Evaluation of The Impact of Vitamin A Fortified Cooking Oil Consumption in Indonesia

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ABSTRACT

Vitamin A deficiency remains a leading cause of morbidity and mortality in Indonesian children and women. In 2011, fortification of unbranded palm oil with retinyl-palmitate began in selected districts on a voluntary basis. As a pre-post evaluation, we assessed consumption of fortified oil, changes in vitamin A intake and retinol status in 2 surveys of women and children, just before fortification started and a year later. Poor households were randomly sampled from twenty-four peri-urban villages in two districts on West Java. Serum retinol (adjusted for sub-clinical infection) was analyzed in cross-sectional samples of lactating mothers and their infants 6-11 months, and children 12-59 months, and in cohorts of children 5-9 years and women 15-29 years, alongside household socio-economic conditions, and individual-level oil and food consumption from single 24-hour dietary recall. Fortified oil improved vitamin A intakes, contributing an average 26%, 40%, 38%, 29% and 35% of daily Recommended Nutrient Intake (RNI) for children 12-23 months, 24-59 months, 5-9 years, lactating and non-lactating women, respectively. Serum retinol was 2-19% higher at endline compared to baseline ($p<0.001$ in infants 6-11 months, children 5-9 years, lactating and non-lactating women; $p=0.057$ in children 24-59 months; non-significant in children 12-23 months). Retinol in breast milk averaged 20.5 µg/dL at baseline, 32.5 µg/dL at endline ($p<0.01$). Deficiency prevalence (retinol <20µg/dL) was 6.5-18% across groups at baseline, and 0.6-6% at endline ($p<0.011$, all groups). In multivariate regressions adjusting for socio-economic differences, fortified oil consumption predicted improved retinol status for children 6-59 months ($p=0.003$) and 5-9 years ($p=0.03$). Although this evaluation without comparison group cannot prove causality, retinyl content measured in oil samples, RNI contributions and the relation between vitamin A intake and serum retinol across age groups provides strong plausibility of oil fortification impacting vitamin A status in Indonesian women and children.

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EXECUTIVE SUMMARY

Background

Until the 1990s vitamin A deficiency (VAD) remained a severe public health problem in Indonesia. Although VAD has been steadily declining over the last three decades in South East Asia, large pockets of sub-clinical vitamin A deficiency appear to persist, particularly among the poor [Rohner 2013].

Since 1978, the government of Indonesia has scaled up the distribution of high-dose vitamin A capsules to young children (6-59 months) to include all areas of the country. This program consistently reaches about 70% of eligible children.

The Global Alliance for Improved Nutrition (GAIN) supported the Indonesian Nutrition Foundation for Food Fortification (KFI; formerly 'Koalisi Fortifikasi Indonesia') to undertake a pilot fortification project, and to scale it up to a national mandatory program for cooking oil fortification with vitamin A. Voluntary fortification of unbranded cooking oil with vitamin A started in two provinces of Indonesia (East and West Java), and an effectiveness study was undertaken in 2 districts of West Java (Tasikmalaya and Ciamis) in July 2011, where one oil refinery, Sinar Alam Permai (PT SAP) has a majority market share of the cooking oil market.

Methods

The objective of this study was to measure the impact of vitamin A fortification of unbranded cooking oil on serum retinol concentrations and VAD prevalence in women and children. Its two cross-sectional surveys also measured prevalence of anemia and iron deficiency based on hemoglobin, serum ferritin, and soluble transferrin receptor (sTfR) concentrations and anthropometry (length/height and weight) for children 6-59 months.

The study was designed with two components: two consecutive cross-sectional surveys, and a cohort followed during the year between the two surveys, and also included in the surveys. Cross-sectional groups were children 6-59 months (n=787 baseline, 812 endline) and lactating mothers (n=324 baseline, 349 endline). Cohort groups consisted of school-age children 5-9 years (n=248 enrolled at baseline, 186 retained at endline and analyzed) and non-lactating women of early reproductive age (15-29 years, n=252 enrolled at baseline, 171 retained at endline and analyzed).

The surveys were conducted in 24 peri-urban villages (12 in each district), selected by two-stage sampling. Within villages, a random sample of eligible subjects was selected from among poor households, defined according to their possession of a government-issued poor-family card. The sample population was thus not selected to be representative of the districts or province, but as a population suspected to be at higher risk of poor vitamin A status. Household and subject sampling framework, eligibility criteria and procedures were the same at baseline and endline.

For the cohort groups, over the year between the two surveys self-reported morbidity was measured, through 2-weekly questionnaires administered by the district supervisor and village nurse/midwife. At these same visits oil samples were taken for quantification of vitamin A levels in oil consumed in households.

This was a collaborative study between the National Institute of Health Research and Development (NIHRD), Ministry of Health, Indonesia (NIHRD-MoH), KFI and GAIN. The baseline survey was supported by NIHRD-MoH and the endline survey was supported by GAIN.

Results

Household socio-economic conditions
Households sampled were generally poor, with 37-53% at baseline, and 26-44% at endline in the lowest expenditure quintile compared to nation-wide expenditure quintiles. Households' socio-economic status improved from baseline to endline, as evidenced by significant decreases in the proportion of households in the lowest expenditure quintile and in households' proportional expenditure on food, as well as improved housing condition scores and household capital asset scores. These shifts were apparent in both the cross-sectional and the cohort groups, suggesting real changes (rather than unintended sampling biases) over the study year.

Vitamin A levels in oil
Oil sample measurements found retinyl palmitate levels averaging at 43.6 IU/g at factory level (n= 20 samples), 28.3 IU/g at distributor level (n= 28 samples), 25.7 IU/g at small stalls (n= 54 samples) and 28.5 IU/g in households (n= 75 samples) – compared to the Indonesian National Standard (SNI) level of 45 IU/g for oil leaving the factory.

Vitamin A intake from fortified oil and from foods
Fortified oil considerably improved VA intakes, measured against Recommended Nutrient Intake (RNI) standards, contributing an average 4% of daily RNI for infants 6-11 months, 26% in children 12-23 months, 40% and 38% in children 24-59 months and 5-9 years and 29% and 35% for lactating and non-lactating women, respectively. This improvement occurred without any increase in overall oil intake in any group, as expected given that the program had not advertised or promoted fortification or oil consumption.
In comparison, vitamin A RNI contributions from animal food consumption (at endline) were estimated at 19% in infants 6-11 months (excluding breast milk consumption), 22-48% in older children, and a low 10% and 14% in lactating and non-lactating women, respectively.

Vitamin A status and its determinants
The study found marked increases in serum vitamin A status from before to after the start of consumption of vitamin A-fortified cooking oil, in all groups and for both serum retinol and retinol-binding protein (RBP) as indicator of VA status. Serum retinol and RBP concentrations correlated significantly and consistently over time. Also the prevalence of VAD fell markedly, by 64% in children 12-23 months to as much as 96% in children 24-59 months, an average reduction of 76% (64-96%) across groups. These improvements were statistically significant in all groups. Furthermore, retinol in breast milk increased by 58% ($p<0.01$).

The contribution of VA from fortified oil on improvements in serum retinol concentration was further assessed in multivariate regression analysis, adjusting for household capital assets, household sanitation, housing condition, expenditures on food (as indicator of poverty) and vitamin A capsule (VAC) supplementation among children 6-59 months. When analyzed at the level of village average outcomes (N=24), the estimated vitamin A RNI contribution from cooking oil (as village average) was positively associated with a larger increase in serum retinol concentration from baseline to endline (as village average), and this effect was significant for children 6-59 months ($p=0.003$) and children 5-9 years ($p=0.03$).

No regression model could adequately predict serum retinol improvements for lactating and non-lactating women, whether analyzed at village level or at individual level. In multivariate regressions, socio-economic variables did not independently influence serum retinol status

Child growth
Child stunting fell in infants 6-11 months (from 26.5% to 9.9%, $p<0.01$) between the 2 surveys. At least in part this may be due to higher socio-economic status of the sample at endline. Also methodological issues cannot be ruled out, because the standard deviation (SD) at baseline was large compared to the endline, suggesting greater error of measurement in the baseline survey. There

was no change in weight for age, and there were no consistent changes in wasting among children 6-59 months.

Self-reported morbidity
Self-reported morbidity decreased over eight months of cohort data analyzed in women 15-29 years ($p<0.001$), but not significantly so in children 5-9 years. In both cohorts, there was a striking relation between lower baseline serum retinol status and higher reported morbidity. The relation between lower baseline serum retinol and higher morbidity weakened over the months, as morbidity declined most in those children and women with worst retinol status at baseline.

Anemia and iron status
There were no significant changes in anemia prevalence in any group, and (except for children 5-9 years) no significant improvements in mean hemoglobin concentrations. Neither did the iron status markers serum ferritin and sTfR concentrations show any consistent significant changes across groups.

Limitations

The study's most important limitation was the absence of a control group, due to which results cannot conclusively be interpreted as demonstrating impact of the oil fortification. The inclusion of a control group was not deemed ethically or operationally feasible, the latter due to a rapid scale-up of fortification of vegetable oil. Socio-economic conditions of sampled households were better at endline than at baseline. In the cohorts, this must have reflected real socio-economic improvements, while in non-cohort groups possibly unintended differences in household sampling may have contributed. Whether secular trends or sampling differences, the socio-economic shifts from baseline to endline are likely to have influenced vitamin A status and health outcomes, although multivariate regressions attempted to adjust for these.

Key data limitations include a simplified dietary recall analyzed using a national food composition table developed over a decade ago, measurement error (of unknown magnitude) of serum retinol and RBP levels in both survey rounds; measurement error of infant and child height especially at baseline, and small sample sizes for vitamin A measurement in household cooking oil which were extrapolated to other households in the same village. While these data limitations did not produce any clear systematic bias in either baseline or endline results or their difference, they likely caused non-differential mis-classification in outcomes. Therefore, we believe that the effect of fortified oil consumption on vitamin A status may in reality have been greater than observable in these data.

Conclusions & Programming implications

In conclusion, this pilot project demonstrates the possibilities of large-scale oil fortification to improve poor people's vitamin A intake and status. Serum vitamin A status improved markedly over the first year of distribution of vitamin A fortified cooking oil in all study groups. This notably included infants 6-11 months, in whom the benefits may reflect improved maternal nutrition during pregnancy and lactation.

Study findings should be interpreted with caution, given the lack of a control group and above mentioned data quality limitations. The vitamin A status results likely reflect at least in part an impact of the fortified oil. The observed changes in child growth are likely a result of significant socio-economic developments (an ongoing secular trend in Indonesia), possibly a sampling difference between the surveys, data quality issues, or a combination of these factors.

Meanwhile, findings suggest that there is much to be gained from improving vitamin A status in Indonesia. Although this report could not show definitive results of the program on functional outcomes, improved vitamin A status is expected to improve immunity and reduce morbidity from infectious diseases, and the observed decline in morbidity in women 15-29 years, and weakening of associations between poor vitamin A status and morbidity over the study year in women and children 5-9 years, are consistent with that. Furthermore the increased concentration of vitamin A in breast

milk provides an important source of the nutrient for small children. While Indonesia no longer meets the WHO criteria for having a severe vitamin A public health problem, fortification is an important population-based strategy to ensure continued and consistent intakes of vitamin A.

To optimize the impact of the oil fortification program that is now launched nation-wide, adequate QA/QC procedures and enactment will be essential to assure universal industry compliance with fortification standards. Further, a comprehensive monitoring and evaluation of availability and consumption of fortified oil, and ideally of health effects, with follow-up surveys in the study area and other sentinel sites will need to be conducted. Health impact evaluations might consider using serum RBP concentration as a cheaper VA status indicator than serum retinol concentration.

Indonesia is a leading global supplier of cooking oil for neighboring countries, which have a vitamin A deficiency burden and universal consumption of palm oil as the major source of fat. The program therefore has tremendous potential to reduce vitamin A deficiency and its associated burden of morbidity and mortality throughout Asia and beyond in a highly cost-effective way.

LIST OF ABBREVIATIONS

AGP	Alpha-1-acid-Glycoprotein
Balitbangkes	Badan Penelitian dan Pengembangan Kesehatan (National Institute for Health Research and Development – NIHRD)
BBIA	Balai Besar Industri Agro (Agroindustrial laboratory in Bogor)
BL	Baseline survey (2011)
CRP	C-reactive protein
EL	Endline survey (2012)
ELISA	Enzyme-Linked Immunosorbent Assay
GAIN	Global Alliance for Improved Nutrition
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit
HAZ	Height for Age Z-score (indicator of stunting)
HH	Household
HPLC	High Performance Liquid Chromatography
IPB	Institut Pertanian Bogor (Bogor Agricultural Institute)
IU	International Units
KFI	Indonesian Nutrition Foundation for Food Fortification
NIHRD	National Institute for Health Research and Development
PT SAP	PT Sinar Alam Permai (member of Wilmar Group)
Puslitbang Gizi	Center for Nutrition Research and Development
QA/QC	Quality Assurance / Quality Control
RBP	Retinol Binding Protein
RNI	Recommended Nutrient Intakes
SAFO	Strategic Alliance for Fortification of Oil (and other staple foods)
SCI	Sub-clinical Infection/Inflammation
SD	Standard Deviation
SUSENAS	Survei Sosioekonomi Nasional (National Socio-economic Survey)
sTfR	Soluble Transferrin Receptor
VA	Vitamin A
VAC	Vitamin A Capsules
VAD	Vitamin A Deficiency
WAZ	Weight for Age Z-score (indicator of underweight)
WHZ	Weight for Height Z-score (indicator of wasting)

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I. BACKGROUND

I.1. Vitamin A deficiency in Indonesia

In Indonesia, subclinical vitamin A deficiency (VAD) was estimated in 1976 to affect up to 50% of pregnant women and preschool-age children, and to be a leading cause of preventable pediatric blindness, as well as to increase susceptibility to severe infection and death [Soekirman et al 2012]. VAD, defined as a serum retinol concentration of less than 20 µg/dL or 0.7µmol/L, persisted as a public health problem, affecting 5%-50% of different population sub-groups according to different studies [Wieringa et al 2003; Herman et al 2006], but showing a steady decline since the mid-1970s [Muhilal 1994; Sandjaja 2013]. Vitamin A status of mothers and infants are closely linked, as a mother with VAD cannot provide sufficient vitamin A to her child to build body stores during the last months of pregnancy nor through her breast milk, resulting in a high risk for deficiency in the infant [Dijkhuizen et al 2001]. The importance of maternal nutrition for child growth has been recently reconfirmed [Black et al 2013].

