeness of dietary recall forms. Procedures for collecting dietary information and estimating amounts consumed were performed as described by Gibson and Ferguson (16). The amounts of all foods, beverages and ingredients of mixed dishes consumed by the respondents were estimated either in household units using marked known-weight (standardized) utensils, or in monetary value. The total volume of food cooked for the respondents' household and the volume of the food consumed by the respondent were estimated to derive the proportion consumed. Household units were converted back to weight equivalent as follows: beverages and foods consumed were listed along with corresponding standardized utensils; the standardized utensils were weighed without and with beverages or foods corresponding to levels indicated by the respondents; and amounts consumed were derived from the difference between the two (16). The same procedure was applied to determine total amount cooked for the households, using known-weight utensils. When a monetary unit was used to describe a portion, these foods or ingredients were converted to weight equivalent as follows: all ingredients and their monetary values were listed. Three to five food vendors were randomly selected from each of the three most frequented markets of the study area for a total of nine to fifteen vendors. For each ingredient, the prices and weights of different portions sold were recorded from each vendor to derive the mean weight per fixed monetary value (16). To estimate the amount of ingredients eaten from mixed dishes with unknown amounts of ingredients (e.g., because the foods were gifts from neighbours or purchased outside the home), standardized recipes of these foods were determined (16). Weights were measured using digital dietary scales with a 10 kg maximum range, and 2 g graduation.

Nutrient intake

Nutrient intake was computed with the VBS Food Calculation System version 3[1], using the Mali food composition table (*Table de composition d'aliments du Mali* (TACAM)) (17) as the primary data source for energy and nutrient content of foods. The United States Department of Agriculture (USDA) Nutrient Database Release 20 (18) was used as a secondary data source when needed. The International Mini List version 2.0 (19) and McCance &

[1] Bas Nutrition Software, Arnhem, The Netherlands, www.bare.nl, last update on August 8, 2004

Widdowson's Composition of Foods (20) were referred to when USDA nutrient values were very different from TACAM or when no suitable food match was found in the TACAM or USDA food composition tables. When the nutrient content of cooked forms of the foods was not available, USDA Retention Factors Release 6 (21) was used to account for nutrients loss during cooking. For this study, the intakes of energy, carbohydrates, protein, fat, and 11 micronutrients (iron, zinc, calcium, Vitamin A, Vitamin C, riboflavin, thiamin, niacin, B6, folate and B12) were estimated.

Evaluation of the 2d-24hDR

A weighed food record on one day (1d-WFR) among a sub-sample of 35 women, and the 95% limits Goldberg cut-offs (22) were used to evaluate energy and micronutrients intake assessed by the 2d-24hDR at group level. The ratio of EI to BMR ranged from 0.8 to 2.75 with average values of 1.5±0.4. The ratio fell within the 95% CI of the Goldberg cut-offs, indicating that the reporting bias in energy intake was not significant at group level. In addition, there was no statistically significant difference between mean (original) intakes estimated by the 2d-24hDR and 1d-WFR for all nutrients (*P*-values > 0.05).

Statistical analyses

Statistical analyses were computed with SPSS 19 for Windows (IBM SPSS Statistics Release 19.0.0.1, SPSS Inc., Chicago, IL, 2011). *P*-values < 0.05 were considered significant for all analyses. Mean intake distributions from the 2d-24hDR were visually checked and tested for normality using Shapiro-Wilk test. Energy and macronutrients were normally distributed but not the micronutrients. These latter were log transformed to normality. One-way ANOVA and general linear model were used to determine intra- (S_w^2) and inter-individual variance (S_b^2), unadjusted and adjusted for cluster effects. Adjusted intra-individual variance was used to determine the number of assessment days needed for the observed estimate of an individual's intake to lie within a specified percentage of the true mean at 95% CI, as follows: d = (Z_α CV_w/D_w) (1), where d is the number of required days per individual, Z_α the normal deviate, e.g. 1.96 at 95% CI, CV_w the within-subject variation, calculated as square root of intra-individual variance (SD)/mean intake, and D_w the specified limit as a percentage of long-term individual intake (1). Values of 10% and 20% were tested in this study. Both intra-and inter-individual variances were used to estimate the number of days required for correctly classifying individuals using the following formula $d = [r^2/(1 - r^2)] \times (S_w^2 / S_b^2)$ (23); where d is the number of days, S_w^2 / S_b^2 is the within/between-subject variance ratio, r is the attenuation factor, defined as the unobservable correlation between observed and true mean nutrient intakes of subjects (23). Higher values of r indicate a lower proportion of misclassification. Values of 0.8 and 0.9 were tested for the present study.

Results

Overall, the women's median age was 30 years. Mean BMI was 23.4 kg/m^2. One-third of the women were not married (including widow/divorced), and two-thirds had fewer than 3 children. Thirty-five percent of them were illiterate (never went to school). Nearly half of the women had no income-generating activity (48%), and among those who did, 8% had formal employment. Detailed description of the diet has been given elsewhere (14). Briefly, all of the women (100%) consumed starchy staples, mainly sorghum and millet, and vegetables. Nuts/seeds were consumed by 36% of the women. The main animal source foods consumed were beef (70%), large fish (47%) and milk/yoghurt (47%). Eggs were consumed by only 8% of the women, and foods such as chicken and other poultry were not consumed by any woman in the sample during the dietary assessment period. Sugar-rich foods, mostly refined sugar and soft drinks, were consumed by 57% of the women, oils and fatty foods by 79% and 25% of the women respectively.

Overall, the intra-individual variance was greater than (or equal to) inter-individual variance for fat, Zn, Ca, Vitamin A and C, and B6 intake. Subsequently, variance ratio was ≥1 for those nutrients, with global range of 0.1-3.9 for all nutrients. Niacin, B12 and folate showed very high values due to the influence of outliers. Adjusting for cluster effect appeared to increase the variance ratios, with range of 0.1 to 6.9 (**Table 1**). Based on within subject variation coefficient and at ±10% limit percentage of the true mean, a minimum number of 7 (folate intake) to 47 dietary assessment days (fat intake) would be required to have an estimate of the true average intake of the sample. A number of 2 to 12 days were estimated for ±20% limit. There was no change in number of days required after adjusting for cluster effect. Using within and between subject variance, minimum of 2-29 and 2-12 replications were required respectively for an attenuation factor of 0.9 and 0.8 between true and observed intake (**Table 2**).

Discussion

The present study reported on estimates of day-to-day variation in energy, macro- and micronutrients intake from the diet of a sample of West African women living in urban areas, with implications for usual intake estimation at group and individual level. The ratio of intraindividual to interindividual variance was equal to or greater than 1 for fat, Zn, Ca, Vitamin A and C, and B6 intake. Less variance was explained by within-subject variation for energy, carbohydrates, protein, iron, thiamin and riboflavin intake. Adjusting for cluster effects increased the variance ratios, due to either a reduction of between-subject variance or increase of within-subject variance. However, the increase was not sufficient to influence the number of days required for assessing usual intake.

The variance ratio of energy intake estimated in our study was the lowest as compared to those estimated in other parts of Africa (East (Kenya), South (Malawi) and North (Egypt)) (7) as well as other developing countries (Bangladesh, Indonesia and Mexico) (6, 9, 10) and some developed countries (US, UK and Canada) (2, 5, 8, 10). Similar trend was observed for micronutrients intake, except for zinc, vitamin C and niacin showing higher values than those of the industrialized countries, albeit lower than those of the other developing countries.

Table 1. Mean (SD) and median (IQ range) nutrients intake with variance components (intraindividual, interindividual and variance ratio) unadjusted and adjusted for cluster effects (n=96)

Nutrients	Mean (SD)	Median (25^{th}-75^{th})	Unadjusted Variance[a]			Adjusted variance[a]		
			intraindividual	interindividual	Ratio	intraindividual	interindividual	Ratio
Energy (kcal)	2149 (561.7)	2157 (1798.8 - 2450.9)	266951	763506	0.4	269146	729012	0.4
Carbohydrates (g)	334.0 (93.4)	330.4 (269.5 - 394.4)	6900	55148	0.1	6484	46612	0.1
Protein (g)	60.7 (18.8)	61.3 (47.1 - 72.6)	361	812	0.4	366	986	0.4
Fat (g)	72.4 (29.3)	69.3 (51.8 - 90.3)	644	194	3.3	644	94	6.9
Iron (mg)	17.5 (7.4)	15.9 (12.1 - 22.8)	0.2	0.8	0.3	0.2	0.8	0.3
Zinc (mg)	10.5 (4.1)	10.0 (8.0 - 12.5)	0.2	0.2	0.9	0.2	0.2	1.1
Calcium (mg)	477.0 (256.9)	431.5 (274.1 - 596.7)	12.6	4.7	2.7	13	6.8	1.9
Vitamin C (mg)	62.3 (25.7)	62.9 (42.9 - 74.6)	9.9	4.5	2.2	9.8	7.4	1.3
Vitamin A (µgRE)	371.2 (245.7)	323.8 (172.1 - 555.3)	13.4	3.4	3.9	13.3	5.4	2.5
Thiamin (mg)	1.0 (0.3)	0.9 (0.8 - 1.2)	0.1	0.3	0.3	0.02	0.1	0.3
Riboflavin (mg)	0.8 (0.3)	0.8 (0.5 - 1.0)	0.1	0.3	0.5	0.1	0.4	0.4
Vitamin B6 (mg)	1.3 (0.4)	1.3 (0.9 - 1.6)	0.1	0.1	0.8	0.1	0.1	1.0
Niacin (mg)	10.5 (4.6)	9.5 (6.5 - 13.9)	0.4	0.0	-[b]	0.4	0.0	-[b]
Vitamin B12 (µg)	1.6 (0.9)	1.4 (0.8 - 2.2)	0.4	0.0	-[b]	0.4	0.0	-[b]
Folate (µg)	130.2 (55.9)	120.7 (90.9 - 163.0)	1.3	0.0	-[b]	1.3	0.0	-[b]

[a] analysis with logtransformed values for all micronutrients
[b] very high values strongly influenced by outliers

Table 2. Number of replicates needed for 95% of observed intakes to lie within specified percentage of true mean and for correctly ranking individuals[a] (n=96).

Nutrients	CV intraindividual (%)	Specified percentage limit of true mean[b]		Attenuation factor r^c	
		±10%	±20%	0.9	0.8
Energy (kcal)	24.1	22	6	2	1
Carbohydrates (g)	24.1	22	6	1	1
Protein (g)	31.5	38	10	2	1
Fat (g)	35	47	12	29	12
Iron (mg)	15.5	9	2	1	1
Zinc (mg)	18.2	13	3	5	2
Calcium (mg)	22.7	20	5	8	3
Vitamin C (mg)	25.7	25	6	6	2
Vitamin A (µgRE)	24.5	23	6	11	4
Niacin (mg)	23.9	22	5	-[d]	-[d]
Thiamin (mg)	46.8	19	5	-[d]	-[d]
Folate (µg)	13.6	7	2	-[d]	-[d]

[a] analysis with logtransformed values for all micronutrients
[b] number of days calculated as : $d = (Z_\alpha\ CV_w/D_w)$, where Z_α is the normal deviate (1.96 at 95% CI), CV_w the within-subject variation, and D_w the specified limit as a percentage of long-term intake (1).
[c] number of days calculated as $d = [r^2/(1 - r^2)] \times (S_w^2/S_b^2)$, where r is the attenuation factor defined as the unobservable correlation between observed and true mean nutrient intake, S_w^2/S_b^2 is the within-to-between-subject variance ratio (24).
[d] not calculated because very high values of variance ratios influenced by outliers

This suggested a lower day-to-day variation in urban Malian diet and subsequently less days required for assessing usual nutrient intake, probably explained by the low diversity of the diet and the restriction in food choices (even when a large range of foods is locally available) due to low living standards (9). However, our study was restricted to 2 recall days, excluding weekend days, to a unique food season and to a relatively limited sample size, whereas most of the above mentioned studies were performed on more than 2 recall days, and/or across different seasons of the year. Thus the overall diet variation was not captured, being the major drawback for the present study.

The higher variance ratio of fat intake compared to other macronutrients suggested an emerging intake of high-fat foods by the women, as shown by the percent of women consuming fatty foods such as fried potato, fried plantain. This should be further closely monitored, in association with the increased risk of overweight and obesity among this sample (published elsewhere, (24)). Among the micronutrients, iron intake showed low within-to-between subject variance ratio and low number of replicates for estimating true average intake at 20% acceptable error. This indicated a consistent daily iron intake from the diet, though the bioavailability of this intake is insufficient for achieving high probability of adequacy, due to the negative influence of phytate on iron absorption from plant-based diets (25). The number of days required for an accurate estimation of the usual nutrient intake is an important issue for designing appropriate nutrition research or interventions. It has been reported that the extent of within-subject variation varies across nutrients, and the greater the intraindividual variance, the greater the number of days (1, 2, 5). This was shown in the present study by the range of days required across nutrients for estimating usual intake at group level: 2-3 days (iron, zinc and folate) to 12 days (fat) at percentage limit of ±20%, and 7-9 (iron, folate) to 47 days at ±10%. Also, as expected, different number of replicates were estimated in this study depending on whether the usual intake is needed for deriving group estimates or for ranking individuals (1, 5). For applicability in designing future research, findings from the present study should be balanced with research purpose, cost, targeted nutrients, desired precision limit and sampling design to derive the best suitable number of required days.

Conclusions

Replicating the 24hDR on two independent days allowed having an estimate of day-to-day variation from urban Malian women's diet. Most of the West African countries (including Mali) are in the lowest human development category and in addition, the diet is still influenced by traditional dietary practices. Such information is useful for assessing the extent of nutrient intake inadequacy risk among women in those areas in comparison to dietary recommendations, and for correctly ranking individuals. To improve the accuracy of these estimates, at least 2 to 6 replications for estimation at group level and 2 to 12 replications for analysis at individual level are required for the 24hDR, on independent days, including weekend days and with a larger sample size.

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Chapter 5

Consumption of, and beliefs about fonio (*Digitaria exilis*) in urban areas in Mali

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Abstract

The study sought to determine beliefs and practices about neglected crops in West-Africa, using fonio (*Digitaria exilis*) as a model to understand how obstacles impede the consumption of this cereal in Bamako, the capital city of Mali. This was a cross-sectional study on food ethnography in three steps: a market survey on availability of fonio, a food consumption survey on utilisation of fonio, and on beliefs and attributes on fonio. The study covered the pre-harvest and post-harvest periods and involved key informants, food vendors, and women of reproductive age in households. Fonio, as all cereals, is available year-long on markets in Bamako, and is abundant from September to May before most of the common cereals mature. More than two-thirds (68%) of the women reported having consumed fonio one to three times a month. Fonio was more consumed as snack (djouka) on working days (62%) than on weekend and special event days, suggesting that encouraging the development of ready-to-serve fonio-based products would help increase the consumption of fonio among women in urban area. The average individual portion size of fonio was 152 g/day, and the contribution to daily energy intake was 16%. A large share of the women was convinced that eating fonio was good for them (95%) and their family members (94%). Also, most of them thought that fonio had good cooking, organoleptic and nutritional qualities and could contribute to diet's variation (91% to 100%). Decision by the women to purchase or prepare fonio in the household could be favourably influenced by factors such as media, household members suffering from anaemia, neighbouring people buying fonio and shortage of other cereals; whereas shortage of fonio products (77%), high cost of fonio products (69%), difficult cooking process (51%), and lack of knowledge about processing and cooking fonio (43%) were likely to limit fonio consumption among the women. Also, in the present study, fonio was perceived to be for rich people by more than half (58%) of the women. Improving cooking process and knowledge of the women about fonio cooking, as well as creating a demand for the women with the household's head and others through media, social and health care services would help increase fonio consumption in Bamako.

Introduction

Malnutrition and numerous deficiency diseases continue to persist in the developing world as a result of deficiencies of essential nutrients in the daily diet (1). Women of reproductive age are mostly affected because of increasing needs during pregnancy and lactation. In Mali, a prevalence of 13% has been reported for women of reproductive age suffering from chronic energy deficiency and 50% for anaemic women in Bamako the capital city (2). Household food insecurity is an important underlying cause of malnutrition and how households cope with scarce food are key determinants in maintaining healthy nutrition (3). Promotion of local and traditional food has been recommended to improve household food security and further contribute to reduction of malnutrition (4). Fonio (*Digitaria exilis),* one of the most ancient indigenous West African cereals is a major part of the diet in some communities in Mali (5-7). Regarded for a long time as a minor crop without economic potential (8), fonio is attracting renewed interest in urban areas of West Africa for its organoleptic and nutritional qualities (9). Recently, the crop has been listed as priority crop for West Africa (10). Because of its short growing cycle, fonio can be harvested in the critical shortage season before major food crops (7) and its contribution to food security has been suggested (11). The food composition table of Mali indicates fonio as second cereal with higher iron content (8.5 mg/100g dry matter) after sorghum (11 mg/100g dry matter) (12). The grain is also an excellent source of methionine, cysteine and leucine, whose concentrations are slightly higher than those defined for the FAO reference protein (13). Fonio-based products and modern recipes have been developed in urban areas in Mali (14). Promoting fonio consumption in urban areas could help improve household food security not only as income source but also as a transition food that could contribute to energy intake of household members during seasonal food shortage. However, fonio consumption is still low, particularly in urban areas. Based on a study in three West African cities, Bamako (Mali), Conakry (Guinea) and Ouagadougou (Burkina Faso), a consumption frequency of less than once a month for 54% of fonio consumers in households has been reported (5). Average amount consumed per person was estimated at 4.4 kg/ year for Mali (6) with a range of 650 to 840 g/year in Bamako (5). Difficult post- harvest processing, high-quality product demand, time consuming cooking process, and high-cost of fonio products were pointed out to explain the low consumption of fonio in Bamako (5, 7-9, 15). However, social factors and cultural beliefs are also important factors influencing food choice and consumption patterns (3, 4). The present study sought to determine the intake of fonio in households and shared beliefs about fonio consumption in urban Mali. The research is part of the FP6/EU/INCO/STREP[1] funded FONIO project, which aimed at upgrading the quality and competitiveness of fonio (*Digitaria exilis*) for improved livelihoods in West Africa.

Methods

A cross-sectional food ethnographic study (3, 4) was performed in Bamako, the capital city of Mali, during the pre-harvest (August-September) and post-harvest (February-July) periods. The study was carried out in three steps: 1) a market survey on availability of fonio, 2) a food consumption survey on utilisation of fonio and 3), a beliefs and attributes study on fonio among women of reproductive age (4).

Informants and respondents

Food vendors

Three categories of food vendors were selected: the street food vendors, the supermarkets food vendors and the markets food vendors. Street food vendors are those who sell ready-to-serve food either at fixed places (restaurants) or at non determined places (road sides). The supermarket food vendors are those who sell food at fixed prices in supermarket. The market food vendors are those who sell foods at bargaining base prices in markets. In total, 40 street food vendors, 15 in restaurants and 25 on road sides were randomly sampled. Five supermarkets were randomly selected, according to geographical position. Three of the most frequented markets of Bamako were visited: Medine, Fadjiguila and Sabalibougou. A total of 63 market food vendors were randomly selected according to the type and diversity of foods sold.

Households

A household was considered as any person or group of persons who share the same living accommodation, who pool some or all of their income and wealth and who take food prepared from a common kitchen or cooking pot (16). A total of 30 households were

[1] Project number 0015403

selected by a convenient sampling (17) based on discussion with key informants (agriculture and demography services staff, community leaders). The average size of the households was 11±6.8 members, and 33.7% of the households had more than 11 members. One food preparer was conveniently selected in each household based on discussion with the members. The preparers are the persons in the households who play a key role in the preparation of the food for all household members.

Women of reproductive age

A total of 108 women of reproductive age (15-49 year-old) were randomly selected in 12 quarters of Bamako using three-stage cluster sampling (18) and the random walk method (19). The women were involved in the survey based on verbal agreement.

Availability study

Availability study was carried out in the pre-harvest period to identify available and consumed fonio products in Bamako. This included market survey and interviews in households.

The market survey was done by discussion with the selected food vendors in markets, supermarkets, restaurants and at road sides. Data such as fonio product names, attributes, seasonality, units sold, price per unit sold, were collected.

Interviews were carried out with the food preparers in the selected households. They gave information on the size of the household, the meal pattern, the main fonio dishes cooked in the household, and the seasonal availability of fonio products.

Food consumption survey

A food consumption survey including a 24-hr recall and a food frequency focused on fonio products were performed with the 108 women of reproductive age during the post-harvest period. Data were collected by well-trained local assistants through semi-structured interviews based on standard questionnaires.

