Impact assessment of the oil and wheat flour fortification program in Côte d'Ivoire

Final report

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Drafted by Christine Northrop-Clewes, Giovanna Raso, Nicholas Mascie-Taylor and Fabian Rohner

Executive Summary

This report summarizes the findings of the end-line survey to assess the impact of the oil and wheat flour fortification programmes in Côte d'Ivoire conducted in households in three communes in Abidjan and the rural area of Bouaflé from 13 October to 27 October 2010. The collaborating partners include: the Centre Suisse de Recherches Scientifique en Côte d'Ivoire, the Institut National de Santé Publique and the University of Cocody, with support from Helen Keller Côte d'Ivoire, the Global Alliance for Improved Nutrition and the Swiss Federal Institute of Technology.

Survey Objectives

(a) To conduct a cross-sectional survey to estimate the differences in vitamin A status of pre-school children (Pre-SAC) and women of reproductive age (WRA) living in households consuming fortified oil relative to those living in households consuming unfortified vegetable oil in two areas in Côte d'Ivoire (Bouaflé and Abidjan).

(b) To conduct an end line cross sectional survey of Pre-SAC and WRA to estimate iron status of Pre-SAC and WRA, and folate status of WRA, in two areas in Côte d'Ivoire (Bouaflé and Abidjan) after 3 years of flour fortification.

Survey Methods

Sample design

A two-stage sampling method was used to select the study population based on probability proportional to size. The sampling area comprised the rural part of Bouaflé health district and the three communes in Abidjan: Treichville, Attécoubé and Abobo. Twenty-six clusters for each area were selected and in each cluster 12 households. WRA were selected using a Kish-table and all Pre-SAC per household were enrolled.

Consent

Ethical authorization was obtained from the National Ethics Committee of Côte d'Ivoire. Written consent was obtained from the head of each household and of the participating WRA.

Questionnaire

Two field teams presented the study objectives and procedures to the head of each randomly selected household. After receiving written informed consent, the household head was interviewed about demographic and SES indicators, knowledge and benefits of fortified products, and use of cooking oil and wheat flour. Eligible and consenting members of the household were then invited to take part in the blood sample collection.

Blood sampling

Intravenous blood samples were taken from the arm veins of WRA and Pre-SAC >12 months of age. After mixing, a drop of blood was tested at the household for hemoglobin using a HemoCue™. At the district health centre, two drops of whole blood were removed to prepare a malaria blood smear, the rest of the whole blood was spun and the plasma separated into 4 aliquots:

a) 200µL for the analysis of CRP, AGP, retinol binding protein (RBP), plasma ferritin and sTfR by an enzyme-linked immunoabsorbent sandwich assay (ELISA).
b) 50-100µL plasma from the WRA was prepared in opaque tubes for folate analysis
c) Two further small tubes of plasma were frozen locally and stored as repository samples at -30°C at CSRS.

Summary of findings

Socio-economic status

Nearly all rural households owned their own homes (95%), whereas 73% of urban households rented. In the urban areas, 98% of houses were made of cement and had electricity, whereas 65% of rural houses were made of cement and only 42% had electricity. Another important difference was that 96% of drinking water in urban areas was from a tap compared to only 12% in the rural households, and 96% of urban houses either had a cement latrine or flush toilet, but 86% of rural households defecated in fields. The majority (92%) of rural families were farmers while 47% of urban householders were employed and 21% were traders.

Fortification

Oil

The legally mandated level for the fortification of oil with vitamin A is 8 µg/g retinol equivalents (RE). Overall 32.2% of oil samples were fortified at the legal level or above, and there was no significant difference in the level of oil fortification between the rural area of Bouaflé (33.5%), and the urban area of Abidjan (30.8%). There is an expected variation in the level of fortification of about 20%, which means any oil containing ≥6.4 µg/g RE of vitamin A was considered to be adequately fortified, and 50% of the oil was in this category, with no rural/urban differences. Concentrations of vitamin A ≤3.2 µg/g RE in oil were considered to be unacceptable, and 35% of urban and 41% of rural samples fell into this category, respectively.

Flour

Iron

The legal level for the fortification of flour with iron is 60 ppm. Overall 69.1% of flour samples were fortified at the legal level or above, and there was a significant difference in the level of flour fortification between the rural area of Bouaflé (47.3%) and the urban area of Abidjan (92.6%; $p<0.001$). There is an expected variation in the level of fortification of about 20%, which means any flour containing ≥48ppm of iron was considered to be adequately fortified, and nearly 100% (99.7%) of urban flour samples were in this category, but only 50% of rural samples. A third of rural samples were considered non-fortified with concentrations of iron ≤24 ppm.

Folic acid

The legally mandated fortification concentration for folic acid in flour is 1.5 mg/kg. Overall 0.9% of flour samples were fortified at the legal level or above and there was a significant difference in the level of flour fortification between the rural area of Bouaflé (1.8%) and the urban area of Abidjan (0%; $p = 0.001$). But as with the other two fortificants, a 20% variation in concentration was deemed acceptable (≥1.2 mg/kg) and overall, 6% of flour samples had this concentration of folic acid with significant differences between rural (9%) and urban (2%). Flour samples with ≤0.6 mg/kg folic acid (considered non-fortified) were found in 64% of urban samples and 39% of rural ones.

Micronutrient status

Vitamin A

Pre-SAC

After correction for the presence of inflammation, identified by elevated concentrations of AGP (>1 g/L) and/or CRP (>5 mg/L), 9.6% of urban and 24% of rural Pre-SAC were identified as VAD. Urban males and females had significantly less VAD than those living in rural areas (males: 13.2% versus 24.9%, respectively, $p<0.001$; females: 6.5% versus 24.8%, respectively,

p<0.001). In rural areas, for both the corrected and non-corrected concentrations of RBP >15% of the children in the population surveyed were VAD which according to the WHO suggests a public health problem amongst that age group, assuming that retinol and RBP cutoffs are equivalent.

WRA
Among WRA there was no significant difference in mean RBP or differences in VAD by locality nor was there any significant age effect. The mean corrected RBP concentration was 1.64 µmol/L, and there was no significant VAD in this population (0.7%).

Folate

All WRA had folate concentrations <10nmol/L and were thus deficient. The geometric mean of the logged folate concentrations was 0.097 nmol/L in the rural and 0.091nmol/L in the urban area (p=0.019).

Iron

Pre-SAC
The mean hemoglobin concentration was significantly higher in urban (104 g/L) than rural children (85 g/l; p <0.001). In contrast, the geometric mean ferritin concentration corrected for inflammation was significantly higher in the rural area (45.3 µg/L and 29.3 µmol/L, respectively; p <0.001). The geometric mean of the sTfR concentrations was also higher in rural (9 mg/L) than urban children (4.6 mg/L; p<0.001).

Hemoglobin concentrations increased with age (0.278 g/L/month), as did ferritin concentrations (0.374 µg/L/month), while sTfR concentrations fell (0.061 mg/L/month).

In the rural area 92.2% of pre-SAC were anemic, and of those, nearly 1 in 5 had severe anemia (<70g/L) compared to 56.3% of children in the urban area with anemia equivalent to 1 in 38 of urban children. The prevalence of moderate anemia (hemoglobin ≥70g/L and <110g/L) in rural children (74%) was significantly higher than in urban children (54%), as was mild anemia (92% vs. 56%, respectively; p<0.001).

Depleted iron stores (ferritin <12 µg/L, after correction for inflammation) were found in 22% of urban children and 7% of rural children, and iron deficiency (sTfR >8.3 mg/L) was found in 11% of urban and 9% of rural children.

WRA
The mean hemoglobin concentration was significantly higher in urban (120 g/L) than rural WRA (105 g/l; p <0.001). The geometric mean ferritin concentrations corrected for inflammation were not different (urban 38.3 µg/L and rural 37.4 µmol/L). The mean of the sTfR concentrations were higher in rural (6.5 mg/L) than urban WRA (4.5 mg/L; p<0.001).

Hemoglobin concentrations decreased with age (0.32 g/L/year), and ferritin concentrations increased (6.4 µg/L/year).

There was little severe anemia (2%) among WRA. The prevalence of moderate anemia (Hemoglobin ≥70g/L <110g/L) in rural WRA (74%) was significantly higher than urban WRA (44%) as was all anemia (78% vs. 45%, respectively; p<0.001).

Depleted iron stores (ferritin <12 µg/L, geometric mean corrected for inflammation) were found in 38.5% of urban and 37.4% of rural WRA. Iron deficiency (sTfR >8.3 mg/L) was found in 4.5% of urban and 6.5% of rural WRA.

Plasmodium infection

Pre-SAC
In the rural area, 43% of Pre-SAC were infected with *Plasmodium falciparum* but significantly fewer urban children were infected (7%; p<0.001). Infections with *P. malariae* and *P. ovale* were minimal but parasitemia was significantly less in the urban area. Fourteen children had double infections. There was a decline in intensity of infection with age of 107 parasites/µL blood/month of age.

WRA
Likewise in WRA, parasitemia with *P. falciparum* was significantly higher in rural (13%) than urban women (7%; p<0.008).

Associations between SES, age, sex and Plasmodium spp., and biomarkers

Households
SES explained up to 27% of the variation in hemoglobin concentrations in Pre-SAC and there was a significant effect on locality. In rural areas where there was no flushing toilet, river water was used for drinking and there was no village mobile phone hemoglobin concentrations in Pre-SAC were 17g/L lower than in urban areas.

Pre-SAC
Concentrations of RBP in Pre-SAC with *Plasmodium* infections (0.85 µmol/L) were significantly lower than uninfected children (0.99 µmol/L; p<0.001).

Using corrected values of RBP as a continuous variable, and correcting for age, and sex urban children still had significantly higher RBP concentrations (+1.2 µmol/L) than rural children. Rural children were 3.2 times more likely to have VAD than urban children, in a stepwise regression.

Nearly all children with *Plasmodium* infection were anemic (96%), compared to 64% without Plasmodium infection. Children without *Plasmodium* infection were much more likely to have depleted iron stores (3.9 times) and to be more iron deficient (2.4 times) than infected children.

Corrected mean ferritin concentrations were 12 µg/L higher in Pre-SAC with *Plasmodium* parasites than those without after correcting for age, sex and locality. Concentrations of sTfR were also higher in *Plasmodium* infected children by 2.6 mg/L. In contrast, children with *Plasmodium* infection had hemoglobin concentrations 9.5 g/L less than those without (p<0.001)

Uninfected children were 3.9 and 2.4 times more likely to be iron depleted and iron deficient, respectively, than *Plasmodium* infected children.

Children without inflammation (CRP or AGP) or *Plasmodium* parasites had the lowest ferritin concentrations (no elevated AGP: 39.6 µg/L; no elevated CRP 42.3 µg/L), while those with elevated acute phase proteins and *Plasmodium* parasites had the highest concentrations of ferritin (elevated AGP + *Plasmodium*: 109.7 µg/L; elevated CRP + *Plasmodium* 119.4 µg/L).

WRA
No analyses are reported for WRA as the number with VAD was too small, or there was no significant difference to report.

WRA living in rural areas were 4.3 times more likely to be anemic than those in an urban area.

Used as a continuous variable, 17.8% of the variance in hemoglobin was explained by SES of which locality was the main predictor, and the rural mean was 18.1 g/L lower than the urban mean (p=0.03).

WRA with *Plasmodium* infection had significantly lower mean hemoglobin concentrations after correction for age and locality (-4.7 g/L; p<0.027). Corrected concentrations of ferritin were put into a regression model but there was no significant difference in ferritin concentrations between WRA with or without *Plasmodium* infection.

WRA without inflammation (CRP or AGP) or *Plasmodium* parasites had the lowest ferritin concentrations (no elevated AGP: 51.4 µg/L; no elevated CRP 52.3 µg/L), while those with elevated acute phase proteins and *Plasmodium* parasites had the highest concentrations of ferritin (elevated AGP + *Plasmodium*: 120.3 µg/L; elevated CRP + *Plasmodium* 111.2 µg/L).

Using folate concentrations as a continuous variable, 5% of the variation was explained by cooking method, as women cooking with charcoal or gas had higher mean folate concentrations than those cooking with wood (both +0.4 nmol/L; p<0.001), i.e. those with higher SES are likely to cook with gas or charcoal.

Impact of the fortification program on biomarkers

Pre-SAC

By calculating the quantity of vitamin A consumed as fortified oil/day, a significant positive relationship (p=0.007) was found between RBP and vitamin A consumption, such that a 1 mg RE/kg additional intake of vitamin A produced an increase in RBP concentration of 0.49 µmol/L.

A significant effect was found of iron fortification on hemoglobin concentration: in a regression model controlling for the effects of age and sex, there was a hemoglobin increase of 0.24 g/L for each additional ppm of iron in the flour. This increase was even more marked if only children free of malaria parasites were included in the analysis.

WRA

There was no significant relationship between mean RBP and vitamin A in the oil, either using all three categories of oil fortification or just the two extreme categories (≥6.4 µg/g and ≤3.2 µg/g RE). The relationship between plasma VAD and concentrations of vitamin A in the fortified oil was not analyzed as the percentage with deficiency was so small.

No significant effect of iron fortification on iron biomarkers was found.

There was a significant difference in mean plasma folate by folic acid in the diet mainly driven by the higher mean in the ≥1.2 mg/kg category of folic acid in the urban samples (p=0.021, logged data). The amount of natural folate in the diet was not evaluated.

Discussion

<u>Oil and biological impact</u>

1. About 50% of oil samples were adequately fortified (≥6.4 µg/g) and 35% were inadequately fortified (≤3.2 µg/g).

2. The significant association between consumption of vitamin A as fortified oil and plasma RBP in Pre-SAC may be explained by the fact that infants are born with no liver stores of vitamin A and are reliant on dietary intake for their vitamin A and so plasma levels are responsive to changes in vitamin A in the diet. WRA have liver stores and plasma vitamin A levels are homeostatically controlled so there was no expected relationship with dietary intake. Further, because of the low prevalence of VAD, suggesting adequate intake, it would be unlikely to see any impact of the fortification program on WRA.

3. Inflammation and presence of *Plasmodium* infection had an impact on circulating levels of RBP, however after correction for inflammation, overall plasma concentrations of RBP in Pre-SAC (0.94 µmol/L) were not dissimilar to those of British Pre-SAC (1.04 µmol/L). Rural/urban differences remained after correction for inflammation and the prevalence of VAD in rural Pre-SAC was 24.3% representing a public health problem as defined by WHO. In contrast only 9.6% of urban Pre-SAC were VAD. The difference in the two residencies may be explained by the fact that there may be less vitamin A in the diets of those in rural areas resulting in dietary VAD. Further, exposure to infected mosquito bites, resulting in *Plasmodium* parasites in the blood, was far greater in the rural area, and may be responsible for many more children being affected by changes in the acute phase proteins resulting in lower plasma RBP concentrations; such relationships have been found by other authors. The residual difference in the VAD may be explained by the fact that although vitamin A is taken up from the diet and transported to the liver, it may become trapped there as the circulating RBP concentrations are lower in an acute phase response and so not enough RBP is available to transport the retinol to the tissues of the body.

<u>Flour and biological impact</u>

1. In the 3 communes of Abidjan, almost 100% of flour samples were adequately fortified with iron (≥ 50ppm), but in Bouaflé only half the flour was acceptably fortified.

2. The usefulness of ferritin as an indicator of iron stores in an area endemic with malaria is disputed, as is the method for correcting ferritin for inflammation. However in Pre-SAC, using corrected ferritin concentrations, and accounting for locality, age and sex, the ferritin concentrations in the infected group were still 12 µg/L lower than those in the non-infected group, which suggests that the correction factors are not completely correcting ferritin, therefore additional correction factors are needed to correct ferritin where clinical malaria and/or *Plasmodium* parasites have been diagnosed.

3. After the effects of age and sex had been removed, the analysis found a significant effect of iron fortification of flour on hemoglobin and sTfR concentrations of pre-SAC. The data on ferritin concentrations were difficult to interpret possibly due to the important confounding role of malaria parasites and general sub-clinical inflammation.

4. Concentrations of sTfR are determined by marrow erythropoietic activity. In this survey a small effect of inflammation on sTfR concentrations was found which suggests that there was some malaria-associated hemolysis resulting in increased erythropoiesis, which in turn increased concentrations of sTfR. Previous studies have suggested sTfR concentrations may not measure iron deficiency in malarious areas, as it is responding both to the rate of erythropoiesis and the deficit in functional iron in the erythron.

5. Only 6% of flour samples had the legal level of folic acid (>1.2 mg/kg) and 42% were fortified between concentrations >0.6 and <1.2 mg/kg. If the folic acid was added at the correct concentration in the premix, and the iron data used to confirm fortification levels suggests it was, then there is a need to ascertain what has happened to the folic acid

over time since the flour left the mill. Folic acid is unstable and loses activity in the presence of light, oxidizing or reducing agents, and acidic and alkaline environments but it is relatively stable to heat and humidity, and it has been suggested 100% of folic acid is still present in flour after 6 months. It is necessary to carry out some investigations on the stability of folic acid by taking random samples from storage facilities, distributers, shops and at households across the country and testing them at an accredited laboratory.

Conclusion

This cross-sectional survey revealed that among children aged 6-59 months, living in households who purchase and consume vitamin A fortified oil, there a was positive association with vitamin A status; an additional intake of 1mg RE translated in a 0.49 µmol/L increase in RBP in this age group. Sub-group analysis revealed that in children 24-59 months the relationship between consumption of fortified oil and RBP concentrations was even more marked as consumption of 1 mg RE oil translated into a 0.58 µmol/L increase in RBP concentration, yet in children 6-23 months although there was an increase of 0.26 µmol/L in RBP concentration/mg RE oil, the relationship was not significant. In contrast, among WRA, vitamin A status was not improved probably due to the initial low prevalence of VAD as measured in the baseline survey of 2007.

With regard to the impact of iron-fortified flour, the data indicated a positive impact on hemoglobin and sTfR levels in all pre-SAC, whether *Plasmodium*-free or not, whilst for ferritin, interpretation of the findings was difficult. Each additional ppm of iron in the flour resulted in a hemoglobin increase of 0.24 g/L.

For WRA, no impact of the iron in the flour on iron indicators was found, whereas there was a moderate but positive association between folic acid content in the flour and plasma folate and hemoglobin concentrations.