The main cause of VAD is insufficient dietary intake of vitamin A, which is found in animal source foods as preformed vitamin A (retinol) and in plant source foods as provitamin A. The predominant diet in Indonesia is rice- and vegetable-based, and provitamin A has low bioavailability [De Pee et al 1995; Campbell 2009]. When access to food is limited, poor households decrease variety and quality of their diets in an effort to satisfy energy needs, which further increases the risk of micronutrient deficiencies such as VAD [West et al 2010; Institute of Medicine 2001; Torlesse et al 2003; Murphy&Allen 2003]. Improving vitamin A status in vulnerable groups is likely to impact many different outcomes such as resistance to infection [Ross et al 1996], and possibly linear growth of children [Ramakrishnan et al 2004; Donnen et al 1998]. Further, several studies have shown that vitamin A supplementation improves the mobilization of iron from stores for erythropoiesis, thereby increasing hemoglobin concentrations and reducing anemia prevalence [Suharno et al 1993] and ferritin and transferrin receptor concentrations [Zimmermann et al 2006].

I.2. Strategies to reduce Vitamin A Deficiency

High-dose vitamin A supplementation to children 6-59 months of age has been shown to reduce their mortality by ~25% [Imdad et al 2011; Beaton 1994], and a semi-annual vitamin A capsule (VAC) distribution program to children in this age group has been implemented in Indonesia since 1978. However, approximately 30% of eligible Indonesian children are not reached by the VAC distribution [Departemen Kesehatan RI 2008 and 2010], and it is speculated that those missed are the children with worst nutritional status, growth faltering and infectious disease prevalence [Berger et al 2007]. Moreover, the impact of VAC on vitamin A status in the children that do receive the capsules is small and transient [Pangaribuan et al 2003]. Strategies to improve vitamin A status of infants below 6 months of age are even more challenging [Ayah et al 2007]. In addition, other age groups at risk for VAD are not targeted by VAC, notably school age children and women of reproductive age. Therefore, large-scale food fortification with vitamin A is a preferred complementary strategy to improve the vitamin A status of the Indonesian population more broadly, as low dose vitamin A is more efficiently absorbed and utilized [Muhilal 1988], particulary in an oil-based media.

Fortification of vegetable oil with vitamin A is considered a cost-effective, simple-to-implement strategy [Allen et al 2006; West&DarntonHill 2008; Martianto et al. 2005; Martianto et al. 2007]. The oil matrix stabilizes retinol and delays its oxidation [Dary&Mora 2002], which supports the evidence that vegetable oil, is a good candidate for vitamin A fortification. Retinyl palmitate is common in vitamin A fortification, and has been used successfully in fortifying sugar, monosodium glutamate and wheat flour [Arroyave G et al 1981; Solon et al 2000] in addition to cooking oil and margarine [Muhilal et al 1988; Solon et al 1996; Dutra-de-Oliveira 1998; Dutra-de-Oliveira 1998a].

I.3. Cooking oil use in Indonesia

Most of the cooking oil produced in Indonesia is exported. Malaysia and Indonesia are the largest exporters in the world, producing enough cooking oil to cover millions of preschool children in some of the highest risk regions of the world [Laillou et al 2013].

The portion for domestic consumption is marketed using two approaches: about 30% is sold in larger quantities of branded oil in supermarkets, much of which is voluntarily fortified with 45 IU/g vitamin A according to the current, voluntary national standard. Approximately 70% of the oil is sold as unbranded and unpackaged oil in small neighborhood shops/stalls (*warung*). Consumers bring their own bottle to be filled from a bulk container or buy small plastic sachets of oil [Soekirman et al 2012]. A small demonstration project sponsored by the Japanese Fund for Poverty Reduction and the Asian Development Bank undertaken in Makassar, South Sulawesi showed that cooking oil fortification with vitamin A improved serum retinol concentrations of school children [Martianto et al. 2007; Martianto et al 2009] and was acceptable to poor consumers.

As of 2014, the Indonesian Ministry of Industry is in the process of rendering fortification of unbranded cooking oil with vitamin A mandatory. This will provide fortified oil to practically all families in Indonesia at risk for VAD. Through the mandatory fortification initiative, it is estimated that by 2015, over 4 million metric tons of unbranded vegetable oil sold to Indonesian consumers will be fortified with vitamin A, reaching more than 200 million Indonesians, of which an estimated 125 million are women of reproductive age and children younger than 12 years. Ultimately, it will be mandatory for all cooking oil to be fortified but the first and most important step is the fortification of unbranded oil as it reaches the majority of the population, in particular the poor.

Cooking oil is transported from the factory to the distribution centers by company vessels. Then the oil is picked up by mobile tank trucks from the distributor to supply several districts, and then to sub-distributors in sub-districts, via several retail levels until finally it reaches the small stalls in villages catering directly to the consumers. At each level below the district distributor (who has a contract with the producer), there is a possibility of mixing the fortified oil with oil from other producers, which is currently still unfortified. This will not be the case anymore when the mandated law is enacted.

As a prerequisite for mandatory fortification of cooking oil with vitamin A, an effectiveness study was conducted in West Java province during 2011-12. The study received support from GAIN and was implemented in cooperation with one of the largest cooking oil producers, who has a majority market share in the two study districts (PT SAP of the Wilmar Group). On a larger scale, as part of the start of the national fortification, KFI worked with two cooking oil producers (Wilmar Group and Musim Mas). Therefore, outside the study area, more areas and people were reached by vitamin A-fortified cooking oil since 2011, but this report presents the findings of the effectiveness assessment in the two districts of West Java.

II. OBJECTIVES

II.1. Overall objective

To measure the impact of fortification of unbranded cooking oil with vitamin A on vitamin A status of pre-school and school-age children, non-lactating women of (early) reproductive age, and lactating mothers.

II.2. Specific objectives

1. To assess the vitamin A status of selected vulnerable population groups, i.e. children 6-11, 12-23, 24-59, 60-108 months (5-9 years) of age; as well as lactating women (mothers of the children 6-11 months), and women of early reproductive age (15-29 years, non-pregnant, non-lactating) by measuring ***concentrations of retinol*** *(and RBP, as secondary vitamin A status marker)* ***in***

serum and breast milk, before the distribution of the vitamin A fortified cooking oil (July 2011) as compared to 12 months after its introduction (July 2012).

2. To determine the presence of acute and chronic inflammation in the selected population groups by ***measuring concentrations of C-reactive Protein (CRP) and α-1-acid-glycoprotein (AGP)*** to facilitate the interpretation of retinol concentrations in serum/breast milk and serum ferritin.

3. To determine the ***prevalence of anemia and iron deficiency*** among the target groups before the distribution of the vitamin A fortified cooking oil as compared to 12 months after its introduction (year 2).

4. To assess the ***household coverage of fortified oil*** by assessing the vitamin A content of vegetable oil sampled from participating households or, if no oil is available at a specific household, from the small stall where that household usually purchases vegetable oil.

5. To ***evaluate the possible effectiveness and/or impact*** of vitamin A fortified cooking oil consumption on the serum retinol concentrations of the target groups by controlling for determinants and confounders such as high-dose vitamin A capsules, other micronutrient supplements, other vitamin A-fortified foods and socio-economic status.

II.3. Study hypothesis

The hypothesis tested by the study was that the fortification of cooking oil with vitamin A will lead to a 10% increase from baseline in mean retinol concentrations in serum and in breast milk, among women 15-29 years, lactating mothers, preschool children, and school children 5-9 years old.

III. METHODS

III.1. Organization of the study

The study was collaboration among three institutions, namely the National Institute of Health Research and Development (NIHRD) of the Indonesian Ministry of Health, the Indonesian Nutrition Foundation for Food Fortification (KFI) and the Global Alliance for Improved Nutrition (GAIN). Other partners in the study were the PT Wilmar group (notably PT SAP), the District Health Offices of Tasikmalaya and Ciamis, Prodia Laboratory, Vit A-Iron Lab (Germany), BBIA Laboratory and GIZ-SAFO.

III.2. Study design

The study worked with PT SAP (Wilmar Group) as the most important refinery of cooking oil in the study area. Although they claimed to have over 50% market share, the actual size of their market share never entirely became clear. The oil was distributed through the normal market system and the effect on serum retinol concentrations and other biological indicators was assessed through a baseline survey prior to distribution of fortified oil in July 2011, and an endline survey in July 2012. The fortified oil produced by PT Sinar Alam Permai (PT SAP) of Wilmar Group in Palembang (South Sumatera province) was shipped by tug-towed barge to their distribution center in Cirebon (West Java province), which supplies Wilmar distributors in several districts of West and Central Java. At the time of loading the barge, the premix was added to the cooking oil. From Cirebon it was transported by truck to various district distributors.

The basic study design was a pre-post evaluation after the introduction of an intervention, i.e. the fortification of unbranded cooking oil. No control group could be included in the study, since the market-based distribution system did not allow for a relevant control area to be included. The study focused on vulnerable age groups: infants and young children, school children, lactating women and non-lactating non-pregnant women of (early) reproductive age (15-29 years). Because of the narrow

age ranges of children 6-59 months in the study, and the short duration of lactation compared to the 1-year interval between the two surveys, it was not possible to do a cohort study for children 6-59 months (for whom results were analyzed in age sub-groups) and for lactating mothers. Therefore, the following study approaches were used:

Two repeat cross-sectional surveys on:
1) Breast-fed infants 6-11 months,
2) Lactating mothers of those infants,
3) Children 12-23 months,
4) Children 24-59 months.

Two longitudinal cohort follow-up rounds on:
5) School-age children aged 60-108 months (5-9 years),
6) Women of early reproductive age (15-29 years).

III.3. Eligibility and sampling of study villages, households and respondents

The sampling universe for this study included poor households with a breastfeeding mother and her infant aged 6-11 months, or at least one child between 12 months and 9 years old, or a woman 15-29 years old, in the districts of Tasikmalaya and Ciamis, in West Java province. These districts were selected based on an anticipated high market share of unbranded cooking oil produced by PT SAP and relatively little exposure to fortified oil at baseline.

Eleven poor peri-urban villages and the largest town were selected from each district, for a total of 24 villages/sites, through a two-stage sampling (four sub-districts in each district, and then 3 villages/sites selected within each sub district), from among villages considered relevant in dialogue with producer PT SAP, based on their expected coverage of villages with the fortified oil.

A household was considered to be poor if they had a government-issued “poor family” card (considering all three types of cards issued by village heads in the years 2009-2011: *PKH* (*Program Keluarga Harapan* or conditional cash transfer), *Jamkesmas* and *Jamkesda* (*Jaminan Kesehatan Masyarakat* and *Jaminan Kesehatan Daerah*, respectively, i.e. health insurance). Thus, the sample population was not selected to be representative of the districts or province, but as a population suspected to be at higher risk of low serum retinol.

From among households listed as poor according to poor-family cards (see above), households were selected for the presence of an infant aged between 6-11 months at planned time of baseline, living with its mother in the household, and predominantly breastfed. Detailed respondent eligibility criteria are listed in **Annex 5**. The targeted sample size (see below) was then realized by random sampling from the total sampling framework of households fulfilling these criteria.

The same random sampling procedure was repeated to sample respondents in other age groups: of all households with a valid poverty card, those with one or more children aged 12-23 months, 24-59 months or 5-9 years, or a non-lactating women 15-29 years were listed and the required number of households then randomly selected. Per household, only one subject per age group could be selected; however the study did allow selection of one or more children, women or a child and a woman from one household into different study groups. In cases where two subjects in the same age group had been selected from one household, the youngest of these two was kept, but the oldest replaced by a subject from a different poor household, additionally sampled randomly from the remaining poor households in the sampling framed. This household sampling procedure, within the same 24 villages, was followed without modification at both baseline and endline surveys.

III.4. Sampling unit and sample size

The sample size was calculated to provide sufficient power to detect an increase of 10% or more in mean retinol concentration in serum or breast milk as main study outcomes, with a confidence interval of 95% (alpha = 0.05) and a statistical power of beta = 0.80, for preschool and school age children and women of early reproductive age. For lactating mothers, the sample was set to detect a serum retinol increase of 20% or more. To control for cluster-design effect (given the two-stage village sampling) and dropout, the calculated sample size was doubled and then increased by 10% (**Table 1**). Since lactating mothers and infants 6-11 months were selected as mother-child pairs, the numbers of samples in those groups are the same.

Table 1. Sample size determination based on mean serum retinol concentrations

Target group	*Expected mean serum retinol (µg/dL)*	*α*	*β*	*SD*	*Expected impact (d)*	*Crude sample size*	*Adjusted sample size**	**Reference for expected mean**
Infants 6-11m	20	0.05	0.80	0.20	0.10	84	**≈ 320**	Dijkhuizen, 2004
Children 12-23m	27.1	0.05	0.80	0.25	0.10	99	**≈ 220**	Webb, 2009
Children 24-59m	27.1	0.05	0.80	0.25	0.10	99	**≈ 220**	Webb, 2009
Children 5-9y	30	0.05	0.80	0.25	0.10	99	**≈ 220**	Nga, 2009
Lactating mothers	25.7	0.05	0.80	0.60	0.20	188	**≈ 320**	de Pee, 1997
Non-lactating women 15-29y	28.6	0.05	0.80	0.30	0.10	142	**≈ 250**	Dijkhuizen, 2004

*The adjusted sample size takes into account a design effect of 2 [Statistics Indonesia, 2007 DHS] and a 10% drop-out [Martianto et al. 2009].

III.5. Study period

Distribution of fortified oil in the study area started in July 2011. Baseline data were collected in June - July 2011 and endline data in June - July 2012. This was purposively planned to minimize bias from high dose VAC distributed to children under five years that takes place every year in February. It takes three to six months for the effect of high dose VAC on serum retinol concentrations to disappear [Gorstein et al 2003, Perlas et al 1996, Mason et al 2011, West et al. 1999]. The original contract for the study ended on 31 January 2013 but was amended to 31 December 2013 to accommodate the long process of data validation.