The 24-hr recall was performed to assess the mean energy intake of the women, the daily portion size of fonio and the contribution of fonio to energy intake. The recall was performed on two non consecutive days following a standardized format (19). Weekends and special event days were excluded. Amount of ingredients eaten from mixed dishes and snacks with unknown amounts of ingredients (gifts or foods purchased outside the household) were determined using the standard recipe method (19). Amounts consumed were estimated in household utensils and monetary values. Conversion factors from household measures and monetary value to weight equivalent (grams) were determined. Weights were measured using digital dietary scales (Soehnle, Plateau Art, Germany) Nr 65086 (22lb), maximum range 10 kg, nearest to 2 g (0.1oz). Food intake was computed by the VBS Food Calculation System version 3[2] using primarily the Mali food composition table TACAM (12), and secondarily the USDA nutrient database release 18 (20) and 20 (21). The International Mini List (IML), version 2.0[3] and the McCance & Widdowson's composition of foods (22) were used when USDA nutrient values were different from TACAM or when no suitable food match was found. USDA Retention factors release 6 was used to determine nutrient losses during cooking (23).

Foods were classified into 13 groups: starchy staples; dairy; legumes and nuts; small fish eaten whole with bones; all other flesh foods and miscellaneous small animal protein; vitamin A-rich dark green leafy vegetables; vitamin A-rich deep yellow; orange and red vegetables; vitamin C-rich fruits; vitamin C-rich vegetables; vitamin A-rich fruits; eggs; all other fruits and vegetables; and all other foods (including sugars, fats, and stimulants such as tea and coffee). The food groups were taken from the standardized analytical research protocol of the Women's Dietary Diversity Project (24).

The frequency questionnaire focused on fonio and fonio products, and was completed to estimate the usual frequency of fonio products consumption in a period of one month. The questionnaire included a list of 15 fonio dishes with associated frequency categories.

Beliefs and attributes study

The beliefs and attributes study consisted of a questionnaire survey with the 108 women of reproductive age. Topics to be included in the questionnaire were identified by a food attribute and pile sort study (4). For this latter study, 26 women different from the 108, were randomly selected from the households involved in the food consumption survey described above. The main selection criteria were to be willing to participate and to have basic knowledge on fonio. The study on food attributes and pile sort was carried out in three

[2] Bas Nutrition Software, Arnhem, The Netherlands, www.bware.nl
[3] Available from: http://www.fao.org/infoods/

steps: pile setting, food difference and food attribute (4). The questionnaire was structured in topics such as knowledge of iron deficiency; diabetes and fonio; outcomes of fonio consumption; fonio attributes; perceived barriers to fonio consumption; information source; people and factors that could enhance fonio consumption; and subjective beliefs about fonio. To determine the perceptions of the women, the questions were reflected as statements and the women were asked to indicate if they agree, disagree, or are neutral. For each question, each response corresponds to a one-point score.

Data analysis

Incomplete data of 6 women were dropped. Descriptives were used to determine average portion size of fonio, mean energy and macronutrients intake, and contribution of fonio to the mean energy intake. Descriptives were also performed to determine the most consumed fonio dishes and the consumption frequency. Beliefs about fonio were reported as the proportion of women who agree with the statements of the questionnaire. All analyses were performed using SPSS 14.0.1 (2005) for windows.

Results

Fonio availability and consumption frequency

Overall, 11 food groups were available on Bamako markets: 1) cereals and cereal products, 2) roots and tubers, 3) legumes, nuts and seeds, 4) fruits and sweets, including beverages, 5) vegetables including leafy vegetables; 6) meat and poultry; 7) fish and fisheries; 8) dairy and eggs; 9) oils and fats; 10) spices; 11) stimulants and others (**Table 1**).

Table 1. Food availability on Bamako (Mali) markets

Food groups	Foods
Cereals	Wheat, maize, rice, millet, sorghum, fonio
Starchy roots and tubers	Potato, sweet potato, cassava, yam (white and yellow), plantain
Legumes/nuts/ seeds	Cashew nuts, groundnut, bambara groundnut (white and red), coconut, cocoa, African locust bean seeds, cowpea (white and red), Hibiscus seed, green peas, baobab seeds, tamarind seeds
Fruits and sweeties	Orange, lemon (yellow and green), tangerine, avocado, Pineapple, melon, Pear, Liana fruit, apple (green, yellow and red), Papaya, plum (yellow and red), nectarine, grape fruit (red and green), dates, banana (yellow and green), Mango, shea fruit, sugar powder, Chocolate, Honey, guinea sorrel juice, orange juice, soft drinks,
Vegetables	Cucumber, tomato (fruit and paste concentrated), okra, onion, shallot, shallot leaves, Hot pepper, sweet pepper green, Egg plant, Bitter tomato, cabbage, lettuce, Parsley leaves, Turnip, Carrot, Beet root, french bean, baobab leaves, Hibiscus leaves, Green leaves,
Meat/poultry	Beef, veal, goat, lamb, pork, chicken, duck
Fish and fisheries	carp (red and grey), pink trout, grouper, sardine, catfish, threadfin, shrimp, freshwater fish, sea crab, gamba
Dairy/eggs	cow milk, yoghourt, cheese, chicken eggs
Oil and fats	sunflower oil, olive oil, palm oil (white and red), peanut oil, soya oil, butter, Shea butter,
Spices	pepper grain, aniseed, garlic, curry, ginger, clove, laurel leaves, vinegar, maggi cube, mustard
Stimulants	Coffea, tea, colanut

Wheat, rice, millet, maize, sorghum and fonio were the most common cereals available in Bamako. Like all cereals in Bamako, fonio is available year-long but is abundant before all other cereals from September (one of the typical shortage months in Mali) to May. On markets, fonio is sold either as whole grain, husked grain, precooked grain, or washed and dried. Two main fonio dishes were sold at the road sides: foyo (fonio couscous) and djouka (mixture of fonio, vegetables and groundnut). The most common fonio products available in supermarkets were dried precooked fonio, djouka and dègué (mixture of fonio and curdled milk). In restaurants, fonio is served either as foyo supplemented with various sauces, or as djouka. At home, the most common fonio dish cooked is foyo, supplemented with various sauces.

Of the 15 fonio- based dishes, djouka, foyo and fini zamé (fried fonio) were eaten by 73 out of 102 women (71%). Among those consumers, foyo and djouka were eaten by 41% and 55%, respectively (**Table 2**). Among those consuming foyo and djouka, 68% reported a consumption frequency of one to three times per month. Few women (8%) reported consumption of more than 10 times per month (Table 2). Fonio was more frequently consumed as snack (djouka) on working days (62%) than on weekend (26%) and special event (baptism and wedding) days (13%).

Table 2. Frequency of consuming fonio among women in Bamako (n = 73)

	Times / month									
	1	2	3	4	5	6	8	10	13	Total
Number of women consuming all fonio dishes	31	12	7	6	3	6	2	4	2	73
	Number of women consuming by dish									Total
Djouka fonio	14	5	4	4	3	3	2	3	2	40
Foyo	15	6	3	2	0	3	0	1	0	30
Fini zamé	2	1	0	0	0	0	0	0	0	3
Days of consumption	Number of women									
	Working days			Weekend days			Event days			Total
	45			19			9			73

Fonio intake and contribution to energy intake

Starchy staples, vitamin C-rich vegetables, all other fruits and vegetables, other flesh foods and miscellaneous small animal protein, and legumes and nuts were the food groups mostly consumed by the women (**Table 3**).

Among starchy staples, rice (38%), wheat products (33%) and millet products (20%) were the most commonly consumed, the least consumed being fonio (5%). Tomato (36%), cabbage (19%) and hot pepper (15%) were the most often consumed vitamin C-rich vegetables, used mainly as ingredients of sauces (percent not shown in table).

Table 3. Number of women consuming food groups and contribution to mean macronutrients intake (n=102)

Food groups	Most consumed foods	Number of women	Contribution to intake		
			Energy	Protein	Fat
All starchy staples	Rice, wheat, millet, fonio, sorghum, yam, cassava, potato, sweet potato, plantain	102	45.7	36.0	12.1
Vitamin C-rich vegetables[a]	Green sweet pepper, tomato, cabbage, dried onion, fried dried shallot, hot pepper	102	1.6	2.4	0.3
All other fruits and vegetables	Cucumber, okra, onion, garlic, tomato paste, bitter tomato, eggplant, pumpkin, courgette, french bean, garlic, ripe banana, pineapple juice unsweetened	101	3.2	4.2	0.3
Flesh foods and miscellaneous small animal protein	Beef, large fish (nile perch, catfish mudfish)	100	6.7	27.3	11.3
All legumes and nuts	African locust bean, groundnut, cowpea	74	10.5	16.8	23.2
All dairy	Milk	49	4.6	9.0	7.6
Vitamin A-rich dark green leafy vegetables[b]	Lettuce, amaranth leaves, green leaves, shallot leaves, sweet potato leaves, fakouhoye leaves, bean leaves, parsley/celery leaves	42	0.4	1.2	0.1
Vitamin A-rich deep yellow, orange and red vegetables[b]	Carrot	17	0.1	0.1	0.0
Vitamin C-rich fruits[a]	Orange, ripe papaya	9	0	0	0
Eggs	Hen egg	8	0.3	0.9	0.6
Vitamin A-rich fruits[b]	Ripe mango, red palm oil	8	0.5	0.0	1.4
Small fish eaten whole with bones	Small fish	6	0.1	0.4	0.0

[a] Vitamin C-rich fruits and vegetables are defined as those with >18 mg/100g in the form eaten
[b] Vitamin A-rich fruits and vegetables are defined as those with >130 RAE/100g in the form eaten

Mean daily energy intake of the women ranged from 525 to 4150 kcal, averaging 2054 kcal (**Table 4**). With 35% sourced from animal foods, mean protein intake of the women contributed 11% of the daily energy intake, being in the range recommended by WHO (25). Energy intake was mostly provided by starchy staples, with cereal-based dishes being the largest contributors to energy intake, and legumes and nuts groups the largest contributors to fat intake (**Table 4**). Protein intake was mostly provided by starchy staples group (36%), other flesh foods and miscellaneous small animal protein group (27%), and legumes and nuts group (17%). Based on the proportion of women consuming fonio products, individual portion size of fonio ranged from 113 to 208 g/day, averaging 152 g/day. The average energy intake from fonio was 321.6±74.6 kcal/day, giving a contribution of 16% to mean daily energy intake.

Table 4. Mean daily macronutrient intake of women (n=102)

	Mean nutrient intake			
	Mean	**SD**	**Range**	**% kcal**
Energy (kcal)	2054.0	716.5	525 - 4150	
Protein (g)	57.7	25.6	12.6 - 136.0	11
Animal protein source (g)	20.1	20.0	0 - 8.6	4.6
Plant source (g)	38.7	20.8	9.2 - 109.9	8.8
Total carbohydrate (g)	310.1	112.3	106.6 - 605.6	57
Total fat	73.2	34.8	1.7 - 181	32
Fonio consumption				
Individual portion size of fonio (g)	152.4	35.3	113 - 208	
Energy intake from fonio (kcal)	321.6	74.6	238.4 - 438.9	

Beliefs and attributes about fonio

According to their background, more than half of the women reported that fonio contains iron and its consumption could help prevent anemia and treat diabetes (**Table 5**). A large share of the women (94%) was convinced that eating fonio is good for them and their family members. Further, most of them thought that fonio had good cooking, organoleptic and nutritional qualities and could contribute to diet's variation. However, some barriers to fonio consumption have been identified.

Table 5. Beliefs about fonio consumption

Topic	Questions	Percent
Knowledge	Fonio is important to treat diabetes	88
	Fonio can prevent anaemia	70.4
	Fonio contains iron	64.8
Outcomes from fonio consumption	Eating fonio is good for my household members	95.4
	Eating fonio is good for me	94.4
Fonio attributes	Fonio has good taste, swells up well during cooking, is pleasant in mouth	99.6
	Fonio is a traditional food and diversifies meals	94.5
	Fonio stimulates appetite, easy digestible, nutritious, healthy, good for weight loosing	92.8
	Eating fonio helps to treat diseases and to prevent stomach problems	91.2
Perceived barriers	Fonio not available throughout the year	76.9
	Fonio not available on markets and restaurants	73.5
	High cost of fonio products	69.4
	Fonio contains impurities, not white, has small size grain, low quality variety	62.8
	Not knowing how to cook fonio	42.6
	Household size too large to prepare fonio	51.9
	Time consuming, hardness of cooking	51.4
	Head of the household does not like fonio	45.4
Information source, people and factors enhancing fonio consumption	The media favourably affect decision to eat fonio	93.5
	Nurse, social workers, favourably affect decision to eat fonio	93.5
	Fonio consumed mostly during important ceremonies, like weddings, funerals or baptism	92.1
	Household members suffering from anaemia favourably affect decision to buy fonio	90.8
	Friends, members of my association, neighbours favourably affect decision to eat fonio	85.8
	People around me buying fonio makes me want to eat fonio	85.2
	Husband, household members, mother-in-law favourably affect decision to eat fonio	83.9
	A shortage of food favourably affects decision to eat fonio	81.5
	Fonio sellers favourably affect decision to buy fonio	79.7
	Fonio consumed mostly in restaurants and when guests in household	74.1
Subjective beliefs	Fonio is for rich people	58.3
	Fonio is for poor people	35.2

These are: 1) seasonal shortage (or frequent unavailability) in restaurants (77%) and on markets (74%); 2) high cost of fonio products (69%); 3) lack of knowledge about processing

(43%); time consuming processing and difficult cooking process (51%); and 5) small size, dirtiness and dark colour (some varieties) of fonio grains (63%). The size of the household (52%) and the apathy of the household head for fonio consumption (45%) were additional barriers reported by the women. Decision to buy or prepare fonio for the household could be favourably influenced by factors such as media (94%), household members suffering from anaemia (91%), neighbouring people buying fonio (85%) and food shortage (82%). The women also reported that they ate fonio mostly in restaurants or when they have guests for dinner at home (74%) and during important celebrations like weddings, baptism (92%). Paramedical staff, friends, husband and family members are persons who could positively or negatively influence fonio consumption. Beliefs such as "fonio is for rich people" were also reported by 58% of the women as factors likely to influence fonio consumption.

Discussion

Food ethnography was performed to determine the contribution of fonio to the dietary pattern, and to identify shared beliefs about fonio consumption in urban Mali.

Fonio is available in shortage season before the most common cereals are harvested. The usual diet is based on cereal, mainly rice, and fonio was reported as consumed by 5% of the women. The same trend has been earlier described for a rural area in Mali, but the main cereal dishes were based on maize and sorghum, and fonio consumption reported by 12% of men and women (26).

This difference could be linked to the relative abundance of almost all common cereals during the food consumption survey period (post-harvest), and also to the contrast of urban vs. rural areas, where dishes are still mostly based on indigenous cereals (8). Consumption frequency (one to three times a month) and average daily individual portion size of fonio (152 g) were higher in the study compared to the frequency of less than once a month and the individual amount of 650 to 840 g/person/year reported earlier in the study on fonio consumption in urban areas (5).

This suggests an increase in fonio consumption since 2004. However, because of the low proportion of women consuming fonio dishes (5%) and small size of daily portion consumed (152 g/day) compared with those of rice (38% and 489 g/day, respectively), the contribution of fonio was about the fifth of the daily energy intake of the women consuming fonio dishes. This small portion size of fonio compared to rice could be explained by the fact that fonio was mostly consumed as snack. No previous studies reported contribution of fonio to energy intake, thus hampering comparisons, but under same conditions (equivalent proportion of consumers and daily portion size), fonio (steam-cooked) would better contribute to energy intake than rice, due to its higher energy content: 223 kcal/100g for cooked fonio as compared to 102 kcal/100g for cooked rice (12).

Fonio has often been reported as a food mostly cooked and consumed during weekends and special events (5, 7-9, 15). A previous study in Bamako also reported that fonio was often consumed outside and cooked in households mostly when there are guests (5). The availability study confirmed that fonio products are mostly served in restaurants and most of the women are likely to consume fonio outside. The food frequency survey revealed that fonio is more often consumed on working days than on event days. This suggests that encouraging development of ready-to-eat fonio-based products would help increase the consumption of fonio among women, especially in urban areas.

Difficult post-harvest processing, time consuming cooking process, and high-cost of fonio products were often reported as common barriers to fonio consumption (5, 8, 9, 15). The results of the present study not only supported previous studies but revealed other factors that were also likely to hamper fonio consumption in Bamako. One of the perceived barriers was the lack of knowledge about processing and cooking fonio. Due to its very small size, fonio is contaminated with sand and dust during post- harvest processing and needs to be carefully washed for several times using a traditional sedimentation method to get a cleaned product, which must be steam-cooked two to three times at least to get a soft-in-mouth and easy-to-digest product (9). This cleaning and cooking process needs some basic ability and knowledge that might not be common among all women, especially in the context of urban areas. Another reported obstacle was the size of the household. This might be linked either to the high cost of fonio products which could not allow purchasing large amounts when the size of the household is large, or to the difficult cleaning and cooking process that might increase cooking time in large size households. Furthermore, the apathy of the household head for fonio consumption (perceived barrier for 45% of the women) might be related to the low quality of cooked fonio served by the women in the households due to their lack of knowledge and skills. On the other hand, factors like food shortage, media, family members suffering from anaemia, neighbourhood opinion, and paramedical staff, family members and friends are likely to influence fonio consumption among the women. Association between these factors and consumption of fonio needs to be tested in further studies. Finally, in the present study, fonio was perceived to be for rich people by more than half of the women. This viewpoint contradicts previous reports on fonio which often postulated fonio to be for poor people (7, 9). These discrepancies in results might be due to the fact that this study was carried out in an urban context and women could have linked their opinion to the high cost of fonio products.

Conclusion

The purpose of this study was to determine shared beliefs that could positively or negatively influence fonio consumption. Results showed that fonio is available year- long on markets in Bamako, as all cereals, but is abundant before most of the common cereals. More than two-thirds (68%) of the women reported consuming fonio one to three times per month. Fonio was more consumed as snack on working days than on weekend and special event days, suggesting that encouraging development of ready-to-eat fonio-based products would help increase consumption of fonio among women in urban areas. Energy intake was mostly provided by starchy staples, with cereal-based dishes being the largest contributors to energy intake. The average individual portion size of fonio was 152 g/day, and its contribution to daily energy intake was 16%. A large share of the women was convinced that eating fonio is good for them and their family members. Further, most of them thought that fonio had good cooking, organoleptic and nutritional qualities and could contribute to diet's variation (91% to 100%). The decision to buy or prepare fonio by women in households might be favourably influenced by factors such as media, household members suffering from anaemia, neighbouring people purchasing fonio and shortage of other cereals; whereas shortage of fonio products, high cost of fonio products, difficult cooking process, and lack of knowledge about processing and cooking fonio are likely to limit fonio consumption among women in urban area. Further, fonio was perceived to be for rich people by more than half of the women. Improving cooking process and knowledge of women about fonio cooking, as well as creating a demand for the women with the husband and others through media, social and health care services would help increase fonio consumption inside households in Bamako. Further research should be designed to examine the effect of these factors on consumption of fonio in urban areas in Mali.

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Chapter 6

Factors predicting consumption of fonio grain (Digitaria exilis) among urban Malian women of reproductive age

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Abstract

Objective: To identify factors influencing intention to consume an indigenous nutritious grain, fonio (Digitaria exilis), among women in Mali.

Design: A cross-sectional questionnaire survey based on the Theory of Planned Behavior and the Health Belief Model.

Setting and Participants: One hundred and eight women (31.5 ±10.5 years), randomly selected by a 3-stage cluster sampling, Bamako, Mali.

Main Outcome Measures: Model constructs scores, intention to consume fonio, and fonio consumption.

Analysis: Correlation and multiple regression, Wilcoxon signed rank and Mann-Whitney tests for score comparison.

Results: Attitudes toward behavior ($\beta = 0.32$, $P < 0.05$) was the best predictor of intention to consume fonio, which was significantly correlated with fonio consumption (*rho* = 0.78, $P < 0.001$). Health value ($\beta = 0.23$, $P < 0.05$) was a significant predictor of health behavior identity, which was significantly correlated with attitudes toward behavior (*rho* = 0.67, $P < 0.001$) and perceived barriers (*rho* = 0.33, $P < 0.001$). The latter formed a significant interaction term between intention and behavior ($\beta = -0.72$, $P < 0.05$).