Recommendations

For Côte d'Ivoire, the following recommendations can be made

1. Maintain the oil and iron fortification program, since it appears to particularly benefit children aged 6-59 months of age; possibly, the iron compound should be revised and a more bio-available form be used in the future.
2. In 2009, with the support of Helen Keller International, approximately 85% of children 6-59 months received vitamin A capsules and this program should be continued, most particularly for children 6-23 months who may not be able to eat enough fortified oil to be effective in reducing VAD.
3. Investigate why there is a difference in the level of iron fortification between the urban and rural areas.
4. Investigate why folic acid levels are so low in the fortified flour at household level
5. Investigate whether cooking practices are destroying the folic acid in the flour and in other folate containing foods commonly eaten, e.g. vegetables.
6. Promote the use of insecticide-treated bed-nets, especially in rural areas

With regard to further research, it will be important to further assess the influence of sub-clinical inflammation and *Plasmodium* parasitemia on assessing iron status; from this analysis, it seems that the currently existing correction algorithms do not fully account for the effects.

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List of abbreviations

AGP **:** α-Acid Glycoprotein
CDC : U.S. Centers for Disease Control and Prevention, Atlanta
CSRS : Centre Suisse de Recherches Scientifiques en Côte d'Ivoire
CRP : C-reactive Protein
ELISA : Enzyme Linked Immunosorbent Assay
ETHZ : Swiss Federal Institute of Technology Zürich
GAIN : Global Alliance for Improved Nutrition
GPS : Global Positioning System
Hb: Hemoglobin
HKI: Helen Keller International
HPLC : High Performance Liquid Chromatography
INSP : Institut National de Santé Publique
MoH : Ministry of Health
LNSP : Laboratoire National de Sante Publique
PIPAF : Programme Ivoirien de la Promotion des Aliments Fortifiés
Pre-SAC: pre-school aged children, 6-59 months
PF: Plasma ferritin
RBP : Retinol Binding Protein
RE : Retinol Equivalent
sTfR **:** Soluble Transferrin Receptor
VAD : Vitamin A Deficiency
WHO : World Health Organization
WRA: Women of Reproductive Age, 15-49 years

1 Introduction

1.1 Micronutrient deficiencies and malnutrition in Côte d'Ivoire

In the autumn of 2007, a team of researchers from the "Centre Suisse de Recherches Scientifiques en Côte d'Ivoire" (CSRS), the Institut National de Santé Publique (INSP) and the University of Cocody, with support from Helen Keller Côte d'Ivoire (HKI), the Global Alliance for Improved Nutrition (GAIN) and the Swiss Federal Institute of Technology (ETHZ), conducted a baseline survey to estimate the prevalence of vitamin A, folate and iron deficiencies. This survey was the most recent nationally representative dataset on the micronutrient status of the Ivorian population (Tschannen et al. 2009); other estimates on these deficiencies were considerably older and not nationally representative (Ake et al. 2001; Rohner et al. 2007; Staubli Asobayire et al. 2001; Yapi et al. 2005), or lacked assessment of certain key nutrients (e.g. folate).

The 2007 survey was designed to provide national estimates and covered a total of 900 households in 9 eco-regions of Côte d'Ivoire. Deficiencies of three micronutrients were estimated for preschool-aged children (6-59 months old; Pre-SAC) and women of reproductive age (15-49 years old; WRA). Whereas vitamin A deficiencies were relatively low in WRA: (0.4%), they were higher in Pre-SAC (15%), but anemia prevalence was high in both populations (WRA: 51%, pre-SAC: 72%). Iron deficiency rates were high among pre-SAC (51%) but surprisingly low for WRA (17%). Folate deficiency among WRA was universally high (86%).

1.2 National policy and fortification program

According to the World Health Organisation (WHO), data from the 2007 Côte d'Ivoire survey reported anemia was a severe public health problem in pre-SAC and WRA (WHO/UNICEF/UNU 2001), but vitamin A deficiency (VAD) was only a public health problem in pre-SAC (WHO 1996). Iron deficiency rates were also high in pre-SAC but not in WRA. The Ministry of Health in Côte d'Ivoire (MoH, Ministère de la Santé et l'Hygiène Publique) adopted multiple strategies to combat the multifaceted etiology of anemia including supplementation, dietary counselling, food diversification and fortification, and the reduction of malaria and other parasitic diseases. One strategy put in practice by the MoH to fight iron-deficiency anemia (IDA) was the supplementation (iron and folate) of pregnant women during prenatal and postpartum consultations. Efforts to reduce VAD in Côte d'Ivoire included the supplementation of pre-SAC, linked to routine immunization programs "EPI-Plus" (WHO 2007). As a complement to the supplementation programs, fortification of wheat flour with electrolytic iron and folic acid and palm oil with vitamin A was recommended and was put in practice in Côte d'Ivoire under the oil and wheat flour fortification program in Côte d'Ivoire (Programme Ivorian de la Promotion des Aliments Fortifiés; PIPAF). Under the PIPAF program, two laws pertaining to the fortification of vegetable oils and wheat flour were passed in 2007, rendering fortification of these two vehicles mandatory.

In autumn 2009, a survey was conducted by researchers from HKI and INSP, with support from GAIN, to estimate the household coverage of fortified vegetable oils and wheat flour available to final beneficiaries, i.e. households, WRA and children aged 6-59 months (unpublished). Samples of vegetable oil and wheat flour were provided by the households, and were subsequently analyzed to estimate their levels of fortification. A total of 2850 households were surveyed. Overall, nationwide household coverage of fortified oil (≥ 6.4 µg/g retinol equivalent [RE]) was found to be approximately 24%, although only 12% was fortified at levels in accordance with the legislation (≥ 8 µg/g RE). In addition, the coverage of the fortified oil was patchy and more concentrated in some regions. In urban and rural zones, 30% of households had flour fortified in accordance with the legislation (60 ppm), and approximately half of the surveyed households consumed flour with slightly lower than mandated levels of iron, 50 ppm.

Since the 2009 survey, however, a major mill has begun fortifying and household coverage was anticipated to be much higher.

1.3 General objectives of the survey

The coverage of the GAIN-funded oil and wheat flour fortification program in Côte d'Ivoire was evaluated in a rural area, Bouaflé and in 3 communes in the urban area of Abidjan. Given that there was significant household coverage of fortified flour and some coverage of fortified oil in 2009, a second survey was planned that included biomarkers. The purpose of this cross-sectional study was to assess end-line micronutrient status to help establish the impact of the PIPAF program.

Fortified oil was on the market prior to the GAIN funded project and 55% of the commercialized vegetable oil was estimated to be fortified at that time (World Bank Institute 2009). The PIPAF Program aimed to increase the coverage of the fortified oil from 55% to 80%. The 2007 baseline survey, and the subsequent coverage survey in 2010 indicated that consumption of fortified oil was relatively heterogeneous, therefore the impact of the program in 2010 was evaluated by assessing the vitamin A status of persons who habitually consume fortified oil compared to those who usually consumed unfortified oil. In contrast, because there was no fortified flour on the market in 2007, the impact of consuming fortified flour on iron status of pre-SAC and WRA, and on folate status of WRA was to be compared to the baseline data. However, because of the complex nature of the analysis at end line, only the end line data is analyzed and reported here. The pre-/post comparison of the impact of the iron and folic acid fortified wheat flour is not considered in this report.

Two general objectives were formulated for this study:

1. To carry out a cross-sectional survey to estimate the differences in vitamin A status of pre-SAC and WRA living in households consuming fortified oil relative to those living in households consuming unfortified vegetable oil in two areas (Bouaflé and Abidjan).

2. To carry out a cross sectional survey to evaluate the iron status pre-SAC and WRA and the folate status of WRA consuming fortified flour in two areas (Bouaflé and Abidjan).

2 Methods

2.1 Study design

The survey was cross-sectional and the data collected was for two different purposes to evaluate the micronutrient status of pre-SAC and WRA consuming fortified flour and in a later analysis, not included in this report, to evaluate the impact of the folic acid and iron fortification program by comparing to the baseline data. In addition, households consuming vitamin A fortified oil were compared with those not consuming the oil. The survey was conducted in two areas, the urban area of Abidjan and the rural area of the Bouaflé health district. The baseline survey carried out in 2007 also included these two areas.

The survey took place in the same season of the year as the baseline study to reduce the influence of seasonal variation (2007:July/August; 2010:October). Also, the survey followed the last vitamin A supplementation round (May 2010) by at least 4 months and preceded the next round of supplementation which was to be done in November 2010.

A two-stage sampling method was used to select the study population based on probability proportional to population size. The sampling area comprised the rural part of the Bouaflé District and the three communes in the urban areas of Abidjan: Treichville, Attécoubé and Abobo. The three communes of Abidjan had been selected randomly in the 2007 survey to represent Abidjan. Twenty-six clusters for the urban and 26 clusters for the rural area were selected independently by a two-stage process, leading to a total of 52 clusters for the pooled sample:

Stage 1: for each area, the total population for each commune was listed and the cumulative population calculated. The sampling interval was then calculated by dividing the total cumulative population size by the total number of clusters. A random number was generated to give the random starting point, and the sampling interval was used to identify the clusters to be selected.

Stage 2: The required number of households within each cluster was randomly selected. The selection of households was done by selecting a random starting point from among the 4 geographical extremities or the center of the village/quarter. From there, the direction to proceed, towards one of the 4 remaining points was randomly selected. Along this defined transect, the first household to the right was selected. Then, subsequent households were selected using the “next nearest household” approach as frequently used in EPI surveys.

In each cluster, a total of 15 households were included in the sample. Within each household, the one participating WRA was determined by selection based on the Kish-Table among all eligible subjects (Kish 1949). All pre-SAC of the household were enrolled.

Eligibility criteria:

- Be of correct age/gender
- For WRA, not pregnant (self-reported)
- Consenting
- No counter indications for blood sampling (e.g. hemophilia, severe wasting)

Exclusion criteria:

- Age not within the specified range
- No WRA present in the household
- For WRA, self-reported pregnancy

- Written informed consent not obtained
- Counter indications for blood sampling

2.2 Field and laboratory procedures

The study protocol and data collection tools (questionnaires and field forms) were developed by researchers from GAIN, the CSRS and the INSP between August and September 2010. The 2-day training of the field teams took place on 11 and 12 October 2010 at the INSP during which 4 teams were identified. Each team consisted of a team leader (supervisor), two investigators, a laboratory technician, a nurse and a driver; all of the team members had been involved in at least one of the previous surveys. The study protocol was then presented to the team members and the method of selection of households and target population reviewed. The use of different tools for data collection (individual and household questionnaires, points of sale field forms, etc.) and the survey material (oil and flour containers, GPS, labels, blood sampling disposables) was reviewed with each team. On the second training day, a pre-test was conducted by the 4 teams in the commune of Adjamé in Abidjan located near the INSP. The objectives were to (i) test the field data collection tools, and (ii) train team members. Thereafter, data collection tools were finalized. The field material was then distributed to each team (see Appendix 2).

The field survey was conducted between 13 October 2010 and 27 October 2010. Two teams were based in Abidjan and two teams were based in Bouaflé. Nurses were recruited locally for each team. Two additional laboratory technicians were recruited locally (1 in Abidjan and 1 in Bouaflé) to help the teams at the laboratory.

The fieldwork was started after the supervisors of each team visited a cluster (village in Bouaflé or commune/quarter in Abidjan) to inform village or commune authorities as well as health authorities about the study. After authorization to work in their village or commune/quarter was obtained, the team members visited one cluster each day to carry out the questionnaire at the household and take samples of oil and wheat flour, as well as collect blood samples from the target groups. Before commencement of the data collection, written informed consent was obtained from each study participant or parent/legal guardian in the case of study participants aged below 18 years after the objectives, procedures, potential risks and benefits of the study had been explained to the household members. It was also made clear that study participants could decide to cease participation at any time in the study.

2.3.1 Household questionnaires and oil and flour sample collection

At the household, the team introduced themselves to the household head and other household members. The study objectives and procedures were presented to the household members. After receiving written informed consent from the household head, using the household questionnaire, the WRA was interviewed on demographic and socio-economic indicators, knowledge and benefits of fortified products, use of cooking oil and wheat flour (frequency and quantity of purchases). The household questionnaire can be found in Annex III. If an oil or flour sample could not be collected from the interviewee, she was asked where the oil/flour used by the household was normally purchased, and if possible, she described the brand and packaging. Finally, the interviewee was asked to accompany a member of the team to the store to purchase the oil or flour.

Oil samples were stored in boxes in the dark and cool at INSP initially, and later at CSRS, before being shipped for analysis. Flour samples kept dry and cool and were immediately shipped to Switzerland on completion of the field-work.

The members of selected households eligible and consenting were invited to participate in the blood sample collection. For this purpose a letter of invitation (labeled with the household ID) was distributed to participants inviting them for blood sampling.

2.3.2 Blood sample collection

Experienced technicians carried out the blood sampling in a central health facility in the village. For each respondent, the technician responsible for phlebotomy checked the labeled blood invitation letter(s) that the WRA brought from her household, removed the matching label(s) for the blood sample(s) and stuck each label to the Vacutainer™ to be used for the blood from the WRA and children. If the technician failed to get blood on the first attempt, with permission they tried for a second attempt. After two unsuccessful attempts, the respondent was thanked but no blood was drawn.

Intravenous blood samples were collected from arm veins in WRA and pre-SAC older than 1 year of age. Infants under 1 year were sampled from the heel. Venous blood samples (4 mL) were drawn into EDTA-treated evacuated tubes (Becton Dickinson, Nyon, Switzerland). After blood collection, the technician gently inverted the Vacutainer™ several times to ensure a homogeneous sample. A Vacutainer™ dispenser was used to extract a drop of blood from the vacutainer and the drop was applied to the hemocuvette for the measurement of hemoglobin using a HemoCue™ (Hb201+,HemoCue, Angelsborg, Sweden). The hemoglobin result was recorded on the invitation form. Subsequently, the Vacutainer™ was placed in a cool box containing cool blocks to ensure that the whole blood sample was stored cold but not frozen, at ~+4°C and in the dark until further processing.

On diagnosis of severe anemia (<60 g/L), a rapid detection of malaria was carried out using a dipstick. With diagnosed parasitemia, antimalarial treatment was administered by the nurse according to established national guidelines. For all severely anemic but malaria-negative subjects, an iron supplementation course according to national guidelines was administered. All participating households received verbal information on how to prevent and treat micronutrient deficiencies. Additionally, light medical treatment was offered for free for minor diseases as diagnosed by the local nurse (mainly analgesics).

2.3.3 Blood sample processing in the field

Collected whole blood samples were transported on cool packs to the district health headquarters every day. On arrival, two drops of whole blood were removed for the preparation of a malaria blood smear and fixed and stained for later microscope reading. The plasma was then separated from the remaining whole blood by centrifugation at room temperature for 10 minutes at 3000 x g, divided into 4 aliquots and stored in labeled Eppendorf-tubes within labeled racks at -20°C until further analysis. When the district headquarters could not provide freezer space, each team carried a portable freezer, allowing storage conditions at -20°C. The aliquots were:

a) 200 µL plasma from each WRA and pre-SAC stored in small tubes for the analysis of C-reactive protein (CRP), α-1 acid glycoprotein (AGP), retinol binding protein (RBP), plasma ferritin, and soluble transferrin receptors (sTfR) by the enzyme-linked immunosorbent sandwich assay (ELISA).

b) For WRA, 500-1000 µL plasma was stored in opaque tubes for analysis of plasma folate concentrations.

c) Two additional small tubes with plasma were stored as repository samples at -30°C at the CSRS in Abidjan.

All aliquoted tubes were frozen locally at -30°C until shipment on dry ice to Germany and Switzerland, respectively where they were then stored at -80°C.

2.3.4 Sample analysis and quality control

(a) Hemoglobin

Hemoglobin was measured using a portable Hemocue™ device and suitable hemocuvettes. Measurements were made at the village central facility, and results recorded on the individual form and communicated to the participating subjects. Quality control was conducted every morning using 2-level controls provided by the manufacturer.

(b) Plasma RBP, ferritin, sTFR, CRP and AGP

DBS-Tech laboratories in Germany analyzed the plasma samples for RBP, ferritin, sTfR, CRP and AGP (Erhardt et al. 2004). All analytes were analyzed in one run from less than 100µL plasma by ELISA. The laboratory is participating in the US Centers for Disease Control and Prevention (CDC) VITAL-EQA inter-laboratory comparison rounds, and has implemented a rigorous internal quality control system.

(c) Plasma folate

The Swiss Vitamin Institute (SVI) analyzed the plasma samples from the WRA for folate concentrations, using the microbiological assay (Lactobacillus caseii ATCC 7469). The SVI uses a standard method as described in the “Schweizerisches Lebensmittelbuch, Januar 2001”, issued by the Swiss Federal Office of Public Health. The laboratory regularly participates in the CDC inter-laboratory comparison rounds.

(d) Malaria parasitemia

After completion of a cluster (usually early afternoon), thick and thin blood films for malaria testing were prepared, stained with Giemsa, and dried for storage. Malaria slides were then examined under a microscope for species-specific *Plasmodium* parasites by experienced laboratory technicians. Parasites were counted against 200 leukocytes (if <10 parasites were identified, counting was continued up to 500 leukocytes). Counts were converted to the number of parasites/µL of blood, assuming a leukocyte count of 8000/µL (WHO 1990).

(e) Analysis of oil samples

The CSRS analysed the vegetable oil samples with a portable iCheck™ CHROMA photometer using iEx™ ELAN disposable reaction vials and cuvettes (BioAnalyt GmbH, Teltov, Germany) to quantify the vitamin A concentration of the oil in mg RE/kg.

(f) Analysis of flour samples

The SVI analyzed the flour samples for iron and folic acid concentrations. For iron, the analyses were conducted using atomic absorption spectrometry after dry ashing using their reference method as described in the “Schweizerisches Lebensmittelbuch, Januar 2001”, issued by the Swiss Federal Office of Public Health. After the extraction of folic acid from the flour, the concentration of folic acid was determined using the same method as described for the plasma folate.

2.3 Ethical Considerations

Ethical authorization for the survey was obtained from the National Ethics Committee of Côte d’Ivoire (Comité National d’Ethique et de la Recherche, CNER). Ethical clearance was necessary because survey volunteers were to be asked a number of SES and health-related

questions and to provide an intravenous blood sample. The study protocol translated into French was presented on 6 October 2010 to the CNER and was approved by the MoH (5713/2010/MSHP/CNER).

Prior to inclusion in the survey, informed written consent was sought from the head of each household on behalf of the pre-SAC within that household, and of the participating WRA. If the household head was unable to read and write, the consent form was read out to them and a thumb/fingerprint was taken as consent to take part in the study in lieu of a signature. Written consent was required for participation, and therefore, a description of the survey's purpose was given, in addition to emphasizing that an intravenous blood sample would be taken; any potential risks were highlighted. The respondents were also told that they were free to withdraw from participation in the survey at any time, even after written consent had been given.