III.6. Recruitment and training

Enumerators for the study were 24 local nutritionists from the District Health Offices working at *puskesmas* (Community Health Centers) in the area. They were trained prior to field implementation of baseline and endline surveys. The three-day trainings covered the overall objectives of the study, eligibility of subjects, questionnaires, informed consent forms and field management. For the endline the same enumerators were used, but two in each district were replaced for health reasons.

A district coordinator was appointed for each district to coordinate the study implementation. They were trained with the enumerators and also spent extra time with the principal investigator and study team including international technical support officers during their monthly supervision.

III.7. Data collection

III.7.a. Questionnaires

A structured questionnaire was developed and used during both baseline and endline surveys to collect data on socio-economic status and composition of the household, single individual-level 24-hour dietary recall and history of disease. Data on socio-economic status included household expenditure, household expenditure on food, including specifically on cooking oil, household capital

assets, sanitary conditions, housing condition, food consumption patterns, access to electricity, family size and highest educational level in the household (see **Annex 2**).

Households of all cohort members (children 5-9 years and women 15-29 years) were visited every two weeks during the study period by the district supervisor and village nurse/midwife for collection of a household oil sample and a morbidity history form. Five types of morbidity were monitored: 1) Acute Respiratory Infection (ARI), 2) Pneumonia, 3) Diarrhea, 4) Measles, and 5) Other. Morbidity history covered the two weeks preceding the visit. In practice, this cohort follow-up was realized to fullest extent only between October 2011 and May 2012, while fewer visits and observations happened in the remaining months. For this reason, the provisional morbidity analysis shown in this report includes data from October 2011 to May 2012 only.

III.7.b. Anthropometry

Anthropometric data were collected on all subjects using standardized equipment for measuring weight and length/height. Recumbent length of infants under 24 months was measured using a length board to the nearest 0.1 cm. Height of children older than 24 months and women was measured using microtoise to the nearest 0.1 cm. All subjects were weighed using standardized digital scales with accuracy of 0.1 kg. All measurements were carried out using WHO standard methods [World Health Organization 2006]. For children under five years, these measurements were converted to Z-scores using international reference weights and heights [WHO 2006]. Body Mass Index (BMI) was calculated for women of reproductive age and lactating mothers.

III.7.c. Blood

Blood collection, aliquoting and analysis of serum retinol concentration were performed by Prodia Laboratory ("Prodia") in Jakarta, an internationally certified laboratory, using the same procedures and methodologies at baseline and at endline.

Venous blood samples (5mL) were collected into plain glass evacuated tubes from the arm in women and children above one year old. Respondents were asked to come to a health facility or central village facility where experienced phlebotomists carried out the blood collection. If the phlebotomist failed to obtain the blood sample on the first attempt, with permission they could try a second time. When both attempts were unsuccessful, the respondent was excluded. Hemoglobin was measured on the spot using a portable Hemocue^{TM} device and suitable hemocuvettes (Hemocue, Aangelsborg, Sweden). Results were recorded on the individual form and communicated to the respondent. Quality control was conducted every morning using 2-level controls provided by the Hemocue manufacturer.

Serum was separated from the remaining whole blood in the field by centrifugation at room temperature for 8 min at 3000-x g and divided into aliquots, which were stored at -80^{0}C until further analysis. At least one, and where possible, two reserve samples were aliquoted. All samples were transferred and stored in labeled Eppendorf-tubes, and kept in labeled racks.

A first tube was used to collect 500 µL serum for the measurement of retinol concentration using HPLC by Prodia. A second tube (small Eppendorf provided by the Southeast Asian Ministers of Education Organization (SEAMEO) Tropical Biology) was used to collect 200 µL serum for the analysis in the Vita Iron Lab, Germany, of retinol binding protein (RBP), C-reactive protein (CRP), alpha-glycoprotein (AGP), ferritin, and soluble transferrin receptor (sTfR) by sandwich enzyme-linked immunosorbent assay (ELISA) [Erhardt et al 2004]. A third tube was used to assess internal and external reliability of serum retinol concentration using HPLC.

III.7.d. Breast milk

Lactating mothers were visited by midwives to collect breast milk samples. For this, at both baseline and endline, all milk from one breast that had not been used for at least 30 minutes was collected during the middle of the morning feeding with a hand-pump. Of this sample, twenty-five ml was then

aliquoted and transported in a bottle covered with aluminum foil, stored at –80^0 C and analyzed for retinol using HPLC in BBIA (Agro-Industry Laboratory) Bogor.

III.7.e. Cooking oil

Fortification levels of the cooking oil were assessed at the factory level, distributor, the small stalls and the household level. At the factory level, this was done through monitoring records of internal QA/QC. For district-level distributors, small stalls and households oil sampling and retinyl-palmitate quantitation was performed by the study group.

All oil analyses were done using a rapid device ("iCheck Chroma"), developed by BioAnalyt GmbH, Germany and validated for use in refined palm cooking oil [Rohner et al 2011].

Twenty-five mL of unused cooking oil was collected every two weeks from 321 of the 339 households in the cohort groups (children 5-9 years and non-lactating women 15-29 years), and households were reimbursed for the value of the oil.

Samples were kept in plastic bottles, which were completely filled, covered with aluminum foil, boxed by village and month, and stored in a dark room in the district "basecamp". At the end of the study, the oil samples were transported to *Puslitbang Gizi* in Bogor and stored at project headquarters.

The total number of unused cooking oil samples collected from the cohort households was 2,905 (**Table 2**). Of these, an average of 5-8 samples per district per month were immediately analyzed, a total of 75 samples over November 2011 to May 2012. Due to funding constraints, only in November-December 2012 (6-18 months after collection), an additional 702 samples were analyzed at the SEAFAST center of IPB. KFI supplied a large number of iCheck Chroma vials and in February-March 2013, the research team analyzed the remaining 1,211 small-stall and 2,128 household samples. Thus, a total of the 4,920 oil samples were measured.

Table 2. Vitamin A level determination from cooking oil samples from households

Analysis of Oil Samples			Level in the Distribution Chain				
Timing after collection	**Location**	**By whom**	**1 Factory**	**2 Distributors**	**24 Small stalls**	**321 cohort house holds**	**Total**
Immediatel y	Field	Study team	20	28	54	75*	**177**
Nov-Dec 2012 (6-18m)	SEAFAST Lab, Bogor	SEAFAST	0	0	702	702	**1,404**
Feb-Mar 2013 (8-21m)	Puslitbang Gizi, Bogor	Study team	0	0	1,211	2,128	**3,339**
Total			**20**	**28**	**1,967**	**2,905**	**4,920**
Samples with undetectable vitamin A concentration (<3 mg RE/kg, corresponding to <10 IU/g)			0 (0%)	0 (05)	1095 (56%)	1742 (60%)	2837 (58%)

*75 samples over Nov. 2011 to May 2012 (ranging from 5 to 6 in Tasikmalaya, and from 5 to 8 in Ciamis (modus 5) per district per month. Over July-October 2011, a period for which the original data were lost and only monthly aggregated results retained, similar numbers of samples were analyzed immediately every month.

III.8. Ethical Considerations of the Study

Ethical authorization for the study was obtained from the NIHRD Ethical Review Committee for the baseline study in its letter of approval number KE.01.05/EC/262/2011 dated May 3, 2011. Ethical approval was also obtained from the Ministry of Health Research Division Ethical Review Committee for the endline survey in 2012 (Number KE.01.05/EC/409/2012 dated May 16, 2012; both attached to this report as **Annex 3**).

Prior to any data being collected, informed consent was obtained from each subject or from a parent if the subject was a child (**Annex 2**). A description of the study and its purpose was given and the respondents were told that they were free to withdraw from the study at any time.
On diagnosis of severe anemia (<7.0 g/dL) at baseline, the subject was excluded from the study and referred to the nearest health center for adequate treatment. Based on assessment by the health center doctor, subjects were included if they showed no evidence of current infectious disease or severe malnutrition. Confidentiality of the subjects was strictly maintained.

During data collection, participants were given a small financial compensation for blood and oil samples, and for morbidity records.

III.9. Data Management and Analysis

III.9.a. Data quality assurance

Quality during data collection
To ensure the quality of data collected, three procedures of data quality control were applied: 1) pre-testing of the questionnaires instrument (outside the study area, in 2011); 2) minimizing inter-observer variation; and 3) supervision.

Inter-observer variation was minimized by trainings of field enumerators prior to both surveys, which standardized understanding and skills of enumerators around the study and their tasks, and equalized enumerator capabilities in data collection and management including identifying and solving problems that might emerge during implementation. Trainings used a data collection manual that consisted of sections on sampling, interview, how to deal with respondent inquiries regarding the nature of the questions, and how to appropriately fill in the questionnaires.

Supervision was provided by the research team and district coordinators, as operational controlling to solve any problems emerging during data collection, and as technical supervision by the research team to district coordinators and enumerators, including checks on completeness and validity of interview/questionnaire responses.

Daily inspections on the completeness of data collection forms/questionnaires was carried out directly in the field to verify whether the identification number had been given to each form and also to check missing or incomplete information/data. The area supervisor was responsible for this task. Reliability check of data was also be conducted to identify the consistency for all inter-related variables. In case of incomplete or poorly administered questionnaires, the supervisor went back to the respective household and completed the questionnaires.

For biological sampling, the primary sources for errors during sample collection are loss, mislabeling and poor storage. Loss of blood and breast milk samples was monitored daily, using the cluster summary sheets and sample summary sheets. Mislabeling was prevented by pre-printing labels using a 5-digit code, using the same pre-printed labels for identification of the subject, his/her forms and his/her blood container (microtainer). During sample processing, pre-printed labels with the same code identified the sub-samples. Attention was furthermore paid to optimum transport and storage conditions at all levels; however, no constant monitoring of the cold chain was conducted.

Quality control in laboratory analyses was, for all biomarkers, applied at two levels: pre-analysis ensuring that the chosen equipment and protocols were precise and adequate, and during analysis ensuring that processes were followed correctly and consistently. For Hemocue, devices were re-calibrated daily and a record of calibration results kept. Vita Iron lab provided quality control of its analyses of ferritin, sTfR, RBP, CRP and AGP. For all biomarkers, baseline and endline samples were analyzed at once after the endline survey, so as to reduce the risk of biases associated with events and developments at the laboratories.

Quality during Data Entry and Cleaning
All data were coded and into an electronic database. Laboratory/biomarker data were double entered, which for hemoglobin occurred at the field site. Also anthropometry data (child age, weight and weight), key respondent identifiers (district, cluster, household) were double entered, whereas other variables were single-entered.

The consolidated dataset was then checked for internal consistency and validity by an external validation team from the School of Public Health at the University of Indonesia in Depok. Based on their feedback, questionnaires were revisited and some of the data was re-entered to correct errors (e.g., household expenditure).

Data were then cleaned for outliers and merged into working databases.

III.9.b. Exclusion of outliers

For serum hemoglobin concentrations, extreme values <5 g/dL were excluded from analysis, leading to exclusion of one child 6-59 months with a recorded hemoglobin concentration of 1.2 g/dL, one child 5-9 years with a recorded hemoglobin concentration of 0 g/dl at endline, and one lactating mother with a recorded hemoglobin concentration of 1.2 g/dL.

For child anthropometric data, extreme values defined as Z-scores below <-4 or above +4 were capped at -4 or +4, respectively (see **Table 23**).

For serum and breast milk retinol measurements or for dietary intake, no outliers were excluded.

III.9.c. Constructed and extrapolated variables

Some of the variables used in the analysis had to be constructed or extrapolated first.

- ***Cooking oil vitamin A content and intake***

Samples of cooking oil were collected at the household level for the cohort groups (children 5-9 years and women 15-29 years), and at village level (point of purchase) for the other groups. For the non-cohort groups, the mean of the vitamin A content found in the samples collected from cohort HHs in their village was used as the content at the HH level.

The individual dietary recall data were analyzed for foods prepared with oil and an estimate was used for the amount of cooking oil in each meal [Badan Penelitian dan Pengembangan Kesehatan 2007; Krisdinamurtirin et al. 1974].

Household expenditure data were collected, including expenditures for cooking oil, as an alternative basis from which to estimate oil intake. From each household data were collected on amount and price of the last purchase of cooking oil and over what period the oil was consumed.

- ***Food consumption data***

The dietary recall provided types and amounts of foods consumed. These were analyzed using the *Nutrisoft* software package [Pusat Penelitian dan Pengembangan Gizi 2008] and Indonesia's official food composition table [Krisdinamurtirin et al., 1974], to obtain vitamin A intake from foods. Because Nutrisoft does not correct for poor bioavailability of (pro-) vitamin A/carotenoids in food, the relative vitamin A intake from plant-source foods – which the study populations consumed in large amounts – is highly overestimated. Therefore, our analyses considered vitamin A intake only from animal-source foods and oil-containing foods.

Oil consumption was then estimated from food intake using the Indonesia's official table for converting dishes containing oil into oil intake amounts, which assumes a fixed average amount of cooking oil in each dish or recipe {Badan Penelitian dan Pengembangan Kesehatan, 2007}.

Vitamin A intake data were converted to percentage of daily age-specific Recommended Nutrient Intakes (RNI) using the reference RNIs in **Table 3** [WHO/FAO 2002].

- ***Contribution to RNI of vitamin A-fortified oil***

To assess the contribution of oil consumption and other sources of vitamin A to the total intake, the percentage contribution of oil and animal foods to daily RNI of vitamin A was estimated for individuals in all groups, at baseline and at endline.

- ***RNI contribution from oil (based on consumption/intake data)***

This was done by multiplying the estimated individual-level cooking oil intake (consumption), with the household-level vitamin A content of oil, and then divided by the RNI for vitamin A for each age group.

- ***RNI contribution from animal foods (based on consumption/intake data)***

As individual intake data of animal foods were available for all subjects, vitamin A intake from animal source foods, expressed as percentage of the daily RNI, was calculated from each individual's vitamin A intake from animal source foods, divided by the daily RNI for the respective age group.