Conclusions and Implications: Fonio consumption could be increased in Bamako through stimulation of positive attitudes, changing men's beliefs, influencing family and neighbors' opinions, and improving the processing and the skills of women who prepare fonio.

Introduction

Sorghum and millets are among the world's most important food crops being the main sources of protein and energy (1) especially for inhabitants of the semi-arid tropics. In Mali, sorghum, millet, maize, rice and fonio are traditionally common cereals which provide the basis for a daily meal in many households (2).

Fonio (*Digitaria exilis)* is considered one of the oldest West African indigenous cereals (3). Up to 250 000 tons of fonio is produced annually on more than 380 000 hectares (4), and its contribution to food security has been suggested (5). Fonio resembles Te'f (*Eragrostis tef*), a traditional cereal cultivated in Ethiopia. Te'f and fonio have some similarities in structural and physicochemical properties (6). The potential for production of fonio in West Africa is high, because it performs better than other cereals under conditions of drought and low soil fertility, and is less susceptible to damage by pests during storage (7). In countries like Burkina Faso, Guinea, Senegal, Mali and Nigeria, fonio is a staple food especially for communities in mountainous areas (3). Its potential for reducing human misery during "hungry times" is valuable because some varieties have a very short growing cycle and are ready to harvest before all other grains. Moreover, when the other cereals are scarce due to a failing harvest, fonio consumption is high (3).

Nutritionally, fonio is regarded as a rich source of energy during the food shortage season (3). The food composition table of Mali indicates fonio as the cereal with the highest content of calcium (41 mg/100g dry matter) compared to the other cereals, and the second for highest concentration of protein (7.1 mg/100g dry matter) and iron (8.5 mg/100g dry matter) after sorghum (11 mg/100g dry matter for both protein and iron) (8). Fonio is also an excellent source of methionine, cysteine and leucine, whose concentrations are slightly higher than those defined for the FAO reference protein (9). Fonio species contain less polyphenols than sorghum (10), hence the high digestibility of protein observed in fonio (11).

Fonio is among the world's best-tasting cereals and has a high diversity of uses in human consumption (3). In many African countries, fonio is traditionally cooked as couscous, various porridges and dishes, and local beverages (11). Also, fonio has been used for bakery products such as cakes, doughnuts and cookies, and has been suggested as a substitute for semolina (a wheat product used to make pastas) (6).

Fonio has a great religious and socio-cultural importance for its producers in many West African communities (3, 12, 13). In Senegal, Mali, Guinea, Burkina Faso and Togo, fonio is reserved particularly for chiefs, royalty and special occasions like *Ramadan* for Muslims (12, 13). For some traditional communities in Togo, fonio is the most important ingredient in women's initiation ceremonies, weddings, and for the traditional baptism of newborn children. In some communities fonio is believed to help prevent blood clotting after women give birth (12) and fonio porridge is recommended for breastfeeding women to stimulate milk production (14). The grain is also reported to be easy to digest (4).

One of the objectives of the Plan of Action of the Food World Summit 1996, was to reduce by half the number of undernourished people before the year 2015. The importance of the production and consumption of traditional under-utilized crops with good nutritional value, which have been abandoned in favour of commercial crops, was highlighted among the recommended strategies to ensure food security in West African households (14, 15). From a nutrition perspective, under-utilized crops such as fonio could increase the availability of energy and nutrients and provide a balanced diet composition, improving food security in low-income rural and urban households. Moreover, fonio can be cultivated in small family farms with minimum costs of water and fertilizers, in land not appropriate for other crops. Therefore, small producers and women may obtain economic benefits from these income-generating activities (15).

During the past 10 years, collaborative efforts have been made to make fonio more competitive on markets in terms of quality and price, by domesticating the seeds, and improving the grain processing techniques at small companies and women's groups through the modernization of existing and the development of new equipment (4). Modern small-scale processing industries for ready-to-cook products have been developed (16). Despite these efforts, fonio is still under-utilized and its consumption is low in urban areas. In urban areas in Mali, fonio accounts for less than 1% of the cereals eaten, and the average consumption is between 0.5 kg to 1.0 kg per person annually (16).

To date, limited research has indicated that difficult post-harvest processing, a time consuming cooking process, and the high-cost of fonio products compared to other cereals like maize, rice and millet, are the main factors explaining low consumption of fonio in urban areas (16). However, social factors and cultural beliefs can also influence food choice and consumption patterns (17). To improve the consumption of fonio as a staple food in urban areas, there is a need to identify social cognitive factors that may positively or negatively influence its consumption, especially in urban contexts. Also, the interrelationships among the potential factors and the consumption of fonio are important issues to be clarified in the process of enhancing production and consumption of a crop that could contribute to household food security in both rural and urban areas.

The Theory of Planned Behavior (TPB) (18, 19) and the Health Belief Model (HBM)(20) have been used to examine factors influencing consumption behavior (21, 22). According to the TPB, behavior is mainly determined by a related intention. Behavioral intention is established based on behavioral attitudes, subjective norms, and perceived behavior (18, 19). The HBM is a health-specific model, which suggests that health behaviors are the result of a set of core beliefs (21).

Sun et al. (23) suggested a new model based on the combination of the TPB and the HBM in their study on variables that significantly predict intention to consume iron-fortified soy sauce in rural and urban areas of China. This new model consists of 12 constructs distributed within internal and external factors (**Figure 1**) (23, 24). Perceived susceptibility is one's subjective perception of the risk of contracting a health condition, and perceived severity expresses the feelings concerning the seriousness of contracting an illness or leaving it untreated. Health value represents the importance of the health consequences of the disease. Perceived barriers are the beliefs about negative aspects of the behavior, while attitudes towards behavior are the positive or negative evaluation of the consequences of the behavior and its importance. Health behavior identity reflects whether the person thinks that performing the behavior is a good thing to do. External control beliefs represent one's perceived ability to perform the behavior, while cues to action are external triggers that stimulate action to perform the behavior. Subjective norms reflect what others think the individual should do and the individual's motivation to comply. In the Attitude-Social Influence-Self Efficacy model (25) the perceived barriers in internal factors are expected to be an interaction term in the relationship between intention and behavior. This relationship was added to the original model of Sun et al. (23) in our study.

Using the combined models of the TPB and HBM, the present study seeks to identify factors influencing the consumption of fonio among women of reproductive age in urban areas in Mali.

Methods

Participants and sampling

In total, 108 apparently healthy women of reproductive age (15-49 year-old) belonging to one Malian sociolinguistic group were randomly selected from 108 households in 12 quarters of Bamako, using a 3-stage cluster sampling, as described by UNICEF (26). Households were selected using the random walk method (27), and all eligible women of the selected households were listed. From this list, one woman was randomly selected in each household for the questionnaire survey. The main selection criteria were willingness to participate and basic knowledge on fonio products as foods. When a selected woman did not fulfill the selection criteria, another woman in the same household was selected. Almost all selected women met the selection criteria.

To identify the items of the model, an attribute-pile sort (APS) study was conducted with 26 key informant women, randomly selected from the previously described list of eligible women based on their willingness to participate, excluding those selected for the main study. Verbal agreements were obtained from the local leaders of the quarters, the heads of households and the women before starting the survey. The study is part of a larger project aiming at upgrading the quality and competitiveness of fonio for improved livelihoods of the stakeholders of the marketing system (28). One objective of the project is to determine the mean nutrient intake and nutritional status of the women of reproductive age in Bamako and the importance of fonio in the dietary pattern. The proposal of the whole survey was approved by the Ethical Committee of the National Institute of Public Health Research of Mali.

Food APS study

The food APS study was conducted in 3 steps: the pile sorting, the food difference and the food attributes, as described in Blum et al. (29). The food groups of the Mali food composition table (8) were used for the pile sorting. The food difference method was based on comparison of fonio with rice and millet. The food attributes method was applied only for fonio and fonio products.

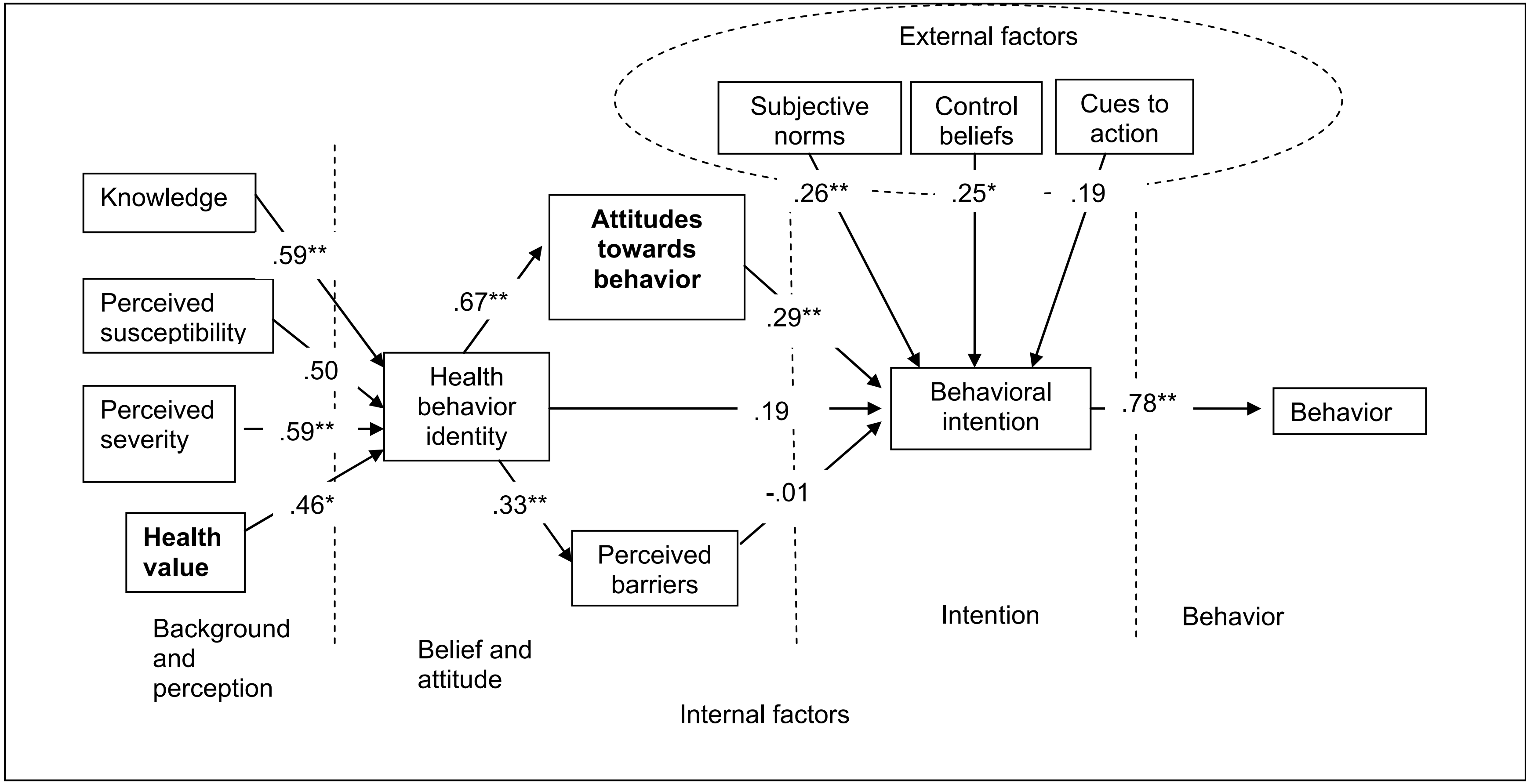

Figure 1. Combined Health Belief Model and Theory of Planned Behavior based on Sun et al. (23) with Spearman correlation coefficients between constructs. ** P<0.05, ** P<0.01*

The women were asked to describe 3 main attributes: (1) the outcomes (advantages and disadvantages) expected when consuming fonio products, (2) the people and the information sources that would enhance their fonio consumption, and (3) the factors that would limit their consumption of fonio.

Questionnaire

The questionnaire consisted of 76 items identified by a literature search and the APS study. The items were categorized according to the 12 constructs of Sun et al.'s model (**Figure 1**) (23). The maximum number of questions per construct was 20 (perceived barriers). External control beliefs had only one question. Items about iron deficiency and diabetes were introduced in background and perception constructs because relationships were expected between these items and fonio consumption based on the answers of the women during APS study. Behavioral intention and behavior are intention to consume fonio and fonio consumption respectively. Socio-demographic characteristics were recorded as well. The questionnaire was translated into French and Bambara (the most common local language) to ensure that locally used terms for iron in food, iron-rich food, iron deficiency, and diabetes were used. Correctness of the translation was checked with back translation into English and French respectively. To ensure that questions were comprehensible for the women and that they were stated in a standardized way by the interviewers, the questionnaire was pre-tested among 10 women different from those participating in the research. No change to the questionnaire was necessary after the pre-test.

Variables measurement

The variables were measured at the respondents' home through structured interviews carried out by well-trained local female assistants (native French and Bambara speakers) based on the questionnaire. To ascertain that the women understood the terms "diabetes", "iron in food", and "anemia", they were asked if they had heard about these terms, and whether they could give an example of food rich in iron before starting the interviews. No woman was removed from the study because all of them were aware of the terms. All questions, except for those related to socio-demographic characteristics, were reflected as statements and the women were asked to indicate their level of agreement using a 5-point

Likert response scale, ranging from strongly disagree to strongly agree. To ensure that respondents understood the Likert scale well, an example question not related to fonio was used to clarify the response scale before each interview. For intention to consume fonio and fonio consumption, a different answer scale was used: no consumption, once a month, 2-3 times a month, once a week, and 2 or more times a week.

The items of most constructs were scored from 1 to 5. However, the items of 2 constructs (attitude towards behavior and subjective norms) consisted of pair questions (behavioral beliefs & evaluation of beliefs, and normative beliefs & motivation to comply, respectively). The scores of the behavioral and normative beliefs ranged from -2 to 2, and scores of the evaluation of beliefs and motivation to comply ranged from 1 to 5. The scores of the pair questions were then multiplied to derive one score for each item. This resulted in a score range of -10 to 10 for each item. For each respondent, the scores within a construct were added, to derive a total score per construct.

Data analysis

Descriptive statistics were performed to examine socio-demographic characteristics of the women, and to calculate the median scores of the constructs. Multiple item constructs were tested for reliability of the questionnaire and internal consistency using Cronbach's α and item-total correlation (30). The items within a construct were regarded as consistent when Cronbach's α was approximately 0.80 or higher and the corrected item-total correlation of all items in a construct was higher than 0.30 (30). The Wilcoxon signed-rank test was used to compare the scores of intention to consume fonio and fonio consumption and to test whether the subjects significantly changed their response in one direction (i.e. score intention > score behavior). A Mann-Whitney test was used to examine whether the scores of women with a high intention and those with a low intention to consume fonio were significantly different. Spearman's correlation (31) was used to test the bivariate association within constructs. Multiple linear regression analyses were performed to examine the contribution of the constructs to intention to consume fonio, and to assess whether the perceived barriers were a significant interaction term in the relationship between intention to consume fonio and fonio consumption. All models were adjusted for age, education and interviewer. Overall, statistical tests were two-tailed and P-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS for Windows (SPSS Inc., version 12.0.1, Chicago, Ill, 2003).

Results

The mean age of the women was just beyond 30 years and 15% of the respondents were adolescent (15-18 year-old). Illiteracy rate was 29%, while 17% reached secondary and higher school. Approximately half of the women (46.3%) went to primary school, but not always completed the education program. Two-thirds of the women were married and 34% had more than 3 children. One-third of them have no children. Two-thirds of the women obtained an income, mainly by commerce activities (**Table 1**).

Cronbach's α coefficients ranging from 0.77 to 0.95 demonstrated high reliability of the multiple item constructs (**Table 2**). Median scores for all constructs ranged from 4 (external control beliefs) to 70 (perceived barriers). The high value of median scores in comparison to the range of possible scores showed that most women tended to agree with the statements.

Table 1. Sociodemographic characteristics of the women (n=108)

Characteristics	Percent
Age	
15 – 18 years	14.8
19 – 34 years	46.3
35 – 49 years	38.9
Education	
Illiterate	28.7
Primary school [a]	46.3
Islam school [a]	8.3
Secondary school or higher [a]	16.6
Ethnic group	
Malinké	21.3
Bambara	18.5
Peulh	12.0
Other (Sarakolé, Senofo, Bamanan etc.)	48.1
Marital Status	
Married	67.3
Separated	2.9
Unmarried	26.9
Widow	2.9
Number of children	
No	33
1 to 3	32.9
4 to 11	34.1
Income-generating activities	66.7

[a] Some of the subjects did not complete the education program.

Note: A household is considered as a group of persons who sleep and eat together.

Almost 40% of the women had consumed fonio at least once a week in the last month, while more than 50% had the intention to consume fonio once or more a week. About 5% did not consume fonio in the past month and 10% had no intention to consume fonio in the next coming month (**Figure 2**).

Intention to consume fonio was highly correlated with fonio consumption (*rho* = 0.78, $P < 0.001$) (**Figure 1**). The Wilcoxon signed-rank test showed that more than 50% of the women had the same scores on intention to consume fonio and fonio consumption. In case of differences, the score on intention to consume fonio was significantly higher than fonio consumption ($P < 0.001$) (data not shown).

Table 2. Internal consistency and median scores of the constructs (n=108)

Constructs	Number of items	Cronbach's α	Median	(25^{th}-75^{th})	Range[a]
Knowledge	12	0.86	50	(46-55)	12; 60
Perceived susceptibility	8	0.77	32	(27-34)	8; 40
Perceived severity	14	0.88	61	(56-65)	14; 70
Health value	7	0.89	33	(31-35)	7; 35
Health behaviour identity	2	0.95	9	(8-10)	2; 10
Perceived barriers	20	0.89	70	(59-79)	20; 100
Attitudes towards behaviour	12	0.93	67	(48-104)	-120; 120
External control beliefs	1	-	4	(2-5)	1; 5
Cues to action	10	0.87	40	(38-43)	10; 50
Subjective norms	8	0.92	34	(27-50)	-120; 120
Intention	1	-			
Behaviour	1	-			

[a] Range refers to the possible scores for each variable

All mean scores of the items within attitudes towards behavior were positive for both the low and high-intention groups, indicating that the general evaluation of the consequences of fonio consumption was positive in both groups. The Mann-Whitney test for differences between the 2 groups showed that the items taste, swelling during cooking, nutritional content, health benefits, weight, and treatment of diseases were more important for the high-intention group ($P < 0.05$) than for the low-intention group (data not shown). The mean scores of subjective norms were also positive. The husband's opinion was more important for the high-intention group than the low-intention group ($P < 0.05$) (data not shown).

Most constructs were significantly correlated with intention to consume fonio, except for perceived barriers, health behavior identity and cues to action (**Figure 1**). Significant correlations were found between background and perception of the respondents and health behavior identity, with knowledge and perceived susceptibility showing the strongest correlation ($rho \geq 0.5$, $P < 0.01$).

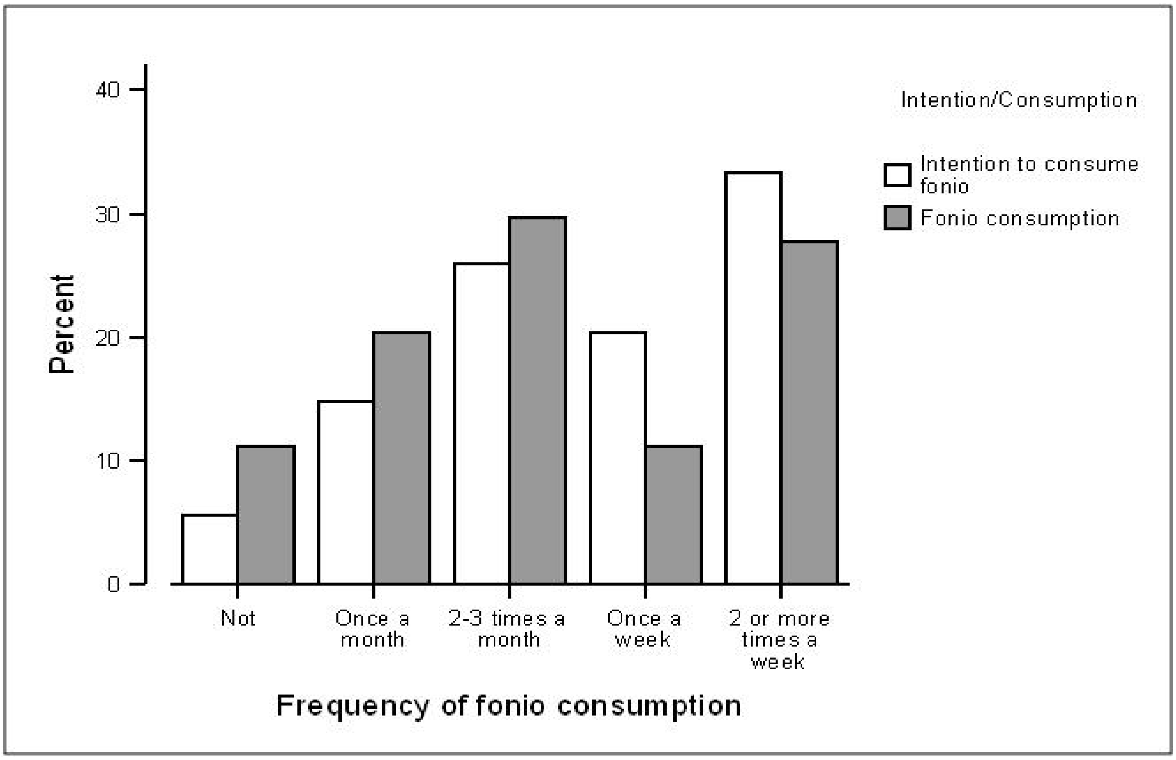

Figure 2. Intention to consume fonio in the coming month, and fonio consumption of the last month (*n*=108).