2.4 **Data management and statistical analysis**

Data were double entered and cross-checked using EpiInfo version 6.04 (CDC, Atlanta, USA). Exceptions were data files received from DBS-Tech laboratories in Germany and the Swiss Vitamin Institute. All data files were transferred and merged into one master file using STATA/IC version 10.1 (StataCorp LP; College Station, TX, USA).

Statistical analysis was conducted using SPSS (version 19, IBM, USA). Continuous data were checked for skewness using the Cox test (coefficient of skewness divided by the standard error of skewness) as well as by examination of the frequency distribution. The relationship between two categorical variables was analyzed by the chi-square test and between continuous variables by independent-sample t-test or one-way ANOVA. Curve estimation included testing for linear and quadratic effects of the continuous independent variables. Sequential multiple regression analyses were used to analyze dependent continuous variables with two or more independent variables and binary and multinomial logistic regression analyses were used to predict group membership based on categorical and continuous independent variables.

Inflammation correction: Ferritin concentrations are increased by inflammation, even in apparently-healthy people, and hence the prevalence iron deficiency is generally under-estimated. WHO recommends serum ferritin concentrations as the best indicator of iron deficiency (WHO/CDC 2007). Therefore, to interpret ferritin in the presence of inflammation, WHO suggest that one or more acute phase proteins should be measured to detect the presence of inflammation but they give no instruction on how to use acute phase proteins to interpret ferritin. Thurnham and colleagues (2010) described a method to estimate the increase in ferritin caused by inflammation in apparently-healthy people, such as the population used in this survey, using two acute phase proteins, CRP and AGP and calculated factors to correct ferritin for the influence of inflammation and we adopted this approach for the data correction.

Retinol concentrations are reduced by the presence of inflammation and hence prevalence of VAD can be over-estimated. A similar correction to that described above can also be done for retinol concentrations (Thurnham et al. 2003). In this study, RBP concentrations were measured and not retinol but there was an excellent correlation between the retinol and RBP concentrations in the CDC VITAL EQA program carried out at the time of this analysis. Therefore, the correction for inflammation proposed for serum retinol was applied to correct the RBP.

For the calculation of the daily vitamin A intake through oil, the following procedure was performed:

- Daily quantity of oil consumed at the household was calculated from the money spent on purchasing oil (using price/L) and the reported frequency of purchase; the amount of oil in liters was then converted to oil in kg (using the conversion factor of 0.8875 g/mL[1])
- The amount of vitamin A consumed per household was obtained using the measured concentration of vitamin A in mg RE/kg multiplied by the amount of oil (in kg) consumed on a daily basis
- The number of "consumption units" was then calculated using a simplified procedure, modified from Gibson (2005), see Table 1. For this, the number of household members in each age group was calculated, and the number of "consumption units" summed together

Table 1: Definition of oil "consumption units" based on household census

	Gibson		Modified for the present study		
Age	Male	Female	Age	Male	Female
>14 y	1	0.9	> 15 y	1	0.9
11-14 y	0.9	0.9	6 – 14 y	0.78	0.78
7-10 y	0.75	0.75	0 -5 y	0.3	0.3
4-6 y	0.4	0.4			
<4 y	0.25	0.25			

- Using the amount of vitamin A consumed in the household through the oil and the total number of consumption units, an average vitamin A consumption for each of the household members was calculated.

[1] At 25°C, see http://www.chempro.in/palmoilproperties.htm

3 Results

3.1 Operational sampling

A series of challenges were met during the operational sampling, and although they are unlikely to have a considerable impact on the findings in this study, they are listed below:

- The survey had to be completed prior to the mass vaccination campaign/vitamin A supplementation program, which was initially scheduled for November, but because of the national election, the vaccination campaign was moved forward to October 28, 2010. Because of the change in schedule, there was a reduced time frame for the survey data collection, and in the last clusters, the village leaders were not prepared ahead of the arrival of the survey teams. As the village leaders did not have time to sensitize the population, the result was additional work for the field teams, but fortunately this had no adverse consequences on the number of households included.
- In the last cluster for one of the teams, there were no hemocuvettes left, therefore seven subjects did not have a hemoglobin concentration measurement.
- One of the HemoCue devices did not work properly and had to be replaced; again, no consequences resulted from this.
- Due to the profound political instability following the elections in late 2010 and the subsequent security situation, shipment of plasma samples to Germany and analysis of the vitamin A concentration in the oil and malaria parasite counts were delayed by over three months.

Four field teams visited the selected households and Table 2 shows the number of oil and flour samples collected by each team. As many households did not have flour on the day of the survey, the teams collected flour samples from the local shop used by the household or from the bakery where the bread was made. The shop or bakery may have been serving many households in a cluster, so the flour sample collected is used in the data analysis to represent each household who purchased from them in that cluster. Collected and analyzed flour samples could be attributed to 82.4% of the households.

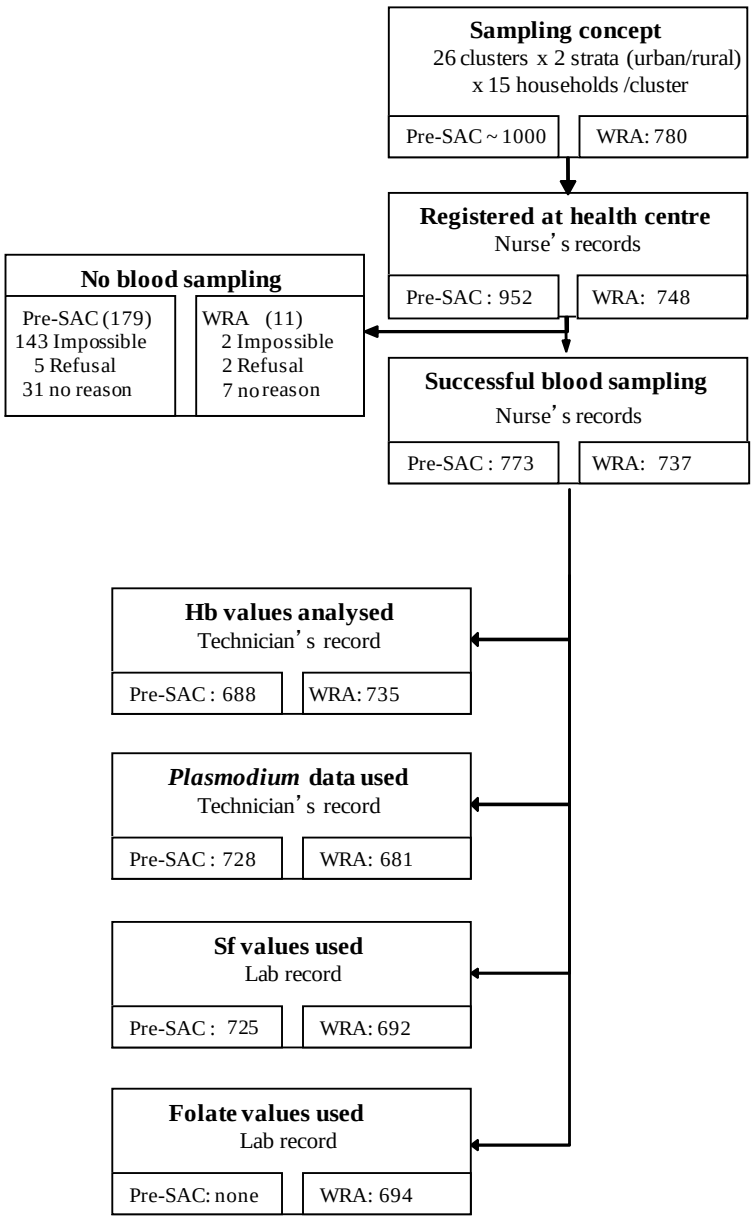
Table 2: Food samples collected by team

Teams	Clusters	Oil	Household Flour	Flour samples from shop assistants*	Flour samples from bakery*
Team 1	13	173	0	29	13
Team 2	13	168	0	22	10
Team 3	13	195	2	02	13
Team 4	13	194	0	02	17
TOTAL	52	730	2	55	53

*note that one of these samples could be assigned to various households in the same village, in case that no flour samples were available at the household.

The sample sizes of children and WRA varied depending on the variable being analyzed (Figure 1), but for most analyses there were about 938 children (sex ratio 0.9:1, male to female) and 714 WRA. The mean age of the children was 30.3 months (range 6-59 months) and there was no significant difference in mean age between boys and girls. The mean age of mothers was 30 years (range 15-49 years) and just over 52% over the sample lived in a rural area.

Figure 1: Flow Diagram of the numbers recruited and samples collected from both the urban (Abidjan) and rural areas (Buoaflé)



3.2 Socio-economic status

The socioeconomic variables are summarized in Table 3. There were very significant differences between urban and rural areas for all the socio-economic variables examined.

About a third of urban household heads were educated up to secondary or a higher level compared with less than 20% of rural household heads. Whereas nearly all rural household heads were farmers, most urban heads tended to work in private industry or as traders.

Almost three quarters of urban households lived in rented accommodation but nearly all rural households owned their property. Seventy percent of urban households lived in houses with less than three rooms while rural houses had more rooms, and almost 50% of households in Buoaflé had 3 or more rooms.

Nearly all urban houses were constructed with cement walls and corrugated iron roofs, whereas about a third of rural houses were made of mud/wood or mud/cement walls and 20% had paper/straw roofs. Cooking was primarily with wood, and to a lesser extent charcoal in rural areas and with gas and charcoal in urban areas. About 96% of urban households had tap water, while in rural areas the main water source was a central pump. Only about 14% of rural households had some form of latrine while all urban households had a latrine.

Almost all urban households had an electrical supply and 91% owned a television. About 52% of rural households reported owning an electrical appliance, a quarter had television and just over half had a radio. Mobile phone ownership was almost universal in urban areas compared with three-quarters of rural households. Land phones were not common in urban households and rare in rural households. Internet access in the home was virtually absent in both urban and rural households, although 50% of urban households had access in their locality. Only about 1 in 17 households owned a car in Abidjan, and motorcycle and bicycle ownership was also low. In rural areas bicycle ownership was high at just over 80%.

Table 3: Summary of socio-economic variables by locality

	Variable	Categories	Rural	Urban	p
Household head	Education	No schooling	40.6	36.2	<0.001
		Primary	42.0	30.9	
		Secondary	16.3	25.6	
		Higher	1.1	7.3	
	Occupation	Farmer	92.2	2.4	<0.001
		Trader	1.1	21.6	
		Government	0.8	7.4	
		Handicraft	0	2.0	
		Private industry	0.5	47.2	
		Retired	1.2	3.7	
		Other	4.2	15.6	
Housing	Status	Owner	95.2	24.4	<0.001
		Rent	1.2	73.1	
		Housed by someone	6.3	2.6	
	Wall	Mud/wood	20.3	0.9	<0.001
		Mud-cement	14.8	0.6	
		Cement	64.9	98.5	
	Roof	Paper/straw	20.2	0	<0.001
		Corrugated iron/tiles	79.8	100	
	Rooms	1	7.1	26.7	<0.001
		2	18.1	44.3	
		3	25.4	20.8	
		4	22.9	4.6	
		5	14.5	1.5	
		6+	12.0	2.1	
	Electricity	Yes	41.6	98.3	<0.001
	Cooking	Wood	90.6	3.3	<0.001
		Charcoal	7.6	25.9	
		Gas	1.3	69.5	
		Electricity	0.6	1.3	
	Water source	River	10.2	0.6	<0.001
		Central pump	65.5	2.9	
		Tubewell	12.2	0	
		Tap water	12.1	96.4	
	Toilet	Open	85.6	0	<0.001
		Pit latrine	1.5	0.8	
		Cement latrine	12.3	55.7	
		Flush toilet	0.6	43.6	
Appliances	Electrical	Yes	51.5	94.9	<0.001
	Fridge	Yes	3.8	24.6	<0.001
	Television	Yes	25.6	91.0	<0.001
	Radio	Yes	52.8	66.5	<0.001
	Fan	Yes	11.2	77.2	<0.001
	Air conditioner	Yes	0.7	2.8	<0.001
	Video recorder	Yes	11.0	55.6	<0.001
Household communication	Phone (land)	Yes	0.4	5.9	<0.001
	Mobile phone	Yes	74.6	97.1	<0.001
	Internet	Yes	0.6	1.0	<0.001
Village communication	Phone	Yes	1.6	49.7	<0.001
	Mobile phone	Yes	91.4	97.8	<0.001
	Internet	Yes	1.9	48.5	<0.001
Transport	Car	Yes	0.7	6.0	<0.001
	Motorcycle	Yes	15.9	3.6	<0.001
	Bicycle	Yes	81.8	2.7	<0.001

3.3 Fortification levels of fortified foods

3.3.1 Oil

The legally mandated level for the fortification of oil with vitamin A is 8 µg/g RE. Overall 32.2% of oil samples were fortified at the legal level or above, and there was no significant difference in the prevalence of adequately fortified oil between the rural area of Bouaflé (33.5%), and the urban area of Abidjan (30.8%). There is an accepted variation in the level of fortification of about 20% by the time the oil reaches the household, which means any oil containing ≥6.4 µg/g RE of vitamin A was considered to be adequately fortified, and 50% of the oil was in this category, with no rural/urban differences. Concentrations of vitamin A ≤3.2 µg/g RE in oil were considered to be fortified at unacceptable levels, and 35% of urban and 41% of rural samples fell into this category, respectively. Any oil that had vitamin A levels >3.2 µg/g RE but < 6.4 µg/g RE fell into a 'grey area' which was not considered to be fortified enough to have a biological effect and so was not included in the adequately fortified category for any calculations. There were significant urban-rural differences mainly in the low and middle categories (Table 4).

Table 4: Percentage of oils fortified according to defined cut-offs

Locality	% Oil samples with vitamin A			p
	≤3.2 µg/g RE	>3.2 to <6.4 µg/g RE	≥6.4 µg/g RE	
Urban	35.0	14.2	50.8	0.003
Rural	41.0	9.2	49.9	
Total	38.0	11.7	50.3	

3.3.2 Flour

Iron

The legal level for the fortification of flour with electrolytic iron is 60 ppm. Overall 69.1% of flour samples were fortified at the legal level or above, and there was a significant difference in the prevalence of fortified flour between the rural area of Bouaflé (47.3%) and the urban area of Abidjan (92.6%; p<0.001). We assumed that some fluctuation during the fortification process of flour is normal, which means any flour containing ≥48ppm of iron was considered to be adequately fortified, and 99.7% of urban flour samples were in this category, but only 50% of rural samples. A third of rural samples were inadequately fortified with concentrations of iron ≤24ppm. There were significant urban-rural differences using the defined cutoffs, nearly all the urban households were in upper category (Table 5). Any flour that had iron levels >24 ppm but < 48 ppm fell into a 'grey area' which was not considered to be fortified enough to have a biological effect and so was not included in the adequately fortified category for any calculations.

Table 5: Percentage of flour fortified according to defined cut-offs

Locality	% Flour samples with iron			p
	≤24ppm	>24 to <48ppm	≥48ppm	
Urban	0.3	-	99.7	<0.001
Rural	33.3	16.6	50.1	
Total	17.3	8.6	74.0	

Folic acid

The legally mandated fortification concentration for folic acid in flour is 1.5 mg/kg. Overall 0.9% of flour samples were fortified at the legal level or above and there was a small but significant difference in the level of flour fortification between the rural area of Bouaflé (1.8%) and the urban area of Abidjan (0%; p = 0.001). But as with the other two fortificants, a 20% variation in fortification was deemed acceptable (≥1.2 mg/kg) by the time the flour reached the household, and overall 6% of flour samples had this concentration of folic acid with significant differences between rural and urban samples. Flour samples with ≤0.6 mg/kg folic acid were inadequately fortified. Concentrations in the range >0.6 to <1.2 mg/kg suggested some fortification but were considered inadequate to have a biological effect. Using the cut-offs, there was marked heterogeneity between urban and rural samples with the poorest fortification concentrations in the rural areas (Table 6).

Table 6: Percent of flour samples with folic acid according to defined cut-offs

Locality	% Flour samples with folic acid			p
	≤0.6 mg/kg	0.6 to <1.20 mg/kg	≥1.20 mg/kg	
Urban	39.1	58.7	2.2	<0.001
Rural	63.5	27.3	9.2	
Total	52.1	42.0	5.9	

3.4 Micronutrient status of Pre-SAC and WRA

3.4.1 Vitamin A status and inflammation of Pre-SAC and WRA

Pre-SAC

In pre-SAC children the overall mean RBP concentration was 0.94µmol/L. The mean RBP concentration in rural areas was 0.12 µmol/L lower than that in urban areas (<0.001) and there was no significant sex difference (Table 7). RBP concentrations in rural males were significantly lower than in rural females, the equivalent difference was not significant in urban areas (Table 7). There was no relationship between RBP concentrations and age of the child.

There was significantly less VAD (defined as RBP< 0.7 µmol/L) in urban than rural areas (p < 0.001), and only in urban areas did more boys have VAD than girls (male/urban: 13.2% versus female/urban: 6.5%, p<0.04). Overall, there was no significant difference in VAD (Table 7).

Table 7: Mean corrected RBP concentrations and prevalence of vitamin A deficiency in preschool children by sex and residency

	Mean µmol/L	P (sex)	VAD (%)	P (sex)
All Male	0.92		19.3	
All Female	0.96	ns	16.5	ns
Rural male	0.85		24.4	
Rural female	0.92	0.022	24.3	ns
Total rural	0.89		24.3	
Urban male	1.00		13.2	
Urban female	1.02	ns	6.5	0.04
Total urban	1.02		9.6	
All pre-SAC	0.94		17.5	

The acute phase proteins CRP and AGP were used to classify inflammation into 4 categories and correction factors were calculated as part of the meta-analysis by Thurnham et al (Thurnham et al. 2003) to correct retinol/RBP concentrations for the presence of inflammation:

1. Normal or no inflammation, no elevated acute phase proteins – no correction.
2. Incubation period defined as a CRP concentration of >5mg/L – correction factor 0.13.
3. Early convalescence defined as a CRP concentration >5 mg/L and an AGP concentration > 1 g/L – correction factor 0.24.
4. Late convalescence defined by an AGP concentration >1g/L- correction factor 0.11.

Using these definitions, there were marked urban-rural differences with significantly higher levels of inflammation (elevated AGP and CRP) in rural areas (Table 8).