Table 3. RNI for vitamin A by age group

Age	*RNI for vitamin A (µg/day)*
Infants up to 6 months	375
Infants 7-11 months	400
Children12-47 months	400
Children 48-59 months	450
Children 5-9 years	600
Lactating women	850
Women of reproductive age	700

Source: [WHO/FAO 2002].

- ***Household daily expenditure quintiles***

The distribution of the study population over expenditure quintiles was expressed in terms of existing national household daily expenditure quintile categories from the 2010 SUSENAS [Badan Pusat Statistik Direktorat Diseminasi Statistik 2010] **(Table 4)**,

Table 4. Quintile category of daily household expenditure, based on SUSENAS 2010

Quintile category	*Daily household expenditure (IDR)*
Q1	< 8,821
Q2	8,821-11,869
Q3	11,870-15,704
Q4	15,705-22,899
Q5	>22,899

- ***Percentage of household expenditures on food***

This is a marker of poverty, as households with lower incomes (and therefore lower expenditures) will spend a larger share of those expenditures on food. This poverty indicator is preferred over absolute values of expenditure, which potentially vary more with household size and with economic inflation over the year.

- ***Household asset score***

The survey questionnaire included questions about ownership of a number of household assets such as a car, motorcycle, refrigerator, computer, hand phone, TV, DVD player and radio. For each of these items, an estimation was made of the average price (**Table 5**), and each item was then assigned a score according to its share in total assets price. This score is included in the regression model to control for socio-economic status.

Table 5. Assets owned by households and their scores

Asset	Estimated price (Rp)	Asset code (% of total asset price)	% Of HH owning the asset
Radio (tuner)	100,000	0.14	37.2
Tape/DVD player	200,000	0.27	94.5
Color TV set	3,000,000	4.09	91.2
Mobile phone	1,500,000	2.05	39.7
Computer (PC/laptop)	5,000,000	6.82	2.3
Refrigerator	3,000,000	4.09	17.6
Motorcycle	10,000,000	13.64	43.2
Car	50,000,000	68.21	1.1
Other assets	500,000	0.68	2.0
Total asset price	**73,300,000**	**100.00**	

The scores for all items were then aggregated into a household score of capital good assets. The higher the score the richer the household. The scores were categorized into quintiles:

Category 1 (poorest) : score <20(68% of study sample at baseline, 60% at endline)
Category 2 (poor) : score 20 to <40 (19% of study sample at baseline, 25% at endline)
Category 3 (moderate) : score 40 to <60 (11% of study sample at baseline, 14% at endline)
Category 4 (rich) : score 60 to <80 (0.5% of study sample at baseline, 1.0% at endline)
Category 5 (richest) : score ≥80 (1.2% of study sample at baseline, 0.8% at endline).

- ***Household sanitation score***

The housing sanitation score is based on a number of variables (**Table 6**). For each variable the lowest score was 1 for the worst condition and the highest was 3 for the best condition. Intermediate conditions were assigned score 2. The housing condition score was obtained by totaling all these scores, in a range of 8-24. The higher the score the better the housing condition.

Table 6. Scoring of household sanitation

Description	Options	Score assigned
Cleanliness of living room	Clean	3
	Not clean	1
Window in living room	Yes, opened everyday	3
	Yes, rarely opened	2
	No window	1
Ventilation in living room	Yes, more than 10% floor area	3
	Yes, less than 10% floor area	2
	No ventilation	1
Lighting in the living room	Sufficient	3
	Not sufficient	1
Cleanliness of bedroom	Clean	3
	Not clean	1
Window in bedroom	Yes, opened everyday	3
	Yes, rarely opened	2
	No window	1
Ventilation in bedroom	Yes, more than 10% floor area	3
	Yes, less than 10% floor area	2
	No ventilation	1
Lighting in the bedroom	Sufficient	3
	Not sufficient	1

- ***Housing condition score***

Similarly, a housing condition score was calculated based on a number of variables, with each assigned a score between 1 and 3 (**Table 7**). The housing condition score was obtained by totaling all these scores with a range of 7-21. The higher the score the better the housing condition.

Table 7. Scoring of household condition

Description	Options	Score assigned
Type of housing	House not on poles	3
	House on poles (*rumah panggung*)	2
	Floating house	1
Type of roof	Concrete roof	3
	Tiled roof	3
	Shingles (*sirap*)	3
	Tin roof	2
	Asbestos roof	2
	Thatched roof (*rumbia*)	1
	Other type of roof	1
Type of ceiling	Concrete	3

	Gypsum	3
	Asbestos	2
	Wood/plywood	2
	Bamboo	1
	Other type of ceiling	1
	None	1
Type of wall	Concrete wall	3
	Wood/plywood wall	2
	Bamboo wall	2
	Other type of wall	1
	None	1
Type of Floor	Marble/ceramic/tiled/cement floor	3
	Broken cement floor	2
	Wood/bamboo/rattan floor	2
	Soil floor	1
Number of bedrooms	3 or more bedrooms	3
	2 bedrooms	2
	1 bedroom	1
Having a separate bedroom	Yes	3
	No	1

- ***Body iron stores***

Body iron stores (mg/kg) were calculated from the soluble transferrin receptor/ferritin ratio using the method of Cook [Cook et al 2003].

III.9.d. Sub-clinical inflammation and correcting serum retinol and ferritin levels

Sub-clinical inflammation (SCI) is defined as serum CRP concentration >5mg/L and/or AGP concentration >1g/L [Thurnham et al 2003 and 2010, Wieringa 2002]. Subjects are grouped into four categories according to their acute phase proteins CRP and AGP: reference (both CRP and AGP normal); incubation (CRP>5mg/L and AGP normal); acute convalescence (CRP>5mg/L and AGP>1g/L) and late convalescence (CRP normal and AGP>1g/L). During sub-clinical inflammation, serum retinol concentrations are decreased while serum ferritin concentrations are increased. Failing to adjust serum retinol and ferritin concentrations for SCI leads to an over-estimation of VAD, and an under-estimation of iron deficiency. Therefore, in this study, the values of serum retinol and serum ferritin concentrations were corrected using meta-analysis correction factors as per **Table 8** [Thurnham et al 2005 and 2010].

Table 8. Factors to correct serum retinol and ferritin concentrations for subclinical infection

Infection phase		Acute Phase Proteins	Correction for retinol	Correction for ferritin
I	Reference	Normal	1.00	1.00
II	Incubation	CRP elevated only (>5mg/L)	1.13	0.77
III	Early convalescence	CRP and AGP elevated	1.24	0.53
IV	Late convalescence	AGP elevated only (>1g/L)	1.11	0.75

III.9.e. Statistical analysis

The clean databases were shared and analyzed using SPSS package version 19.0. Student t-tests (paired for continuous variables in cohort groups; unpaired/independent samples for all outcomes in cross-sectional groups and for binary/proportional variables in cohort groups), were used to test differences between baseline and endline results, averaged within age groups.

A multivariate linear regression was used to test the effect of fortification on serum retinol and RBP concentrations, controlling for confounders and other determinants. The key outcome measures were adjusted serum retinol concentrations at endline, and the endline-minus-baseline shift in adjusted serum retinol concentration, as a function of vitamin A intake from oil, as well as confounders of this relationship including socio-economic factors and vitamin A intake from non-oil sources, in particular animal-source foods.

Vitamin A intake from oil was entered in these regressions as estimated percentage of RNI contribution from oil (as described above). The following socio-economic factors were included: percentage of household expenditures on food, household capital asset score, housing condition score and house sanitation score. As expected confounders of the shifts in serum retinol and RBP concentrations following oil fortification, we furthermore included as covariates:

- percentage RNI contribution from animal foods (for all groups);
- coverage with VAC at the February 2012 round, five months before the endline survey (for children 6-59 months);
- Age, for children 6-59 months, since within this age range food intake patterns and nutritional needs and determinants change rapidly as the children grow;
- Adjusted serum retinol concentration at baseline: at individual level for children 5-9 years and women 15-29 years, and as village averages for other groups.

Apart from serum retinol concentration at baseline, covariates considered to be relatively stable over the study period were generally taken from the endline (rather than baseline) survey, in view of a perceived overall better quality of the endline survey. By exception, the percentage household expenditure on food was taken as endline-minus-baseline shift.

Given the cross-sectional nature of the measurements in children 6-59 months and lactating mothers, and the cohort data available for children 5-9 years and women 15-29 years, regressions were ran at both the level of individual respondent outcomes and of village-average outcomes, to exploit all relevant options to detect a possible effect of oil fortification on improvement in serum retinol status with optimal statistical power (**Table 9**).

Table 9. Multivariate regression designs to detect determinants of retinol status improvements from baseline to endline.

Unit of analysis	Groups included	Rationale
Village (N=24, 23 degrees of freedom)	All groups	• For non-cohort groups, this was the lowest level at which baseline-to-endline shifts could be evaluated. • Village-level covariates should suffer less from measurement imprecision (e.g. in retinol status, oil consumption, and vitamin A levels in oil). • Village-level determinants should be relevant also for breastfed infants, whose nutrition is determined by nutrition of their mothers rather than by their own oil and food intake.
Individual (N=157 or 166 degrees of freedom)	Cohorts only	Most powerful analysis, but possible only for cohorts (children 5-9 years and women 15-29 years), in which baseline and endline measurements can be linked for each individual respondent.

IV. RESULTS

IV.1. Respondents and their characteristics

IV.1.a. Sample size at Baseline (BL) and Endline (EL)

All analyses were limited to those subjects with both interview and blood data available (**Table 10**). For cohort members, analyses were limited to subjects who participated in both baseline and endline. Drop-out from the cohorts was considerable, mainly due to refusals (at endline) to blood drawing, migration out of the area and absence from home during data collection days. No records were kept on numbers and reasons of refusal and drop-out.

Table 10. Targeted and realized numbers of respondents

Group	Targeted sample size	Subjects with blood data		Subjects with complete interview data		Subjects with both blood and interview data	
		Baseline	Endline	Baseline	Endline	Baseline	Endline
Infants 6-11 m	320	343	356	324	344	318	335
Children 12-23 m	220	248	241	241	239	236	236
Children 24-59 m	220	243	251	235	244	233	241
Children 5-9 y	220	260	189	248	186	248(186)*	186
Lactating mothers	320	354	359	324	349	324	349
Women 15-29 y	250	274	173	252	171	252(171)*	171

*Numbers in brackets are the numbers of matched records, as these are cohort groups for which only subjects with complete data at both baseline and endline were used.

The set-up of data collection and data entry was organized differently during baseline, paid for by the Government, and endline, paid for with GAIN funds. At baseline, respondents were interviewed first and then convened for blood collection, at which stage some refused. At endline, blood samples were drawn before interviews were held. In addition, during baseline, enumerators were also responsible for data entry, while dedicated data entry clerks were hired for the endline. During the baseline survey, some respondents were sampled for blood yet not interviewed (against protocol), and a number of questionnaires got misplaced and lost so the subject had to be excluded from analysis. This did (almost) not happen during endline.

The drop-out among cohort groups from baseline to endline may relate to refusal of blood drawing, moving out of the study area and some respondents having been absent from their home during the data collection days. Unfortunately, no records are available on numbers and reasons of refusal, but anecdotal information suggests that many parents could not stand to see their child crying at the blood drawing. It is also of note that cohort participants were paid a small financial compensation for blood drawing (baseline and endline), for bi-weekly morbidity exams and any oil samples taken during the bi-weekly visits (with which most cohort members complied faithfully throughout the year), but not for the endline interview.

IV.1.b. Characteristics of study groups, at baseline and endline

Children from the cross-sectional sample populations were evenly distributed over the sexes at both baseline and endline. However, infants 6-11 months sampled were older at endline compared to baseline (p=0.01), while the average age of children 24-59 months sampled was lower (p=0.04). The cohort groups, as expected, were exactly a year older at endline than at baseline (**Table 11**).

Household socio-economic indicators generally improved from baseline to endline, except for sanitation. Nevertheless, the large percentage of households in the lowest expenditure quintile (37-53% at baseline, and 26-44% at endline) compared to national quintiles (from 2010 SUSENAS) confirms that the study sample remained a relatively poor population. There were no consistent patterns of difference between baseline and endline in young children's coverage of vitamin A supplementation capsules and ever-breastfeeding history.