Concerning the constructs within 'belief and attitude', health behavior identity was significantly correlated with perceived barriers ($rho = 0.33$, $P < 0.001$) and attitudes towards behavior ($rho = 0.67$, $P < 0.001$). This latter construct showed a significant correlation with intention to consume fonio ($rho = 0.29$, $P < 0.01$). From the external factors, subjective norms ($rho = 0.26$, $P < 0.01$) and control beliefs ($rho = 0.25$, $P < 0.05$) showed significant correlations with intention to consume fonio (**Figure 1**).

The relative contribution of the variables to behavior is shown in **Table 3**. Roughly 66% of the variance in fonio consumption could be explained by intention to consume fonio and perceived barriers (Model 4). Only 5% of the variance in intention to consume fonio could be explained by 'Belief and attitude', of which attitudes towards behavior was the only significant predictor (Model 2; standardized $\beta = 0.32$, $P < 0.05$). The variables within 'Background and perception' accounted for 34% of the variance in health behavior identity, where only health value was a significant predictor (Model 1; standardized $\beta = 0.23$, $P < 0.05$). Subjective norms from the external factors seemed to be the best predictor for intention to consume fonio (standardized $\beta = 0.28$, $P < 0.05$, not shown in the table), but significance disappeared after adjusting for age, education, and interviewer (Model 3). In model 4, perceived barriers were a significant interaction term (standardized $\beta = -0.72$, $P < 0.05$) in the relation between intention to consume fonio and fonio consumption. In the high-barrier-group (score on perceived barriers > 70, n = 54) the association between intention to consume fonio and fonio consumption was lower (*rho* = 0.69, $P < 0.001$) compared to the low-barrier group (score on perceived barriers ≤ 70, n = 54, *rho* = 0.87, $P < 0.001$).

Discussion

The core objective of our study was to identify factors influencing the consumption of fonio among women of reproductive age in urban areas in Mali, using the adjusted model of Sun et al. (23). Intention to consume fonio was a good predictor of fonio consumption among women of reproductive age. A greater intention to consume fonio was directly associated with positive attitudes and through these, was indirectly influenced by health behavior identity. The latter was associated with health value. Finally, the association between intention and behavior was lower when perceived barriers were higher.

Our study confirmed the results of previous studies stating that the behavior is mainly determined by a related intention. Intention accounted for 66% of the variance in the behavior in our study. No similar previous studies on fonio or other cereals has been reported to allow comparison, but the predictive power of behavior in our study was higher in comparison with previous studies using the TPB model (31-33).

Table 3. Predictors of health behavior identity (model 1), intention to consume fonio (models 2 and 3) and fonio consumption (model 4) among women of reproductive age in Mali (n=108)

Models[a]	**Standardized *β***	***P***	**R^2**	**Adjusted R^2**
Model 1				
Dependent variable: health behaviour identity			0.39	0.34
Knowledge	0.22	0.089		
Perceived susceptibility	0.08	0.466		
Perceived severity	0.19	0.128		
Health value	0.24[b]	0.011		
Model 2				
Dependent variable: intention to consume			0.11	0.05
Health behaviour identity	0.03	0.785		
Perceived barriers	-0.07	0.500		
Attitudes towards behaviour	0.32[b]	0.016		
Model 3				
Dependent variable: intention to consume			0.12	0.07
External control beliefs	0.19	0.071		
Cues to action	-0.01	0.918		
Subjective norms	0.25	0.068		
Model 4				
Dependent variable: fonio consumption			0.67	0.66
Age	0.15	0.011		
Perceived barriers	0.34	0.050		
Intention to consume fonio	1.39	0.063		
Perceived barriers - Intention to consume fonio	-0.72[b]	0.037		

[a]all models are adjusted for age, education and interviewers effect

[b]standardized *β* for these variables were significant (*P* < 0.05)

The proportion of women who intended to consume fonio once a week was higher than those consuming fonio once a week in the past month, indicating the potential role of a nutrition education program for promoting the consumption of fonio in urban areas. Increasing fonio consumption in urban areas may strengthen livelihoods of the women who develop modern small-scale processing industries for ready-to-cook fonio, while contributing to energy supply for households especially during the food shortage season.

Concerning the internal factors, the study showed that attitude measures were the best predictor of behavioral intention, confirming the findings of Nejad et al. (22). The most important items reported within positive attitudes were good taste, pleasantness in the mouth, easy digestibility, healthiness, nutritious quality, meal diversification, contribution to weight maintenance, and appetite stimulation. Those beliefs were also reported in previous studies (2-5, 12, 13, 16). The positive mean scores of the items and the correlation of the construct with intention to consume fonio suggested that these qualities should be emphasized to stimulate fonio consumption in urban areas. The women also liked fonio because of its traditional value. This confirmed the results of Konkobo-yameogo et al. (16) who reported that in urban areas in Mali and Burkina Faso, fonio is also considered as an African food and the consumers like it because it is a food traditionally included in their dietary pattern.

Health behavior identity appeared to be a good mediating variable for stimulating a positive attitude towards fonio consumption. This indicates that women will evaluate the consequences of fonio consumption more positively, when they agree that fonio is good for them and their household members. Previously, Sun et al. (23) also found a significantly positive correlation between health behavior identity and attitudes towards iron-fortified soy sauce consumption. Health behavior identity was also positively associated with perceived barriers, indicating that women perceive more barriers when they are convinced that consuming fonio is good for them. Conversely, the association between intention and behavior is lower when the perceived barriers are higher. Sun et al. (23) also demonstrated that Chinese women perceived more barriers when they agree that iron-fortified soy sauce was good for them and their household members.

The results of this study indicated 8 significant barriers to consuming fonio, including availability in city markets, restaurants or canteens, lack of consistent supply throughout the year, high cost of fonio products, hard texture coupled with time-consuming cooking process, a lack of knowledge related to preparing and cooking, poor quality of fonio (dirty and filled with sand) cooked at home, large family size requiring a larger amount of fonio, and an apathetic head of household. Our results not only support the barriers reported by Konkobo-yameogo et al. (16) but revealed that other factors are also likely to limit fonio consumption in Bamako. One of them was the lack of knowledge about processing and cooking. Indeed, due to the very small size of fonio grains, threshing, removing husks and hulls, and milling are time-consuming: it takes nearly 1 hour to peel just 1 to 2 kilos of fonio by hand (4). After a complete dehulling, the grain is still contaminated with sand and dust, and needs to be carefully washed for several times using a traditional sedimentation method to get a cleaned product. This latter product must be steam-cooked 3 times at least to get a soft-in-mouth and easy-to-digest product (4). This cleaning and cooking process requires some basic ability and knowledge (16) that might not be common among all women, especially in urban areas. Another reported bottleneck was the size of the household. This factor could be related either to the high cost of fonio products compared to rice, which would make it difficult to afford large amounts to feed the entire household, or to the difficult cleaning and cooking process that might increase cooking time in large size households. The apathy of the household's head for fonio consumption might be related to the low quality of the fonio-based foods served by the women in the households due to their lack of knowledge about cleaning and cooking. Reducing these barriers might help increase fonio consumption in the households in Bamako.

Concerning the factors of background and perception, knowledge and susceptibility factors showed the strongest correlations with health behavior identity, but the value to health was the best predictor. Sun et al. (23) also showed that health value was highly correlated with health behavior identity in rural areas, and in urban areas, knowledge, susceptibility and severity were significantly correlated with health behavior identity. The items of health value comprised importance of health in the life, survival, growth, intelligence and school performance of the children, importance of income for survival, importance of weight maintenance, and their own survival. So, whether women think that fonio is good for them was mainly determined by general value perceptions of health rather than specific health issues such as iron deficiency or diabetes. Almost all of the previous studies have used the behavioral models to understand the consumption behavior of specific nutrient rich foods such as iron-fortified foods (23), soy products (33), dairy products (32), legumes and vegetables (31). Our study was concerned with an indigenous cereal of which consumption has been often associated with cultural and religious beliefs, hence the link with a particular micronutrient was not as evident. And although previous studies on fonio reported the potentialities of fonio in the treatment of diabetes, stomach diseases and blood losses after women give birth (12), no scientific evidence of the relationship between fonio consumption and the treatment of the aforementioned diseases has been demonstrated so far. Therefore, an intervention aiming at increasing the intention to consume fonio needs to positively influence the background and perception of the women, with an emphasis on the general value of health associated with fonio consumption.

Concerning the external factors, the subjective norms were correlated with the behavioral intention but they were not good predictors. Our results confirmed the findings of Rah et al. (33) who also showed that the subjective norms were correlated with the behavioral intention, but predicted it more weakly than the attitudes. Opinions of the family, the neighbors and the husband were the most important items included in this factor in our study. In the context of Africa, values of extended family and community also significantly influence the behavior of the individual (34, 35). In Mali, the husband has a crucial role in decision-making within the household. For example, in the Demography and Health Survey (36), the women reported that the husbands alone make the decisions regarding daily household purchases (63.4%), and which food to cook each day (33.1%). In our sample, 67% of the women were married, and most of them agree that the opinion of their husband is very important for them. Thus, nutrition education regarding fonio should first address positive attitudes, but also influencing men, family and the neighborhood's beliefs about fonio consumption would be an important step for the promotion of fonio to be more efficacious.

Cues to action (from external factors) composed with items such as "food shortage, media, important ceremonies, special events, presence of guests for dinner, and invitation to restaurant" were not significantly associated with intention to consume fonio. Our results did not confirm those of Sun et al. (23) who found a positive association between cues to action and behavioral intention. Given that the median score of the items was positive and that previous literature reported fonio as a shortage food (3, 13), a special event food (2, 12, 16) and a food mostly consumed in the restaurants (16), it was a bit surprising that this relationship was not significant in the current study.

The high reliability of the multiple constructs (0.77 to 0.95) and the positive correlation within most of the constructs suggested the adequacy of the model for the purpose of our study. However, some limitations need to be considered for future research. First, we found that almost 40% of the women consumed fonio once a week or more. This frequency is much higher than the 10% for several times/week previously reported for urban areas in Guinea (Conakry) and Burkina Faso (Ouagadougou) (16). Hebert et al. (37) concluded that self-report of dietary intake could be biased by social desirability or social approval, especially when correct or desirable answers are easily recognizable. So, being aware of the study purpose could have created social desirability bias in the answers of the women, and they could have overestimated their fonio consumption. Secondly, almost all women tended to agree with the statements, as shown by the high values of the median scores. Hofstede and Hofstede (38), interested in the 'individualism versus collectivism' dimension of cultural behavior, showed that West African countries can be characterized as more collectivistic, where direct confrontations are considered as rude and undesired and the word 'no' is seldom used, as it is considered confrontational. Consequently, for the Likert scale, this could indicate that people from West African countries have a tendency to agree (positive score). In Mali, no research has been done on response patterns to Likert items. In the present study, the influence of cultural behavior on the answers was minimized by carefully training the respondents in order to understand the Likert scale, and by using a 5-point Likert scale to allow for variation, even if the answers are only positive. Third, Ajzen and Fishbein (39, 40) recommended that researchers should always conduct a pilot study, in which open-ended questions are used to obtain significant beliefs from a sample similar to the one that will be used in the main study. Although this recommendation has been followed up in our study through the food APS study, a large part of the variance in the behavioral intention could not be explained, suggesting that some factors could have been left unexplored.

Overall, our study revealed that the women living in urban area in Mali had positive attitudes towards fonio consumption and a positive evaluation of the consequences of fonio consumption on their health. However, barriers such as time-consuming cooking process and lack of knowledge related to preparing and cooking fonio should be reduced to ensure that intention leads to effective fonio consumption.

Implications for research and practice

Baranowski et al. (41) emphasized the importance of using theory to develop nutrition-based interventions. The results of the present study suggested the potential role of a nutrition education program for promoting the consumption of fonio in urban areas in Mali. An increase in fonio consumption may strengthen livelihoods of fonio producers, processors and retailers, while contributing to dietary energy supply for households especially during the food shortage season. The model of Sun et al. (23) was useful for identification of factors that could be important in promoting fonio consumption among women of reproductive age in Bamako. This could be achieved through stimulation of positive attitudes, influencing men, family and neighbors' opinions, and improving the processing and the know-how of women who prepare fonio. Future research should be designed to investigate whether promoting fonio in urban areas would help improve economic benefits and household food security, and further studies using behavioral models should pay attention to identifying more specific beliefs.

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Chapter 7

Effect of dephytinization of fonio with intrinsic wheat phytase on iron absorption in Beninese women

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Submitted

Abstract

Background: Fonio is an indigenous West African cereal which may contribute to household food security. However, low iron content and high phytate in processed fonio products make fonio based meals a poor source of bioavailable iron.

Objective: We compared the iron bioavailability from ferrous sulfate fortified fonio meals , with and without reducing phytic acid by native phytase from wheat flour.

Design: Using a crossover design, sixteen Beninese women were fed non-dephytinised (FFP) and dephytinised fonio porridge(FWFP) fortified with 4 mg isotopically labeled (^{57}Fe)- and (^{58}Fe)-ferrous sulfate. Iron absorption was measured based on erythrocyte incorporation of stable isotope labels 14 d later.

Results: Phytate-to-iron molar ratio decreased from 23.7:1 in FFP to 2.7:1 in FWFP. Iron fortification reduced phytate:iron ratio from 1.9:1 in FFP to 0.3:1 in FWFP . Geometric mean (95% CI) iron absorption ratio significantly increased from 2.6 (0.8-7.8) in FFP to 8.3 (3.8-17.9) in FWFP meal (paired *t* test, $P < 0.0001$). Three out of 16 subjects had iron deficiency anemia. Serum ferritin negatively correlated with iron absorption from both meals, but significantly only from FWFP ($r = -0.64$, $P < 0.001$). In the regression with ferritin and meal type as predictors, only ferritin was a significant predictor of iron absorption (standardised $\beta = -0.57$, 95%CI (-0.77, -0.19), $P < 0.01$).

Conclusions: Dephytinisation and fortification with 4 mg ferrous sulfate significantly increased fractional iron absorption by 3.2-fold. The technical feasability of this strategy in a home-fortification perspective needs to be further explored with regard to users' compliance in developing countries' household conditions.

Introduction

Fonio (*Digitaria exilis*) is an indigenous West African cereal, with potential for contribution to household food security because of the early maturing of some varieties (1). In semi-arid areas, fonio represents a major part of the diet. In subhumid zones, fonio is stored for long periods, to be used as staple food during food shortage season (2). In consumption areas, fonio is mainly consumed as couscous or porridge made from grain or flour (3). In urban areas in Mali, fonio is consumed at least once per week by one-third of the women (4). However, processing of fonio reduces the initially high iron content to 0.8 - 1.8 mg/100g on dry weight basis (5). In addition, as most plant-based foods, fonio grains contain phytic acid which forms complexes with iron, thus reducing its bioavailability (6, 7). Home processing such as soaking, malting, cooking, germination, fermentation, parboiling did not sufficiently reduce the phytate content in fonio (5) to the optimal phytate-to-iron molar ratio (<0.4:1) to achieve a significant increase in iron absorption from pure cereal-based meals (8). Low iron content combined with high levels of phytate indicate that there is a need to increase both iron content and bioavailability for fonio-based diets to contribute to the daily intake of iron. Iron fortification of staple foods has been recommended as a strategic option to increase the content of available iron in the foods. Ferrous sulfate has been the standard iron compound used in iron absorption studies and the bioavailability of other iron compounds are expressed relatively to its bioavailability (9). Water soluble compounds like ferrous sulfate may be suitable for low iron fortification levels in iron-deplete populations, as it has been shown that iron absorption from ferrous sulfate is well up-regulated in iron deficiency (10). However, because iron absorption can be strongly inhibited by phytic acid in cereals-based foods (11), reducing phytate levels might be essential for an improved iron absorption from iron-fortified fonio-based diets. Phytic acid can be degraded by enhancing the activity of intrinsic phytase present in a large range of plant-derived foods, or by adding microbial phytases (12, 13). Previously it has been shown that adding wheat to cereal or legume-based complementary food mixtures completely degraded phytate at optimal incubation conditions (14). The efficacy of this technique in improving iron absorption from different food matrices needs to be further explored (15). The objective of the present study was to measure the effect of dephytinisation of iron-fortified fonio porridge with intrinsic wheat phytase on iron absorption from test meals labelled with stable iron isotopes.

Methods

Subjects

A group of 16 apparently healthy young Beninese women aged 18 to 30 years (confirmed through birth certificate or other official documents) were recruited between September 2010 and January 2011, from neighborhood communities of the University of Abomey Calavi. Inclusion criteria were: *i*) body weight < 65 kg (measured according to standard procedures (16)), *ii*) no pregnancy (confirmed by rapid pregnancy test using Nancy HCG kit, 3H Medical Products, China) and no breastfeeding, *iii*) no reported chronic medical illnesses, *iv*) no reported symptoms of malaria in the last 2 months (fever, headache, stomach ache, diarrhoea, nausea, vomiting), *v*) no recent malaria (negative blood smear response based on Giemsa stained microscopy following standard guidelines (17), *vi*) no intake of vitamin and mineral supplements in the last 2 weeks, *vii*) no blood donation in the last 6 months, *vii*) no iron medication or supplementation during the study, *ix*) no reported allergy to gluten. A sample size of 16 subjects was estimated, based on expected mean increase in bioavailability of 30% (18) with 95% power and a significance level of 5%, using a standard deviation of 0.35 (log) iron absorption (19) and accounting for a conservative drop out of 2 participants. Before enrolment, study participants received a full explanation of the study in written form and orally during group discussion sessions. Written informed consent was obtained from all subjects before starting the study. The study protocol was approved by the National Provisional Ethical Committee for Public Health Research in Benin (Comité National Provisoire d'Ethique pour la Recherche en Santé Publique, CNPERS) and by the Medical Ethical Committee of the University of Wageningen (Medisch Ethische Toetsingscommissie, METC-WU).

Study design

Subjects were given 2 labeled meals on consecutive days using a cross over design (**Figure 1**). Iron absorption was based on erythrocyte incorporation of iron stable isotope labels 14 days after intake of labelled test meals. The meals consisted of a single fonio flour porridge (FFP) and a mixed fonio-wheat flour porridge (FWFP), both fortified with 4 mg iron.

Two weeks before the test, participants were dewormed with anthelminth (Zentel, 400 mg Albendazole tablets, laboratoire Glaxosmithkline, France).

On the first day (day 1), participants were randomly assigned to 2 equal groups: the 1st group receiving 240 g FFP with 3 mg $^{58}FeSO_4$ and 1 mg $FeSO_4$ with natural isotopic composition and the 2nd group receiving 240 g FWFP with 4 mg $^{57}FeSO_4$. In addition, they consumed 50 ml of drinking water used to rinse the bowl after the test meal was consumed. Meals were served to participants from 7.00 to 9.00 am after overnight fasting (no food after 8.00 pm and no drink after 12.00 am on the evening before day 1). Meals were consumed under standardized conditions and close supervision. No food was allowed to the participants within 3 hours after test meal consumption. After this period each participant received a breakfast package which the subject was allowed to consume ad libitum.