Table 8: Prevalence of normal and elevated acute phase proteins [APP] (C-reactive protein [CRP] and α-1 acid glycoprotein [AGP]) by rural/urban and the effect of correction for inflammation on overall prevalence of vitamin A deficiency (VAD) among rural and urban pre-school children

Variable	Urban (%)	Rural (%)	Total (%)	p
Elevated AGP (>1 g/L)	27.8	68.7	49.8	<0.001
Elevated CRP (>5 mg/L)	18.5	53.3	37.2	<0.001
Normal CRP and AGP	68.7	26.9	46.2	<0.001
Elevated CRP	3.6	4.4	4.0	
Elevated CRP and AGP	14.9	49.0	33.2	
Elevated AGP	12.8	19.7	16.6	
*VAD	9.6	24.3	17.8	<0.001

*VAD defined as RBP <0.7 µmol/L, corrected using meta-analysis of Thurnham et al. (2003)

The overall mean RBP concentration for the Pre-SACs after correction for inflammation was 0.94 µmol/L, with boys having a lower mean value (0.92 µmol/L) than girls (0.96 µmol/L). When the corrected RBP concentrations were used to determine the prevalence of VAD based on a RBP cutoff <0.7 µmol/L, urban males and females still had significantly less VAD than those living in rural areas (males: 13.2% versus 24.9%, respectively, p<0.001; females: 6.5% versus 24.8%, respectively, p<0.001) but there was no significant sex difference in the prevalence of corrected VAD (19.3% and 16.5%, males and females respectively). For both the overall corrected and non-corrected concentrations of RBP, >15% of the children in the population surveyed were VAD which according to the WHO suggests a public health problem amongst that age group (WHO 1996), assuming that retinol and RBP cut-offs are equivalent. However, separating VAD by locality, the majority of the public health problem was in the rural areas.

WRA

Among WRA there was no significant difference in mean RBP or differences in VAD by locality nor was there any significant age effect. The mean corrected RBP concentration was 1.64 µmol/L (n= 692), and there was no significant VAD in the population (0.7%) (Table 9).

Using the definitions for inflammation as described above, 83% of WRA had normal concentrations of the acute phase proteins and there were no marked urban-rural differences.

Table 9: Effect of the acute phase proteins (C-reactive protein; CRP and α-1 acid glycoprotein; AGP) on vitamin A deficiency (VAD) among women of reproductive age (WRA).

Variable	Urban (%)	Rural (%)	Total (%)	p
Raised AGP (>1 g/l)	8.7	12.1	10.3	ns
Raised CRP (>5mg/L)	13.0	13.4	13.2	ns
Normal CRP and AGP	83.9	82.5	83.2	
Elevated CRP	7.4	5.4	6.5	
Elevated CRP and AGP	5.6	8.0	6.6	ns
Elevated AGP	3.2	4.1	3.6	
*VAD	0.3	1.3	0.7	ns

*VAD defined as RBP <0.7 µmol/L, corrected using meta-analysis of Thurnham et al. (2003)

3.4.2 Plasma folate status in women of reproductive age

There were no significant urban-rural mean differences using either arithmetic or geometric means nor were there any age effects. All WRA had low folate levels with concentrations <10nmol/L (Table 10).

Table 10: Mean folate concentrations (nmol/L) for women of reproductive age

Arithmetic Mean (nmol/L)	Geometric mean (nmol/L)	Low folate (% <10nmol/L)
0.28	0.09	100.0

Using mean or logged folate values gave similar results. The rural sample had marginally higher mean folate concentrations, 0.29 nmol/L versus 0.28 nmol/L, than the urban areas (p=0.07), but the geometric mean (correcting for skewness of data) of logged folate values were significantly higher in the rural, 0.097 nmol/L versus 0.091 nmol/L (p=0.019) in the urban group.

3.4.3 Iron status of Pre-SAC and WRA

Pre-SAC

The mean hemoglobin concentration was significantly higher in urban than rural children, while plasma ferritin concentrations (unlogged and logged data) were significantly higher in rural than urban children (Table 11). Prevalence of depleted iron stores (defined as ferritin concentrations < 12 µg/L) was significantly higher in urban than rural children. The mean sTfR concentration in children in the rural area was greater than the cut-off (>8.3mg/L) and there were significant differences between those with iron deficiency by locality (Table 11 and Table 12).

Markers of inflammation:

In a similar way to that described for RBP, the acute phase proteins CRP and AGP were used to classify the effect of inflammation on ferritin concentrations using correction factors calculated as part of the meta-analysis by Thurnham et al (Thurnham et al. 2010):

Normal or no inflammation, no elevated acute phase proteins – no correction
Incubation period defined as a CRP concentration of >5mg/L – correction factor 0.77
Early convalescence defined as a CRP concentration >5 mg/L and an AGP concentration > 1 g/L – correction factor 0.53
Late convalescence defined by an AGP concentration >1g/L- correction factor 0.75

Using these definitions, corrected ferritin (logged and unlogged) was significantly higher in rural than urban children.

Table 11: Means of hemoglobin (Hb), ferritin and soluble transferrin receptors (sTfR) by locality of preschool children

Variable	Urban		Rural		p	Total population	
	*Arith mean	^Geo mean	Arith mean	Geo mean		Arith mean	Geo mean
Hb g/L	104.6	-	85.0	-	<0.001	94.3	
Ferritin corrected µg/L**	38.3	29.3	59.5	45.3	<0.001	49.7	34.7
sTfR mg/L	5.1	4.6	10.6	9.0	<0.001	8.09	6.29

*Arith mean = arithmetic mean¸

^geo mean = geometric mean

**Ferritin corrected (Thurnham et al. 2010).

The majority of rural children were anemic (92.2%; hemoglobin < 110 g/L) compared with just over half of the urban children (56.3%). Nearly 1 in 5 rural children had severe anemia compared with only 1 in 38 urban children (Table 12). The percentage of those with depleted iron stores and corrected depleted iron stores was small but was significantly higher in urban than rural children. Prevalence of iron deficiency was small and there were no significant differences (elevated sTfR values) by locality.

Table 12: Prevelance of pre-school children who were anemic, had depleted iron stores and were iron deficient by locality

Locality	Anemia				Depleted iron stores		Iron deficient	
	Hb <70	Hb ≥70 <110	Hb >110		Ferritin corrected <12 µg/L		sTfR >8.3 µg/mL	
	%	%	%	p		p	%	p
Urban	2.6	53.7	43.7	<0.001	22.1	<0.001	10.7	ns
Rural	17.8	74.4	7.8		6.9		8.7	
Total	10.6	64.6	24.8		13.9		9.7	

Hemoglobin concentration increased significantly with age (0.278 g/L/month), as did ferritin concentrations (0.374 µg/L/month), while sTfR concentrations fell on average by 0.061 mg/L/ month. Stepwise binary logistic regression analyses revealed that after removing the effects of the age and sex of the child, locality remained significant for hemoglobin and ferritin concentrations but not for sTfR concentrations. The combination of age and locality was able to correctly predict 92.3% of anemic children and 37% of normal children (overall 78.6% correctly predicted).

WRA

Mean hemoglobin concentrations were significantly higher in urban than rural WRA, while the opposite was found for concentrations of sTfR. There were no significant differences in corrected mean ferritin (unlogged or logged) by locality (Table 13).

Table 13: Means of hemoglobin, ferritin and soluble transferrin receptors (sTfR) by locality of women of reproductive age

Variable	Urban	Rural	p	Total population

	Arith* Mean	**Geo mean	Arith Mean	Geo mean		Arith Mean	Geo mean
Hemoglobin g/L	119.6		105.3		<0.001	113.2	
Ferritin corrected µg/L	53.2	38.5	51.0	37.4	ns	52.1	38.0
sTfR mg/L	4.8	4.5	7.0	6.5	<0.001	5.8	5.3

*Arithmetic mean

** Geometric mean

Just over three-quarters of rural WRA were anemic compared with less than half of the urban sample (Table 14). There were no significant differences in the prevalence of depleted iron stores (unlogged or logged) or corrected depleted iron stores (unlogged or logged) by locality.

Table 14: Prevalence of women of reproductive age who were anemic, had depleted iron stores and were iron deficient by locality

Locality	Anemia				Depleted iron stores		Iron deficient	
	<70	≥70 <120	>120		Ferritin corrected <12µg/L		sTfR	
	%	%	%	p	%	p	%	p
Urban	0.5	44.1	55.4	<0.001	10.1	ns	6.3	ns
Rural	4.2	73.5	22.3		9.6		9.6	
Total	2.2	57.3	40.6		9.8		7.8	

Hemoglobin concentrations fell by, on average, 0.32 g/L/year of age among WRA, while ferritin concentrations increased, on average, by 6.4 µg/L/year of age. Stepwise binary logistic regression analyses revealed that, after removing the effects of age, locality remained significant for hemoglobin concentration. The combination of age and locality was able to correctly predict 75.1% of anemic WRA and 58.6% of normal WRA (overall 65.3% correctly predicted).

3.5 *Plasmodium* parasites in study participants

Malaria transmission occurs for most of the year in Côte d'Ivoire; the main parasite is *Plasmodium falciparum,* but *P. malariae* and *P. ovale* are also found. In pre-SAC, the prevalence of the three species were significantly higher in rural than urban areas, but there were no differences in the intensity of infection (arithmetic or geometric means) by locality Table 15). There were 14 pre-SAC with double infections: six with *P. ovale* also had *P. falciparum*, while eight of the 24 children with *P. malariae* also had *P. falciparum* There was a highly significant linear decline in intensity of infection with age in children: an increase in age by 1 month was associated with a fall, on average, of 107 parasites/µL blood. There was no significant difference in prevalence or intensity between boys and girls before or after correcting for age.

Table 15: Intensity of Plasmodium infection in preschool children

Locality	*P. falciparum*	*P. malariae*	*P. ovale*	*Plasmodium spp.*

	%	Arith Mean*	Geo Mean†	%	Arith Mean*	Geo Mean	%	Arith Mean*	Geo mean	%
Urban	7.3	2391	657	0.2	176	177	0	-	-	7.5
Rural	43.0	4486	1134	4.4	3213	604	1.1	4253	2347	45.8
p value	<0.001	ns	ns	<0.001	ns	ns	0.03	-	-	
Total	27.4	4242	1064	2.6	3134	574	0.6	4253	2347	29.1

*Arithmetic mean (parasites/µL blood),
†Geometric mean (parasites/µL blood)

Among WRA, the prevalence of *P. falciparum* was significantly higher in the rural areas, but there were very few WRA with *P. malariae (*n=3) and none with *P. ovale* (Table 16). There were no double infections in WRA. There was no association between intensity and age in WRA.

Table 16: Intensity of malarial infection in women of reproductive age

Locality	*P. falciparum*			*P. malariae***			*Plasmodium* spp
	%	Arith Mean*	Geo mean†	%	Arith Mean	Geo mean	%
Urban	6.8	10137	419	0.5	80	81	7.3
Rural	12.7	799	303	0.3	96	97	13.0
p value	0.008	ns	ns	ns	ns	ns	
Total	9.5	4369	343	0.4	85	86	9.9

*Arithmetic mean (parasites/µL blood),
†geometric mean (parasites/µL blood),
**no *P. ovale* found

3.5.1 Associations between biological biomarkers and *Plasmodium* spp. parasites

Pre-SAC – iron biomarkers

As stated earlier, 45.8% of rural and 7.5% of urban children had *Plasmodium* spp., infection, and there were strong associations between *Plasmodium* parasitemia and the biomarkers. On average, those with *Plasmodium* parasites had mean hemoglobin concentrations 16.9 g/L lower than those without infection and this difference increased to 18.0 g/L after correcting for age and sex of the child. To test whether the effect of *Plasmodium* spp., on hemoglobin levels was similar in both urban and rural children locality was also included in the analysis, which showed no difference by residency, and indicated that children with *Plasmodium* parasites had a lower mean hemoglobin concentration of 9.5 g/L (Table 24). In addition, *Plasmodium* positive children had a weak but just significant negative linear association between hemoglobin and intensity of parasitic infection with *Plasmodium spp.* (b = -0.000188, p = 0.03).

Children with *Plasmodium* parasites had higher mean ferritin levels (uncorrected and corrected) and these differences remained after correcting for age, sex and locality of the child (Table 17). In *Plasmodium* positive pre-SACs, mean sTfR was also significantly higher and the difference remained significant after correcting for age, sex and locality of the child. *Plasmodium* intensity was significantly positively associated only with uncorrected ferritin concentrations (b=0.0009, p = 0.014). No association was found between sTfR and intensity of infection.

Table 17: Effect of *Plasmodium spp*. parasites in Pre-SAC on the iron biomarkers

Variable	*Plasmodium spp.*	p	Difference between malaria – ve and +ve	p

	-ve†	+ve		Before correction	After correction for age, sex and locality	
Hemoglobin g/L	100.2	83.3	<0.001	+16.9	+9.5	<0.001
Corrected Ferritin µg/L	42.5	62.9	<0.001	-20.4	-12.0	<0.001
Log Corrected Ferritin*	28.4	50.1	<0.001	-21.7	-**	<0.001
sTfR mg/L	6.4	11.2	<0.001	-4.8	-2.6	<0.001
Log sTfR	5.4	9.5	<0.001	-4.1	-**	<0.001

† -ve: no *Plasmodium* parasites; +ve: positive *Plasmodium* parasites counts; *geometric mean ; ** not possible to compute because of log scale

Nearly all children with *Plasmodium* parasites were anemic (95.5%) compared with less than two thirds (64.2%) of those without infection (Table 18). Severe anemia was three times greater in children with *Plasmodium* parasites. A binary logistic regression analysis correctly predicted 91.7% of anemic children and 44.2% of normal children (overall 79.9% correctly predicted) on the basis of the child's age, sex, locality and *Plasmodium* prevalence. A child with *Plasmodium* parasites was 6.0 times (95% CI odds ratio 3.1 – 69.6, p<0.001) more likely to have anemia than a child free of *Plasmodium* infection, after taking into account, age and sex of the child and locality.

Table 18: Effect of presence or absence of *Plasmodium* infection on anemia, depleted iron stores and iron deficiency in pre-school children

***Plasmodium* spp. present/ absent**	**Anemia (g/L)**				**Depleted iron stores**		**Iron deficient**	
	<70	**≥70<110**	**>110**		**Ferritin corrected <12µg/L**		**sTfR**	
	%	**%**	**%**	**P**	**%**	**p**	**%**	**p**
-ve†	6.1	58.1	35.8	<0.001	19.4	<0.001	11.8	0.01
+ve	18.8	76.5	4.6		3.9		5.8	
Total	10.6	64.6	24.8		13.9		9.7	

† -ve: no *Plasmodiu*m infection; +ve: positive *Plasmodium* counts

Children without *Plasmodium* parasites were much more likely to have depleted iron stores (using corrected ferritin concentrations) and to be iron deficient. A binary logistic regression revealed that uninfected children were 8.9 (95% CI 2.6 – 30.3, p<0.001) and 3.9 (95% CI 1.8 – 8.2, p<0.001) times more likely to have depleted iron stores (for uncorrected and corrected, respectively) than infected children. Uninfected children were 2.4 times more likely (95% CI 1.2 – 4.7, p=0.014) to be iron deficient than infected children.

Mean plasma ferritin concentrations were all significantly higher in children with a *Plasmodium* infection in both rural and urban areas (Table 19).

Table 19: Effect of presence or absence of *Plasmodium spp*. in pre-school children on ferritin concentrations by locality

Variable	Urban			Rural		
	-ve*	+ve*	p	-ve*	+ve*	p
Corrected Ferritin (µg/L)	35.5	70.8	<0.001	57.0	61.7	ns
Log Corrected Ferritin (µg/L)	24.0	48.4	<0.001	39.1	49.8	0.013

* -ve: no *Plasmodium* infection; +ve: positive *Plasmodium* counts

Pre-SAC – vitamin A biomarkers

Concentrations of RBP in those Pre-SAC with *Plasmodium* parasites (0.85 µmol/L) were significantly lower than those without parasites (0.99 µmol/L; p<0.001). After correcting for age, sex and locality, there was still a significant difference between those with *Plasmodium* parasites and those without (difference +0.09 µmol/L; p< 0.001).

WRA – iron biomarkers

After correcting for malarial status, the analyses revealed that WRA with *Plasmodium* parasites had a significantly lower mean hemoglobin concentration, and the difference remained significant after correcting for the age and locality of the WRA (Table 20). Mean uncorrected ferritin levels were significantly higher in WRA with *Plasmodium* parasites before and after correcting for age and locality. No other differences were significant.

Table 20: Effect of the presence or absence of malarial parasites on the iron biomarkers in women of reproductive age

Variable	*Plasmodium* infection		P	Difference between *Plasmodium* –ve and +ve		p
	-ve†	+ve		Before correction	After correction for age and locality	
Hb (g/L)	113.9	107.1	0.003	6.8	+4.7	0.027
Corrected Ferritin (µg/L)	51.6	57.3	ns	-5.7	-7.2	ns
Log Corrected Ferritin (µg/L)*	37.5	42.3	ns	-4.8	-**	ns
sTfR (mg/L)	5.8	5.8	ns	-	0.31	ns
Log sTfR (mg/L)	5.3	5.4	ns	-0.1	-**	ns

† -ve: no malaria; +ve: positive malaria counts; *geometric mean; ** not possible to compute because of log scale

Approximately 10% of WRA were infected with *Plasmodium* parasites, and anemia prevalence was found to vary significantly between WRA with and without *Plasmodium* infection. Over three-quarters of WRA with *Plasmodium* parasites were anemic compared with just over half without infection. After correcting for age and locality, WRA with *Plasmodium* parasites were twice as likely to have anemia (95% CI 1.1 – 3.6, p=0.003) than those free of infection (Table 21).

Table 21: Effect of presence or absence of *Plasmodium spp*. on iron biomarkers in women of reproductive age

***Plasmodium* infection**	**Anemia (Hb g/L)**				**Depleted iron stores corrected**		**Iron deficient**	
	<70	**≥70 <120**	**>120**		**Ferritin <12µg/L**		**sTfR**	
	%	**%**	**%**	**p**	**%**	**p**	**%**	**p**
-ve[†]	2.1	55.5	42.4	0.014	10.1	ns	8.0	ns
+ve	2.9	72.9	24.2		7.5		6.0	
Total	2.2	57.5	40.3		9.8		7.8	

[†] -ve: no malaria; +ve: positive malaria counts

Corrected mean ferritin levels were significantly higher in WRA with *Plasmodium* parasites in urban areas but there was no significant difference in means in rural areas (Table 22).