Table 11. Characteristics of respondents at baseline and endline, and significance of difference

		Infants 6-11 m	Children 12-23 m	Children 24-59 m	Children 5-9 y*	Lactating mothers	Women 15-29 y*
Age (mean, months or years)	N at BL	318	236	233	186	324	171
	BL±SD	8.1±1.8	17.1±3.8	38.3±10.0	6.9±1.1	28.9±6.7	23.0±6.6
	N at EL	335	236	241	186	349	171
	EL±SD	8.5±1.8	18.1±3.2	36.2±10.0	7.9±1.1	29.1±6.4	24.0±6.6
	p-value	0.010	0.04	0.044	<0.001	0.63	<0.001
Sex (M/F ratio)	N at BL	318	236	233	186		
	BL ratio	49.1%	45.8%	47.6%	44.6%		
	N at EL	335	236	241	186		
	EL ratio	50.1%	50.8%	51.0%	44.6%		
	p-value	0.78	0.27	0.27	1.0		
Ever breastfed	N at BL	318	236	211			
	BL±SD	96.5%	96.6%	53.1%			
	N at EL	335	236	42			
	EL±SD	99.7%	96.6%	38.1%			
	p-value	0.010	1.0	0.076			
VAC receipt in February preceding survey	N at BL	318	236	236			
	BL±SD	26.4%	92.4%	92.7%			
	N at EL	335	236	246			
	EL±SD	28.7%	95.8%	95.4%			
	p-value	0.52	0.12	0.81			
Mean HH capital asset score (possible range: 0-100) [+]	N at BL	318	236	233	186	324	171
	BL±SD	11.1±10.5	12.0±12.2	11.9±12.2	11.7±12.6	11.1±10.5	12.8±13.3
	N at EL	335	236	241	186	349	171
	EL±SD	13.2±9.2	13.4±12.6	13.7±13.3	11.7±11.0	13.1±9.1	14.8±11.4
	p-value	0.016	0.21	0.14	0.95	0.009	0.039
Mean Housing condition score (possible range: 7-21) [+]	N BL	318	236	233	186	324	171
	BL±SD	18.4±2.8	18.5±2.3	18.4±2.3	18.4±2.4	18.4±2.7	18.4±2.7
	N EL	335	236	241	186	349	171
	EL±SD	19.2±1.4	18.9±1.6	18.9±1.5	18.9±1.5	19.2±1.4	18.9±1.5
	p-value	<0.001	0.009	0.016	0.006	<0.001	0.005
Mean HH sanitation score (possible range: 8-24) [+]	N BL	318	236	233	186	324	171
	BL±SD	20.2±3.6	20.2±3.7	20.3±3.7	18.4±4.0	18.8±4.0	19.3±4.2
	N EL	335	236	241	186	349	171
	EL±SD	20.5±3.2	20.2±3.6	20.1±3.6	17.8±3.8	18.9±3.7	19.0±3.9
	p-value	0.29	0.69	0.65	0.072	0.72	0.48
HH expenditure quintile (% in lowest quintile)	N BL	318	236	233	186	324	171
	BL	52.8%	44.6%	50.2%	51.1%	52.5%	37.4%
	N EL	335	236	241	186	349	171
	EL	38.2%	44.1%	33.2%	43.5%	38.7%	25.7%
	p-value	0.0002	0.23	<0.001	0.12	0.0003	0.02
HH expenditure on food as % of total expenditure	N BL	318	236	233	183	321	171
	BL±SD	71.1±39.5	70.6±18.3	70.3±12.8	70.3±12.2	71.4±39.5	68.5±12.1
	N EL	335	236	241	183	349	171
	EL±SD	64.8±11.8	67.0±11.8	67.2±12.9	67.9±12.3	64.9±12.0	65.0±12.4
	p-value	0.004	0.02	0.009	0.026	0.003	0.003
Highest level of education in HH	N BL	318	236	233	186	324	169
	N EL	335	236	241	186	349	169
Incomplete primary school	BL %	2.5	2.6	2.5	5.1	2.5	3.0
	EL %	1.7	2.1	2.9	5.1	1.7	3.0
Completed Primary school	BL %	43.7	55.7	58.5	53.7	43.7	59.2
	EL %	42.4	43.3	49.8	53.7	42.4	59.2
Completed Junior High School or more	BL %	53.8	41.7	39.0	41.2	53.8	37.8
	EL %	55.9	54.6	47.3	41.2	55.9	37.8

* For these cohort groups, p-value of baseline-to-endline differences in continuous variables was based on paired T-tests, and on unpaired T-tests for binary/proportional variables; for cross-sectional groups, p-values for all outcomes from unpaired T-tests.

[+]The higher the score the better the house or household's condition.

Unless indicated, values shown are mean ±Standard Deviation (SD); p-values denote statistical significance of the difference between baseline and endline.

IV.2. Cooking Oil

IV.2.a. Vitamin A content of cooking oil

Originally fortified at the Indonesian National Standard (SNI) level of 45 IU/g oil, the fortification level of the cooking oil leaving the factory (according to factory records) was 43.6 IU/ g (± SD 2.5). This declined to 28.3 IU/g (±7.7) at distributor level, 25.7 IU/ g (±10.5) at small stalls, and 28.5 IU/g (±12.0) in 75 'fresh' oil samples collected over the study period from 75 out of the 339 cohort households, and analyzed immediately (**Figure 1a**). This is in contrast with samples stored 6 – 21 months before i-Check analysis (see **Table 2**), which found lower vitamin A content: 13.2 IU/g ±13.1 at household level and 11.7 IU/g±12.1 at small stalls (**Figure 1b**).

Samples with vitamin A content below the i-Check assay's lower detection limit of <3 mg RE/kg, corresponding to <10 IU/g, were included with value 1 mg RE/kg, i.e. 3.3 IU/g in all calculations of means, standard deviations (**Figures 1+2**) and RNI contributions (**Table 12**). Vitamin A content below the detectable level was not found in any of the factory or distributor samples, but it was found in 56-60% of samples in small stalls and households (**Table 2**). The vast majority of undetectable values were from small stall and household samples analyzed after a 6-18 month storage, except for 3 freshly analyzed household samples.

A random sub-set of oil samples collected from cohort households during monthly monitoring revealed considerable variation over the months of the year, and between the two districts (**Figure 2**). This variation likely reflected dilution with unfortified oil in the distribution chain.

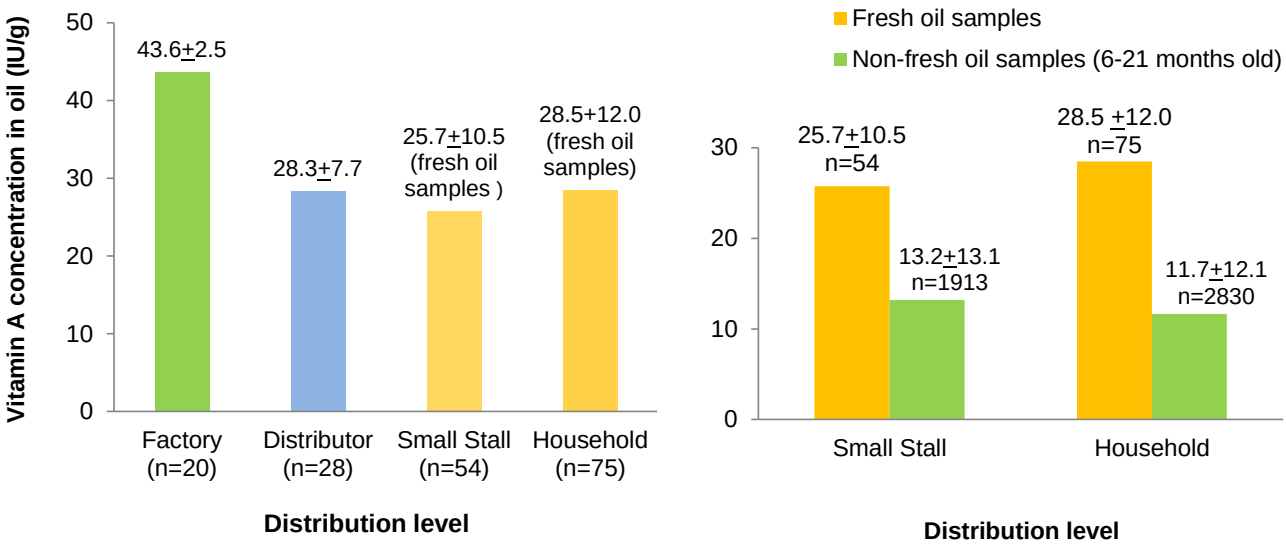

Figure 1. Vitamin A content of cooking oil at different distribution levels.

(a) oil samples analyzed immediately after collection;

(b) comparison between samples analyzed immediately after collection ('fresh') and samples analyzed 6-21 months after collection, from households and small stalls

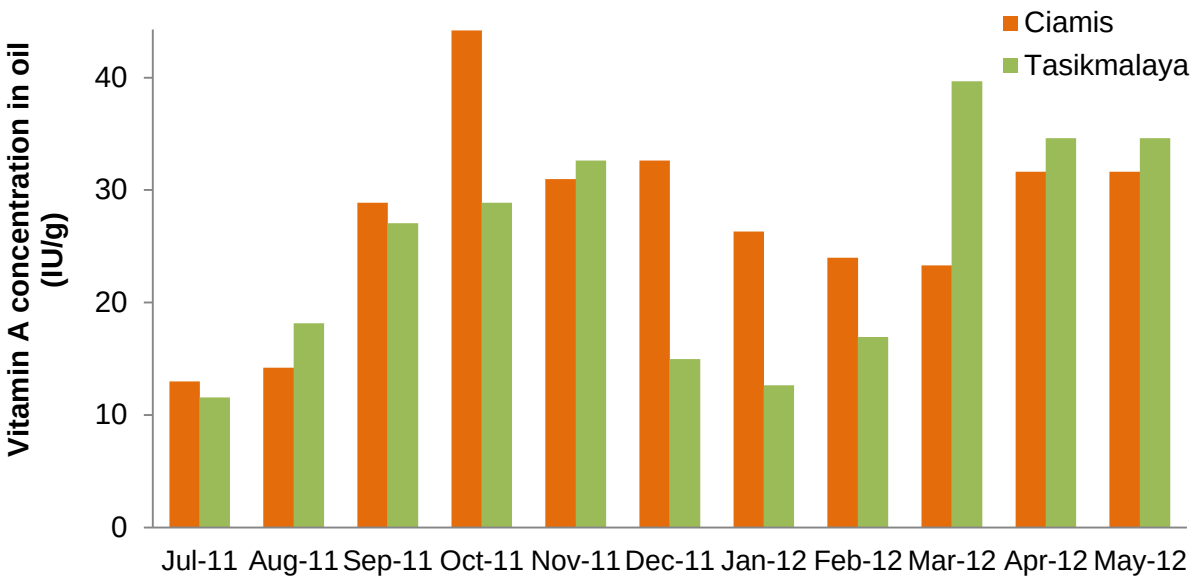

Figure 2. Mean vitamin A content of cooking oil samples from households collected monthly, and analyzed immediately

Note to Figure 2: Over Nov. 2011 to May 2012, the samples per district per month ranged from 5 to 6 in Tasikmalaya, and from 5 to 8 in Ciamis (modus 5), for a total of N=75. Over July-October 2011, a period for which the original data were lost, similar numbers of samples were analyzed immediately every month.

IV.3. Consumption of cooking oil and foods containing vitamin A, and their contributions to RNI

Based on the single 24-hour dietary recall of all foods containing oil, the mean oil consumption at baseline ranged from 2.4 mL/capita/day for infants to 31.5 mL/capita/day for lactating mothers. A year later the range was 1.7-29.8 mL/capita/day. There were no significant differences in consumption of cooking oil between baseline and endline, as expected given that fortification had not been advertised or promoted in any way (**Table 12**). According to respondents, oil purchased by the household is consumed within a few days (mean 4, median 3; interquartile range 2-7 days; **Table 12**).

Mean vitamin A intake was calculated as percentage of the RNI for vitamin A by age group to assess the contribution of the oil consumption to total intake (**Table 13**). At baseline, no fortified cooking oil was available, so the RNI contribution from oil was set at 0.

The fortified oil available at endline was estimated to contribute 26% of daily RNI for children 12-23 months, increasing to 40% and 38% for children 24-59 months and 5-9 years, in whom RNI is relatively low. RNI contributions were 29% and 35% for lactating mothers and women 15-29 years, respectively. The RNI contribution from fortified oil was only 4% of RNI in breastfeeding infants, in whom vitamin A intake via breast milk was not estimated.

For animal foods, the mean RNI contributions for vitamin A were below 50% in all groups, and it was as low as 10-19% for lactating women. RNI contribution from animal foods was generally lower at endline than at baseline, except in children 12-59 months – this reflected lower reported food intakes at endline compared to baseline (see **Annex 9**).

Consumption of vitamin A-fortified cooking oil improved overall vitamin A intakes considerably, except among the youngest infants, who only consume very small amounts of prepared foods. Overall vitamin A intake, from animal foods and fortified oil combined, at endline averaged 73% of daily RNI at 12-23 months, 82% at 24-59 months, 60% at 5-9 years, and 39% and 49% in lactating mothers and women 15-29 years, respectively.

At the average vitamin A level in oil in households (freshly analyzed samples) of 27 IU/g, to risk vitamin A intake above 100% of recommended RNI through fortified oil alone, daily oil consumption would have to be above 48 grams for children 6-11 or 12-23 months, above 54 grams for children 5-9 years and above 103 and 85 grams for lactating and non-lactating women, respectively. Based on the population distributions of oil consumption in 2012, 0% of infants 6-11 months, children 5-9 years and lactating women, and 1.8% of children 12-23 months, 2.3% of children 24-59 months and 1.0% of women 15-29 years had such high oil intakes to risk an above-recommended vitamin A intake. However, no single individual in any group had oil consumption that exceeded the guidance level for long-term intake of 1,500 µg retinol equivalent/day, equal to half the upper limit for adults.

Table 12. Mean availability of cooking oil (mL/capita/day) at household level at baseline and endline

Households with	2011										2012									
	HH size		Volume of oil purchased (ml)		Money spent on oil (IDR)		How long does the oil last (day)		Availability (ml/cap/day)		HH size		Volume of oil purchased (ml)		Money spent on oil (IDR)		How long does the oil last (day)		Availability (ml/cap/ day)		p value for difference, baseline to endline
	N	Mean	*N*	Mean	*N*	Mean	*N*	Mean	*N*	Mean	*N*	Mean	*N*	Mean	*N*	Mean	*N*	Mean	*N*	Mean	
Lactating mother and infant 6-11m	*324*	4.6	*319*	365	*319*	4,053	*319*	4.3	*319*	25.3	*349*	4.7	*349*	480	*349*	5,655	*349*	5.2	*349*	24.5	0.86
Child 12-23 m	*241*	4.4	*239*	346	*239*	3,833	*239*	4	*239*	25.8	*239*	4.6	*239*	456	*239*	5,378	*239*	4.6	*239*	29.3	0.86
Child 24-59 m	*235*	4.5	*233*	403	*233*	4,387	*233*	4.9	*233*	23.7	*244*	4.6	*244*	369	*244*	4,375	*244*	4.2	*244*	24.7	0.44
Child 5-9 y	*186*	4.9	*181*	353	*181*	4,969	*183*	4.1	*181*	24.1	*186*	4.8	*181*	365	*181*	4,398	*183*	4.2	*181*	23.7	0.76
Non-lactating women 15-29 y	*171*	4.3	*168*	409	*168*	4,421	*168*	4.5	*168*	25.5	*171*	4.4	*168*	416	*168*	4,970	*168*	4.6	*168*	26.5	0.35

Table 13. Oil consumption and Mean vitamin A intake as percentage of age-adjusted RNI by study group, based on consumption/dietary recall data

	Children 6-11m*	Children 12-23m	Children 24-59m	Children 5-9y	Lactating mothers	Non-lactating women 15-29y
RNI for Vitamin A (microgram/day)	***400***	***400***	***450***	***600***	***850***	***700***
Oil consumption (g/day) at **baseline (all samples)**	2.4	13.5	22.3	24.8	31.5	29.1
Oil consumption (g/day) at **endline (all samples)**	1.7	12.5	21.5	27.4	29.8	29.4
Mean % RNI Vit A intake from **animal foods** at **baseline**	32	43	42	28	19	27
Mean % RNI Vit A intake from **animal foods** at **endline**	19	48	42	22	10	14
Mean % RNI Vit A intake from **oil** at **endline (all oil samples)**	**4**	**26**	**40**	**38**	**29**	**35**
Mean % RNI Vit A total intake at **endline**: **animal foods + oil** (all oil samples)	**23**	**73**	**82**	**60**	**39**	**49**

* Animal foods intake excludes intake from breast milk.
RNI contribution calculations for oil use vitamin A content results from the subset of household and small-stall oil samples that were immediately analyzed (see **Figure 1a**).
Source for RNI: WHO/FAO, 2002.