On day 2, within the same timeframe and under identical conditions as day 1, the two groups of participants received the labelled test meals FFP and FWFP with alternate composition and iron compound following a cross over design.

The day before day 1 (day 0) and on day 16, 8 ml venous blood samples were collected from the participants in K_2EDTA tubes and in normal tubes, between 7.00 am to 8.00 am after overnight fasting. Blood withdrawal was performed by two experienced technicians in a private room at the biomedical analysis laboratory of the Army Hospital of Cotonou (Benin).

During the test period, to ensure that participants were fasted, the evenings before days 1, 2, and 16, participants were lodged in a centre in the neighbourhood of the University of Abomey Calavi, and dinner was served from 6.30 to 7.45 pm.

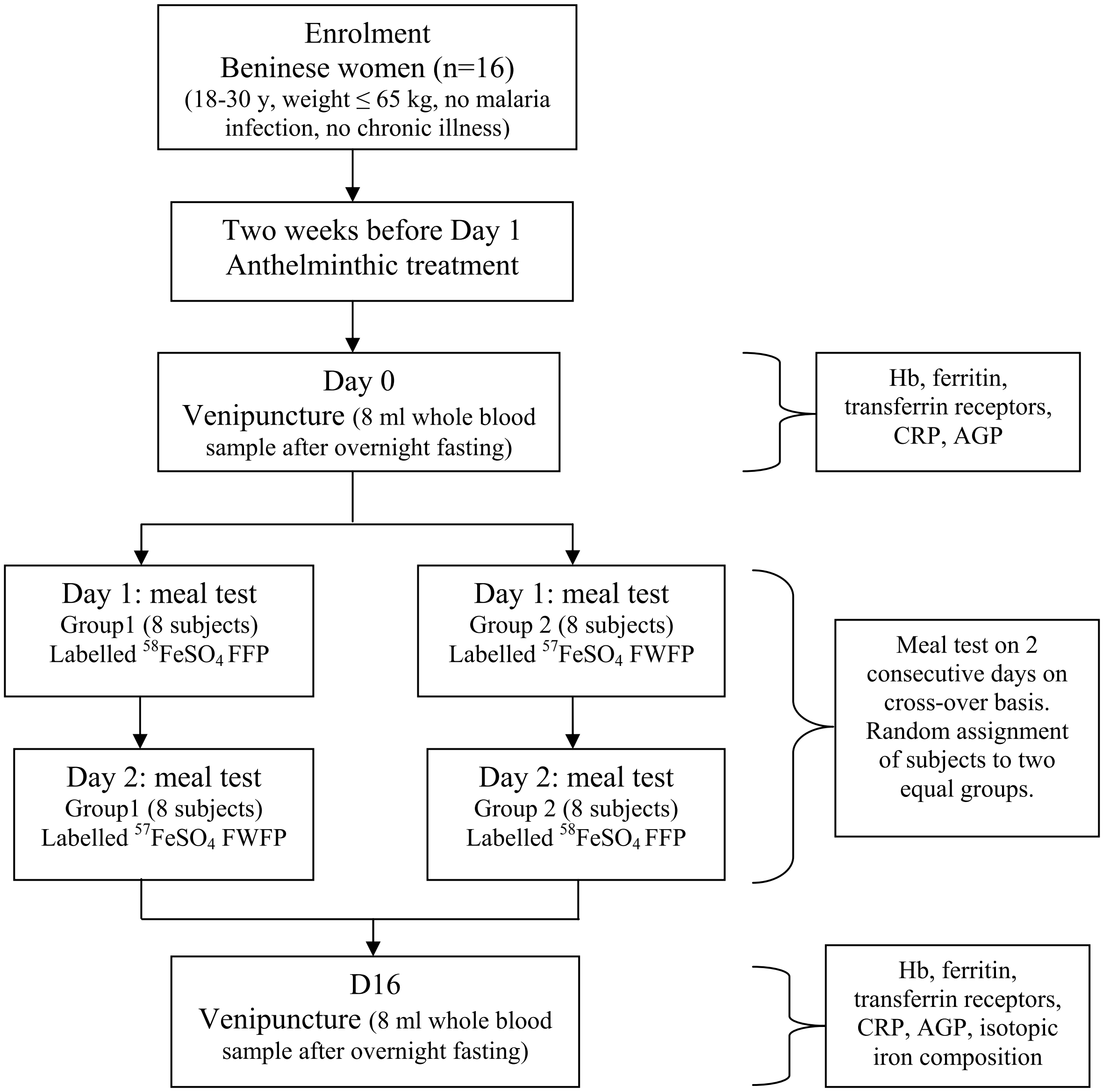

Figure 1. Experimental design of iron absorption test with fonio porridge using stable iron isotope. FFP, Fonio flour porridge; FWFP, fonio + wheat flour porridge; ^{57}FeSO4 and ^{58}FeSO4, ferrous sulfate isotope 57 and 58.

Test meals

Test meals consisted of 2 fonio porridges of 240 g: single fonio flour porridge (FFP) and mixed fonio-wheat porridge (FWFP, ratio 3:1, weight-to-weight). The meals were prepared in bulk at the Division of Human Nutrition of Wageningen University (Netherlands), using adapted recipes previously developed by Egli et al (5, 14). Fonio flour was diluted in water, at a water-to-flour ratio of 20 ml-to-1g. The mixture was added to boiling water cooked for 22 minutes from boiling. The pH of the cooked porridge was adjusted to approx. 5 by adding citric acid at incubation temperature (50°C). The porridge was divided into 16 portions of 240 g which were immediately cooled on ice to restrain wheat intrinsic enzymatic activity. For the mixed fonio-wheat meal, the same recipe was used and the wheat flour was added directly to the porridge at incubation temperature, after adjusting pH to approx. 5. After mixing homogeneously, 16 portions of 240 g of the mixed porridge were set in the incubator for 3h at 50°C under constant stirring at 115 rpm (Innova 44, incubator shaker series, New Brunswick Scientific Co., Inc, Eppendorf company, USA), after which they were immediately cooled on ice. Porridge portions were stored at -18°C and sent by express courier to Benin one week before the test. Each evening before day 1 and day 2, 16 portions were thawed overnight in a fridge at 4 -5°C. On the test days, thawed portions were quickly warmed up for 1 min in a microwave and iron isotope solutions were added quantitatively before consumption. Weights were measured using electronic scales S4001 (capacity 4000g, precision 0.1g, Denver instrument, NY, USA) for flours, and CP 324S (max 320g, precision 0.1mg, Sartorius, Germany) for iron isotope solutions.

Analysis

Phytate and iron in the test meals

Iron concentration in the test meals was analysed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), after microwave digestion with hydrofluoric acid (40%), HNO_3 (65%), and H_2O_2 (30%). Phytate content was determined as inositol hexakis-phosphate (IP6) according to Van Veldhoven and Mannaerts (20), liberated from phytate degradation using a modified method of Makower (21).

Measurements of blood indicators

Haemoglobin (Hb) was measured immediately after blood collection, using an automated Sysmex counter (KX 21-N, Sysmex, cyanide-free reagent used for Hb detection, normal values range 11.50-15 g/dL). A 3-level Eightcheck-3WP control material provided by the manufacturer was used for quality check. Overall anemia was defined as Hb < 12 g/dL, moderate anemia as Hb < 10 g/dL and severe anemia as Hb < 9.0 g/dL. A sandwich enzyme-linked immunosorbent assays (ELISAs) technique was used for the simultaneous measurement of SF, sTfR, CRP and AGP (22). Iron deficiency was defined as SF <12 µg/L or sTfR > 8.5 mg/L, and iron deficiency anemia as Hb < 12 g/dL and SF <12 µg/L. CRP > 10 mg/L and AGP values > 1 g/L were used to identify existing inflammation.

Isotopic composition in blood samples

Whole blood samples were mineralised and separated as described by Schoenberg and von Blanckenburg (23). Mineralisation was done with concentrated HNO_3 (65%) and H_2O_2 (30%), using a microwave digestion system with temperature control. Iron was then separated from matrix elements by anion-exchange chromatography using resin AG 1-X8, 100-200 mesh (Bio-Rad) and precipitated with ammonium hydroxide (NH_4OH). Isotopic analysis was performed using a high-resolution, multicollector inductively coupled plasma mass spectrometer (Neptune; Thermo-Finnigan) at the Institute of Geology and Mineralogy of the University of Cologne, Germany using Cu-doping to correct for internal mass bias (23). Each isotopically enriched solution was measured in triplicate using the bracketing technique (23). One third of the samples were re-measured as external duplicates. Analysis was done under chemical blank monitoring using 57Fe or 58Fe indicator solutions as quality control.

Calculation of Fe absorption

Calculation of iron absorption was based on the shift in the isotopic ratios after a 14-d incorporation period as described by Walczyk et al. (24). Circulating iron was calculated on the basis of the blood volume, which was estimated from the participant’s height and weight according to Brown et al. (25). Isotopic ratios were calculated according to the principle of isotope dilution (26) and taking into account that isotopic labels are not monoisotopic (27). A ratio of 80% incorporation of the absorbed iron into red blood cells was assumed for our sample of young women.

Statistical analysis

Statistical analyses were performed using PASW statistic 18 (Release 18.0.3; IBM SPSS Inc., Chicago, IL, 2010). P values < 0.05 were considered significant. Visual check and Shapiro-Wilk test were used to check for normality of the distribution. Normally distributed data were expressed as mean ± SD, non-normally data distributed as median (interquartile range) and log transformed data as geometric mean ± SD. Fractional iron absorption distribution was log-transformed to normality and corrected to a value corresponding to serum ferritin concentration of 15 µg/L (28). Log-transformed and corrected fractional iron absorption from FFP and FWFP meals were compared using paired t tests. Pearson's correlation was used to test for association between (log) serum ferritin and log fractional iron absorption from different meals. Linear regression was used to test the effect of meals and serum ferritin levels on iron absorption, with log fractional absorption from FWFP meal as dependent variable and log serum ferritin and meal type (non-dephytinised/dephytinised) as independent variables.

Results

Participants

Sixteen participants were recruited, but one did not complete the test because suffering from malaria on day 16. Participants were aged 24 years on average, with mean (±SD) BMI of 21.2±3.3. Mean (±SD) hemoglobin and body iron stores concentration was 11.9±1.4 g/L and 3.5±4.9 mg/kg respectively. Median (25^{th} - 75^{th}) ferritin and transferrin receptor was 22.9 (12.7 – 74.8) µg/L and 6.0 (5.2 - 7.6) mg/L. Seven subjects had moderate anemia. Three subjects had iron deficiency anemia and one subject was iron deficient but not anemic. Mean (±SD) CRP and AGP concentration was 3.4±7.8 mg/L and 0.8±0.2 mg/L respectively. CRP and AGP concentration was elevated for two (14.7 and 28.2 mg/L) and three participants (1.05, 1.04 and 1.06 g/L) respectively.

Phytate-to-iron molar ratio of test meals

Average iron content in fonio flour was 1.5 (range 0.6 - 4.6) mg/100g dry weight basis. The expected iron concentration in the portion (240 g) of fonio flour porridge and fonio-wheat flour porridge was respectively 0.3 mg and 0.5 mg wet weight basis. Mean (±SD) phytate concentration per FFP and FWFP meal portion was respectively 96±16.2 mg and 16.2±4.4 mg wet weight. Phytate-to-iron molar ratio decreased from 23.7:1 in FFP meal to 2.7:1 in FWFP meal. FFP meal with iron fortification showed phytate:iron of 1.9:1, which decreased to 0.3:1 in FWFP meal.

Iron absorption from test meals

Geometric mean (95% CI) iron absorption from FFP and FWFP meal was 2.6% (0.8 - 7.8) and 8.3% (3.8 - 17.9) respectively (**Figure 2**).

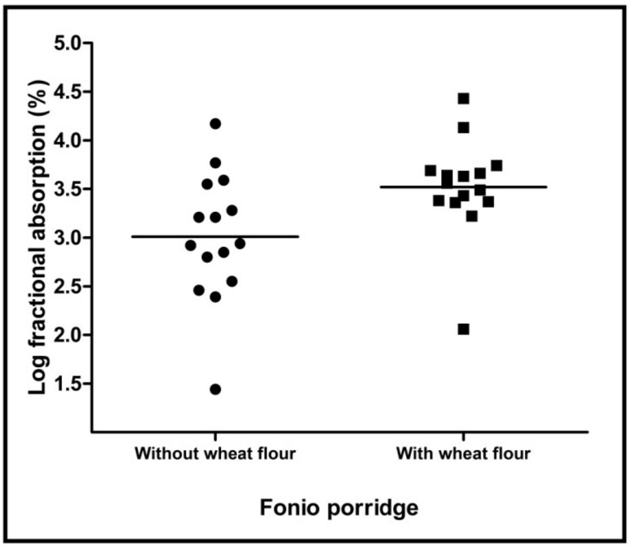

Figure 2. Comparison of (log) fractional iron absorption between non-dephytinised (without wheat flour, FFP) and dephytinised (with wheat flour, FWFP) fonio porridge (n=15). Black lines show mean log fractional iron absorption from each meal. Log fractional absorption corrected to a value corresponding to serum ferritin concentration of 15 µg/L (28). Geometric mean (95% CI) of iron absorption ratio from FFP and FWFP meals is 2.6 (0.8 – 7.8) and 8.3 (3.8 – 17.9) respectively. Log fractional iron absorption was significantly different between meals (paired *t* test, $P < 0.0001$).

Fractional iron absorption in FWFP meal was 3.2-fold higher than in FFP meal (paired *t* test, $P < 0.0001$). The difference remained significant after correction for ferritin concentration (paired *t* test, $P < 0.05$). As expected, serum ferritin negatively correlated with iron absorption but significantly only for FWFP (FWFP meal $r = -0.64$, $P < 0.01$; FFP meal $r = -0.19$,

$P > 0.05$;) (**Figure 3**). Also, in the regression with ferritin and meal type as predictors, only ferritin was a significant predictor of iron absorption (standardised $β$ = -0.57, 95%CI (-0.77, -0.19), $P < 0.01$).

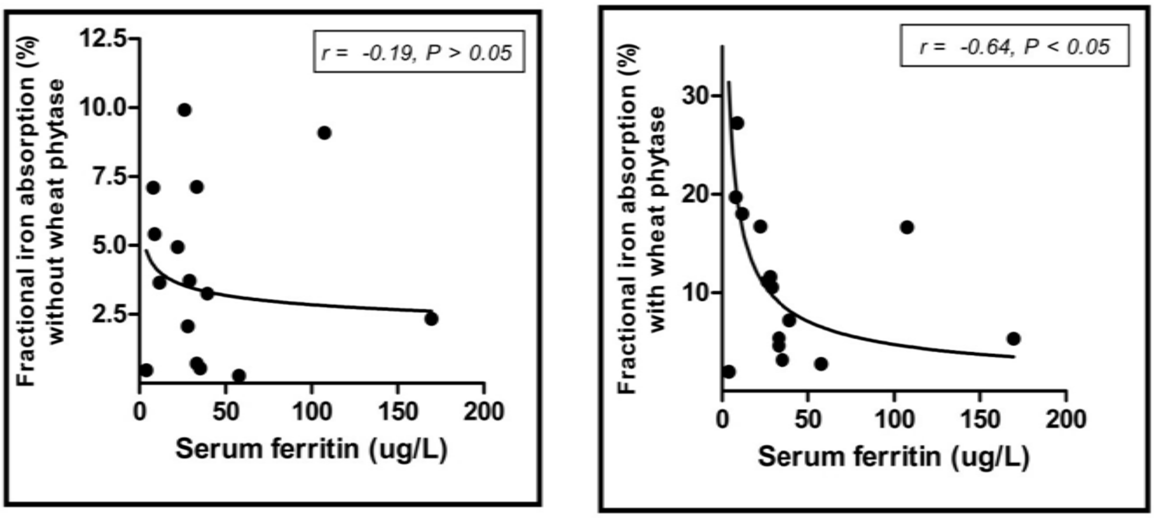

Figure 3. Iron absorption against serum ferritin concentration from non-dephytinised (without wheat flour) and dephytinised (with wheat flour) fonio porridge (n = 15). Black lines represent non-linear log-log fitting regression lines

Discussion

The objective of the present study was to investigate the effect of dephytinisation with intrinsic wheat phytase and iron fortification on iron bioavailability from fonio meals, using stable iron isotopic labels. Main findings were that dephytinisation with intrinsic wheat phytase reduced phytate-to-iron molar ratio from 24:1 to 3:1, iron fortification decreased the molar ratio to 0.3:1, and dephytinisation plus fortification increased iron absorption from 2.6% to 8.2% in fonio porridge.

Inhibition of iron absorption by phytate in plant-based meals is dose-dependent at very low concentrations of 2-10 mg/ meal (8). Thus, phytate:iron molar ratio should be preferably lower than 0.4:1 to achieve a significant iron absorption when no enhancers of iron absorption are added to the meal. The molar ratio phytate-to-iron of 23.7:1 reported in this study was higher than the range of 12-21:1, 9:1, 5:1 and 4:1 reported respectively for sorghum flour, refined maize porridge, wheat flour porridge and rice flour porridges (29, 30). The relative importance of the inhibitory effect of phytate on iron absorption is emphasized in the present study by the fact that 4 mg iron fortification of fonio meals without dephytinisation resulted in phytate-to-iron ratio of 1.9:1, and mean iron absorption of 2.6%. This would provide only 7.6% of the 1.46 mg/day median absolute requirement for iron absorbed among menstruating non-pregnant non-lactating women. This indicates that fortification alone is not sufficient to overcome the negative effect of phytic acid in cereal-based foods.

An essential finding from this study was that adding wheat flour to fonio porridge before consumption reduced the phytate content, decreasing phytate-to-iron ratio from 23.7:1 down to 0.3:1 in iron-fortified fonio porridge. This result confirmed evidence from a previous study showing that adding whole wheat grain to cereal-based complementary foods completely degraded phytate in a relatively short time (14). Reducing phytate content in cereal-based foods is challenging in low-income countries because home processing practices often do not achieve a sufficient degradation to significantly improve the iron absorption. In addition, exogenous phytases are often costly and also, their use is restricted in some countries because some of the microbiases are derived from genetically modified species of *Aspergillus Niger* (30). Therefore, the use of locally available and widely used cereals as wheat for dephytinisation is a promising practice that needs to be further explored in developing countries with other traditional cereal-based porridges.

Most important finding from this study was that complete reduction of phytate content with intrinsic wheat phytase, and fortification significantly increased iron absorption ratio by 3.2-fold. It has been shown that dephytinization significantly increased absorption by 2-fold from low-tannin Sudan sorghum porridges, but phytate degradation was achieved with commercial phytase (31). Also, significant increases in fractional absorption of iron (1.7-fold) and zinc (1.5-fold) in adults have been previously reported for cereal-based foods dephytinized using commercial phytase and wheat flour intrinsic phytase respectively, but without fortification (15, 32). Previous studies on the inhibitory effect of phytate have also reported 2-12 fold increase of iron absortpion from different dephytinised meals (18, 33, 34). These substantial achievements, together with our results, adressed the double issue of the low level of bioavailable iron in cereal-based meals caused by the negative effect of phytate on absorption, and the low content of native iron particularly in fonio meals due to losses during processing.

A high level of fortification (4 mg ferrous sulfate/ 0.5 mg native iron in 24 g dry weight fonio flour) has been used to modify phytate-to-iron ratio in fonio porridges. A practical issue in the context of low income countries is that such a fortification level can be very costly and difficult to apply. Particularly in mass fortification, this level may be too high in view of the amount of fonio consumed. But with that low native iron content in processed fonio products, this proof of concept study showed that this fortification level was necessary to achieve a significant increase in iron absorption. With a lower iron content, the magnitude of improving effect of wheat would be less, but we did not test this. Home fortification of specific foods for a target population has the advantages to be cost-effective, while adressing the needs of a clearly defined group of the population (9, 35). In addition, products are usually fortified in final form, immediately before consumption, limiting the effects of fortificants on sensory properties (9). Such fortification form is likely to be the best suitable for increasing iron content in fonio meals, and should be further explored for assessing cost and technological feasability.

In addition, iron ferrous sulfate used in the present study is reported to affect sensory properties of the fortified meals, reducing their acceptability by the consumers. However, compared to other iron compounds, ferrous sulfate is the cheapest iron compound showing the highest bioavailability (9), and the option of using encapsulated formula has been suggested to overcome the barrier of sensory modifications of the fortified meals (36).