Table 22: Effect of presence or absence of *Plasmodium* infection on ferritin concentrations by locality in women of reproductive age

Variable	**Urban**			**Rural**		
	-ve*	**+ve***	**p**	**-ve***	**+ve***	**p**
Corrected Ferritin (µg/L)	51.5	69.7	0.032	51.1	49.0	ns
Log Corrected Ferritin (µg/L)	37.4	49.0	ns	37.3	38.3	ns

*-ve: no *Plasmodium* infection; +ve: positive *Plasmodium* counts

WRA – vitamin A biomarkers

Mean concentration of RBP in those WRA with *Plasmodium* parasites (1.62 µmol/L) was not significantly different to those without parasites (1.61 µmol/L; p<0.001).

3.5.2 Plasmodium spp., inflammation, ferritin and sTfR

Pre-SAC

There was a highly significant relationship between mean ferritin and AGP/presence of *Plasmodium* parasites and CRP/presence of *Plasmodium* parasites. Children without inflammation, as measured by no elevated AGP and no *Plasmodium* parasites had the lowest mean ferritin concentration of all four groups (post-hoc test, p<0.001), while those with *Plasmodium* parasites with or without elevated AGP had the highest means (post-hoc tests, p<0.001;Table 23). For CRP, those with normal CRP concentrations and negative for *Plasmodium* infection had a significantly lower mean ferritin and sTfR concentrations, while the group with elevated CRP and *Plasmodium* parasites had a significantly higher mean than those with normal CRP and no *Plasmodium* infection (Table 23). Children with elevated AGP concentrations and *Plasmodium* parasites had significantly higher sTfR concentrations than the concentrations in each of the other groups (post-hoc tests). Conversely in those with normal AGP concentrations and no *Plasmodium* infection, sTfR values were significantly lower than all other groups. Likewise, those with elevated CRP concentrations and *Plasmodium* infection had significantly higher sTfR concentrations than the concentrations in each of the other groups (post hoc tests; Table 23). A regression analysis showed that for each 1mg/L increase in CRP, sTfR increased by +0.137 mg/L (p<0.001) and for AGP each 1g/L was associated with an increase in sTfR concentrations of 6.76 mg/L (p<0.001).

Table 23: Effect of acute phase proteins and presence/absence of *Plasmodium* parasites on ferritin concentrations among pre-school children.

	Plasmodium	Ferritin µg/L	p	sTfR mg/L	p
AGP					
Normal	No	38.5	<0.001	5.53	<0.001
Raised	No	48.6		7.50	
Normal	Yes	65.3		9.09	
Raised	Yes	61.9		11.95	
CRP					
Normal	No	39.0	<0.001	5.90	<0.001
Raised	No	52.7		7.34	
Normal	Yes	58.7		9.91	
Raised	Yes	65.3		12.04	

WRA

For WRA, those with raised AGP had significantly higher mean ferritin concentrations (post hoc tests both p<0.001;Table 24) with or without infection. For CRP there was no significant difference among the four means. There was no significant difference between sTfR concentrations in those with or without elevated CRP or AGP concentrations. However, a regression analysis found there was a significant association between sTfR and AGP (b=+1.12, p=0.027).

Table 24: Effect of acute phase proteins and presence/absence of *Plasmodium* parasites on ferritin concentrations among women of reproductive age.

	Plasmodium	Ferritin µg/L	p
AGP			
Normal	No	50.5	ns
Raised	No	60.0	
Normal	Yes	55.1	
Raised	Yes	65.7	
CRP			
Normal	No	51.4	ns
Raised	No	51.3	
Normal	Yes	53.9	
Raised	Yes	65.9	

3.6 Relationship between plasma RBP and vitamin A in the oil

Pre-SAC

There was no significant relationship found between mean RBP concentrations of pre-SAC and vitamin A in the oil, either using all three categories of oil fortification, i.e. ≥6.4 µg/g, >3.2 to <6.4 µg/g, or ≤3.2 µg/g RE or just the two extreme categories (≥6.4 µg/g and ≤3.2 µg/g RE).

In contrast, there was a significant positive relationship between RBP concentrations and daily vitamin A consumption from the oil (p=0.029), so that for each 1 mg RE/kg increase in vitamin A consumption, RBP increased, on average by 0.37 µmol/L.

WRA

As there was little VAD in the WRA there were no associations found with prevalence or mean RBP concentrations.

There was also no significant relationship found between mean RBP concentrations of WRA and vitamin A in the oil, either using all three categories of oil fortification, i.e. ≥6.4 µg/g, >3.2 to <6.4 µg/g, or ≤3.2 µg/g RE or just the two extreme categories (≥6.4 µg/g and ≤3.2 µg/g RE). The relationship between plasma VAD and concentrations of vitamin A in the diet was not analyzed as the percentage of WRA with deficiency was so small. In the WRA, no significant association was found between vitamin A in the oil in the group as a whole or in urban and rural localities separately.

3.7 **Impact of iron fortification on biological status**

Pre-SAC

In order to test whether iron fortification of flour had any impact on hemoglobin concentrations, a sequential multiple regression analysis was undertaken, which removed the effects of age, and sex and tested whether the three categories of iron fortification had an effect on hemoglobin concentration. Those Pre-SAC consuming flour with <24 ppm iron had hemoglobin concentrations 14.3g/L lower, those consuming flour with ≥24 – 47.9 ppm iron, had hemoglobin concentrations 15.7g/L lower than those in the group consuming flour with >48 ppm iron ($p<0.001$ for both categories). Repeating the analyses but correcting for the presence of *Plasmodium* parasites in the model, those Pre-SAC consuming flour with <24 ppm iron had hemoglobin concentrations 11g/L lower, and those consuming flour with 24 – 47.9 ppm iron, had hemoglobin concentrations 10.7g/L lower than those in the group consuming flour with ≥48 ppm iron ($p<0.001$ for both categories; Table 25).

The analysis was then repeated using only children who were negative for *Plasmodium* parasites, the effects of age and sex were removed and the regression model tested whether the three categories of iron fortification had an effect on hemoglobin concentration. Those Pre-SAC consuming flour with <24 ppm iron had hemoglobin concentrations 15.1 g/L lower, and those consuming flour with 24 – 47.99 ppm iron had hemoglobin concentrations 16.2 g/L lower than those consuming flour with ≥48 ppm iron ($p<0.001$ for both categories). These results show that for both *Plasmodium*-free and all Pre-SAC that there is an association between the categories of iron fortification and hemoglobin concentration.

Table 25: Differences in hemoglobin concentrations in pre-SAC according to the fortification level of iron in wheat flour

Category of iron fortification of flour	***Difference in Hemoglobin (g/L)***		**Difference in hemoglobin (g/L) without malaria**
	After correction for age and sex	After correction for age, sex and Plasmodium	After correction for age and sex
<24 ppm	-14.3	-11.7	-15.1
≥24 – 47.99 ppm	-15.7	-10.7	-16.2
≥48 ppm	-*	-*	-*
p	0.001	0.001	

*reference mean concentration

The analyses were repeated using iron fortification levels as a continuous variable. With all children in the model and after removing the effects of age and sex, hemoglobin increased by +0.238 g/L for each unit (ppm) increase in iron ($p<0.001$). Additionally, removing the presence of Plasmodium spp., from the model, hemoglobin increased by +0.171g/L for each unit (ppm) increase in iron ($p<0.001$). Repeating the analysis using only Plasmodium-free children in the model, the regression coefficient was +0.254 g/L increase in hemoglobin ($p<0.001$). These

analyses show that there was a linear improvement in hemoglobin as iron fortification levels increased.

In order to test whether iron fortification of flour had any impact on ferritin and sTfR concentrations, a sequential multiple regression analysis was undertaken, which removed the effects of age, and sex and tested whether the three categories of iron fortification had an effect on ferritin and sTfR concentrations. Those Pre-SAC consuming flour with <24 ppm iron had ferritin and sTfR concentrations 15.7 µg/L and 4.3 mg/L higher, and those consuming flour with ≥24 – 47.9 ppm iron had ferritin and sTfR concentrations 11.5 µg/L and 3.5 mg/L higher respectively than those in the group consuming flour with >48 ppm iron (p<0.001 for all categories; Table 26). Repeating the analyses but correcting for the presence of *Plasmodium* parasites in the model, non-significant differences were obtained (Table 26).

The analysis was then repeated using only children who were negative for *Plasmodium* parasites, the effects of age and sex were removed and the regression model tested whether the three categories of iron fortification had an effect on ferritin and sTfR concentrations. Those Pre-SAC consuming flour with <24 ppm iron had ferritin and sTfR concentrations 18.1 µg/L (n was very small, 14) and 3.2 mg/L higher, those consuming flour with ≥24 – 47.99 ppm iron had ferritin and sTfR concentrations 1.1 g/L (p = 0.02) and 4.9 mg/L (p <0.001) higher respectively than those consuming flour with ≥48 ppm iron.

Table 26: Differences in ferritin and sTfR concentrations in Pre-SAC according to the fortification level of iron in wheat flour.

Category of iron fortification of flour	***Difference in Ferritin µg/L***		**Difference in ferritin without *Plasmodium* spp.**
	After correction for age and sex	After correction for age, sex and Plasmodium	After correction for age and sex
<24 ppm	15.7	9.0	18.1
≥24 – 47.99 ppm	11.5	-0.1	1.1
≥48 ppm	-*	-*	-*
p	0.001	ns	0.02
Category of iron fortification of flour	***Difference in sTfR***		**Difference in sTfR without *Plasmodium* spp.**
	After correction for age and sex	After correction for age, sex and *Plasmodium spp.*	After correction for age and sex
<24 ppm	4.3	3.0	3.2
≥24 – 47.99 ppm	3.5	1.4	4.9
≥48 ppm	-*	-*	-*
p	<0.001	<0.001	<0.001

Using iron fortification levels as a continuous variable, further regression models were undertaken. With all children in the model and after removing the effects of age and sex, ferritin decreased by -0.216 µg/L for each unit (ppm) increase in iron in the flour (p = 0.002). No further relationships were found with ferritin. Repeating the analyses for sTFR, after removal of the effects of age and sex, sTfR decreased by -0.075 mg/L for each unit increase in iron (p<0.001). Removing the effects of age, sex and presence of *Plasmodium* parasites revealed that sTfR decreased by -0.051mg/L for each ppm of iron in the flour (p<0.001). Finally using only children

who were Plasmodium negative in the model, sTfR decreased by -0.058 mg/L/ppm iron in the flour, after removing the effects of age and sex (p<0.001).

WRA

The same analyses as described for the pre-SAC were undertaken (a) using all three categories of level of fortification, i.e. ≤24 ppm, ≥24 – <48 and ≥48 ppm of iron (b) the extreme categories only i.e. ≤24 ppm and ≥48 ppm and (c) iron fortification levels as a continuous variable. For all three analyses no significant effect of iron fortification was apparent on hemoglobin concentration.

Stepwise binary logistic regression analyses were then used to test whether iron fortification levels could predict anemic and normal (non-anemia) status and multinomial logistic regression tested whether iron fortification could predict severe anemia, anemia and normal status. In both sets of analyses the effects of locality and malaria were removed before testing for iron fortification based on (a) three categories: <24 ppm, ≥24 – <48 and ≥48 ppm, (b) the extreme categories only i.e. ≤24 ppm and ≥48 ppm and (c) iron fortification levels as a continuous variable. In all the analyses no significant effect of iron fortification was found.

Likewise using the same analyses as described above, there was no apparent significant effect of iron fortification on ferritin concentrations. However, unlike the other two iron biomarkers there was a significant effect of iron fortification on sTfR concentrations. Those WRA consuming flour with <24 ppm iron had sTfR concentrations 1.1 mg/L higher, those consuming flour with ≥24 – 47.9 ppm iron had sTfR concentrations 1.7mg/L higher than those in the group consuming flour with >48 ppm iron (p<0.001;

Table 27). Repeating the analyses but correcting for the presence of *Plasmodium* parasites in the model, those WRA consuming flour with <24 ppm iron had sTfR concentrations 1 mg/L higher (p<0.001), and those consuming flour with 24 – 47.9 ppm iron had sTfR concentrations 1.8 mg/L higher (p < 0.001) higher than those in the group consuming flour with ≥48 ppm iron (

Table 27).

The analysis was then repeated using only women who were negative for *Plasmodium* parasites, and the regression model tested whether the three categories of iron fortification had an effect on sTfR concentrations. Those WRA consuming flour with <24 ppm iron had sTfR concentrations 1.1 mg/L higher, those consuming flour with 24 – 47.99 ppm iron had sTfR concentrations 1.8 mg/L (p <0.001) higher than those consuming flour with ≥48 ppm iron. These results show that for both WRA *Plasmodium*-free and all women that there is an association between the categories of iron fortification and sTfR concentrations.

Table 27: Differences in ferritin and sTfR concentrations in WRA according to the fortification level of iron in wheat flour

Category of iron fortification of flour	*Difference in Ferritin*		Difference in ferritin without malaria
		After correction Plasmodium	
<24 ppm	-3.8	-5.8	-3.1
≥24 – 47.99 ppm	4.5	4.1	6.0
≥48 ppm	-*	-*	-*
p	ns	ns	ns

Category of iron fortification of flour	*Difference in TfR*		Difference in sTfR without malaria
		After correction Plasmodium	
<24 ppm	1.1	1.0	1.1
≥24 – 47.99 ppm	1.7	1.8	1.8
≥48 ppm	-*	-*	-*
p	<0.001	<0.001	<0.001

3.8 Impact of folic acid fortification on hemoglobin and folate concentrations

Pre-Sac

Hemoglobin:

Originally, three categories of folic acid fortification were specified but as there were very few samples with a folic acid concentration above 1.2 mg/kg, two categories of fortification were used for the following analyses: ≤ 0.6 mg/kg and > 0.6 mg/kg. In order to test whether folic acid fortification of flour had any impact on hemoglobin concentrations, a sequential multiple regression analysis was undertaken, which removed the effects of age and tested whether the two categories of folic acid fortification had an effect on hemoglobin concentration. Those Pre-SAC consuming flour with ≤0.6 mg/kg had hemoglobin concentrations 4.8 g/L lower than those consuming flour with >0.6 mg/kg folic acid (p = 0.004). Correcting for the presence of Plasmodium parasites in the model resulted in no significant difference between the groups (Table 28).

Table 28: Differences in hemoglobin concentrations according to the fortification level of folic acid in wheat flour among pre-SAC

Category of folic acid fortification of flour	*Difference in Hemoglobin (g/L)*		
			***Plasmodium* –ve**
	After correction for age and sex	**After correction for age, sex and *Plasmodium***	**After correction for age and sex**
≤ 0.6 mg/kg	-4.8	0	-4.6
>0.6 mg/kg	-	-	-
P	0.004	ns	0.025

The analysis was then repeated using only children who were negative for *Plasmodium* parasites, the effects of age and sex were removed and the regression model tested whether the two categories of folic acid fortification had an effect on hemoglobin concentration. Those

Pre-SAC consuming flour with ≤ 0.6 mg/kg folic acid had hemoglobin concentrations 4.6 g/L lower, than those consuming flour with 0.6 mg/kg folic acid (p = 0.025). These results showed that for both *Plasmodium*-free and all Pre-SAC that there was an association between the categories of folic acid fortification and hemoglobin concentration.

The analyses were repeated using folic acid fortification as a continuous character. With all children in the model and after removing the effects of age and sex, hemoglobin increased by +5.41 g/L for each unit increase in folic acid (p = 0.036). Additionally, correcting for the presence of Plasmodium parasites in the model, the effect was not significant. Repeating the analysis using only Plasmodium-free children in the model, the regression coefficient was a 7.21 g/L increase in hemoglobin (p=0.042).

Folate:
No plasma folate levels were measured among pre-SAC.

WRA
Hemoglobin:
Those WRA consuming flour with ≤ 0.6 mg/kg folic acid had hemoglobin concentrations 3.0 g/L lower, than those consuming flour with >0.6 mg/kg folic acid (p = 0.047). However after controlling for *Plasmodium* there was no significant difference and no significant differences was found when the analyses were restricted to *Plasmodium*-free WRA (Table 29).

Table 29: Differences in hemoglobin concentrations in WRA in those consuming flour adequately and inadequately fortified with folic acid.

Category of folic acid fortification of flour	***Difference in Hemoglobin (g/L)***		
			***Plasmodium* –ve**
	After correction for age and sex	**After correction for age, sex and *Plasmodium***	**After correction for age and sex**
≤ 0.6 mg/kg	-3.0	-2.10	-1.89
>0.6 mg/kg	-	-	-
	0.047	ns	ns

Plasma folate:
There was a no significant difference in mean plasma folate concentrations by folic acid in the flour in the total sample or urban and rural samples when analysed separately. However an analysis of variance with locality and folic acid categories revealed significantly lower means in the ≤0.6 mg/kg (-0.73) and >0.6 to <1.20 mg/kg (-0.79) categories compared with the ≥1.20 mg/kg category (reference value set to 0, p=0.031). There was also a significant interaction effect indicating that urban and rural means were not consistent across the three folic acid fortification categories, ≤0.6 mg/kg, >0.6 to <1.20 mg/kg and ≥1.20 mg/kg (unlogged p=0.004, logged p=0.007; Table 30).

Table 30: Mean plasma folate by dietary flour folic acid categories (geometric means in brackets)

Locality	**Folic acid in flour**			**p**
	≤0.6 mg/kg	**0.6 to <1.20 mg/kg**	**≥1.20 mg/kg**	
Urban plasma folate (nmol/L)	0.30 (0.098)	0.25 (0.082)	1.03 (0.452)	ns
Rural plasma folate (nmol/L)	0.30 (0.096)	0.31 (0.102)	0.23 (0.092)	ns
All plasma folate (nmol/L)	0.30 (0.097)	0.26 (0.087)	0.36 (0.120)	ns

Regression analysis of continuous data of plasma folate status (unlogged and logged) versus continuous folic acid levels in the flour did not reveal any significant associations.

3.9 Associations of fortification levels and micronutrient status with socio-economic variables

3.9.1 Vitamin A fortified oil and socio-economic variables

Multinomial logistic regression analysis was used to test how well the three categories of vitamin A fortification in oil could be predicted using the socio-economic variables. When all variables were entered together (full model), overall 57.4% of the three categories were correctly predicted: nearly three-quarters in the ≥6.4 category, about half in the ≤3.2 category and under 20% in the >3.2 to <6.4 category (Table 31). A stepwise procedure revealed that 11 variables were significant predictors, and included in order of those most significant: locality, having a village phone, water source for drinking, toilet facilities, head of household educational level and occupation of head of household.