IV.4. Concentrations of C-reactive Protein (CRP) and α-1-acid glycoprotein (AGP)

To adjust serum retinol and serum ferritin concentrations for fluctuations associated with concurrent subclinical infections/inflammation (SCI), concentrations of CRP and AGP in serum – both markers for infection – were measured (**Table 14**). Based on CRP and AGP concentrations, about a quarter of subjects had SCI, without difference between baseline and endline.

Table 14. Mean concentrations of C-reactive protein (CRP) and alpha-glycoprotein (AGP) and prevalence of subclinical infection (SCI) at baseline and endline

	Baseline				Endline				p-value
	CRP (mg/L)	AGP (g/L)	SCI n (%)	n	CRP (mg/L)	AGP (g/L)	SCI n (%)	n	
Infants 6-11m	2.7	0.8	65 (21.5)	303	2.9	0.84	77 (24.0)	321	0.45
Children 12-23m	2.8	0.8	60 (26.4)	227	3.0	0.88	64 (28.4)	225	0.63
Children 24-59m	1.7	0.9	56 (25.1)	223	2.2	0.89	62 (26.7)	232	0.69
Children 5-9y	1.5	0.8	32 (18.3)	175	1.1	0.82	27 (16.5)	164	0.66
Lactating mothers	2.7	1.0	65 (21.5)	303	2.6	1.00	80 (24.5)	326	0.36
Non-lactating women 15-29y	1.9	0.8	40 (23.5)	170	2.3	0.87	52 (30.8)	169	0.13

When comparing subjects with and without SCI, combined over the two surveys, serum retinol, RBP and ferritin concentrations were indeed significantly different ($p<0.001$), except for serum retinol concentrations in children 5-9 years and women 15-29 years (**Annex 6**). Unadjusted values for serum ferritin concentrations were significantly higher than adjusted values among all age groups ($p<0.001$), while unadjusted serum retinol concentrations were significantly lower than adjusted serum retinol concentrations. Therefore, serum retinol, RBP and ferritin concentrations were adjusted for SCI.

In contrast, breast milk retinol concentrations were not adjusted for SCI, since there is no consensus that such adjustment should be done. Moreover, among breast milk samples analyzed here, there was no difference in vitamin A levels between lactating mothers with and without SCI (results not shown).

IV.5. Serum retinol concentrations

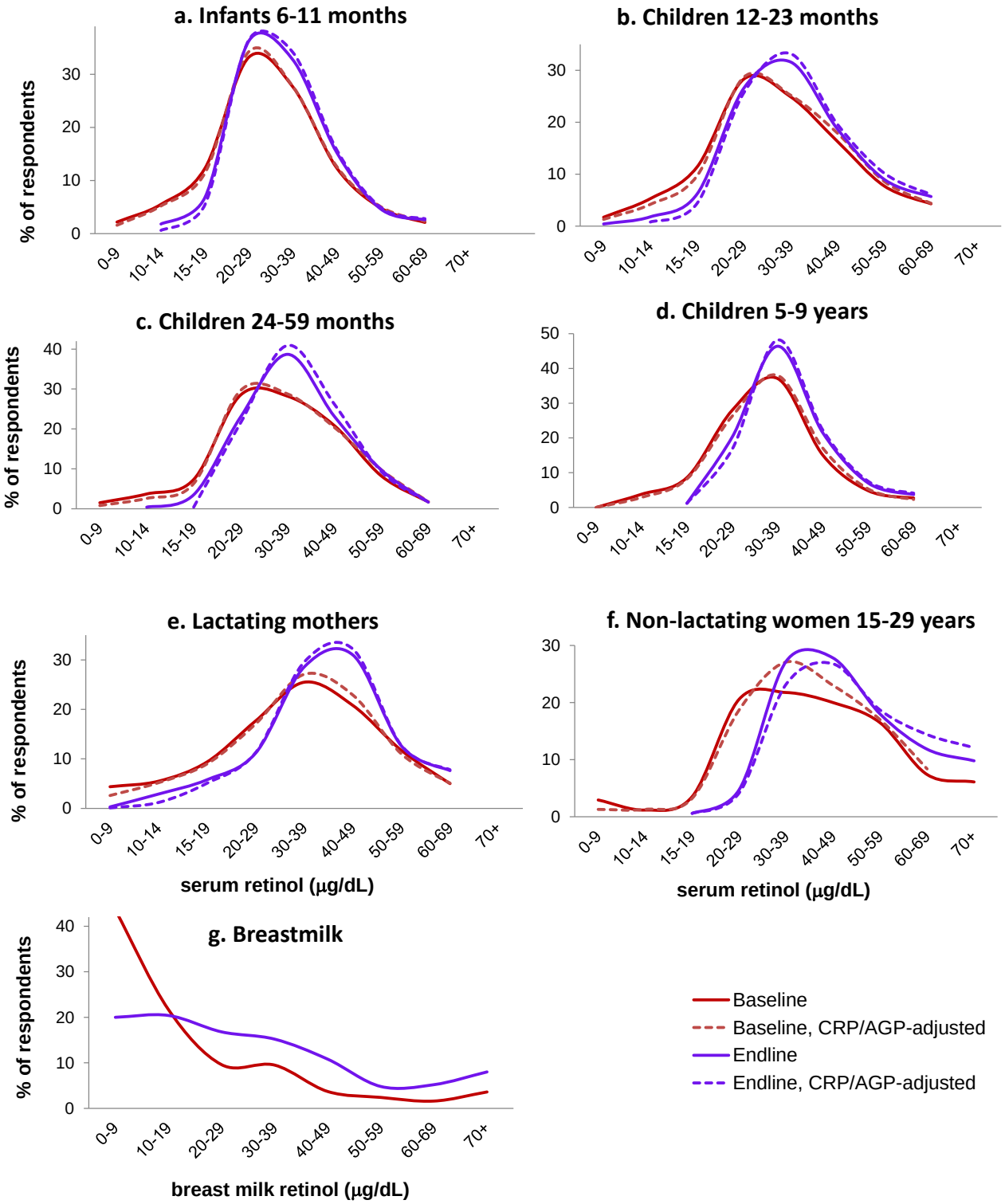

Figure 3. Serum and breastmilk retinol concentrations (µg/dL) at baseline and endline
a. Infants 6-11 months, b. children 12-23 months, c. children 24-59 months, d. children 5-9 years, e. lactating mothers, f. non-lactating women 15-29 years; g. breast milk.

Figure 3 shows population distributions of serum retinol concentration at baseline and endline. Marked improvements (shifts to the right) are apparent in all groups, with the most marked improvement on the left-tail i.e. among individuals with lowest retinol concentration at baseline.

Mean serum retinol concentrations at baseline ranged from 30.7 µg/dL among children 6-11 months and lactating mothers, to 42.7 µg/dL among non-lactating women (**Table 15**). At endline, mean serum retinol concentrations among all groups were higher, and the increase from baseline was significant among all groups except for children 12-23 and 24-59 months. The mean increase ranged from 0.7 µg/dL among 12-23 months-old children to 8.1 µg/dL among non-lactating women.

Table 15. Mean serum retinol concentrations (µg/dL) among subjects at baseline and endline, adjusted for CRP and AGP

Group	Baseline		Endline		mean diff	%	Sig. (2-
	n	Mean±SD	n	Mean±SD	[95% CI]	diff	tailed)
Infants 6-11m	303	30.7±12.6	321	34.5±12.2	3.8 [1.8-5.7]	12.3	<0.001
Children 12-23m	227	34.2±14.5	225	35.0±10.5	0.7 [-1.6-3.1]	2.2	0.529
Children 24-59m	223	36.0±14.1	232	38.3±11.7	2.3 [-0.1-4.7]	6.4	0.057
Children 5-9y	159	34.3±12.6	159	39.4±12.4	5.1 [2.7-7.6]	14.9	<0.001
Lactating Mothers	303	30.7±12.6	326	34.7±12.4	4.0 [2.1-6.0]	13.1	<0.001
Non-lactating women 15-29y	168	42.7±19.2	168	50.9±16.6	8.1 [4.5-11.8]	19.1	<0.001

At baseline, prevalence of VAD (defined as serum retinol concentration <20 µg/dL, **Table 16**), after adjustment for subclinical infection based on CRP/AGP-levels, was highest among the youngest children 6-11 months (18.2%), and around 10% among children 2-9 years. Lactating mothers had a VAD prevalence almost twice that of non-lactating women (10.0% vs. 5.3%).

VAD prevalence decreased significantly in all groups, overall from 12.2% at baseline to 2.9% at endline. The largest decrease was found among children 24-59 months (from 9.9% to 0.4%, or a 96% proportional decrease from baseline), while children 12-23 months showed the smallest (but still 64% proportional, and significant) decrease.

Table 16. Prevalence of Vitamin A deficiency (serum retinol <20µg/dL, after CRP/AGP adjustment) at baseline and endline

	Baseline (%)	Endline (%)	P-value
Infants 6-11m	18.2	6.0	<0.001
Children 12-23m	15.2	5.4	<0.001
Children 24-59m	9.9	0.4	<0.001
Children 5-9y	10.9	1.2	<0.001
Lactating mothers	10.0	2.1	<0.001
Non-lactating women 15-29y	5.3	0.6	0.011
Overall	**12.2**	**2.9**	<0.001

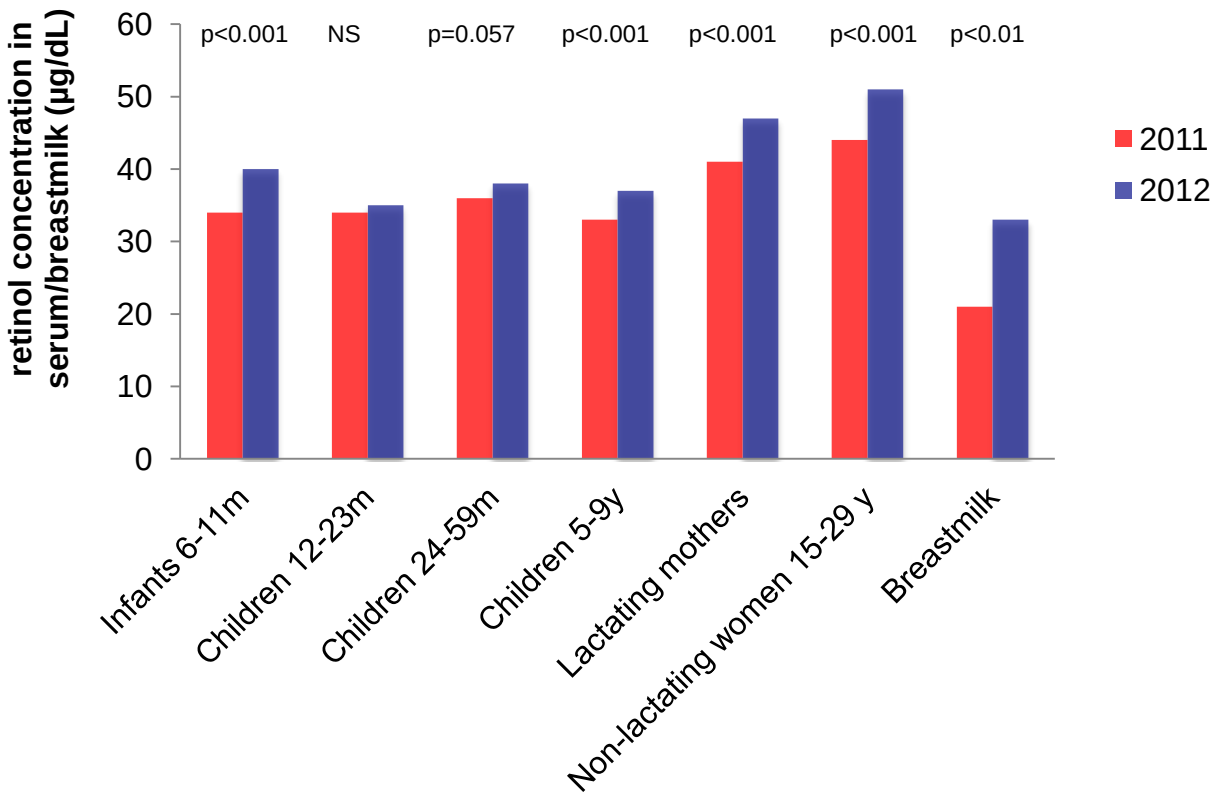

Figure 4. Retinol concentrations in serum and breast milk (µg/dL) at baseline and endline

Breast milk samples were collected from 233 mothers at baseline and 253 at endline. A marked shift to the right was apparent (**Figure 3g**). At baseline the vitamin A concentration averaged 20.5 µg/dL (SD=30.9) and at endline 32.5 µg/dL (SD=27.6), a 58.5% increase (p<0.01, **Figure 4**).

IV.6. Multivariate regressions of determinants of serum retinol

Multivariate regressions identified (village-average) vitamin A RNI contribution from cooking oil as a positive predictor of the (village-average) improvement in serum retinol concentration from baseline to endline for several of the groups (**Table 17**). This effect was significant in children 6-59 months (p=0.003) and children 5-9 years (p=0.03), but not in lactating or non-lactating women 15-29 years. For children 6-59 months, besides RNI from oil also Vitamin A capsule coverage significantly predicted greater serum retinol level improvements (p=0.032). In contrast, the RNI contribution from animal foods, when considered alongside the effect of fortified oil, was negatively associated with serum retinol improvement in children 5-9 years. This may reflect that the improvement in retinol status thanks to the fortified oil was greatest in those beneficiaries and villages with least vitamin A intake from non-oil foods. There were no significant effects of any socio-economic covariate in any of the models (**table 17**).