This study was based on a single meal design, which could have overemphasized the effect of phytate reduction on iron absorption (8). Cook et al. previously reported significantly lower increase in iron absorption from total diet (2.5-fold) compared to increase from a single meal (5.9-fold) (28). In addition, we cannot exclude that other unknown components of wheat flour might have caused the increased iron absorption. However, phytic acid is the main inhibitor of iron absorption (8) and the significantly reduced level of phytic acid means that the natural phytase in wheat is most likely responsible for the improvement in iron absorption.

Investigating the effect of phytase on iron absorption from different food matrices has been recently emphasized (15). Fonio is the most ancient west African traditional cereal and to date, this is the first study investigating the effect of phytase and iron fortification on iron absorption from fonio meals among women using stable isotope technique. Findings from this study indicated that dephytinisation using intrinsic wheat phytase is a promising processing practice to overcome the negative effect of phytate on iron absorption. In addition, iron fortification seemed a necessary step to increase the amount of absorbed iron from fonio meals. The technical feasability of this technology with regard to users' compliance need to be further explored in developing countries household conditions, as wheat phytase can reach its optimal activity only within a limited range of temperature and after more than 1h of incubation.

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Chapter 8

General discussion

This thesis aimed to upgrade the nutritional value of fonio, a traditional West African staple grain. To achieve this objective, processing (dephytinisation and fortification) have been explored as entry points for strategies in a value chain approach targeting West African women of reproductive age living in urban areas. Prior to this, some specific research questions have been investigated to define the nutritional context for operating the value chain strategies, as well as the acceptability of fonio among our study population.

This chapter summarizes the main findings of the research and discusses outstanding methodological questions about the quality of the studies. In addition, the main results are discussed in the context of existing literature. The chapter ends with suggestions for future research and the main implications of this research for public health in West Africa.

Main findings

The main results of the research are presented in **Table 1**. The study population was represented by apparently healthy women of reproductive age (15-49 y) living in an urban area in Mali (chapters 2-6) and Benin (chapter 7). Investigating the nutritional context of the research showed one-quarter of the women with iron deficiency based on ferritin concentration, and 19% overweight women in Mali. About half of the women were at risk of inadequate iron intake and a similar share was at risk of overall inadequacy of micronutrient intake. Intakes of grains, vegetables and legumes/nuts were significantly associated with iron intake but not with the probability of adequacy of iron intake. The assessment of fonio acceptability showed that fonio is consumed one to three times/month by 68% of the women mainly as snack on working days, and on a lesser extent as main dish on weekend days. Average daily portion (152 g/day) consumed was small compared to rice, the main staple cereal of the diet. Increasing the acceptability of fonio in urban areas should focus on stimulating positive attitudes, influencing household's heads, family and neighbors' opinions, and improving the processing skills of the women. Phytate degradation with native wheat phytase and iron fortification of fonio porridges decreased phytate-to-iron molar ratio and significantly improved iron absorption from fonio porridges from 2.6% to 8.2%.

Table 1. Upgrading the nutritional value of fonio in a value chain approach: main findings related to the specific research questions

Main findings	Chapter
Nutritional context	**2**
Prevalence of iron deficiency and overweight (obesity) was 25% and 19% (9%) respectively	
Nutrient intake and adequacy; day-to-day variation in diet	**3; 4**
Iron probability of adequacy was 0.54 and intake from grains, nuts/seeds and leafy vegetables groups was significantly correlated with iron intake (*rho* = 0.3; P < 0.05)	
Mean probability of adequacy for 11 micronutrients (MPA) was 0.47±0.18 and individuals with higher intake from nuts/seeds and leafy vegetables were more likely to have MPA above 0.5	
Intra-to-interindividual variance ratio was ≥ 1 for fat, Zn, Ca, Vitamin A and C, and B6 intake; and <1 for energy, carbohydrates, protein, iron, thiamin and riboflavin intake. At least 2 - 6 replications for an estimation of accurate usual intake at group level, and 2 - 12 replications for correctly ranking individual are required on independent days, including weekend days and with a larger sample.	
Acceptability of fonio	**5; 6**
Intention to consume fonio was significantly correlated with fonio consumption (*rho* = 0.78, *P* < 0.001).	
Positive attitudes were the best predictors of intention to consume fonio (*β*=0.32, *P* < 0.05).	
Subjective norms (men's beliefs and neighbors' opinion) influenced intention to consume fonio (*rho* = 0.26, *P* < 0.001). Perceived barriers (difficult processing and lack of skills in cooking fonio meals), negatively interacted between intention to consume and fonio consumption (*β* = -0.72, *P* < 0.05)	
Processing fonio	**7**
Dephytinisation with native wheat phytase reduced phytate-to-iron molar ratio from 24:1 to 3:1 and iron fortification decreased the molar ratio to 0.3:1	
Dephytinisation and fortification increased iron absorption ratio from 2.6% to 8.2% in fonio porridges	

INTERNAL VALIDITY

The main methodological issues discussed in this chapter are related to the cross-sectional design used in **chapters 2-6**, namely bias in selection of the study population and measurement bias.

Selection of study population

The sample described in **chapters 2-6** was selected using a three-stage cluster sampling design developed by UNICEF for multiple indicator cluster surveys in developing countries (1). Main limitations likely to increase the risk of selection bias concerned the design effect, the non-random selection and the non-response bias.

Design effect

The design effect in a cluster sampling is the factor accounting for the variance between different clusters (2, 3). It is estimated by the ratio of the variance of the outcome variable under cluster sampling design to the variance under simple random sampling. Contrary to simple random sampling, data are usually dependent in cluster sampling design because individuals within clusters are often linked by environmental, social or genetic factors (4). Thus, overlooking the design effect in sample size calculation or data analysis leads to an underestimation of the variance of the outcome variable. In this thesis, chapters 2-4 provided estimates (mean and prevalence) for nutritional and iron status, as well as micronutrient intake and adequacy. Previous estimates of the variance of these parameters were not available, so cluster design effect was not taken into account for sample size estimation or data analysis. Estimated design effect ranging 0.8 to 2.7 have been reported from previous studies using 3-stage cluster sampling, though with larger cluster size and higher number of clusters than in our study (2, 5). This suggested that a sample size 2 times larger than estimated was required for our study. Thus, the variance estimates reported for the above-mentioned outcomes were likely to be underestimated, and the confidence interval overestimated. The influence of the design effect has been reported to be reduced for regression coefficients (6), thus the probability of overestimation of the confidence interval of the correlation and regression coefficients reported in chapters 2-6 was low.

Non-random selection bias

The sample selection was performed by a three-stage cluster sampling design and non-random selection bias may have occurred within each step (1, 7, 8).

In the first step, to ensure a good representativeness of the Malian population, 9 clusters over a total of 72 clusters (1% of Bamako population) were excluded due to over representation of international experts and industries. Also, a probability proportional to size procedure was applied for clusters selection, to take into account the variation in clusters size.

In the second step, the random walk (by spinning bottle at a central point) was used for selecting the households (1, 7, 8). This did not assume a strictly equal probability of selection for each household because the selection stopped when the number of desired respondents was reached (1). In addition, households nearby the starting point were more likely to be oversampled, while those at the edge of the clusters would be underrepresented. However, in lack of a comprehensive map of the study area and complete household addresses and lists as was the case for our research, this method has proven evidence for providing virtually unbiased samples of households (1). In addition, to have a good approximation of the central point of the selected clusters, key informants were consulted and when necessary a rapid tour of the clusters was done.

In the last step, selection bias was likely to occur because often more than one woman in the selected households met the eligibility criteria. To minimize the risk of self-selection, a random selection was done from a list of all eligible women in the selected households.

Thus, care has been taken to minimize the risk of non-random selection bias along the sampling procedure through a systematic approach.

Non-response bias

The non-response bias is a systematic error reducing the representativeness of the sample. The evaluation of the effect of non-response is often difficult because no or only limited data are available for the non-respondents (9).

For the study reported in **chapter 2**, only 66 women out of the 108 selected actually showed up for the blood sample collection session, although all the women in the sample verbally agreed to participate after detailed explanation of the purposes of the study. Trying to collect a blood sample from non-respondents from door to door was not successful and, no clear explanation was given as reason to this refusal. A probable reason might be the strong influence of some common beliefs linking any blood sample withdrawal to the screening for HIV-AIDS in developing countries communities, as HIV-related stigma is a worldwide common phenomenon (10).

This refusal caused a non-response rate of 39%, being a major limitation of the study. Particularly because the sample has been selected with a cluster sampling design, some clusters could have been overrepresented by chance. Consequently, the outcome variables estimated from this sub-sample might be unlikely to reflect the actual estimates of the total sample. However, the non-response rate seemed to be equally distributed across the clusters (3-5%) except for 2 clusters for which the non-response rate was 1% and 6% respectively. Also, from the background information of the sample, it appeared that the sub-sample of respondents were not very different from non-respondents regarding their socio-demographic characteristics: median age of 28y and 30y for non-respondents and respondents groups respectively; illiteracy rate of 17% and 18%; average number of children of 1 (maximum of 10) and 2 (maximum of 11), 14% and 17% married women. In addition, none of these socio-demographic variables appeared to confound the relationships among the outcome variables included in analyses in chapters 2-6. Therefore, a difference in socio-demographic status between respondents and non-respondents was not likely to have an effect on the findings. Lastly, regarding practical issues for research in developing countries, systematic data on women of reproductive age are scarcely reported from West African countries and, although the generalizability could be hampered in our research due to methodology settings, the information provided can be used as starting point for improving future research design.

Measurement bias

Measurement bias could have occurred in chapters 2-6, particularly regarding outcome variable accuracy (chapter 2) (11), diet assessment (chapters 3 and 4) and acceptability of fonio (chapters 5 and 6).

Outcome variable

In **chapter 2**, measurement bias could have happened because the dependent outcome variable systematically misclassified individuals. Investigating the relationship between weight status and iron deficiency, ferritin was used as unique indicator of iron deficiency because serum transferrin receptors showed zero prevalence of iron deficiency among overweight/obese women. Ferritin is a sensitive biomarker of iron deficiency (12) but can artificially increase in inflammatory conditions, reducing its accuracy in detecting depleted iron stores (13, 14). An implication is the probable underestimation of the prevalence of iron deficiency, limiting the power of our study in detecting significant associations between iron deficiency and increasing body mass index. However, to identify individuals with artificially high ferritin levels, C-reactive protein (CRP) was measured, and 5% of the sample showed simultaneously high CRP (> 20 mg/L) and ferritin levels (> 65 mg/L). To limit their confounding effect, data of those respondents were excluded from further analysis in chapter 2. The correction factor suggested by Turnham et al. (15) was not used to adjust for the confounding effect of inflammation on ferritin because this was estimated from HIV-infected individuals and application for apparently healthy population has not been reported.

Bias in assessment of diet

Systematic bias and random errors are the main limitations for the validity of dietary assessment methods and the precision of the estimates (16). Systematic errors usually appear when the respondents misreport the foods consumed, or under/over- estimate the amounts consumed, or when amounts consumed and intake are incorrectly estimated from a non-systematic procedure.

In **chapter 3**, the adequacy of nutrient intakes was estimated using a duplicate 24-h dietary recall assessment (2d-24hDR). In the context of high illiteracy rates and shared plate eating settings even in urban areas in Mali, systematic errors were likely to occur from the respondents who might not be able to recall or report accurately the foods or amounts consumed. Also, because amounts consumed were estimated in households measures, systematic errors could have occurred when converting from households units to standard units (grams). To minimize these errors, a systematic procedure (17) was carefully used at each step during data collection and data handling. In addition, using the 95% limits

Goldberg cut-offs (18) to evaluate energy intake assessed by the 2d-24hDR indicated that reporting bias in energy intake was not significant at group level (**chapter 4**).

Since nutrient intakes from the 2d-24hDR were calculated from food composition tables, there was also a probability of systematic error at this step. Systematic errors are more likely to occur by the use of food composition tables particularly when food composition data are outdated, or when they do not provide the appropriate nutrient information for the foods as consumed (17, 19). The national food composition table of Mali (TACAM) (20) was used as first source of information for our research, based on the assumption that it would provide a good representation of locally available and traditional foods. A systematic procedure was used to improve the reliability of the food composition database used for the study, including: an internal evaluation of the quality of the TACAM (21); a substitution of missing nutrient values with values from secondary tables; the use of retention factors to account for nutrient loss during cooking. Therefore, systematic errors related to the use of food composition data were not likely to be a potential issue for the validity of the dietary assessment.

The most common random error occurring in dietary assessment is the non or inaccurate adjustment for the day-to-day variation in individual diets (22, 23). Day-to-day variation leads to an over or under estimation of the intake, decreasing the accuracy of the prevalence of inadequate intake (24). In **chapters 3 and 4**, the estimates of nutrient intakes were not adjusted for day-to-day variation because the 2d-24hDR was restricted to 2 recall days excluding weekend days, to a unique food season and to a limited sample size, considering the purposes of our research. However, with the use of independent days it was possible to have an estimate of day-to-day variation from the diet, thus allowing to provide further information on number of days needed to have accurate estimation of the usual energy, macronutrient and micronutrient intakes for analysis at individual or population level (chapter 4). Also, in chapter 3, to account for day-to-day variation in estimating the probability of micronutrients adequacy, the ISU method was used to transform and adjust the intake distributions (25).

Thus in general, the main probable sources of systematic errors were carefully monitored during the dietary assessment. But because it was not possible to capture the overall intra-individual variation from the diet by the 2d-24hDR, a very accurate information on the usual nutrient intake was unlikely to be achieved from this research.

Correlated measurement error is a specific issue related to **chapter 4** where food group intake was associated with nutrient adequacy estimated from the same dietary intake data. Correlated errors increase the probability of spurious associations between variables (26). Lower but significant correlation coefficients between dietary diversity score and nutrition adequacy have been reported from independent dietary assessment as compared to coefficients estimated from association between indicators from same dietary intake data (27). Thus, the estimates of the correlation coefficients between food groups probability of adequacy reported in this study were likely to be artificially increased.

Bias in assessment of acceptability

In **chapter 6**, a behavioural model was used to assess the acceptability of fonio. The response bias is the major limitation for the reliability of social cognitive models used to predict behaviour (28). The response bias occurs when the respondents give the response they think suitable for the research, or by social desirability to comply with cultural norms, regardless of their personal feelings. Response bias often hides the true relationships between variables (28). The study in chapter 6 showed that most of the respondents tended to agree with the statements of the model. We suspected that being aware of the purpose of the research could have created a response bias among the women. Also, in many West African cultures, saying ‘’no’’ is perceived as a direct confrontation, which is an undesirable social behaviour (29, 30). This could have led the women to answer by social desirability resulting in a reduced variation in answers. Thus flawed association between constructs could have occurred in the model. However, to reduce this risk, the respondents were carefully trained before the interviews to understand the likert scales. Also, a 5-point likert scale was used instead of the 3-point likert scale, to allow for variance in answers, even when positive answers were given.

EXTERNAL VALIDITY

In the present research the value chain for nutritional goals has been used as approach for adding nutritional value to fonio, focusing on social acceptability, home-processing and fortification as strategic entry points (**Figure 1**).

In this section, the validity of the findings will be considered within the context of existing literature by reviewing the nutritional context, as well as the strategies used to add value to fonio. Future research and practical implications for public health will also be discussed.

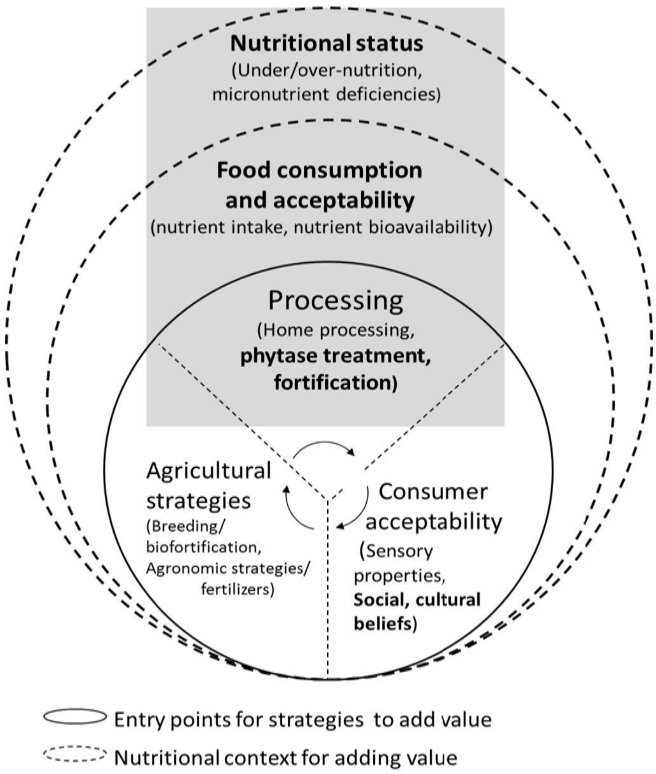

Figure 1: Conceptual framework for adding value to a product: the value chain approach for nutritional goals. Adapted from Hawkes and Ruel (21)

Nutritional context

Iron deficiency and nutritional status

Chapter 2 of this thesis addressed the need of documenting the nutritional context in which the value chain will be operated, by providing updated information on the nutrition and iron status of Malian women of reproductive age. Specific biochemical indicators like serum ferritin (SF) and transferrin receptors (sTFR) were used to provide accurate estimate of iron deficiency.

In previous studies in West African countries using SF, prevalences of 17%, 40% and 16% have been observed in urban areas among menstruating women in Benin (31), women of reproductive age in Cote d'Ivoire (32) and pregnant women in Ghana (33). These estimates, together with the prevalence of 25% observed in our sub-sample of Malian women are in the range of the 17-32% rate of iron deficiency derived by halving prevalence of anemia among West African women of reproductive age (34). This showed that depleted iron store

is a common public health issue among women in West African urban areas. Using sTFR, 9% of Malian women were iron-deficient, which was lower than the 35% reported in Cote d'Ivoire (32). The high cost and expertise demand associated with the use of a variety of specific indicators limited systematic information on the trends of iron deficiency prevalence among West African women. This is shown by the fragmented information gathered in the above-mentioned studies, and the lack of data based on sTFR in most of those studies.
Documenting iron status among vulnerable groups with accurate biochemical indicators is an essential step for designing and monitoring appropriate nutrition intervention programs (35). Efforts are being made in West African countries to add at least ferritin concentration to the measure of hemoglobin for detecting iron deficiency, as shown by the studies mentioned above. Yet, the high prevalence of infections and inflammation in West African countries remains major drawback for the use of SF as only indicator of iron deficiency (36). Implications for the present research have been discussed previously in measurement bias. CRP and AGP level has been most often used to exclude data confusing the actual estimates of iron deficiency prevalence. By doing this, the estimate of iron deficiency prevalence using ferritin is also likely to be spuriously lowered (37). Using correction factor to adjust for the confounding effect of inflammation on ferritin, more precise identification of iron deficient and iron-replete individuals has been reported in HIV-infected adults population (15, 38). This method needs to be further tested in apparently healthy West African populations living in high infections settings.

Chapter 2 also addresses the emerging issue of increased body mass index or body fat as a risk factor of iron deficiency. This issue has often been investigated in industrialized countries, but changes in lifestyles and shift of traditional to transitional diet in urban areas of emerging and developing countries suggest an increased probability of association between weight status and iron deficiency in those settings. This has been demonstrated among women and children from transition countries like Morocco, Thailand, India and Mexico (39, 40). A number of hypotheses has been mentioned to explain this association, the most probable being the impairment of iron absorption and metabolism due to the inflammation process associated with hepcidin function in obesity, as described in number of studies reported in chapter 1.

Findings from this study showed an increasing body mass index among urban Malian women, with a total overweight prevalence of 19%, being at the lower side of the range of 18-33% reported for West African women (41). However, iron deficiency was not associated with increased body mass index among oursample. In addition to the small sample size addressed above in internal validity, a probable hypothesis for this lack of association might be that the inflammation process related to obesity did not achieve the critical level inducing imbalanced iron absorption in our sample. There is a need of more documentation on this issue from West African urban areas and more evidence about the underlying mechanisms of this association.