Table 31: Vitamin A concentration of oil in households correctly predicted using socio-economic variables

Model	Vitamin A in fortified oil (µg/g)				p
	≤3.2	>3.2 to <6.4	≥6.4	Total	
	%	%	%	%	
Full	48.8	17.7	73.1	57.4	<0.001
Stepwise	50.4	15.0	72.1	57.3	<0.001

3.9.2 Iron fortified flour and socio-economic variables

Similar analyses as were done for the oil were carried out for iron and folic acid in flour. When all the socio-economic variables were entered together, overall, 83.5% of the three iron fortification categories were correctly predicted with nearly all households in the highest category (≥48ppm) predicted (Table 32). Using a stepwise procedure, 14 variables were significant predictors, the most significant of which included locality, water source, occupation of the head of household, having a mobile phone, number of rooms and roof construction.

Table 32: Iron concentration of flour in households correctly predicted using socio-economic variables

Model	Iron in fortified flour (ppm)				p
	≤24	>24 to <48	≥48	Total	
	%	%	%	%	
Full	55.3	67.0	97.1	83.5	<0.001
Stepwise	48.4	59.4	91.0	81.5	<0.001

3.9.3 Folic acid fortified flour and socio-economic variables

For folic acid overall, 72.3% of the three categories were correctly predicted with better prediction in the lower two categories. When a stepwise procedure was used, 11 variables were significant (Table 33) which in order of significance included having a village mobile phone, education of the head of household, water source, number of rooms and ownership of an electrical appliance.

Table 33: Folic acid concentration of flour in households correctly predicted using socio-economic variables.

Model	Folate in fortified flour (mg/kg)				p
	≤0.6	>0.6 to <1.20	≥1.20	Total	
	%	%	%	%	
Full	77.2	71.5	36.1	72.3	<0.001
Stepwise	76.7	61.4	30.4	67.4	<0.001

3.9.4 Association between vitamin A status and socio-economic variables

Pre-SAC

Multinomial logistic regression was used to test how well VAD could be predicted using the socio-economic variables. When all variables were entered (full model) 30.3% of the VAD group was correctly predicted compared with 88.7% of the normal group (Table 34). A stepwise regression revealed that only locality was a significant predictor and rural children were 5.0 times more likely to have VAD than urban children (95% CI 3.3-7.6). When the corrected RBP concentration was used only 1% of VAD children were correctly predicted and the stepwise procedure revealed that locality was the only significant predictor with rural children being 3.2 times more likely to be VAD than urban children (95% CI 2.0-5.0).

Table 34: Percentage of pre-school children correctly predicted as vitamin A deficient (VAD) using socio-economic indicators

Variable	Model	Normal %	Deficient %	Total %
VAD	Full	88.7	30.3	71.7
	Stepwise	100.0	0.0	70.8
Corrected RBP	Full	99.8	1.0	82.6
	Stepwise	100.0	0.0	82.6

When RBP was analysed as a continuous variable, about 15% of the variation was explained by the socio-economic variables and locality was the best predictor. After removing the effects of the other variables, urban children had a significantly higher mean RBP concentration, on average, than rural children (+0.12µmol/L, p<0.001) but there was no significant difference between boys and girls.

WRA

No analyses were undertaken for WRA as the number of women with VAD was too small. In a multiple regression analysis with RBP as a continuous trait, no socio-economic variable showed any association with RBP.

3.9.5 Association between iron status and socio-economic variables

Pre-SAC

Using all socio-economic variables, 90.8% of anemic children were correctly predicted but only 51.3% of children with normal hemoglobin (Table 35). Using a stepwise approach, the main findings were that children living in rural areas, in a household not having a flush toilet, using a river as the main water source and no mobile phone were much more likely to be anemic (odds ratio ranged from 1.7 to 10.4). The model with these four variables was nearly as good as the full model in predicting anemic and normal children.

The socio-economic variables were poor at predicting depleted iron stores (uncorrected and corrected) and only locality was a significant predictor with urban children being 5.7 times and 3.5 times more likely to have depleted iron stores (95%CI:2.1 – 6.0) for corrected ferritin, respectively (Table 35).

Table 35: Iron stores of pre-school children correctly predicted using socio-economic variables

Variable	Model	Normal (%)	Deficient (%)	Total (%)
Anemia	Full	51.3	90.8	80.9
	Stepwise	51.3	88.8	78.9
Depleted iron stores corrected (ferritin < 12µg/L)	Full	99.6	1.3	88.6
	Stepwise	100.0	0.0	88.6

Multiple regression analysis revealed that the socio-economic variables explained 27% of the variation of hemoglobin (continuous) and there was a significant effect of locality. A sequential multiple regression analysis, which removed all the other socio-economic variables before testing for locality, found that rural children had, on average, a significantly ($p = 0.012$) lower mean hemoglobin (-17 g/L) than urban children.

The socio-economic variables explained between 9.5% and 16.7% of the variation of ferritin. Rural children, on average, had a higher geometric mean ferritin concentration of 66 µg/L ($p=0.012$) than urban children (29.3 µg/L), after correcting for the other socio-economic variables.

WRA

For WRA, all the socio-economic variables together correctly predicted 76.6% who were anemic and 53.1% who were normal (Table 36). The stepwise procedure revealed that only two variables were significant, locality and having access to a village internet system. WRA living in rural areas were 4.3 times more likely to be anemic (95%, CI 3.0-6.2) if they did not have access to the internet, and no access in rural areas was associated with a 1.6 times greater risk of anemia (95%CI: 1.0 – 2.4).

When analyzed as a continuous variable, 17.8% of the variance of hemoglobin was explained by the socio-economic variables of which locality was the main predictor and the rural mean was 18.1 g/L lower than the urban mean ($p=0.003$) after taking into account all the other SES variables.

Depleted iron stores (categorical, uncorrected and corrected) were poorly predicted by the socio-economic variables and in the stepwise model no socio-economic variable was significant (Table 36)

Table 36: Iron status of women of reproductive age correctly predicted using socio-economic variables

Variable	Model	Normal (%)	Deficient (%)	Total (%)
Anemia	Full	53.1	76.6	67.2
	Stepwise	75.0	58.8	65.3
	Stepwise	-	-	-
Depleted iron stores corrected (ferritin < 12µg/L)	Full	100.0	6.7	90.2
	Stepwise	-	-	-

3.9.6 Association between folate concentrations and socio-economic variables

WRA

No categorical analyses were undertaken as all women had low folate levels. When analyzed as a continuous variable, 5% of the variation was explained and the only SES variable which

showed a significant association was cooking method and women cooking with charcoal or gas had a higher mean folate level than those cooking with wood (both +0.4 nmol/L, $p<0.001$).

4 Discussion

The current survey was designed to assess the impact of the GAIN-funded oil and wheat flour fortification program in two areas of Côte d'Ivoire: a rural area, Bouaflé and 3 communes in the urban area of Abidjan. Specifically, to estimate the differences in vitamin A status of pre-SAC and WRA living in households consuming fortified oil relative to those living in households consuming unfortified vegetable oil, and the impact of consuming fortified flour on the iron status of pre-SAC and WRA, and on folate status of WRA.

4.1 **Socio-economic issues**

As part of the survey, a questionnaire collected data on the socio-economic status of the households and showed significant differences in all aspects among those living in rural and urban areas. From the demographic data, analysis showed that there were significant differences between urban and rural households in the construction of their houses and the availability of electricity. Nearly 99% of urban houses were made of cement and had electricity, but only 65% of rural houses were made of cement and 42% had electricity. In a more affluent urban home, food can be stored and prepared in a way that minimizes the dangers of contamination because of the presence of a refrigerator, gas stove and tap water in the house. In the poorer rural households, where there is no refrigerator, food is cooked outside over a wood fire and water is carried from a central water pump, appropriate food hygiene is a much more difficult to achieve. Personal hygiene is also more problematic in rural areas, where 86% of the respondents are defecating in the open and there is no convenient access to water to wash their hands. The consequence of these environmental conditions may be that vulnerable poorer households will be exposed to more pathogenic organisms than more affluent households, which might lead to e.g. more diarrheal episodes and helminth infections, especially in pre-SAC. Although this was not tested in this survey, others have found evidence that the presence of a latrine, especially in combination with access to clean water has a positive effect in reducing the incidence of diarrhea (Bateman et al. 1995; University of Pretoria - Centre for Environmental Economics and Policy in Africa 2010), and the presence of soil-transmitted helminths in children. In addition, the presence of inflammation can depress the appetite of those exposed, the consequence of which will be reduced uptake of iron and vitamin A from the food so reducing the effectiveness of any intervention program such as the fortification program in Côte d'Ivoire.

4.2 **Oil and RBP concentrations**

Infants have little or no stores of vitamin A in their livers at birth and are consequently solely reliant on dietary vitamin A to build up their liver stores over the early years of life. As a consequence, they are very vulnerable to deficiencies in vitamin A in the diet.

The survey found an interesting significant positive relationship between the daily consumption of vitamin A from the fortified oil and RBP concentrations in all pre-SAC, and in those 24–59 months old, as there was an increase in RBP concentration of 0.49 µmol/L and 0.58 µmol/L per every 1mg RE/kg of oil consumed, respectively. Although historically, the fortification of margarine with vitamin A has been a success story in many western countries, there are few national oil fortification programs, which have shown a positive effect of the fortification of oil with vitamin A on plasma RBP or retinol. The results suggest that children 24–59 months were consuming enough of the oil on a daily basis to have a positive impact on plasma RBP. In the younger children (6–23.9 months), although there was also a positive relationship between consumption of the fortified oil and RBP concentrations, the relationship was not strong enough to be significant suggesting the children were not eating enough of the oil. However, some of the younger children will also have been breast-feeding and may have been consuming breast

milk with an improved vitamin A content as a result of the fortified oil eaten by the mothers, but as breast milk samples were not collected it is not possible to ascertain whether this happened.

No relationship was found between the fortified oil consumed and RBP concentrations in the WRA. Given that there was no VAD in women either baseline or end line, the lack of a relationship was not unexpected. WRA have liver stores of vitamin A, and the circulating vitamin A concentrations are homeostatically controlled so there is no expected direct relationship between intake and plasma concentrations.

4.2.1 Inflammation and malaria, and the interpretation of RBP

Poorer diets, poorer environmental conditions and higher inflammation rates in rural areas are driving the higher prevalence of VAD. Circulating concentrations of retinol and RBP are reduced by inflammation, and in such situations plasma RBP or retinol are not good indicators of vitamin A status, especially if exposure to inflammation is high, as in malaria endemic areas like Côte d'Ivoire. Inflammation is accompanied by an acute phase response during which various acute phase proteins may be increased or decreased. In this survey, we measured two acute phase proteins: CRP and AGP both of which increased in the presence of inflammation. The main function of RBP is as a transport protein to carry retinol around the body, but during inflammatory episodes, RBP also acts as a negative acute phase protein when the circulating concentrations of RBP and therefore retinol are reduced. Correction of RBP concentrations for the presence of inflammation, using the factors from the meta-analysis developed by Thurnham et al (Thurnham et al. 2003) adjusted the overall mean RBP concentration of Pre-SAC to 0.94 µmol/L, close to the mean retinol concentration for UK children of 1.04 ± 0.28 µmol/L reported from the British Pre-School survey from 1995 (Gregory et al. 1995), and provided a clearer picture of the prevalence of VAD in the Pre-SAC in Côte d'Ivoire. In the urban area, the prevalence of VAD, after correction for inflammation, was 9.6%, very close to that of the UK Pre-SAC (10%), but in the rural areas 24.3% of children were still VAD, representing a public health problem as defined by WHO (WHO 1996). The residual difference in the VAD between the two areas may be explained by the difference the in exposure to *Plasmodium* parasites, i.e. 46% Pre-SAC in the rural and 7.5% in urban areas had parasites, and although vitamin A is taken up from the diet and transported to the liver in all children, in rural children the vitamin A may become trapped there as the circulating RBP concentration is lower in response to the *Plasmodium* infection, hence not enough RBP is available to transport the retinol to the tissues of the body. An alternative explanation may be that clinical malaria is present in many rural children, but the correction factor is only designed to correct for inflammation where there is no clinical sickness, hence it is not able to correct RBP concentrations completely.

Residency was a significant predictor of VAD and rural children were 3.2 times more likely to have VAD than urban children. This finding was supported by further statistical analysis, which showed when using RBP as a continuous variable, 15% of the variation in RBP was explained by socio-economic variables, with locality as the best predictor. One possible reason for the difference in VAD between the urban-rural areas is the exposure of the rural Pre-SAC to more mosquito bites and consequently *Plasmodium* parasites. Data from a study which distributed insecticide-treated bed-nets in Côte d'Ivoire in 2008 found that even after the distribution of insecticide-treated bed-nets to WRA, the mean biting rates and entomological inoculation rates for *Anopheles gambiae s.s.*, the main malaria vector in central Côte d'Ivoire was 4.1 bites/person/night in July, corresponding to mean entomological inoculation rate of 148 infective bites/person/year (Koudou et al. 2010). As the public health messages were forgotten, the use of bed-nets went down and as a result children under 5 years were being bitten regularly with each infective bite probably leading to an inflammatory response, even if no clinical malaria developed. At the time of the survey reported here, use of bed-nets was low in the rural areas

(personal communication) and so children would be exposed to mosquito bites and the resulting consequences. Those in the urban areas would be more, but not completely protected in cement houses with glass windows and corrugated iron roofs, plus *Plasmodium* transmission pressure is often lower in urban settings.

Côte d'Ivoire is a country with stable malaria transmission and children become infected early in life. These young children experience more severe disease symptoms during the first five years of life, part of which is due to their own body's response to malaria. For example, malaria fever is associated with high levels of a cytokine, tumor necrosis factor (TNF). This cytokine is released by macrophages when *Plasmodium* infected red blood cells rupture, and large numbers of parasites enter the blood stream. The cytokine also stimulates an acute phase response. Therefore the hypothesis of the etiology is that as children get older and their humoral immune system matures, they develop a natural immunity to malaria and the disease becomes less severe, even though they continue to be bitten by mosquitoes, consequently there is less impact on RBP. Adult women have more immunity to *Plasmodium* infection, due to a fully developed humoral immunity, and hence can recover from inflammation episodes far more quickly, i.e. it is transient.

4.3 Flour

4.3.1 Iron fortification and iron biomarkers

In the 2009 coverage survey in Côte d'Ivoire, approximately 30% of flour was fortified in accordance with legislation (60 ppm), with approximately half of the surveyed households consuming flour with slightly lower than mandated levels of iron (50 ppm). Since the 2009 survey, a major mill has started fortifying flour and in the current end line, coverage of flour with concentrations of iron ≥ 48 ppm in Abidjan was almost 100%, but the situation in Bouaflé was different with only half the flour being acceptably fortified. The difference between the coverage of adequately fortified flour in the rural and urban areas cannot be explained and will need further investigation.

Pre-SAC

Hemoglobin:

There was a significant difference between the hemoglobin concentration of urban and rural Pre-SAC, although anemia rates were high in both groups. The WHO classification of anemia in a population states that if >40% of the population have anemia, (as defined for each population group), then the problem is of severe public health significance (WHO/UNICEF/UNU 2001). Overall in Côte d'Ivoire, 75.2% of Pre-SAC had hemoglobin concentrations <110 g/L indicating a severe public health problem, even after 3 years of flour fortification with electrolytic iron. However, in children who were negative for *Plasmodium* spp., and consuming flour with <24 ppm or ≥ 24-<48 ppm iron, the hemoglobin concentrations were 15.1g/L and 16.2g/L less respectively than that of children consuming flour with >48ppm iron (after correction for other potential confounders), which suggests that adequately fortified flour is needed to have the some measurable impact on hemoglobin concentrations. In addition, when using the iron fortification data as a continuous variable and considering children who were *Plasmodium*-free, hemoglobin concentrations increased by 0.254 g/L for each unit (ppm) increase in iron showing a positive linear improvement in hemoglobin as iron fortification increased.

Hemoglobin concentrations in pre-SAC were significantly lower in those with *Plasmodium* infection, even after correcting for sex and age. Nearly all children who had *Plasmodium* parasites were anemic and severe anemia was three times greater in those with *Plasmodium* infection. Severe anemia is one of the most lethal complications in children infected with *Plasmodium spp.,* but the pathogenesis of this anemia is not completely understood.

Experimental data from malaria-infected humans and animal models suggest that uninfected red cells have a shortened life span and these cells may be vulnerable to early destruction by phagocytosis and/or complement activation, thus contributing to the development of anemia (Waitumbi et al. 2000). Ironically, therefore, the destruction of much of the red cell mass in malaria may not be due to the direct effect of the parasite but may be the result of the immunologic mechanisms designed to defend the body from this invader.

In contrast to vitamin A fortification of oil, the calculation of daily additional iron intake from wheat flour could not be calculated, since it was impossible to obtain wheat flour consumption estimates at the household (and hence, individual) levels.

Ferritin:
Rural children had higher mean ferritin concentrations (66 µg/L) than urban children (46 µg/L), and of the socio-economic variables, only locality was a significant predictor of depleted iron stores with an urban child 3.5 times more likely to have depleted iron stores using corrected ferritin concentrations. Overall, the socio-economic variables explained between 9.5 and 16.7% of the variation in ferritin

Circulating concentrations of ferritin are increased by inflammation, and the usefulness of ferritin as an indicator of adequate iron stores in malaria endemic areas like Côte d'Ivoire has been controversial. Like RBP, ferritin can be corrected using factors derived from a meta-analysis (Thurnham et al. 2010), but there is debate over the usefulness and others have suggested other methods, e.g. using ferritin:sTfR ratio. In this survey, we used the meta-analysis correction method but subsequent statistical analysis showed significant relationships between ferritin, AGP and/or CRP and *Plasmodium* infection. These findings raise doubts about the usefulness of ferritin as a marker of iron stores in malaria-endemic regions and give rise to the question of whether the correction algorithms proposed are correcting sufficiently. The meta-analysis, where the correction factors were derived, used only apparently-healthy people and did not consider those with clinical conditions, e.g. malaria or even *Plasmodium* positive sub-clinical malaria. In Pre-SAC, the presence of *Plasmodium* parasites had a significant effect on corrected ferritin concentrations, as even after the additional corrections for age and sex, children without *Plasmodium* infection had ferritin concentrations 12 µg/L lower than those infected with parasites, which suggests that the meta-analysis correction factors are not completely correcting ferritin when clinical malaria and/or presence of *Plasmodium* parasites are reported, therefore additional correction factors are needed for such situations. Data from another survey in West Africa also found a similar situation and calculated new adjustment factors for children infected with *Plasmodium* spp., with or without elevated acute phase proteins (personal communication, data not published).