In the cohorts of children 5-9 years and women 15-29 years, regressions with the same predictor variables but run at the level of individuals did not obtain a good fit – that is, these models did not identify any significant predictors of serum retinol improvement at the level of individual study participants. Generally, regression models obtained good fit (as indicated by a high R^2) only when conducted at the level of village-averaged outcomes.

When adding baseline serum retinol concentration as additional covariate, that variable became the dominant predictor of the retinol shift: the lower the baseline serum retinol the more likely an upward shift over the subsequent year, and vice versa (p<0.001 for all groups in the village-level analysis,

top-right quadrant of **Table 17**). This effect was strong and significant in all age groups when analyzed at village level (top-right quadrant of **Table 17**), and in the cohort groups also when analyzed at individual level (bottom-right quadrant of **Table 17**). This added covariate markedly improved model fit for all age groups. The effect of baseline retinol status overrode the effect of RNI contributions from oil, which in these expanded models was no longer independently significant.

The effect of baseline retinol concentration can be explained by two mechanisms. First, there is 'regression to the mean' – the phenomenon by which variables that fluctuate randomly within individuals or populations, will tend to average out when measured repeatedly. Secondly, the impact of fortified oil may have been strongest in those people and villages with lowest baseline serum retinol concentrations. Particularly in Tasikmalaya district, villages varied widely in average baseline serum retinol at baseline, and this variation (among the same villages, with subjects sampled according to the same criteria) had nearly disappeared at endline.

Table 17. Multivariate regressions of determinants of improvement in serum retinol concentration from baseline to endline

	Model 1								Model 2							
	Child 6-59m		**Children 5-9y**		**Lactating mothers**		**Non-lactating women 15-29y**		**Child 6-59m**		**Children 5-9y**		**Lactating mothers**		**Non-lactating women 15-29y**	
VILLAGE-LEVEL ANALYSIS:	*Beta*	*p-value*	*Beta*	*p-value*	*Beta*	*p-value*	*Beta*	*p-value*	*Beta*	*p-value*	*Beta*	*p-value*	*Beta*	*p-value*	*Beta*	*p-value*
Serum retinol, baseline									**-0.74**	**0.00**	**-0.64**	**0.003**	**-0.83**	**0.000**	**-0.74**	**0.000**
RNI % contribution from oil, endline	**0.64**	**0.003**	**0.52**	**0.03**	0.44	0.17	0.10	0.67	**0.32**	**0.06**	0.25	0.20	0.18	0.25	0.03	0.86
RNI % contribution from animal foods, endline	-0.20	0.10	**-1.11**	**0.001**	0.01	0.98	0.40	0.11	-0.09	0.57	-0.51	0.08	-0.07	0.63	0.17	0.31
% received Vitamin A supplement capsule in February 2012	**38.9**	**0.032**							-0.03	0.90						
Household capital asset score, endline	1.4	0.08	0.18	0.43	0.27	0.82	-0.35	0.26	-0.02	0.92	0.20	0.27	0.09	0.55	-0.31	0.13
House sanitation score, endline	-2.5	0.18	0.23	0.22	-0.31	0.90	-0.20	0.47	0.08	0.66	0.06	0.70	0.02	0.87	-0.01	0.96
House condition score, endline	-6.8	0.20	-0.10	0.59	-10.4	0.15	0.16	0.51	-0.16	0.27	-0.05	0.70	0.01	0.96	0.15	0.36
Expenditure on food as % of household expenditure, change endline minus baseline	-0.19	0.63	-0.29	0.10	0.00	0.60	-0.09	0.73	-0.12	0.39	-0.21	0.11	-0.07	0.67	0.01	0.95
Age, endline	0.10	0.94							-0.06	0.65						
Degrees of freedom	*23*		*23*		*23*		*23*		*23*		*23*		*23*		*23*	
Adjusted R2 of model	44%		44%		-1%		-4%		67%		67%		61%		57%	
INDIVIDUAL-LEVEL ANALYSIS:																
Serum retinol, baseline											**-0.63**	**0.000**			**-0.72**	**0.000**
RNI % contribution from oil, endline			0.01	0.91			0.04	0.62			-0.04	0.57			0.04	0.51
RNI % contribution from animal foods, endline			-0.06	0.53			0.13	0.14			-0.04	0.55			0.03	0.62
Household capital asset score, endline			-0.02	0.83			-0.04	0.65			0.12	0.15			-0.02	0.72
House sanitation score, endline			0.02	0.85			-0.12	0.15			0.03	0.67			-0.02	0.69
House condition score, endline			0.01	0.90			0.10	0.22			0.01	0.92			0.03	0.65
Expenditure on food as % of household expenditure, change endline minus baseline			-0.04	0.69			0.07	0.39			0.04	0.62			0.09	0.12
Degrees of freedom			*157*				*166*				*157*				*166*	
Adjusted R2 of model			-3%				3%				35%				52%	

Notes: Beta represents the standardized coefficient for the covariate. Adjusted R^2 indicates the proportion of each study group's variation in retinol improvements that is explained by the model. Serum retinol improvements analyzed on CRP/AGP-adjusted values. Yellow highlights indicate statistically significant predictors.

IV.7 Anemia and iron status

Hemoglobin concentrations were similar at baseline and endline, the only significant difference being a slight increase among children 5-9 years (12.6 to 12.9 g/dL, P<0.01). Among lactating mothers, an increase from 13.3 g/dL to 13.5g/dL was borderline significant at p=0.05 (**Table 18**). Anemia prevalence did not decrease significantly in any group.

Table 18. Anemia prevalence (hemoglobin <11 g/dL, %) and hemoglobin concentrations (g/dL), baseline and endline by group (g/dL)

Group	Anemia prevalence (%)				Hemoglobin Concentrations						
					Baseline		Endline				
	BL	EL	% diff	P	n	Mean ±SD	N	Mean ±SD	mean diff [95% CI]	% diff	p
Infants 6-11m	42.5	38.0	-4.5	0.25	310	11.2±1.3	326	11.3±1.7	0.1 [-0.2-0.3]	0.6	0.55
Children 12-23m	39.3	41.6	2.3	0.62	221	11.3±1.4	228	11.3±1.6	0.0 [-0.3-0.3]	0.2	0.88
Children 24-59m	16.4	16.0	-0.4	0.89	225	12.1±1.4	230	12.1±1.4	0.0 [-0.2-0.3]	0.2	0.87
Children 5-9y	14.5	9.7	-4.8	0.17	174	12.6±1.1	174	12.9±1.1	0.3 [0.2-0.5]	2.6	0.00
Lactating mothers	16.8	13.4	-3.4	0.22	310	13.3±1.5	344	13.5±1.5	0.2 [0.0-0.5]	1.7	0.05
Women 15-29y	9.8	9.6	-0.2	0.96	157	13.7±1.4	157	13.6±1.6	-0.0 [-0.3-0.2]	0.2	0.83

Serum ferritin concentrations were similar at baseline and endline, except for an increase in children 5-9 years (p=0.004) and non-lactating women (p=0.011) (**Table 19**).

Table 19. Adjusted serum ferritin concentrations (µg/L), baseline and endline

	Baseline		**Endline**		**mean diff [95% CI]**	**% diff**	**Sig. (2-tailed)**
Group	**n**	**Mean±SD**	**n**	**Mean±SD**			
Infants 6-11m	303	25.3±25.4	321	27.6±27.5	2.4 [1.8-6.6]	9.4	0.26
Children 12-23m	227	20.2±20.7	226	20.1±19.5	-0.1 [-3.8-3.6]	-0.5	0.96
Children 24-59m	223	31.5±22.8	223	33.6±22.7	2.1 [2.1-6.3]	6.7	0.32
Children 5-9y	152	47.3±25.8	152	53.9±32.4	6.6 [2.1-11.1]	14.1	0.004
Lactating mothers	303	25.3±25.4	326	27.6±27.4	2.3 [-1.8-6.5]	9.2	0.27
Non-lactating women 15-29y	168	49.7±35.1	168	58.1±41.4	8.5 [2.0-14.9]	17.1	0.011

Soluble transferrin receptor concentrations were slightly higher at endline than at baseline in all groups except non-lactating women. These differences were significant among infants 6-11 months old (0.97 mg/L or 12.2%; p<0.01), children 12-23 months old (0.8 mg/L or 9.5%; p=0.023) and lactating women (0.93 mg/L or 11.8%; p<0.001). In contrast, there was a significant decrease in transferrin receptor concentration among non-lactating women (-10.8 mg/L or 65.2%, p<0.001) (**Table 20**).

Table 20. Soluble Trans-ferrin receptor concentrations (mg/L), baseline and endline

	Baseline		Endline		mean diff [95% CI]	% diff	Sig. (2-tailed)
Group	n	Mean±SD	n	Mean±SD			
Infants 6-11m	303	7.9±2.7	321	8.9±3.5	0.97 [0.47-1.46]	12.2	<0.001
Children 12-23m	227	8.5±3.6	226	9.3±3.9	0.8 [0.1-1.5]	9.5	0.023
Children 24-59m	223	6.8±2.4	223	7.1±2.9	0.3 [-0.2-0.8]	4.6	0.214
Children 5-9y	154	5.6±1.2	154	5.7±1.5	0.1 [-0.2-0.3]	1.6	0.50
Lactating mothers	303	7.9±2.7	326	8.9±3.5	0.93 [0.4-1.4]	11.8	<0.001
Non-lactating women 15-29y	168	16.5±35.6	168	5.7±3.7	-10.8 [-16.2,-5.3]	-65.2	<0.001

Table 21 shows the change of body iron stores among study groups. No significant changes were observed in body iron stores in children under 59 months and lactating women. However, body iron stores increased by 0.5 mg/kg (p=0.036) among 5-9 year children, and by 0.7 mg/kg (p=0.036) among women 15-29 years.

Table 21. Body iron stores (mg/kg), baseline and endline by study group

	Baseline		Endline		mean diff	% diff	Sig. (2-tailed)
Group	n	Mean ±SD	N	Mean ±SD	[95% CI]		
Infants 6-11 m	303	2.1±3.7	321	1.9±3.9	-0.15 [-0.7-0.5]	-7.1	0.633
Children 12-23 m	227	1.0±4.3	226	0.6±4.1	-0.4 [-1.1-0.4]	-38.2	0.347
Children 24-59 m	223	3.5±3.6	232	3.9±3.4	0.4 [-0.4-0.9]	6.7	0.434
Children 5-9 y	154	5.8±2.5	154	6.3±2.7	0.5 [0.03-0.9]	8.5	0.036
Lactating mothers	303	2.1±3.7	326	1.9±3.9	-0.1 [-0.7-0.5]	-6.2	0.676
Non-lactating women 15-29 y	168	6.0±3.0	168	6.6±3.6	0.7[0.04-1.3]	11.1	0.036

IV.8 Child growth

Table 22 shows height-for-age and weight-for-height Z-scores (HAZ and WHZ scores), and the prevalence of stunting and wasting, among sub-groups of children 6-59 months.

These results are after capping of extreme values <-4 and >+4 HAZ and WHZ, as shown in **Table 23**.

From baseline to endline, HAZ scores tended to be slightly higher at end line than baseline, and this was significant for children 12-23 months (p=0.018). Stunting prevalence was lower at endline in infants 6-11 months (from 26% to 9%, p<0.01), and children 12-23 months (from 39% to 27%, p=0.004). WHZ scores for children 6-23 months was lower, however the prevalence of wasting did not change significantly.

A correlation was found between HAZ, WAZ and WHZ scores, as expected for these interrelated measures (**Figure 5**). However, individual-level data (**Figure 6**) showed a relatively wide variation in HAZ scores (but not WHZ scores) especially in the baseline survey and notably for infants 6-11 months. This indicates sub-optimal quality and precision of the height and length measurements in the baseline survey, a limitation that should be taken into account when interpreting HAZ scores and their apparent shift.

Table 22. Mean Z-scores for height for age (HAZ) and weight for height (WHZ), and prevalence of stunting and wasting, by age group at baseline and endline

	Group	Baseline		Endline		
		N	Mean±SD	N	Mean±SD	p-value*
HAZ	6-11 months	324	**-0.90** ±1.74	342	**-0.72** ±1.19	0.12
	12-23 months	240	**-1.60** ±1.64	238	**-1.29** ±1.17	0.018
	24-59 months	236	**-1.77** ±1.28	244	**-1.67** ±1.01	0.38
WHZ	6-11 months	324	**0.16** ±1.50	342	**-0.16** ±1.14	0.002
	12-23 months	240	**-0.020** ±1.45	238	**-0.27** ±1.08	0.031
	24-59 months	236	**-0.27** ±1.13	244	**-0.28** ±1.03	0.91
Stunting	6-11 months	324	**26.5%**	342	**9.9%**	0.000
	12-23 months	240	**39.2%**	239	**26.8%**	0.004
	24-59 months	236	**42.8%**	246	**38.6%**	0.35
Wasting	6-11 months	324	**4.9%**	342	**4.4%**	0.73
	12-23 months	240	**5.8%**	239	**5.0%**	0.69
	24-59 months	236	**3.4%**	246	**4.5%**	0.54

* Comparisons were not age-adjusted. Mean ages increased from baseline to endline samples in the 6-11 months and 12-23 months groups: 8.1 to 8.5 months (p<0.001); 17.1 to 18.2 months (p<0.001).

Table 23. Number of outlying HAZ and WHZ scores capped by age group, baseline and endline

	HAZ				WHZ			
	Baseline		Endline		Baseline		Endline	
	< -4	*+4*	*< -4*	*+4*	*< -4*	*+4*	*< -4*	*+4*
Child 6-11m	27	2	3	2	0	4	2	1
Child 12-23m	25	2	2	0	0	2	1	0
Child 24-59m	15	0	6	0	0	0	0	1
TOTAL	**67**	**4**	**11**	**2**	**0**	**6**	**3**	**2**

Note: Capping at -4 for values below -4, and at +4 for values above +4.