Iron intake and adequacy

Chapter 3 examined the nutritional context of the research in terms of intake and adequacy of iron in association with the food groups consumed from the diet. A range of 88-90% has been reported for the prevalence of inadequate intake of iron among women of reproductive age in resource-poor settings, including one West African country (42). In the present research, the prevalence of inadequate intake was 46%, being lower than the range reported above. Also, this estimate was lower than the 74% observed among women in urban Burkina Faso (43), though the usual iron intake of the latter (23.6 mg) was higher than the usual intake of 17 mg of the Malian women. These discrepancies may be related to the different bioavailability levels applied to the iron requirement, which was assumed to be 5% for Burkina Faso and 10% for our sample’s diet, based on FAO/WHO estimates of iron bioavailability according to usual diets (35).

Findings from this study also showed that grains, nuts/seeds, and vegetables were the food groups most likely to contribute to iron intake and to overall micronutrient probability of adequacy (MPA), with higher daily intakes from nuts/seeds (> 25 g) and vitamin A-rich vegetables (> 88 g) likely to induce MPA higher than 0.5. However, none of the intake of these food groups appeared to significantly improve iron probability of adequacy, suggesting that a single increase of the diet diversity is not enough for improving iron intake. Owing to the fact that more than 75% of the dietary iron came from plant-based food groups, with 40% from grains, the amount of available iron absorbed from plant-based foods and especially grains is probably an important factor influencing this association. Thus, increasing the availability of iron from a variety of staple foods could be an option for improving iron status among women in Mali, in addition to diet diversification for improving overall micronutrient adequacy.

Strategic entry point for adding nutritional value to fonio

Acceptability of fonio

Investigating consumer-oriented strategies is one of the key entry point of the value chain approach. In the present research, we assessed the socio-cultural acceptability of fonio for documenting potential actions to be included in a behavioral change communication program. This was reported in **Chapters 5 and 6**. A number of positive social and cultural attributes have been related to the consumption of fonio in West Africa (44-48). In previous studies and the present research as well, the most important attributes often reported were the good cooking, organoleptic and nutritional qualities, softness, easy digestibility, healthfulness, contribution to weight maintenance, appetite stimulation, contribution to meal variation, and traditional value of fonio. Health-related attributes were also reported about fonio, being its potential in diabetes and stomach diseases treatment, and blood loss preventing after birth delivery (44). However, scientific evidence of the relationship between fonio consumption and the treatment of these diseases has not been demonstrated so far. Also, most of these attributes were reported in qualitative research, and have never been associated to the consumption of fonio using a systematic method.

In the used behavioral model (49), intention to consume fonio in urban areas was influenced by positive beliefs and attributes mentioned above. Subjective norms, namely husbands' and neighborhood opinion, were also more likely to motivate intention to consume fonio. This pointed out the relative importance of the opinion of the household's head as well as the social environment in the dietary behavior in Malian urban areas. The model also allowed identifying potential barriers to fonio consumption, being seasonal shortage, time-consuming processing and cooking, the high costs of fonio products in urban markets, and the lack of skills in cooking fonio. Those obstacles have been described in previous studies (45, 46), and appeared to have a significant interaction between intention to consume and fonio consumption. These findings indicated that the more the women have intention to consume fonio, the more likely they will face some obstacles for consuming fonio.

An important issue related to the use of behavioral models is the ability to explain the variance in the outcome variables (50). A large variance unexplained suggests that better measures of the model should be designed or more predictors should be added to the model (51). In our study, the model has been carefully designed by associating a comprehensive literature search to qualitative research to collect as many beliefs as possible, and by evaluating the internal consistency and the reliability of the model. Despite this, a large part of the variance in the behavioral intention was not explained, suggesting that some predictors could have been left unexplored. However, the amount of variance a model has to explain for it to be judged acceptable has not been reported (52). Also, the behavioural models often meant to identify factors that need to be influenced to achieve substantial changes in behaviour and the lack of association between the predictors and the behavioural intention does not necessarily question the reliability of the model (52). Thus, although the explained variance of our model is not very high, the identified determinants are relevant to use in a behavioral change program.

Processing

Processing as key strategy for adding value to fonio is documented in **chapter 7.** Explicitly, the effect of phytate degradation with intrinsic wheat phytase and fortification on iron absorption was investigated using stable isotope technique. Phytate at very low concentration can strongly inhibit iron absorption in cereal-based meals and the molar ratio phytate-to-iron should be preferably lower than 0.4:1 to achieve a significant iron absorption (53). Results from our study showed a phytate-to-iron molar ratio of 24:1 in unprocessed fonio flour porridges, being higher than the range of 12:1-21:1, 9:1, 5:1 and 4:1 previously estimated for sorghum, maize, wheat and rice flour porridges respectively (54, 55). Adding wheat flour to fonio porridges significantly decreased the ratio to 3:1. This result confirmed the findings of Egli et al. (56) who demonstrated earlier that adding whole wheat flour to cereals-based complementary foods completely degraded phytate in a relatively short time. However, the phytate-to-iron ratio reached with only dephytinisation did not appear sufficient for achieving significant iron absorption from fonio meals in our study. Indeed, a significant increase of iron absorption ratio by 3.2-fold was achieved after fortification of the dephytinised fonio porridges with 4 mg ferrous sulfate.

It has been shown that dephytinization significantly increased absorption 2-fold from low-tannin Sudan sorghum porridges, but the phytate degradation was achieved by exogenous phytase microbiase (57). Also, 1.7-fold and 1.5-fold significant increases in fractional absorption of iron and zinc respectively in adults have been previously reported for cereal-based foods dephytinized using wheat flour, but without fortification (58). These substantial achievements from the previous and this research emphasized the need to adress the double issue of the low level of bioavailable iron in cereal-based meals (caused by the negative effect of phytate on iron absorption), and the low content of native iron, particularly in fonio meals due to losses during processing.

An issue with single-meal iron absorption study is overemphasizing the effect of phytate reduction on iron absorption (53). Cook et al. (59) previously reported significantly lower increase in iron absorption from total diet (2.5-fold) compared to increase from a single meal (5.9-fold). Therefore, lower increase in iron absorption ratio than reported in this research could have been observed in the context of multiple meal study.

Value chain for adding nutritional value to fonio

The value chain is the process in which a set of related activities and actors add value to a product as it moves through the chain (60). Particularly targeting nutritional goals, the value chain aims at improving nutritional outcomes of vulnerable groups, by addressing the lack of access to foods, and the low and inadequate intake of nutrients from foods. For the present research, implementing the value chain for nutritional purposes targeted the need to set fonio as a key crop in development policy, by adding nutritional value to fonio products for the improvement of women's iron status, while contributing to household food security for fonio stakeholders.

The use of the value chain approach for targeting nutritional goals in developing countries is relatively recent and achievements are still being documented. In order to improve vitamin A intake and status in Mozambique and Uganda, a two-years project has been implemented, using the value-chain approach to increase the availability of orange-fleshed sweet potato (61). In Uganda, with the ultimate goal of improving sustainable livelihoods in rural communities by strengthening key value-chain stakeholders of beans, the approach is currently being tested within a 5-years research project to enhance the nutritional value and marketability of beans (62). The Renewed Efforts Against Child Hunger project recently launched in Sierra Leone aims at scaling up demand for nutritious foods by leveraging local farmers production. The value-chain approach will focus essentially on identifying food-based interventions with potential to increase the incomes of smallholder farmers and improve the nutritional status of the family members, particularly mothers and young children (63).

The definition of an explicit outcome-oriented nutrition goal is a potential strength for a value chain targeting nutrition goals, with the basic assumption that different components of the chain hold potential for solutions (60). In Mozambique and Sierra Leone, emphasis was on increasing the availability of nutritious/nutrient-rich foods like orange-fleshed sweet potato. In Uganda and in Mali (this research), increasing respectively the nutritional quality of a nutrient-rich food and the nutritional value of a staple food was on focus. Overall, strategic entry points identified by the other projects comprised improving production, improving postharvest handling, supporting trader-level marketing, and increasing demand through consumer-oriented activities. In this research focusing on the nutritional aspect of fonio, strategies to add nutritional value to fonio included processing to increase iron bioavailability and uptake from fonio; and consumer-oriented research for investigating socio-cultural acceptability of fonio. Thus, consistently with our research, the aforementioned projects based on clearly defined nutrition-oriented outcomes, and a variety of strategies to reach their goal, although seeking different objectives.

Basically, the value chain applied to nutrition is defined by different terms, according to the field of application (60). This established the conceptual difference between this research and the other projects. The latter used the concept as a process for intervening in a value chain to achieve nutritional goals, of which the nutrition specific term is the value chain development for enhanced nutrition, often called the value chain approach. It involved functioning of various stakeholders and coordination of the different components of the chain to produce sustainable nutritional achievement. The present research puts emphasis on the value added to the product, measured in nutrition or dietary terms in a value chain. Thus specifically, it would be defined as “value-for-nutrition”. However, the most important consideration in leveraging the different value chain concepts for nutritional goals is to generate and achieve a nutritionally-added value, in addition to other benefits like economic value. The value added in the present research can be measured technically by the decreased molar ratio phytate-to-iron in fonio porridge, and the increased iron absorption ratio.

Nevertheless, a nutritionnally improved product that is not culturally acceptable and not economically valuable could not be seen as a benefit product for the value chain, especially for fonio smallhoders and processors (60). As strategic entry point adopted for this research, the social-cultural value of fonio as staple food has been assessed, but not that of the added-value product, neither its sensorial acceptability and economic value.

CONCLUSIONS

The value chain approach is a set of strategies through which values are added to products for creating benefits for stakeholders. Targeting nutrition goals, the value chain is relevant when contributing to improved nutritional outcomes among vulnerable groups. In this thesis we explored value chain strategies as possible solutions to existing nutritional problems among West African women, using fonio as product.

Investigating the nutritional context for operating value chain strategies, we found that overweight/obesity and iron deficiency were simultaneously prevalent among urban Malian women of reproductive, but they were not associated.

Assessing the socio-cultural acceptability of fonio as entry point for consumer-based strategies in the value chain, we suggested that promoting fonio consumption should emphasize positive attitudes and opinions of men, family and neighbors, while strengthening skills of women in cooking good quality fonio meals.

Exploring processing as strategy for adding nutritional value to fonio showed that dephytinisation with native wheat phytase appeared to be a relevant strategy for achieving significant increase in iron absorption from fonio meals only if associated with iron fortification.

IMPLICATIONS FOR FUTURE RESEARCH AND PUBLIC HEALTH

In the following, future research questions and the implication of our findings for public health are discussed.

Future research

Nutrition interventions for reducing iron deficiency in developing countries should further explore the association between increasing body fat mass and iron status. A clear definition of the nutrition problem is the key starting point for operating value chain for nutrition goals. Targeting women in our research, iron deficiency and increased body weight have been identified as existing nutritional problems in Malian urban areas. Evidence has been reported from industrialized countries and some transition countries that overweight is also a risk factor of iron deficiency. It was not the case among our sample, but the evidence of these two morbidity factors evolving simultaneously in West African countries is requesting for more attention for this problem in public health debates. Therefore, we suggest that future research on nutrition-oriented approaches for improving iron status in developing countries should evaluate more closely weight status as risk factor of iron deficiency.

The technical feasibility of plant-based meal dephytinisation with native wheat phytase through home processing pathways needs to be investigated at household level. Substantial removal of phytate from cereal-based foods is challenging in West African countries. Wheat is a locally available and widely used cereal and its potential for dephytinisation has been proven to be higher than most other cereals. In this research, significant decrease of phytate was achieved from fonio meal using wheat flour phytase, but in experimental conditions. The practical feasibility of this technology in West African living conditions needs to be further studied, as wheat phytase can reach its optimal activity only within a limited range of temperature and after more than 1h of incubation. Although the potential usefulness of the method for industrial production of complementary food was demonstrated in an earlier research, the suitability of application at household level is still unknown. Yet, exogenous phytases are not commonly used in most West African countries. In addition, central processing of cereals (including fonio) is still not common nor wellorganised in most West African countries and most processing activities are performed at household level. Thus, home processing should be explored as option for operating cereal-based meal dephytinisation with wheat phytase, through appropriate field trials at household level in order to standardize the procedure.

The impact of value-added fonio products on fonio smallholders' income and livelihoods should be assessed. Leveraging agriculture has been recently emphasized as a sustainable approach for achieving better nutrition among vulnerable groups in developing countries. To operationalize this concept, the value chain approach for nutrition goals is being increasingly implemented in developing countries. A core characteristic of the value chain approach is to add value to products for creating double benefits of increasing income for smallholder farmers and improving the nutritional status of vulnerable populations. Regarding fonio as value chain product, efforts have been made recently to upgrade fonio market value by enhancing productivity, improving post-harvest technologies, quality of exportable fonio products, and promoting women's associations processing fonio in urban areas. In this research we added nutritional value to fonio, with the expectation to boost fonio demand and purchase, so producers will be encouraged to allow larger areas for fonio cultivation and production and as such increase their income. However, the benefits of the above mentioned interventions for stakeholder's income, and hence, nutrition improvement through increased income has not been evaluated, as also did not our research. To bring forward the value chain approach relevance, there is a need to investigate *i*) the long-term effect of operating this concept on stakeholders household food security, in terms of economic value-added, demand/production of the (nutritional and other) value-added products and income; and *ii)* whether the income translate into an improvement of the nutritional situation of vulnerable populations.

Implication for Public Health

Fonio as a traditional cereal might not be an appropriate food for improving iron status through iron fortification. With the increasing nutrition and health consequences related to the global food crisis, the potential contribution of traditional foods to alleviation of poverty, nutritional deficiencies and health problems has been emphasized. A number of previous qualitative and anecdotal studies has claimed fonio as having potential for contributing to food security and nutrition/health among vulnerable groups. The contribution of fonio to household food security during shortfalls has been recently reported. However regarding contribution to nutritional outcomes, it appeared from our research that *i*) average daily frequency consumption (68% consuming 1 to 3 times/month) and daily portion size of fonio (152 g) was relatively low as compared to other staples like rice and millet, *ii*) native iron content of processed fonio products was very low (1.5 mg/100g dry weight basis), and *iii*) a high level of fortification (4 mg/240 g meal portion) was necessary to achieve 3.2-fold increase in iron absorption after dephytinisation. This increase is significant but might not be enough as improved nutritional outcome to balance the costs involved in improving the iron value in fonio meal. Therefore, fonio may not be the preferred food vehicle for fortification to improve nutrition. Results of our study also indicate that more care must be taken when claiming the potential of traditional foods like fonio to contribute to improved nutritional outcomes, especially iron status.

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Summary

English, -Dutch, - French

Summary

Fonio *(Digitaria exilis)* is the most ancient West African cereal representing a key crop in the food supply during crop shortfall periods. During the past decades, efforts have been made to improve the genetic potential of fonio as well as post-harvest processing techniques. However, less is known about the potential of fonio to contribute to nutritional and health-related problems in West Africa. The present thesis focused on the nutritional value of fonio. Particularly, the value chain has been considered as strategic approach to add nutritional value to fonio for improvement of West African women nutritional outcome. Samples of women of reproductive age (15-49 y-old) randomly selected in Bamako and Cotonou, the largest cities of Mali and Benin (West Africa) were involved in the research.

The value chain for nutrition is relevant only if contributing to resolution of existing nutritional problems. The nutritional context for operating the value chain approach in this research was explored in chapters 2-3 by evaluating nutritional problems faced by Malian women in urban areas.

In **Chapter 2** the prevalence of iron deficiency, assessed by serum ferritin and transferrin receptors (sTFR), was determined in relation to body mass index (BMI). To account for the confounding effect of inflammation on ferritin concentration, C-reactive protein was measured and data of individuals with artificially high ferritin levels were removed from non-descriptive analysis in this chapter. Mean ferritin and sTFR levels were 39.3±51.0 µg/L and 4.6±3.6 mg/L respectively. Based on these two indicators, iron deficiency prevalence, was 25% and 9% respectively. Average BMI was 23.4±4.9 kg/m^2, and 19% of women were overweight, of which 9% obese, but increased body mass index was not associated with iron deficiency. A probable hypothesis for this lack of association might be that the inflammation process related to obesity did not achieve the critical level inducing imbalanced iron absorption in our sample.

Chapter 3 assessed iron intake and adequacy among urban Malian women, as well as food groups substantially contributing to these, using a duplicate 24-h recall dietary assessment. We found that with mean daily iron intake of 16.1±8.8 mg, the median iron probability of adequacy was 0.54. Grains, nut/seeds, and vitamin C-rich vegetables significantly correlated with mean iron intake (r = 0.3, P <0.05), but not with iron probability of adequacy. None of meat and fish groups were correlated with iron intake and probability of adequacy. Based on the fact that more than 75% of the dietary iron came from plant-based food groups, with 40% from grains, we suspected that low iron bioavailability from the grains is probably an important factor limiting the correlation between food groups intake and iron adequacy. Thus increasing availability of iron from grains could be an option for improving iron status of women in Mali.

Given that a 24-h dietary recall was designed for the purposes of the research, the ability of the method to provide an estimate of day-to-day variation in intake was discussed, with implication for assessing usual intake from Malian diets in **chapter 4**. Overall, the 24-h provided good estimates of nutrient intake at group level, but within-to-between subject

variance ratio was ≥ 1 for fat, Zn, Ca, Vitamin A and C, and B6 intake. Less variance was explained by day-to-day variation for energy, carbohydrates, protein, iron, thiamin and riboflavin intake. Adjusting for cluster effect appeared to increase the variance ratio. The number of days required across nutrients for estimating usual intake was 2-3 (iron, zinc, folate) to 12 (fat) at percentage limit of ±20% for group level, and 1-2 (energy, protein, iron) to 12 (fat) for correctly ranking individual using an attenuation factor of 0.8. To improve the accuracy of the 24-h recall in assessing usual intake, weekend days should be included, with a larger sample size.

Strategies to upgrade nutritional value of foods through a value chain approach comprise agricultural strategies, processing, and consumer-oriented activities to enhance acceptability. Chapters 5-7 focused on investigating fonio acceptability as entry point for consumer-oriented activities, and processing as strategy for adding nutritional value to fonio products.

Socio-cultural factors influencing fonio consumption in urban areas were identified and integrated in the combined model of Theory of Planned Behaviour and Health Belief Model to assess the acceptability of fonio as staple grain in Malian urban areas (**chapters 5 and 6**). From our results, it appeared that positive attributes often reported were good cooking, organoleptic and nutritional qualities, contribution to meal variation, and traditional value. Potential barriers to fonio consumption identified were seasonal shortage, time-consuming processing and cooking, the high costs of fonio products in urban markets, and the lack of skills in cooking fonio. In the behavioral model, intention to consume strongly correlated with fonio consumption ($r = 0.78$, $P < 0.001$). Fonio consumption was influenced by positive beliefs and attributes mentioned above ($\beta = 0.32$, $P < 0.05$). Subjective norms, namely the opinion of the husband, family and neighbors were also more likely to influence intention to consume fonio ($r = 0.26$, $P < 0.001$). Perceived barriers appeared to have a significant interaction between intention to consume and fonio consumption ($\beta = -0.72$, $P < 0.05$).

In **chapter 7**, an iron absorption study using stable isotope was performed, comparing the effect of iron-fortified fonio flour porridge *vs* iron-fortified fonio-wheat flour porridge on iron absorption among young women in Benin. We found that dephytinisation with intrinsic wheat flour phytase reduced phytate-to-iron molar ratio from 23.7:1 to 2.7:1 in fonio porridges, iron fortification decreased the molar ratio to 0.3:1, and dephytinisation plus fortification increased iron absorption from 2.6% to 8.2%.

In **chapter 8**, based on our main findings and considering existing literature, we suggested that *i*) dephytinisation with native wheat phytase is relevant for achieving significant increase in iron absorption from fonio meals only if associated with iron fortification; *ii*) promoting fonio as a staple food in Malian urban areas should emphasize positive attitudes and opinions of men, family and neighbors, while strengthening skills of women in cooking good quality fonio meals; *iii*) the technical feasibility of plant-based meal dephytinisation with native wheat flour phytase needs to be investigated at household level through home processing pathways; *iv*) further research should: - investigate the long-term effect of operating value chain approach on stakeholders' household food security, in terms of economic value-added, demand and production of the (nutritional and other) value-added products; and - examine whether the income translate into an improvement of the nutritional situation of vulnerable populations.

Samenvatting

Fonio (Digitaria exilis) is een traditioneel West Afrikaans graangewas met een belangrijke rol voor voedselzekerheid in periodes van voedselschaarste. In de afgelopen jaren zijn veel inspanningen gedaan om het genetische potentieel en de verwerkingstechnieken van fonio te verbeteren. Minder bekend is of fonio kan bijdragen aan het verbeteren van voeding- en gezondheidsproblemen in West Afrika. Deze thesis richt zich op de voedingswaarde van fonio. De ketenbenadering wordt hierbij gebruikt als een strategische benaderingswijze om voedingswaarde toe te voegen aan fonio om zo de voedingstoestand van West-Afrikaanse vrouwen in reproductieve leeftijd te verbeteren. Bij deze studie waren vrouwen in reproductieve leeftijd (15-49 jaar) betrokken die willekeurig geselecteerd zijn in Bamako en Cotonou, de grootste steden in Mali en Benin (West Afrika).