In the Pre-SAC, in order to test whether the level of iron fortification had any effect on ferritin concentrations, regression analysis was used and ferritin concentrations in those consuming flour with <24 ppm and ≤ 24 – 47.99 ppm were significantly higher compared to those consuming flour with 48 ppm of iron, when the effects of age and sex were removed. This relationship was contrary to what might be expected, but once the correction for the presence of *Plasmodium* parasites was included there were no significant differences among the groups, which suggests the presence of parasites, was increasing ferritin concentrations and masking the true relationship. To overcome this limitation, the analyses were repeated on children who were *Plasmodium* negative and similar results were obtained. However, the number of children in the group consuming flour with <24ppm iron was very small (n = 14) and the result may not be reliable. Finally, using iron fortification levels as a continuous variable, and removing the effects of age and sex, ferritin concentrations were found to decrease by 0.216 µg/L for each unit (ppm) of iron in the fortified flour. Correcting for *Plasmodium* parasites in a similar regression analysis,

and using only children negative for *Plasmodium* parasites no relationships were found. As before, these relationships are difficult to interpret, but where there was infection or inflammation, i.e. especially the rural areas, there were apparently less children with depleted iron stores because the ferritin was acting as an acute phase protein and increasing in concentration and so confounding any interpretation of what is happening in response to the fortification program.

sTfR:
Overall mean sTfR concentrations were within the accepted range, using the Ramco™ classification. Various commercial assays give different normal ranges for their assays because up until recently there has been no international standard for sTfR. WHO have now established an international standard, but to our knowledge the commercial companies have not yet adjusted their assays accordingly (Thorpe et al. 2010).

The usefulness of sTfR in diagnosing iron deficiency in the presence of inflammation has been debated. The most important determinant of sTfR seems to be marrow erythropoietic activity. In this survey concentrations of sTfR, CRP AGP and ferritin were elevated and hemoglobin concentrations were decreased in children with *Plasmodium* infection compared to those without. In Pre-SAC, there were strong relationships between the acute phase proteins and ferritin and hemoglobin, and unexpectedly with sTfR, which suggests that sTfR was responding to inflammation, but this relationship did not exist in the WRA. Ooi et al (Ooi et al. 2009) assessed the effect of inflammation on sTfR and found a positive association with CRP in children 0.4 months to 18 years and the association persisted even after accounting for the age-dependency of sTfR, which we also found. A similar acute phase reactant response of sTfR was also reported in children with a high load of infection and inflammation (Wians et al. 2001) but Verhoef et al (Verhoef et al. 2001) did not find any association between CRP and sTfR in children 6-36 months in Kenya. Iron deficiency stimulates an increase in sTfR levels due to the compensatory increase in erythropoiesis. Conversely, inflammation can decrease sTfR production, thus lowering its serum level in inflammation. Because there is an opposing effect of iron deficiency and inflammation on sTfR production, the relative severity of iron deficiency and inflammation can affect the relationship between sTfR and CRP in a population sample, such as in Côte d'Ivoire. As the area is also endemic for malaria and many children were *Plasmodium* parasite positive, there may also have been some malaria-associated hemolysis resulting in increased erythropoiesis, which in turn increased concentrations of sTfR. Verhoef et al (Verhoef et al. 2002) suggest that sTfR concentrations in malarious areas measure the rate of erythropoiesis and the deficit in functional iron in the erythron and hence, may not be suitable for detecting iron deficiency in individuals with malaria. The sTfR data therefore needs to be interpreted with care.

In the Pre-SAC, in order to test whether the level of iron fortification had any effect on sTfR concentrations, regression analysis was used and sTfR concentrations in those consuming flour with <24 ppm and ≤ 24 – 47.99 ppm were significantly higher compared to those consuming flour with 48 ppm of iron, when the effects of age and sex were removed, and when the additional effects of the *Plasmodium* parasites were removed. Likewise, using children who were negative for *Plasmodium* parasites those consuming flour with <24 ppm and ≤24 – 47.99 ppm had significantly higher sTfR concentrations. sTfR concentrations tend to be higher in response to iron deficiency, but bearing in mind the interpretation of sTfR in the presence of malaria parasites, it appears that adequately fortified flour is needed to have the some measurable impact on sTfR concentrations. In addition, when using the iron fortification data as a continuous variable and considering children who were *Plasmodium*-free, sTfR concentrations decreased by 0.058 mg/L for each ppm increase in iron showing a linear improvement in sTfR as iron fortification increased.

Malaria and iron:
Many trials of iron fortification in children in malarial-endemic areas of Africa have been ineffective (Rohner et al. 2010; van Stuijvenberg et al. 2008) or have had only limited effect (Andang'o et al. 2007; Wegmuller et al. 2006). Apart from the effect on the inflammatory system and plasma biomarkers already discussed, malaria may also induce iron deficiency through reduced iron absorption or iron loss after hemolysis as well as sequestration of iron in macrophages of the mononuclear phagocyte system. Although the children in this survey were apparently-healthy and not showing clinical signs of malaria at the time of the blood sampling, those with *Plasmodium* infection were already producing an acute phase response stimulated by an increase in cytokine concentrations, such as interleukin-6 and TNF (Lyke et al. 2004; Wenisch et al. 1999), which also stimulate hepatic hepcidin production. High circulating hepcidin concentrations can reduce iron absorption from the gut by blocking the iron-transporter ferroportin and can increase iron sequestration in the reticuloendothelial system (Weiss 2009). The resulting hypoferraemia limits the iron available for erythropoiesis and contributes to anemia. A study by Cercamondi et al (Cercamondi et al. 2010) confirmed this hypothesis as they showed that afebrile malarial parasitemia decreased dietary iron absorption. The effect appears to be due to the low-level inflammation modulation of serum hepcidin and may help explain why iron supplements and iron fortification of staple foods or complementary foods may be less effective in malarial endemic areas.

WRA
Hemoglobin:
There was a significant difference between the hemoglobin concentrations of urban and rural WRA, although anemia rates were high in both groups. Overall in Côte d'Ivoire, 59.5% of WRA had hemoglobin concentrations <120 g/L indicating a severe public health problem, even after 3 years of flour fortification with electrolytic iron. No association between iron fortification levels in the flour and hemoglobin concentrations could be found among WRA. An important limitation is that we were not able to quantify the daily flour and hence, additional iron intake. This lack of quantification and the fact that a poorly bioavailable iron fortificant is being used may partly explain the lack of these findings, but still, the anemia of WRA in such a setting needs to be further elucidated.

Ferritin:
In WRA, the presence or absence of malarial parasites had a significant effect on uncorrected ferritin concentrations but after correction there was no significant difference in ferritin concentrations between WRA with *Plasmodium* parasites and those without. With less than 10% prevalence, few had depleted iron stores in this age group and iron deficiency fails to explain the high rates of anemia.

4.3.2 Folic acid and folate

The situation with regard to the fortification with folic acid was much less satisfactory as overall only 6% of flour samples had the legal concentration of folic acid (>1.20 mg/kg). Forty two percent of flour was fortified to an acceptable level (>0.6<1.2 mg folic acid/kg) but >50% of flour was considered unfortified. Unfortified flour (folic acid < 0.6 mg/kg) was collected from 63.5% of the Bouaflé households and 39% of urban households. If the folic acid was added at the correct concentration in the premix, and the iron data suggests it was as the flour in the urban area was almost all adequately fortified with iron, it appears that were some losses of folic acid during production, storage or distribution. Normally the iron spot test is used in the mill to make sure the flour is adequately fortified with the assumption that if the concentration of iron is correct so is the folic acid, but perhaps there are issues of degradation of folic acid. Data from DSM suggest that folic acid is unstable and loses its activity in the presence of light, oxidizing or

reducing agents, and acidic and alkaline environments. However, it is relatively stable to heat and humidity (DSM); thus, premixes, baked products, and cereal flours, retain almost 100% of the added folic acid after six months of storage and over 70% of folic acid added to wheat flour is normally retained during bread baking.

Plasma data seems to confirm the fact that the flour folic acid was not at the expected concentration, as all WRA, whether from the rural or urban areas had low mean plasma folate concentrations, with concentrations <10nmol/L. Analysis of the data found a significant difference in mean folate concentrations by folic acid levels in the flour, which was driven by the higher mean folate concentration found in women consuming flour with >1.2 mg folic acid/kg flour, but when a regression analysis was done using continuous plasma folate data versus continuous flour folic acid content data, no associations were found, i.e. folate intake was not reflected in plasma folate concentrations.

4.4 **Recommendations**

For Côte d'Ivoire, the following programmatic recommendations can be made:

1. Maintain the oil fortification program, since it appears to benefit all children aged 6-59 months of age
2. In 2009, with the support of Helen Keller International, approximately 85% of children 6-59 months received vitamin A capsules (Helen Keller International 2012) and this program should be continued, most particularly for children 6-23 months who may not be able to eat enough fortified oil to be effective in reducing VAD.
3. Investigate why there is a difference in the level of iron fortification between the urban area of Abidjan and the rural areas of Bouaflé.
4. Investigate why folic acid levels are so low in the fortified flour at household level by examining whether storage, distribution, packaging or cooking practices are destroying the folic acid in the flour and in other folic acid containing foods commonly eaten, e.g. vegetables.
5. Promote the use of insecticide-treated bed-nets, or other anti-malarial measures especially in rural areas
6. Encourage other public health measures, such as making clean water available for households in rural areas

Research wise, the following recommendation seems appropriate: It is important to further assess the influence of the acute phase response and *Plasmodium* parasitemia on the assessment of iron and vitamin A status, as from this analysis, it seems that the current correction algorithms, which are based on apparently-healthy people and not those with clinical illness, do not fully account for the influence of positive parasitemia and clinical malaria.

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ANNEX I: Study sites

Zone		*N° Grappe*	*Localité*
Rural	Centre-Ouest	11	ALLAHOU BAZI
	Centre-Ouest	12	BAONFLA
	Centre-Ouest	13	BAZI
	Centre-Ouest	14	BAZIAFLA
	Centre-Ouest	15	BEGBESSOU
	Centre-Ouest	16	BLE
	Centre-Ouest	17	BOKASSO
	Centre-Ouest	18	BOZI-I
	Centre-Ouest	19	YOHO
	Centre-Ouest	20	DEGBEZRE
	Centre-Ouest	21	DIADIENOUFLA
	Centre-Ouest	22	DIANFLA
	Centre-Ouest	23	FETEKRO
	Centre-Ouest	24	KAMI
	Centre-Ouest	25	KOUBI
	Centre-Ouest	26	KRINGABO
	Centre-Ouest	27	NANGREKRO
	Centre-Ouest	28	N'DOUFFOUKANKRO
	Centre-Ouest	29	OUANZANOU
	Centre-Ouest	30	PANGBANKOUAMEKRO
	Centre-Ouest	31	PATIZIA
	Centre-Ouest	32	SINFLA
	Centre-Ouest	33	SUEFLA
	Centre-Ouest	34	TANGONOBOUITA
	Centre-Ouest	35	TUANKRO
	Centre-Ouest	36	ZOOLA

Zone		*N° Grappe*	*Localité*
Urban	Abidjan	37	Treichville/Craoné
	Abidjan	38	Treichville/Jacques Aka
	Abidjan	39	Treichville/Notre Dame
	Abidjan	40	Attécoubé/Cité Fermont
	Abidjan	41	Attécoubé/Espoir
	Abidjan	42	Attécoubé/Jerusalem I
	Abidjan	43	Attécoubé/Lagune
	Abidjan	44	Attécoubé/Némantoulaye
	Abidjan	45	Attécoubé/Santé III
	Abidjan	46	Abobo/ABOBO-SUD 2ème TRANCHE
	Abidjan	47	Abobo/ABOBO-TE
	Abidjan	48	Abobo/AGBEKOI
	Abidjan	49	Abobo/AGNISSANKOI AVOCATIER
	Abidjan	50	Abobo/AGOUETO
	Abidjan	51	Abobo/AKEIKOI
	Abidjan	52	Abobo/ANONKOI-KOUTE
	Abidjan	53	Abobo/BANCO 1& 2
	Abidjan	54	Abobo/CLOUETCHA KENEDY
	Abidjan	55	Abobo/AVOCATIER N'GUESSANKOI
	Abidjan	56	Abobo/PLAQUE 1& 2
	Abidjan	57	Abobo/SAGBE CENTRE
	Abidjan	58	Abobo/SOGEFIHA HABITAT
	Abidjan	59	Abobo/CENT DOUZE HECTARES
	Abidjan	60	Abobo/HOUPHOUET BOIGNY
	Abidjan	61	Abobo/SAGBE-NORD
	Abidjan	62	Abobo/SANS MANQUER

ANNEX II: Material list for each team

Liste des documents	**Matériel de laboratoire**
Guide de l'équipe d'enquête	1 pair de gants
Questionnaire ménage	coton désinfectant
Questionnaire Individuel	
Fiche de synthèse point de vente farine/grappe	aiguille
Feuille d'identification des points de vente/zone	Epicraniens
Feuille récapitulative départementale	vacutainer 4 mL
Lettre d'information	coton
Liste de matériel	sparadrap
Lettre d'invitation	DIFF safe
Fiche de Kish	plastique de pesé
Liste de préparation des étiquettes	hémocuvettes
Fiche de suivi des médicaments	boîte de kleenex
Fiche contact (chercheurs, superviseur, DD, ect)	bandelettes pour le test du palu
Crayons	lames GE/GF
taille-crayons	pipettes pasteur
bics bleus	tubes noirs pour folate, pré numérotés
Gommes	tubes pour plasma, 0,2 mL
	tubes pour plasma, 0,5 mL
	portoirs de stockage, tubes noirs
	portoirs de stockage, tubes 0,2 mL
	portoirs de stockage, tubes 0.5 mL

Liste des médicaments	**Liste de petit matériel**
Amodiaquine + artesunate cp enfant (0-11 mois)	Autocollants menages
Amodiaquine + artesunate enfant (1an à 5 ans)	Autocollants farine
Amodiaquine + artesunate cp adulte	Global Positioning System (GPS)
Ferrostrane sirop	Entonnoir
Fer 60 mg adulte , cp	Piles GPS
Paracetamol 500 cp adulte	Piles torches
Paracetamol enfant (Effadol sirop)	bol gradué
Paracetamol enfant (doliprane 300 mg sachet)	bol de prélèvement de farine
Alben 400 mg cp	Louche de prélèvement pour farine
Vitamine C 500 mg cp	Flacons de prélèvement huile
	Essuie tout
	Carton de conservation pour huile
	Scotch grand format
	Carton rangement farine
	Thermomètre électronique

ANNEX III: Household questionnaire

Coller code du ménage

5.1

Nom Localité :	Date :
Initiales de l'enquêteur :	

1) Au chef de ménage, expliquer / rappeler l'objectif de l'enquête et ce qui se passera
2) S'assurer qu'au moins un membre du ménage est une femme âgée de 15-49 ans / enfant 6-59 mois *(au cours du remplissage de la fiche de ménage)*
3) Demander le consentement (répondre aux questions, faire signer la procédure) :
4) Sélectionner une femme en age de procréer et des enfants éligible (ou un des deux si l'autre absent)
5) Remplir le questionnaire en interviewant par priorité : a) la femme participante b) par la mère de l'enfant participant c) par le chef de ménage d) par un autre membre du ménage.

NB : Toutefois, pour des questions individuelles (sections Participants, Santé et habitudes nutritionnelles) seulement relever des informations relatives aux participants !

Formulaire de consentement

Nom et prénoms de la Femme en age de procréer :

JJ/ MM/ AAAA (date de naissance) **Age (années révolues)**

Nom et prénoms des enfant :

JJ/MM/AAAA (Ddn) **Age (mois révolus)** **Sexe (M/F)**

[Si dans un ménage il y a plus que 3 enfants de 6-59 mois, continuer au verso, et clairement indiqué sur recto.]

JJ/MM/AAAA (Ddn) **Age (mois révolus)** **Sexe (M/F)**

JJ/MM/AAAA **Age (mois révolus)** **Sexe (M/F)**

Je confirme par la présente que les personnes ci-dessus originaires de mon ménage participent volontairement à l'étude « Enquête sur le statut nutritionnel de la population ivoirienne ». J'ai été informé oralement et par écrit sur les objectifs, les risques et les avantages de l'étude. Je suis conscient de la possibilité qu'ils ont de se retirer de l'étude à tout moment sans donner de raison et sans subir de préjudices.

Nom du Chef du ménage ou représentant légal :_____

(Préciser la filiation avec l'enfant)

Lieu, date: _____

Signatures ou empreintes de doigt :

Chef du ménage / représentant légal	Femme participante

Recensement de la famille

Coller code du ménage

	Nom **/ Prénom**	Age		Sexe (M/F)	Cible 1 (6 – 59 mois) ☑	Cible 2 (15 – 49 ans) ☑
		Mois	Ans			
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
Total Famille						

(voir table de Kish pour sélectionner la femme cible : Entourer la/les cibles choisies)

Si plus de 25 personnes dans le ménage, continuer au verso !

Questionnaire ménage	**1) Qualité du répondant** : □ femme participante □ mère de l'enfant participant □ chef de ménage □ autre membre du ménage, préciser :…….	
	2) Nom et prénom du chef de ménage : (Nchmen) **Contact (tél, BP, etc.)**	
	3) Savez vous lire ? (1=oui / 2=non)	
	4) Savez vous écrire ? (1=oui / 2=non)	
	5) Quel est le niveau de scolarisation du Chef du ménage? 1=non-scolarisé / 2=primaire / 3=secondaire / 4=supérieur / 5=autre, préciser :…….	

