Effect of three micronutrient supplementation strategies during pregnancy on maternal hemoglobin and iron status

Statistical Analysis Plan (Addendum to "Main" SAP, version 3)

Contents

Approval

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Version History Log

This table will detail the version history for this document, including the key elements of the changes to the versions.

Version	Date implemented	Details of significant changes
1	May 27, 2014	This is the first version. It is intended as an addendum to the main SAP, Version 3. As a result, there are various aspects of the analysis that have been described in the main SAP Version 3 and will not be repeated here. Both the main SAP Version 3 and this current SAP (on Hb and markers of iron status outcomes) will guide the analysis described in this document.
2	Nov 03, 2014	This SAP has been revised from the previous (original) version dated May 27, 2014. The revision was to remove overlaps in the analyses described in this SAP and those performed at UC Davis by Dr. Brietta Oaks as part of her dissertation. Specifically, all analyses examining the associations among variables when considering women of the three groups as one cohort have been removed, as those associations have been examined in the dissertation. The revised SAP now focuses on the effects of the intervention on maternal hemoglobin and iron status, and inflammatory (CRP and AGP) markers.

1. Study objectives

The objectives of these present analyses are to:
Determine the effects of intervention on maternal hemoglobin (Hb), iron status, and inflammatory markers.

Specific objectives are to determine:

1) If, at 36 weeks of pregnancy, there were differences in Hb and iron status (zinc protoporphyrin (ZPP) and soluble transferrin receptor (TfR)) among women who received either LNS, multiple micronutrient (MMN) capsules, or iron-folic acid (IFA) capsules during pregnancy.
2) If, at 36 gestational weeks, there were differences in the prevalence of low and high Hb and low iron status among women who received either LNS, MMN, or IFA during pregnancy.
3) The effect of the intervention on the prevalence of elevated C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP).
4) Whether baseline values and gestational age at enrolment modified the effects of the intervention on Hb and iron (ZPP and TfR) status and inflammation among women at 36 gestational weeks.

2. Hypotheses

a) Women who receive IFA during pregnancy will have higher Hb and lower TfR and ZPP at 36 gestational weeks (gw) compared to the MMN and LNS groups.
b) The percentage of women with Hb below 100 g/L will be lower in the IFA group at 36 gw compared to the MMN and LNS groups.
c) The percentage of women with Hb above 130 g/L will be higher in the IFA group at 36 gw compared to the MMN and LNS groups.
d) The percentages of women with elevated ZPP and TfR will be lower in the IFA group compared to the MMN and LNS groups.
e) At 36 gw, the prevalence of elevated CRP or AGP will be greater in the IFA group than the MMN or LNS groups.

3. Outcome measures

The following outcomes will be analyzed, by intervention group, at 36 gw:

1. Continuous outcomes will include:
   Blood hemoglobin (Hb) concentration in g/L, measured by using the Hemocue autoanalyzer).

a. Zinc protoporphyrin (ZPP) concentration in μmol/mol heme, analyzed on washed erythrocytes by using the hematofluorometer of the Aviv Biomedical Co. (Lakewood, NJ, USA).
b. Soluble transferrin receptor (TfR) concentration in mg/L analyzed immunoturbidimetrically on a Roche Integra autoanalyzer.

c. C-reactive protein (CRP) concentration in mg/L, analyzed immunoturbidimetrically on a Roche Integra autoanalyzer.
d. Alpha-1 acid glycoprotein (AGP) in g/L, analyzed immunoturbidimetrically on a Roche Integra autoanalyzer.

2. Binary outcomes will include the percentage of women with:
a. Low Hb
b. High Hb
c. High ZPP
d. High TfR
e. High CRP
f. High AGP

4. Definitions

a. Low Hb, used to define anemia: Hb <100 g/L,
b. High Hb, used to define excessive Hb: Hb >130 g/L.
c. In exploratory analyses, the proportion of participants with Hb < 90 g/L or < 110 g/L, and > 145 g/L, will also be examined.
d. High ZPP representing iron deficiency: >60 μmol/mol heme.
e. High TfR used to define iron deficiency: >4.4 mg/L
f. High CRP used to define an inflammatory response: >5.0 mg/L.
g. High AGP used to define an inflammatory response: >1.0 g/L.