Een ketenbenadering voor voedingsverbetering is alleen relevant wanneer het bijdraagt aan een oplossing voor bestaande voedingsproblemen. De voedingsproblemen van Malinese vrouwen in urbane gebieden zijn onderzocht en beschreven in hoofdstuk 2 en 3.

In **Hoofdstuk 2** is de prevalentie van ijzertekorten, vastgesteld aan de hand van serum ferritine en serum transferrin receptor (sTfR) concentratie, onderzocht in relatie tot de body mass index (BMI). Om het verstorende effect van infecties op ferritine concentratie te voorkomen, zijn alle vrouwen met een verhoogde C-reactive proteine concentratie en een daarmee samenhangende kunstmatig verhoogde ferritine concentratie van analyse uitgesloten. Gemiddelde SF and sTFR concentraties waren 39.3±51.0 µg/L en 4.6±3.6 mg/L. Gebaseerd op deze parameters was de prevalentie van ijzertekorten 25% en 9% respectievelijk. Gemiddelde BMI was 23.4±4.9 kg/m2 en 19% van de vrouwen had overgewicht waarvan 9% obees. Verhoogde BMI was echter niet geassocieerd met ijzertekorten. Een mogelijke verklaring voor de afwezigheid van dit verband is dat het ontstekingsprocess gerelateerd aan obesitas in onze onderzoeksgroep wellicht nog niet de kritische waarde heeft bereikt waarop het de absorptie van ijzer aantast.

Hoofdstuk 3 beschrijft de ijzer inname van urbane Malinese vrouwen, of deze voldoende is, en welke voedselgroepen substantieel bijdragen aan de ijzerinname, gebaseerd op data van een 24-h recall uitgevoerd op twee dagen. Wij vonden dat met een gemiddelde dagelijkse ijzerinname van 16.1±8.8 mg, de kans op een voldoende ijzerinname 54% was. Granen, noten/zaden en vitamine C rijke groentes droegen significant bij aan de ijzerinname (r = 0.3, P <0.05), maar niet aan de kans op een voldoende inname. Geen van de vlees of vis voedselgroepen droegen bij aan de ijzerinname of aan de kans op een voldoende inname. Gebaseerd op de vinding dat meer dan 75% van het ijzer uit het dieet afkomstig is van plantaardige voedingsmiddelen, waarvan 40% van granen, verwachten we dat de lage beschikbaarheid van ijzer waarschijnlijk een belangrijke oorzaak is van de afwezigheid van een correlatie tussen de consumptie van voedselgroepen en de kans op een voldoende ijzerinname. Daarom zou het verhogen van de beschikbaarheid van ijzer in granen een mogelijke optie zijn voor het verbeteren van de ijzerstatus van vrouwen in Mali.

Aangezien in deze studie een 24-hr recall werd gebruikt aangepast aan de studie doelstellingen, is besproken in **hoofdstuk 4** hoeverre de gebruikte methodologie de dag-tot-dag variatie in inname kan meten en de gevolgen daarvan voor het meten van gebruikelijke nutriënt inname van Malinese diëten. Algemeen genomen gaf de 24-h recall goede schattingen van nutriënt inname op groepsniveau, maar de binnen–tot-tussen persoonsvariatie ratio was ≥1 voor vet, Zn, Ca, vitamines A en C, en B6 inname. De dag-tot-dag variatie verklaarde een kleiner gedeelte van de totale variatie voor energie, koolhydraten, eiwit, ijzer, thiamine en riboflavine inname. Correctie voor een cluster effect deed de variatie ratio stijgen. Het aantal dagen nodig voor het schatten van gebruikelijke inname varieerde van 2-3 (ijzer, zink, foliumzuur) tot 12 (vet) binnen een nauwkeurigheid van ±20% op groepsniveau. Daarnaast zijn 1-2 dagen (energie, eiwit, ijzer) tot 12 dagen (vet) nodig voor het juist classificeren van individuen gebruik makend van een attenuatie factor van 0.8 (welke corrigeert voor de meetfout in de methode). Om de accuraatheid van de 24-hr recall te vergroten, moet een grotere onderzoeksgroep bij de studie betrokken worden en ook moeten weekeinde dagen meegenomen worden.

Strategieën om de voedingswaarde van fonio te verbeteren gebruik makend van een ketenbenadering, omvatten landbouwkundige strategieën, voedselbereidings-methoden en consumentgeoriënteerde strategieën ter verbetering van de acceptatie van fonio. In de Hoofdstukken 5-7 worden de acceptatie van fonio als startpunt voor consument-georiënteerde activiteiten en voedselbereiding als strategie ter verbetering van de voedingswaarde van fonio beschreven.

Sociaal-culturele factoren die de fonio consumptie in Malinese urbane gebieden beïnvloeden werden geïdentificeerd gebaseerd op een gedragsmodel dat de 'Theory of Planned Behaviour' en de 'Health Belief Model' combineert (**Hoofdstuk 5 en 6**). Onze resultaten gaven aan dat de volgende positieve attributen vaak werden gerapporteerd: goede kookeigenschappen, organoleptische en voedingskundige kwaliteiten, bijdrage aan variatie in de maaltijden, en de traditionele waarde van fonio. Potentiele barrières voor het consumeren van fonio waren de seizoensmatige beschikbaarheid, het tijdverslindende verwerkings- en kookproces, de kosten van fonio producten op de urbane markten en de afwezigheid van vaardigheden in het bereiden van fonio. In het gedragsmodel was de intentie om fonio te eten sterk geassocieerd met de actuele fonio consumptie ($r = 0.78$, $P < 0.001$). Fonio consumptie was ook geassocieerd met bovengenoemde attributen ($\beta = 0.32$, $P < 0.05$). Subjectieve normen, met name de opinie van de echtgenoot, familieleden en buren, waren ook gerelateerd aan de intentie om fonio te eten ($r = 0.26$, $P < 0.001$). Barrières zoals ervaren door de respondenten vormden een significante interactieterm in the relatie tussen intentie om fonio te eten en de daadwerkelijke consumptie van fonio ($\beta = -0.72$, $P < 0.05$).

In **Hoofdstuk 7** is een ijzerabsorptie studie (gebruik makend van stabiele isotopen) beschreven, waarin bij jonge Beninese vrouwen het effect van een ijzer verrijkte pap gemaakt van fonio meel vergeleken wordt met dat van een ijzer-verrijkte pap gemaakt van fonio- en tarwemeel. Wij vonden dat in fonio pappen de fytaat-verlagende werking van de intrinsieke fytase uit tarwe de fytaat:ijzer molaire ratio reduceerde van 23.7:1 tot 2.7:1, terwijl de ijzerverrijking de molaire ratio verlaagde tot 0.3:1. De gecombineerde verlaging van fytaat met ijzerfortificatie verhoogde de ijzerabsorptie van 2.6% tot 8.2%.

In **Hoofdstuk 8** gebaseerd op onze belangrijkste bevindingen in het licht van bestaande literatuur, komen wij tot de volgende conclusies en suggesties: *i*) verlaging van fytaat door natuurlijke fytase in tarwe is alleen relevant voor een significante verhoging van de ijzerabsorptie uit fonio maaltijden wanneer het gecombineerd wordt met een ijzerverrijking; *ii*) het promoten van fonio als basisvoedsel in urbane gebieden in Mali moet de positieve attributen benadrukken en ook de opinies van mannen, familieleden en buren, terwijl de vaardigheden van vrouwen in het bereiden van fonio van een goede kwaliteit verbeterd moeten worden; *iii*) de technische haalbaarheid van het verlagen van fytaat in plantaardig voedsel door middel van natuurlijke fytase in tarwe moet verder onderzocht worden op huishoudniveau met gebruikmaking van voedselbereidingsmethoden gebruikt in het huishouden; *iv*) verder onderzoek moet zich richten: - op de langetermijn effecten van een ketenbenadering op voedselzekerheid op huishoudniveau met name op het gebied van toevoegen van economische waarde, productie van en vraag naar producten, met een verhoogde (voedings of anderszins) verhoogde waarde.

Résumé

Le fonio, *(Digitaria exilis),* céréale la plus ancienne de l'Afrique de l'Ouest, joue un rôle critique dans la sécurité alimentaire pendant les périodes de soudure alimentaire. Au cours des dernières décennies, des efforts ont été menés pour améliorer le potentiel génétique du fonio, de même que les techniques post-récoltes. Cependant, peu de travaux ont été publiés concernant la contribution du fonio à la résolution des problèmes nutritionnels de santé publique en Afrique de l'Ouest. La présente recherche s'est intéressée à la valeur nutritionnelle du fonio. Plus spécifiquement, la chaine des valeurs a été considérée comme approche stratégique pour ajouter de la valeur nutritionnelle au fonio afin de contribuer à l'amélioration de l'état nutritionnel des femmes en Afrique de l'Ouest. Des échantillons de femmes en âge de procréer (15-49 ans) ont été sélectionnés au hasard à Bamako et Cotonou, les deux plus grandes villes du Mali et du Benin (Afrique de l'Ouest).

La chaine des valeurs pour l'amélioration nutritionnelle ne serait pertinente que si contribuant à la résolution des problèmes nutritionnels. Le contexte nutritionnel d'opérationnalisation de la chaine des valeurs dans le cadre de la présente recherche a été exploré à travers les chapitres 2-3 par une évaluation des problèmes nutritionnels auxquels sont confrontées les femmes vivant en milieu urbain au Mali.

Dans le **chapitre 2**, la prévalence de la carence en fer évaluée par la ferritine sérique et les récepteurs de transferrine, a été déterminée, en relation avec le statut nutritionnel évalué par l'indice de masse corporelle (IMC). Pour tenir compte de l'effet du statut inflammatoire sur la concentration en ferritine, la concentration en protéine c-réactive a été mesurée et les données des individus présentant des concentrations en ferritine artificiellement élevées ont été exclues des analyses d'inférence dans ce chapitre. Les concentrations moyennes en ferritine et récepteurs de transferrine étaient de 39,3±51,0 µg/L et 4,6±3,6 mg/L respectivement. La prévalence de la carence en fer basée sur ces 2 indicateurs était de 25% et 9% respectivement. L'IMC moyen était de 23,4±4,9 mg/kg^2 et 19% des femmes présentaient un surpoids corporel, dont 9% obèses, mais aucune corrélation n'a été relevée entre la prévalence d'IMC élevé et celle de la carence en fer. Une hypothèse probable à ce manque d'association serait que le processus inflammatoire induit par l'obésité n'aurait pas atteint l'étape critique entrainant une absorption inadéquate du fer par l'organisme dans le cas de notre échantillon.

Le **chapitre 3** a évalué l'apport en fer et son adéquation parmi les femmes maliennes vivant en milieu urbain, ainsi que les groupes d'aliments contribuant substantiellement à cette adéquation, en utilisant comme méthode d'évaluation du régime alimentaire un double rappel de 24 heures non consécutif. Nous avons trouvé qu'avec un apport journalier moyen en fer de 16,1 ± 8,8 mg, la probabilité médiane d'adéquation en fer était de 0,54. Les groupes alimentaires des céréales, noix/légumineuses, légumes riches en vitamine C étaient significativement corrélées avec l'apport moyen en fer (r = 0,3, P <0,05), mais pas avec la probabilité d'adéquation en fer. Aucun des groupes de viande et poisson n'était associé avec l'apport ou la probabilité de d'adéquation en fer. Considérant que plus de 75% du fer

alimentaire est d'origine végétale, avec 40% de contribution des céréales, nous émettons l'hypothèse que la faible biodisponibilité du fer provenant des céréales et légumineuses est probablement un important facteur limitant de la corrélation entre l'apport alimentaire et l'adéquation en fer. De ce fait, l'amélioration de la biodisponibilité du fer provenant des céréales et légumineuses consommées pourrait être une option pour améliorer le statut en fer des femmes au Mali.

Dans le **chapitre 4**, étant donné qu'un double rappel de 24h a été utilisé dans le cadre de la présente recherche, la capacité de cette méthode à fournir une estimation de la variation intra-individuelle dans l'apport alimentaire a été explorée, avec les implications pour l'évaluation de l'apport nutritionnel habituel des régimes alimentaires maliens. Dans l'ensemble, le rappel de 24h, a permis d'obtenir de bonnes estimations des apports nutritionnels moyens au niveau du groupe, mais les ratios de variance intra-/inter-individuelle étaient ≥ 1 pour les apports en lipides, Zn, Ca, vitamine A, C, et B6. Une moindre part de la variance a été expliquée par la variation intra-individuelle de l'apport en énergie, glucides, protéines, fer, thiamine et riboflavine. L'ajustement pour l'effet de la méthode d'échantillonnage en grappes a semblé accroître la valeur des ratios. Selon les nutriments, le nombre de jours recommandés pour estimer l'apport habituel au niveau groupe varie de 2-3 (fer, zinc, acide folique) à 12 (lipides), pour une limite de précision de ± 20% ; et de 1-2 (énergie, protéines, de fer) à 12 (matières grasses) au niveau individu pour un facteur d'atténuation de 0,8. Pour améliorer la précision du rappel de 24h à évaluer l'apport habituel, les jours de week-end devraient être inclus, avec un échantillon plus large.

Les stratégies d'amélioration de la valeur nutritionnelle des aliments grâce à l'approche chaine de valeur comprennent les procédés agricoles, la transformation alimentaire, et les actions visant à améliorer l'acceptabilité des produits par les consommateurs. Les chapitres 5-7 ont examiné l'acceptabilité du fonio comme point d'entrée stratégique pour des actions orientées vers le consommateur, et la transformation alimentaire comme stratégie pour ajouter de la valeur nutritionnelle aux produits du fonio.

Pour évaluer l'acceptabilité du fonio comme céréale de base au Mali, les facteurs socio-culturels influençant la consommation du fonio en milieu urbain ont été identifiés et intégrés dans un modèle comportementale combinant la théorie du comportement planifié et le modèle des croyances relatives à la santé (**chapitres 5 et 6**). De nos résultats, il est apparu que les attributs positifs souvent rapportés au fonio étaient ses bonnes qualités culinaires, organoleptiques et nutritionnelles, sa contribution à la variation des repas, et sa valeur traditionnelle. Les obstacles potentiels à la consommation du fonio étaient la pénurie saisonnière, le long temps de transformation et de préparation, le coût élevé des produits de fonio sur les marchés urbains, et le manque d'habilité des femmes à préparer des produits de fonio de qualité. Dans le modèle comportemental, l'intention de consommer était fortement corrélée à la consommation du fonio (r = 0,78, P <0,001). La consommation du fonio a été influencée par les croyances et attributs positifs mentionnés ci-dessus (β = 0,32, P <0,05). Les normes subjectives, telles que l'opinion du mari, de la famille et des voisins sont également des facteurs influençant l'intention de consommer le fonio (r = 0,26, P <0,001).

Les obstacles perçus semblent avoir un effet interactif significatif entre l'intention de consommer et la consommation du fonio (*β* = -0,72, *P* <0,05).

Dans le **chapitre 7**, une étude d'absorption du fer utilisant les isotopes stables a été effectuée, en comparant l'effet de deux bouillies de fonio déphytinisées et enrichie/non-enrichie en fer sur la biodisponibilité du fer, avec un échantillon de jeunes femmes en âge de procréer au Bénin. Nous avons constaté que: la déphytinisation avec la farine blé a réduit le ratio molaire phytate:fer de 23,7:1 à 2,7:1 dans les bouillies de fonio ; l'enrichissement en fer a réduit le ratio molaire à 0,3:1 ; la fortification plus la déphytinisation ont amélioré l'absorption du fer de 2,6% à 8,2%.

Dans le **chapitre 8**, sur la base de nos principaux résultats et tenant compte des travaux existant, nous suggérons que *i*) pour les aliments à base de fonio, la déphytinisation avec la phytase de la farine de blé ne serait pertinente pour obtenir une augmentation significative de l'absorption du fer que si associée à une fortification en fer; *ii*) les programmes de promotion du fonio comme aliment de base en milieu urbain au Mali devraient mettre l'accent sur les croyances et attributs positifs, les opinions des chefs de ménage, de la famille et du voisinage, tout en renforçant les capacités des femmes à cuisiner des produits de fonio de bonne qualité; *iii*) la faisabilité technique de la déphytinisation des aliments d'origine végétale avec la phytase intrinsèque de la farine de blé devrait être explorée au niveau ménages par le biais des procédés de transformation alimentaire domestique ; *iv*) des recherches futures devraient : - étudier l'effet de l'opérationnalisation à long terme de l'approche chaine de valeur sur la sécurité alimentaire des ménages des acteurs, en termes de valeur économique ajoutée, de production et de demande des produits à valeur (nutritionnelle et autres valeurs) ajoutée ; - et investiguer si le revenu ajouté se traduit par une amélioration de la situation nutritionnelle des populations vulnérables.

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Nadia Fanou-Fogny was born in Cotonou, Republic of Benin (West Africa) on June 23rd 1974. After completing the secondary school in 1993, she studied Chemistry, Biology and Geology for two years at the *Faculté des Sciences et Techniques* of the *Université d'Abomey-Calavi* (UAC), Benin. In 1996, she joined the *Faculty of Agronomic Sciences* of UAC, where she graduated as Agricultural Engineer (*Ir Agronome*) in 2000 with major in Nutrition and Food Sciences. From 2001 to 2004, she worked as assistant of the coordinator for the NGO *Réseau de Développement d'Agriculture Durable*. In 2005-2006 she obtained a Masters degree (*Diplôme d'Etudes Approfondies*) in Nutrition and Food Sciences with major in Human Nutrition at the Département de Nutrition et Sciences Alimentaires of UAC, after which she worked as PhD fellow in Mali and at the Division of Human Nutrition of Wageningen University for the FONIO project (European Union /INCO No 0015403) presented in this thesis.

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EDUCATIONAL PROGRAMME

Discipline specific activities

Courses

Basic statistics and advanced statistics for nutritionists, Wageningen University, 2006
Training on food consumption survey, Wageningen University, 2006
Production and use of food composition data in nutrition, Wageningen University, 2007
Food perception and preference, Wageningen University, 2007

Conferences

Symposium Quality: from soil to Healthy people, Wageningen University, 2006
Symposium Fortification with iron, Wageningen University, 2007
Symposium Nutrition intervention for reduction of iron deficiency anemia, Department Nutrition and Food Sciences, FSA, University of Abomey Calavi, Benin, 2008
Workshop FANTA Women's dietary diversity project, AED/USAID, Washington DC, USA, 2008
Workshop Fonio SIAGRI, Institute of rural economics, Bamako, Mali, 2008
Workshop on conservation and utilization of traditional vegetables, National Institute of Agronomic Research, Cotonou, Benin, 2008
African Nutritional Epidemiology Conferences, Cairo, Egypt, 2008 and Nairobi, Kenya, 2010
Wageningen Nutritional Sciences Forum, Wageningen University, The Netherlands, 2009
7th International conference on Diet Activity and Methods, NCI, Washington DC, USA, 2009
19th International conference on Nutrition, IUNS, Bangkok, Thailand, 2009

General courses

Introduction and Advanced endnote course, Graduate school VLAG, Wageningen University, 2006
PhD introduction week 16th edition, Graduate school VLAG, Wageningen University, 2006
Basic statistics, Wageningen University, 2006
Scientific publishing, Graduate school VLAG, Wageningen University, 2009
Ethics in health research in sub-saharan Africa, Institute of Applied Biomedical Sciences, Cotonou, Benin, 2011
Analysis using R, Graduate school VLAG, Wageningen University, 2012

Optional courses and activities

Research proposal writing and presentations, Division of Human Nutrition, Wageningen University, 2006
Advanced statistics for nutritionists, Division of Human Nutrition, Wageningen University, 2006
PhD retreats and meetings, Division of Human Nutrition, Wageningen University, 2007
Workshop on conservation and utilization of traditional crops, INRAB, Cotonou, Benin, 2008
International Nutrition Club and Methodology club meetings, Division of Human Nutrition, Wageningen University, 2009 and 2011
Retreat chairgroup nutrition and health, Division of Human Nutrition, Wageningen University, 2011

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