Socioéconomie ménage	Observation	6) De quel type est le mur de votre logement ? 1=pas de mur /2 =bois / 3=argile / 4=mixture d' argile et ciment / 5=ciment /6=autres :…….	
		7) De quel type est votre toit ? 1=pas de toit / 2=papo-paille / 3=tôle ondulée / 4=tuile / 5=dalle / 6=autre :……	
		8) Combien de chambre à coucher avez-vous dans votre ménage ? (nombre)	
		9) Avez-vous de l'électricité dans votre ménage ? (1=oui / 2=non)	
		10) Avez vous des appareils électroménagers ? (1=oui / 2=non)	
		11) Quels appareils électroménagers? (1=oui / 2=non) réfrigérateur télévision radio ventilateur Climatiseur Vidéo / DVD	___ ___ ___ ___ ___ ___
		12) Quels sont vos moyens de communication à la maison? (1=oui / 2=non) téléphone fixe téléphone portable internet	___ ___ ___
		13) Quels sont vos moyens de communication *au village / dans la ville* ? (1=oui / 2=non) téléphone fixe téléphone portable internet	___ ___ ___
		14) Pour faire la cuisine, qu'est-ce que vous utilisez ? 1=électricité / 2=gaz / 3=charbon / 4=bois / 5=autre préciser …….	
		15) Quel moyen de déplacement possédez-vous ? (1=oui / 2=non) autres préciser …….. voiture mobylette vélo autres préciser	___ ___ ___ ___
		16) Quel est votre statut d'occupation du logement ? 1=propriétaire / 2=locataire / 3=logé / 4=autre préciser …….. ;	
		17) Quel est l'occupation <u>du chef du ménage</u> ? 1=agriculteur / 2=pêcheur / 3=marchand, commerçant / 4=retraité / 5=fonctionnaire / 6=artisan / 7=handicapé / 8=employé privé / 9=autre préciser :……	
		18) Quel est votre source principale pour l'eau <u>potable</u> ? 1=robinet / 2= puits privés / 3= pompe/forage public / 4= puits publics / 5=autres préciser…….	
		19) Est-ce que votre ménage dispose des toilettes ? 1=chasse d'eau / 2=latrines sol en ciment / 3=latrines sol en terre/sable / 4=pas de toilette	
		20) Est-ce que vous dormez sous moustiquaire? (1=oui / 2=non)	

21. Quelle quantité d'huile achetez-vous d'habitude POUR L'UTILISATION DANS LE MENAGE ? …… (quantité) 99=ne sait pas INDIQUER LE PRIX D'HUILE ACHETE EN F CFA :	
22. Avec quelle fréquence achetez-vous cette quantité d'huile? Tous les ……. Jours (Indiquer le nombre de jours)	
23. Est ce que vous avez actuellement de l'huile à la maison? (1=oui / 2=non) →SI NON, CONTINUER AVEC Q 28	
24. Si oui, est-ce que nous pouvons prendre un peu d'huile pour une analyse au laboratoire? (1=oui / 2=non) →SI NON, CONTINUER AVEC Q 28	

Observation	**25. Marque d'huile?** 1=Dinor / 2=Palme d'Or / 3=Toulor / 4=Goutte d'Or / 5=Autre:……. / 6=non-indiqué	
	26. Conditionnement? 1=emballage original (bouteille, berlingot, bidon 10/20 L) / 2= en vrac (déconditionné, sachet, bidon) / 3=autre, préciser :	
	27. Mode de Stockage ? 1=à l'abris de la lumière / 2=à la lumière / 3=non observable → COLLER L'AUTOCOLLANT CORRESPONDANT AU MENAGE SUR LE FLACON AVEC L'HUILE → CONTINUER A Q 32	

28. Si huile pas présente ou Q 24=non : Où est-ce que vous achetez habituellement l'huile? → IDENTIFIER LEMAGASIN ; IDEALEMENT DEMANDER A QUELQU'UN D'ALLER CHERCHER A LA BOUTIQUE, ACHETER LA MARQUE → COLLER L'AUTOCOLLANT CORRESPONDANT AU MENAGE SUR LE FLACON	
29. Quelle est la marque d'huile achetée? 1=Dinor / 2=Palme d'Or / 3=Toulor / 4=Goutte d'Or / 5=Autre:……. / 6=non-indiqué	
30. Dans quel emballage est l'huile achetée? 1=emballage original (bouteille, berlingot, bidon 10/20 L) / 2= en vrac (déconditionné, sachet, bidon) / 3=autre, préciser :	

31. Est-ce que vous utilisez au moins une fois par semaine de la farine de blé pour préparer des plats? (1=oui / 2=non) →SI NON, CONTINUER AVEC Q 40	
32. Si oui, quel type des plats préparez-vous le plus souvent <u>pour votre ménage</u>? 1=Pain / 2=Beignets/Galettes / 3=Gâteau / 4=Poisson frit / 5= Autre: …… SI LA REPONSE EST 4 ou 5 → CONTINUER AVEC Q 40	
33. Quelle est la marque de farine que vous utilisez habituellement pour la préparation des plats? NE PAS PROPOSER LA REPONSE 1=Farine GMA / 2=Farine LMCI / 3=Farine MMCI / 4=Autre:……. / 9=ne sait pas	
34. Quelle quantité de farine achetez-vous d'habitude? INDIQUER LA QUANTITE EN KG DE FARINE ACHETEE	
35. Avec quelle fréquence achetez-vous cette quantité de farine? Tous les …. Jours (indiquer le nombre de jours)	
36. Est ce que vous avez actuellement de la farine à la maison? (1=oui / 2=non) →SI NON, CONTINUER AVEC Q 40	
37. Si oui, est-ce que nous pouvons prendre un peu de farine pour une analyse au laboratoire? (1=oui / 2=non) →SI NON, CONTINUER AVEC Q 40	

Observation	**38. Marque de farine?** 1=Farine GMA / 2=Farine LMCI / 3=Farine MMCI / 4=Autre:……. / 9=ne sait pas	
	39. Conditionnement? 1=Farine de pâtisserie / 2= farine panifiable, en vrac (sachet) / 3= autre, préciser : → COLLER L'AUTOCOLLANT CORRESPONDANT AU MENAGE SUR LA BOITE AVEC LA FARINE CONTINUER AVEC Q42	

40. Est-ce que votre ménage consomme régulièrement du pain? (1=oui / 2=non) →SI OUI, IDENTIFIER LE BOUTIQIER / LA BOULANGERIE. COLLER AUTOCOLLANT DU MENAGE SUR LA FICHE FARINE ET SAUTER A Q42. →SI NON, CONTINUER AVEC Q41	Identification du Boutiquier / Boulanger :
41. Est-ce que votre ménage consomme régulièrement des beignets ou galettes? (1=oui / 2=non) →SI OUI, IDENTIFIER LA VENDEUSE. COLLER AUTOCOLLANT DU MENAGE SUR LA FICHE FARINE. →SI NON, CONTINUER AVEC Q42	Identification de la Vendeuse :

42. Est-ce que vous utilisez souvent des cubes pour préparer les sauces? (1=oui / 2=non) → SI NON, LE QUESTIONNAIRE EST TERMINE	
43. Marque de cube? 1=Jumbo / 2=Cube Maggi / 3=Maggi Tablette / 4=Autre:…….…. / 9=ne sait pas	

ANNEX IV: Individual questionnaire

Enregistrement :

Nom de l'infirmer :_____/Contact :_____

Date : _____/_____/ 2010 (jj/mm)

Heure : _____ :_____ (hh :min)

Consentement obtenu ?		☐
Poids	**kg**	
Taille	**cm**	

Remarques sur la santé :	Date de dernières règles:
Prise de médicament (dernière semaine) *Lister :*	Prise de supplément Vitamine A : (noter la date – voir cahier de santé) Prise d'autres fortifiants :

Prélèvement sanguin *Initiales du technicien : _____*

Résultat du prélèvement	Echantillon obtenu ☐	Sinon, remarques :
Résultat Hb		g / L
Si inférieur à 60 g / L, faire le test rapide de dépistage paludéen		

Résultat Test Palu	Positif	Négatif	*Remarques*
Traitement antipaludéen	Ok ☐		*Prescription. :*
Supplément en fer		Ok ☐	*Prescription :*
Conclusion : 1) Si le test Palu = positif, faire un traitement antipaludique sans supplémentation en fer 2) Si le test Palu négatif, prescrire supplémentation en Fer et sans traitement antipaludique			

Traitements

Autres	Prescription / Ordonnance (barre la mention inutile, détailler)

Emargement Infirmier que le sujet a reçu les traitements ci-dessus et des informations adéquates

_____

ANNEX V: Information to participants

Enquête sur le statut nutritionnel de la population ivoirienne

(enfants en âges préscolaire et femmes en age de procréation)

Qu'aimerions-nous examiner?

En Côte d'Ivoire, la population souffre de l'anémie due à un manque de fer dans l'alimentation. Cette anémie a des conséquences graves pour la croissance de l'enfant et la capacité de travail de l'adulte. Aussi, le tiers des enfants de moins de 5 ans manquent de Vitamine A. Ce manque a aussi des conséquences sérieuses pour la croissance de l'enfant et les yeux. La cécité (être aveugle) peut être due au manque de Vitamine A dans la nourriture. Les carences en Acide Folique chez les femmes en âge de procréer sont très répandues.

Troisièmement, la malnutrition sévit en Côte d'Ivoire. Beaucoup d'enfants n'ont pas assez à manger et sont pénalisés, souvent pour le reste de leur vie.

C'est pourquoi des organisations internationales et l'Etat Ivoirien ont commencé à enrichir depuis 2008 l'huile végétale avec la Vitamine A et la farine de blé avec le fer et l'acide folique. Maintenant, ils sont intéressés de connaître mieux l'effet de ces aliments enrichis sur le statut nutritionnel en Côte d'Ivoire.

L'équipe qui vous contacte est chargée d'établir le statut nutritionnel et d'évaluer si dans votre ménage, l'huile et les mets à base de farine de blé sont enrichis. C'est pourquoi nous vous demandons de participer à cette étude en tant que représentant des populations vivant en Côte d'Ivoire. Dans chaque foyer, nous travaillons avec une femme âgée entre 15 et 49 ans et tous les enfants en âge préscolaire (½ à 5 ans).

L'objectif est de :

- Recueillir un échantillon d'huile et un échantillon de farine de blé que vous consommez ;
- Mesurer l'état nutritionnel général (âge, hauteur, poids) ;
- Mesurer le taux de fer, de Vitamine A et de l'Acide Folique dans le sang des participants.

Que ferons-nous au long de cette étude?

Tout d'abord, nous vous demandons de consentir à l'étude, par écrit ou empreinte de doigt. Nous vous poserons ensuite quelques questions sur les habitudes nutritionnelles, comme « Combien de fois achetez-vous l'huile par semaine ? ». Aussi, des questions sur l'état de santé s'ensuivent, et nous répondrons bien sûr à vos questions à vous. La maman devrait répondre pour les enfants présents.

Ensuite, le poids et la taille de chaque personne seront relevés. Nous effectuerons une prise de sang chez chacun. Une piqûre intraveineuse (au bras ou au talon chez les enfants très jeunes) sera effectuée pour cela.

Que se passera-t-il avec les données?

Grâce aux analyses que nous ferons sur le sang, nous pourrons établir le statut en fer, déterminer la prévalence d'anémie et mesurer le manque en Vitamine A. Nous observerons aussi si le parasite du paludisme serait présent dans votre sang. Toutes les données personnelles seront codées et traitées confidentiellement. Nous nous engageons de ne pas faire de tests de VIH/SIDA dans votre sang.

Quels sont les risques et les avantages de participer à cette étude?

L’étude ne comporte que des risques mineurs. Les rares complications lors de la prise de sang, telles que saignement, hématome et phlébite (inflammation de la veine) seront prise en charge. La prise de sang sera exécutée par des personnes qualifiées et en utilisant du matériel sûr, nouveau et stérile.

Si vous ou un des enfants sont très anémié, ils recevront une supplémentation en fer par l’infirmier le plus proche. Les participants ayant la fièvre et autres signes de paludisme recevront immédiatement un traitement. Pour les maladies qui seront découvertes lors des analyses ultérieures au laboratoire, nous aurons besoins de prendre votre contact dans un souci purement médical afin de vous retrouver et vous traiter. Avec ces traitements, le manque aigu va normalement disparaître, mais vous devriez aussi prendre en compte les conseils nutritionnels.

Retrait de l’étude

Si vous ne voulez pas poursuivre votre participation à cette étude, vous pouvez à tout moment refuser et vous retirer sans fournir d’explications et sans préjudice.

Si vous avez d’autres questions?

N’hésitez pas à nous contacter si vous avez d’autres questions.

Chef de l’équipe : _____ Numéro :_____	Infirmier local : _____ à _____ Numéro :_____
Investigateurs: Dr. Giovanna Raso, Centre Suisse de Recherches Scientifiques, Abidjan, Tél. : 23 47 27 90 (secrétariat), Tél. mobile: 01 46 60 87 Dr. Odile Aké, Institut National de Santé Publique, Abidjan, Tél. mobile: 03 70 87 83	**Responsable médical** Dr. Odile Aké, Institut National de Santé Publique, Abidjan, Tél. mobile: 03 70 87 83

ANNEX VI: Invitation form

Coller le code du ménage :

Lieu : _____

Nom participante : _____

[Si dans un ménage, il y a plus que 3 enfants, continuer en bas de cette page.]

Noms des enfants : 1._____

2._____

3._____

Contact (chef de ménage) **:** _____

Nous vous remercions pour votre aimable collaboration. Veuillez vous présenter au lieu indiqué le plus vite possible après avoir reçu cette invitation. Vous seriez reçu par un Infirmier et un Technicien expérimenté. Une prise de sang sera effectuée et quelques mesures seront effectuées sur place.

Amener les cahiers de santé !

Si vous en avez besoin, vous recevrez des traitements pour des malaises légers et une prise en charge adéquate pour une anémie sévère ou une attaque palustre.

Après avoir compléter tous les étapes, vous recevrez gratuitement une collation composée de l'huile fortifié pour votre ménage.

Moi, enquêteur, affirme que le consentement éclairé a été obtenu :

Date : _____ Signature :_____

ANNEX VII: Point of sale form

Date :…./……/……. **Localité :**…….

1. Copier les noms des boulangers/vendeuses de la fiche de synthèse dont on prélèvera un échantillon de farine
2. Coller une étiquète de ID FARINE dans la case indiqué
3. Coller la même étiquette sur un pot de prélèvement vide
4. Coller les étiquètes de tous les ménages qui consomment le pain/gallettes de cette boulangerie/vendeuse dans les cases dans la même colonne.
5. Procéder à échantillonner la farine et noter la marque de la farine échantillonnée

	Colle ici ID échantillon farine	Colle ici ID échantillon farine	Colle ici ID échantillon farine	Colle ici ID échantillon farine	Colle ici ID échantillon farine
Nom Boulangerie / Vendeuse					
Marque farine					
Coller dans la colonne correspondante toutes les codes de ménage qui consomment cette farine					

ANNEX VIII: Summary form flour point of sale

Date :…./……/…….
N° Zone :
(**1**.Centre Ouest **2**. Abidjan) ☐

Localité :……

N° Grappe : ☐☐ **N° Equipe:** ☐

1. **Après avoir complété la grappe, noter ci-dessous les points de vente de chacun des 15 ménages enquêtés**
2. **Identifier les Boulangeries qui fournissent les Boutiquiers, si nécessaire**
3. **Identifier les échantillons requis**
4. **Copier les noms des boulangers/vendeuses dont on prélèvera un échantillon de farine sur la fiche FARINE**

ID ménage	Boutiquier	Boulanger	Vendeuse
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			

ANNEX IX: Activity summary form

Projet « Etude de l'impact du programme de fortification en Fer, Ac. Folique et Vitamine A en Côte d'Ivoire » (PIPAF)
CSRS, INSP, GAIN

Nom du département		N° du département

Contact et sensibilisation

Date	Personnes	Contact	Résultats

REMARQUES SUR LE DEROULEMENT DE LA MISSION	
DATE	**OBSERVATION**

INFORMATIONS SUR LES GRAPPES										
No grappe	Nom de la localité/quartier	Coordonnées (°N /°O)	Date sensib.	Date Enquête	Nombre Ménage	Nombre d'échantillon d'huile	Nombre d'échantillon de farine ménage	Nombre d'échantillon de farine vendeuse	Nombre d'échantillon de farine boulanger	Observations

ANNEX X: Kish table for selection of individuals at household level

Explication : Dans chaque ménage, récenser tous les habitants du ménage. Ensuite, lister les femmes éligibles (Femmes entre 15 – 49 ans), en ordre croissant de l'âge (du plus jeune au plus âges). Ensuite, regarder le correspondant des chiffres dans le tableau le Kish croisé avec le dernier chiffre du numéro du ménage. Le chiffre sortant de cette opéaration est le numéro de l'indivue à enquêter sur la liste des personnes éligibles.

TABLE DE KISH

		Dernier chiffre du numéro de ménage									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**
Nombre de personnes éligibles de la sous strate dans le ménage	**1**	1	1	1	1	1	1	1	1	1	1
	2	2	1	2	1	2	1	2	1	2	1
	3	1	2	3	1	2	3	1	2	3	1
	4	1	2	3	4	1	2	3	4	1	2
	5	4	5	1	2	3	4	5	1	2	3
	6	3	4	5	6	1	2	3	4	5	6
	7	5	6	7	1	2	3	4	5	6	7
	8	2	3	4	5	6	7	8	1	2	3

ANNEX XI: Timetable of the project

	Activités à réalisées	Date prévues	Date de réalisation	Parties responsables	Output mesurable	Remarques
1	Finalisation du protocole, traduction et soumission au comité d'éthique	15 Aug -30 Sept 2010	15 Aug -06 Oct. 2010	Chercheurs de GAIN, CSRS et INSP	Protocole d'étude, outils de collecte	
2	Commande de matériel	15 Sept - 10 Oct. 2010	15 Sept - 10 Oct. 2010	Chercheurs de GAIN, CSRS et INSP	Matériel disponible	
3	Accord du comité d'éthique	11 Oct. 2010	16 Octobre 2010	Chercheurs de GAIN, CSRS et INSP	Lettre d'accord du Ministère de la santé	
4	Formation des équipes et enquête pilote	12 - 13 Oct. 2010	11 au 12 Octobre 2010	Chercheurs de GAIN, CSRS et INSP	Guide de collecte, 4 équipes formées	
5	Collecte de terrain	14 Oct. – 05 Nov. 2010	13 au 27 Octobre 2010.	4 équipes de collecte/ DD de Bouaflé et Lagune 2 / Chercheurs du CSRS et INSP	Données recueillies sociodémographiques et alimentaires, les prélèvement sanguins, les Echantillons d'huiles et de farines	La campagne de vaccination et de supplémentation en Vitamin A a occasionné une réduction de la durée de la collecte de données sur le terrain.
9	Saisie et traitement des données	1 Nov. – 31 Déc. 2010	22 nov.- 31 Déc. 2010	Chercheurs de du CSRS et INSP	Bases de données disponibles	
10	Analyse des échantillons de sang, d'huile et de farine	1 Déc.– 28 Fév. 2011	1 Déc – 31 Mai 2011	Chercheurs de GAIN, CSRS et INSP		Les analyses d'huile et l'analyse de goutte épaisse et frottis ont eu un retard important due à la crise socio-politique. Les analyses de sang ont eu aussi un retard dû au laboratoire en Allemagne.
11	Analyse statistique	1 Mar – 30 Mar 2011	1 Juin-	Chercheurs de GAIN, CSRS et INSP		
	Rapport préliminaire	1 Avril– 31 Mai 2011	18 Mai-	Chercheurs du, CSRS et INSP		
	Validation interne	1 – 30 Jun 2011				
12	Diffusion de résultats	31 Juil. 2011				
13	Rapport final (données et financier)	31 Août 2011				