5. Basis for the analysis: Intention to treat

The primary analysis will be by intention-to-treat. That is, all women receiving the supplements will be included in the analysis, regardless of the number of days during pregnancy the supplement was consumed. Data on participants who were lost to follow-up because of death, travel from the study site, or refusal to continue with the study will be included in the analysis if available

6. Statistical software

Analyses will be performed using SAS version 9.3.

7. Outliers

Outliers will be visually inspected by creating box and whisker plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers which are clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers which are plausible or possible will be kept.

8. Data transformation

Distribution of outcome variables and key baseline variables will be inspected for normality and transformed as necessary. If no suitable transformation is found, normalized ranks will be calculated, or categories will be created.

9. Analytical approach

Baseline characteristics of women will be summarized as means ±SD (continuous variables) or frequencies (binary variables) and presented as shown in **Table 1**.

Outcome variables will be analyzed using mixed effects (continuous outcomes) and logistic (binary) regression models, with Tukey-Kramer adjustment for multiple comparisons. Along with the group comparisons, pairwise differences in means (continuous outcomes) and relative risks (binary outcomes) with their 95% CI and p-values will be calculated. Relative risks will be determined using Poisson regression (1). Each outcome will be analyzed twice, first without any covariate adjustments, and then with adjustment for covariates significantly associated with the outcome in a bivariate analysis. This means there may be different sets of covariates for each outcome. The results of the analysis of continuous and binary outcomes will be presented as shown in **Table 2** and **Table 3**, respectively.

10. Covariates

The covariates to be included in the ANCOVA or logistic regression models will be derived from the list below. Each variable that shows a statistically significant association with an outcome ($P<0.1$) will be included in the model.

1. Maternal BMI at enrollment
2. Inflammatory markers (CRP and AGP) at enrollment
3. Primiparity
4. Season at enrollment
5. Either Hb, or anemia at enrollment
6. ZPP at enrollment
7. TfR at enrollment
8. Gestational age at enrolment
9. Age
10. Education
11. Assets index
12. Housing index
13. HH food insecurity index

11. Interaction

We will assess the effect modification by gestational age at enrolment and the baseline values of the outcomes measure. Where the effect modification is significant (alpha <0.10), we will

include an interaction term between treatment and the effect modifier in the mixed-effect or logistic regression model. Each effect modifier will be considered separately in the models to avoid collinearity. Potential effect modifiers to be examined (as continuous variables where possible) are:

1. Anemia status at enrollment
2. ZPP at enrollment
3. TfR at enrollment
4. CRP at enrollment
5. AGP at enrollment
6. Gestational age at enrolment

Results of these sub-group analyses will be presented as shown in Table 4.

Reference

1. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. Am J Epidemiol 2005;162(3):199-200. doi: 10.1093/aje/kwi188.

Table 1
Baseline characteristics of women who completed the study

Variable	**IFA** ($\bar{x}$ ± SD) [n]	**MMN** ($\bar{x}$ ± SD) [n]	**LNS** ($\bar{x}$ ± SD) [n]
Number of participants	xxx	xxx	xxx
Age (y)	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]
Years of formal education	xx.x ± x.x [xx]	xx.x ± x.x [xx]	xx.x ± x.x [xx]
Married or cohabiting (% [n])	xxx.x [xxx]	xxx.x [xxx]	xxx.x [xxx]
Proxy for socioeconomic status	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.x ± x.x [xxx]
Primiparous women (% [n])	xxx.x [xxx]	xxx.x [x]	xxx.x [xxx]
Weight (kg)	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]
Height (m)	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.x ± x.x [xxx]
BMI (kg/m^2)	xx.x ± x.x [xxx]	x.x ± x.x [xxx]	x.x ± x.x [xxx]
Women with a low BMI (< 18.5 kg/m²) (% [n])	xxx.x [xxx]	xxx.x [xxx]	xxx.x [xxx]
Mid upper arm circumf. (cm)	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]
Triceps skinfold (cm)	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]
Gestational age at enrolment (wk)	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]
Positive rapid test for malaria (% [n])	xx.x [xxx]	xx.x [xxx]	xx.x [xxx]

Table 2: Adjusted and unadjusted differences between groups in continuous variables measured at baseline and 36 gestational weeks

Variable		**IFA [n]**	**MMN [n]**	**LNS [n]**	**P-value**	**Comparison of IFA and MMN**		**Comparison of IFA and LNS**		**Comparison of MMN and LNS**	
						P-value	**Difference in means or medians (95 % CI)**	**P-value**	**Difference in means or medians (95 % CI)**	**P-value**	**Difference in means or medians (95 % CI)**
Hb (g/L) **($\bar{x}$ ± SD) [n]**	Baseline	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
	36 GW	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
ZPP µmol/mol heme (geometric mean (95% CI) [n]	Baseline	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
	36 GW	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
TfR (mg/L) (geometric mean (95% CI) [n]	Baseline	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
	36 GW	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
AGP (g/L)	Baseline	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
	36 GW	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
CRP (mg/L)	Baseline	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)
	36 GW	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.x (x.x, x.x) [x]	x.xxx	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)	x.xxx	x.xx (xx to xx)

Table 3 Differences between groups in the proportions of women with Hb, ZPP, TfR, AGE or CRP above or below specified cutoffs.

		IFA n (%)	**MMN n (%)**	**LNS n (%)**	**P-value**	**Comparison of IFA and MMN**		**Comparison of IFA and LNS**		**Comparison of MMN and LNS**	
						Risk ratio (95 % CI)	**P-value**	**Risk ratio (95 % CI)**	**P-value**	**Risk ratio (95 % CI)**	**P-value**
Hb < 100 g/L	Baseline	x (x.x)	x (x.x)	x (x.x)	0.xx		0.xx		0.xx		0.xx
	36 gw	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx
Hb > 130 g/L	Baseline	x (x.x)	x (x.x)	x (x.x)	0.xx		0.xx		0.xx		0.xx
	36 gw	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx
ZPP > 60.0 µmol/mol heme	Baseline	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx		0.xx		0.xx
	36 gw	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx
TfR > 4.4 mg/L	Baseline	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx		0.xx		0.xx
	36 gw	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx
High AGP (1.0)	Baseline	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx		0.xx		0.xx
	36 gw	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx
High CRP(>5.0)	Baseline	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx		0.xx		0.xx
	36 gw	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx	x.xx (x.xx - x. xx)	0.xx

Subgroup analysis of continuous outcomes at 36 gestational weeks[1]

Outcome variable	IFA[2]	MMN[2]	LNS[2]	P[3]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
					Mean difference or RR	p	Mean difference or RR	p	Mean difference or RR	p
Outcome										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Outcome										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Outcome										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Outcome										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Outcome										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx

[1]IFA= Iron-Folic Acid group received 60 mg iron plus 400 µg folic acid; MMN=Multiple Micronutrients group received 1-2 RDAs of 18 vitamins and minerals (including 20 mg iron); LNS=Lipid-based nutrient supplement (LNS) group received LNS with same micronutrients as the MMN group, plus another four minerals (Ca, P, K and Mg) as well as macronutrients. All three supplements were intended for daily consumption.

[2]Data are means ± SE for continuous effect modifiers, or means ± SE [n] for binary effect modifiers.

[3]P-values with asterisks are for interaction. P-values without asterisks compare all three groups in each stratum.