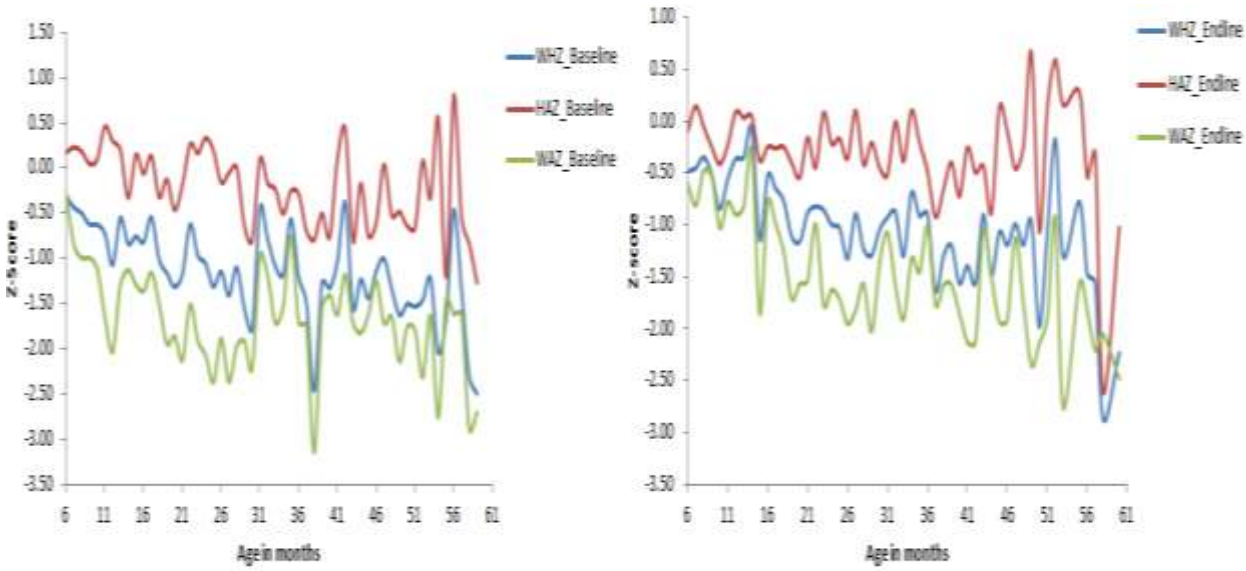

Figure 5. Mean Z-Scores of Height-for-Age (HAZ), Weight-for-Age (WAZ) and Weight-for-Height (WHZ) of children 6-59 months.
(left) baseline; (right) endline.

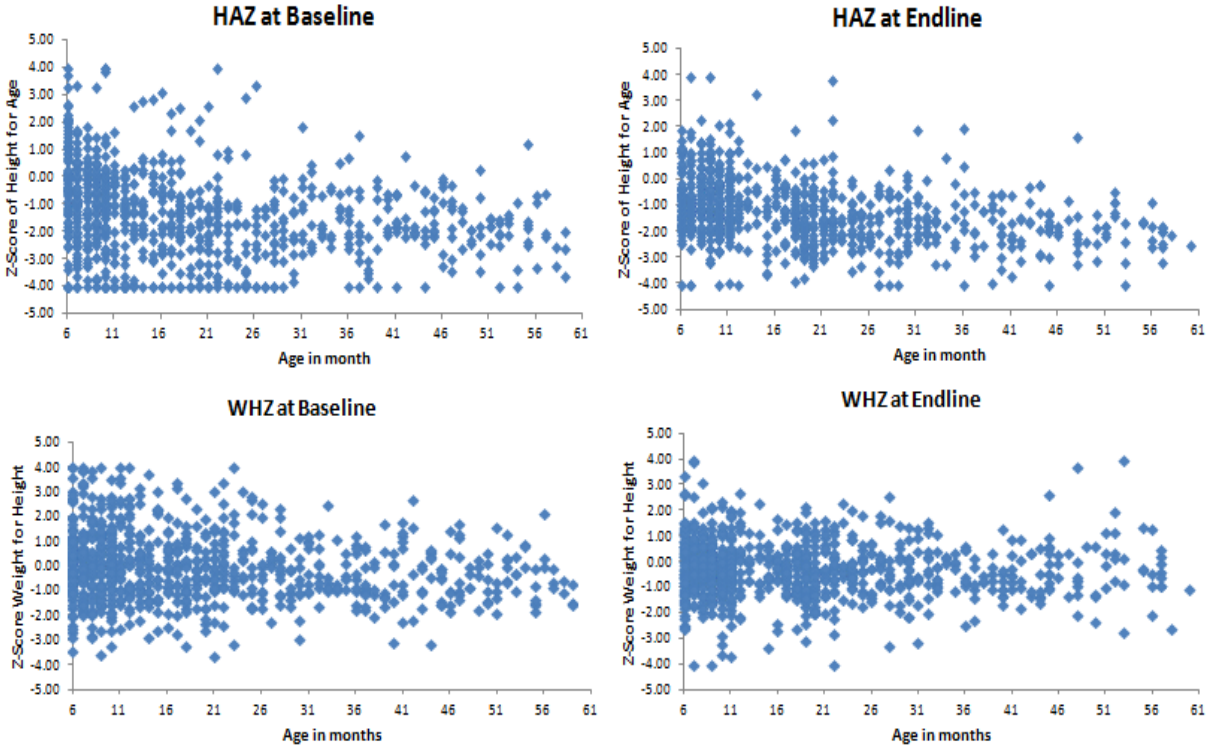

Figure 6. HAZ and WHZ scores of children 6-59 months by age, baseline and endline survey

IV.8.a. Clustering of poor child growth and poor vitamin A status

Stunting and HAZ, and to a lesser extent wasting and WHZ, were associated with poorer serum retinol status at baseline in infants 6-11 months and children 12-23 months, but not in children 24-59 months (**Tables 24-25**). This clustering reflects shared risk factors for poor nutritional status and poor growth: poverty, poor hygiene and poor overall nutrition. The vitamin A/growth clusterings were weaker and less significant at endline than at baseline, reflecting most likely that vitamin A status improved most in those children who had worst baseline vitamin A and growth status.

Table 24. Clustering between low mean HAZ and VAD (defined as CRP/AGP-adjusted serum retinol concentration<20µg/dL) among children 6-59 months old

Group	Time	Vitamin A status	n	Mean HAZ	p-value
Infants 6-11 m	Baseline	Deficient	55	-1.26	0.065
		Normal	248	-0.78	
	Endline	Deficient	20	-0.74	0.98
		Normal	301	-0.73	
Children 12-23 m	Baseline	Deficient	30	-1.58	0.97
		Normal	197	-1.59	
	Endline	Deficient	11	-1.39	0.73
		Normal	213	-1.26	
Children 24-59 m	Baseline	Deficient	19	-1.84	0.74
		Normal	204	-1.74	
	Endline	Deficient	2	-2.16	0.53
		Normal	228	-1.70	

Table 25. Clustering of low mean (CRP/AGP-adjusted) serum retinol concentration with stunting among children 6-59 months old

Group	Time	Stunting	N	Mean serum retinol concentration (µg/dL)	p
Infants 6-11 months	Baseline	Yes	80	28.56	0.074
		No	223	31.48	
	Endline	Yes	34	34.90	0.84
		No	287	34.44	
Children 12-23 months	Baseline	Yes	90	37.04	0.017
		No	137	32.36	
	Endline	Yes	57	34.84	0.91
		No	167	35.03	
Children 24-59 months	Baseline	Yes	94	35.60	0.71
		No	129	36.31	
	Endline	Yes	94	38.24	0.93
		No	136	38.39	

IV.9 Morbidity

Based on self-reported morbidity collected in the cohorts, children 5-9 years were ill more often than women 15-29 years (**Table 26 & Figure 7**). Among the children, acute respiratory infection (ARI) was the most common illness, with a prevalence twice as high as the other reported causes combined. ARI was also the major cause of morbidity among women, in most of the months studied. In both age groups diarrhea and pneumonia were uncommon, and occurring only in some months.

The proportion of subjects reporting no illness during the past month (based on two-weekly records) increased over the 8 months analyzed in women ($p<0.001$ in Pearson chi-square, <0.001 in likelihood ratio test and $p=0.001$ in linear-by-linear association test) but not significantly in children 5-9 years ($p>0.10$ in all three statistical tests) **(Figure 7).**

Table 26. Number of episodes of illness reported over the past month, children 5-9 years and non-lactating women 15-29 years, between October 2011 and April 2012

	Children 5-9y			Women 15-29y		
	Reported illness		Max. episodes of illness	Reported illness		Max. episodes of illness
ARI	78	32.6%	6	52	20.6%	6
Pneumonia	3	1.3%	3	2	0.8%	1
Diarrhea	4	1.7%	2	4	1.6%	1
Measles	1	0.4%	1	0	-	0
Other	29	12.1%	2	36	14.3%	3
Any morbidity	**100**	**41.8%**	**8**	**84**	**33.3%**	**6**
No morbidity	139	58.2%		168	66.7%	
Total cohort	**239**	**100%**		**252**	**100%**	

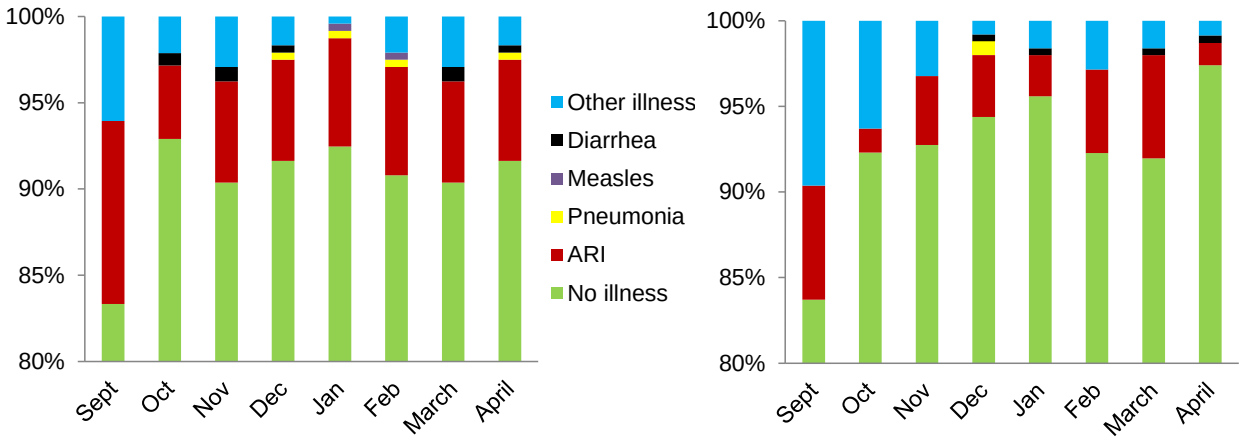

Figure 7. Self-reported morbidity in the past month, by calendar month
(left) children 5-9 years, (right) women 15-29 years.

Preliminary analysis indicates that there is a striking correlation between worse serum retinol status and higher self-reported morbidity (**Figure 8** & **Table 27**), which was significant in children 5-9 years but not in women 15-29 years (**Table 27**). The relation between lower serum retinol concentration and higher morbidity weakened over the months in both groups, as morbidity fell most in those children and women with worst retinol status at baseline. This finding calls for further study.

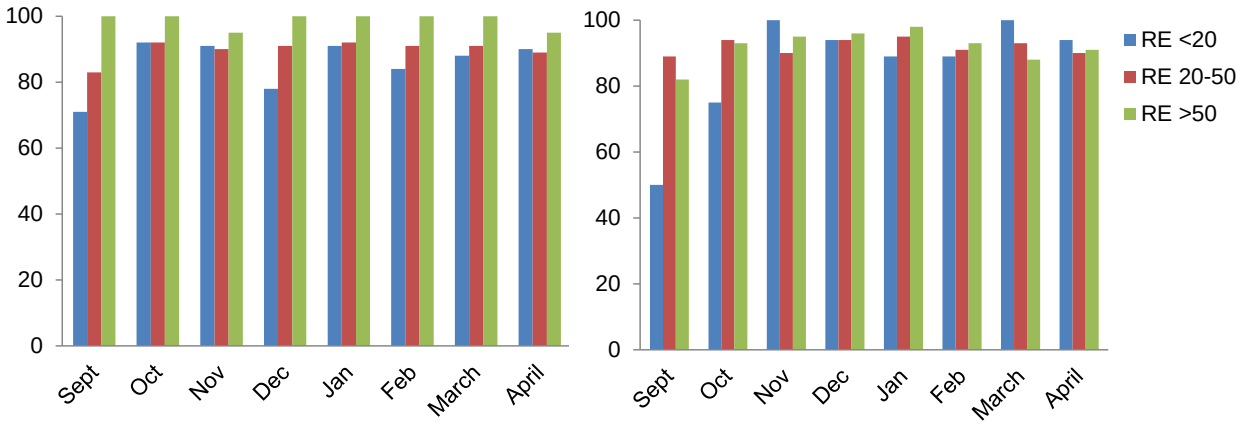

Figure 8. Proportion of cohort without reported morbidity by month and baseline serum retinol (RE) concentration
(left) children 5-9 years, (right) women 15-29 years.

Table 27. Relationship between mean monthly number of episodes of illness, averaged over eight months, and baseline serum retinol concentrations

Serum retinol concentration (µg/dL)	Children 5-9 years Mean number of episodes of illness	N	Non-lactating women 15-29 years Mean number of episodes of illness	N
< 20	0.91	32	0.89	18
20-50	0.74	187	0.56	158
> 50	0.2	20	0.53	76
Total	**0.72**	**239**	**0.57**	**252**
p-value for linear trend (ANOVA)	0.038		0.31	

V. DISCUSSION

This study found marked improvements in serum retinol concentration in poor communities in West Java after oil fortification, with vitamin A deficiency reduced by between 64-96% across age groups studied. Fortified oil contributed an estimated 4-40% of RNI requirements for vitamin A among the differentage groups. Increases in serum retinol concentrations >10% ($p<0.05$) were found in all groups studied, except among children 12-23 months. Retinol concentration in breast milk increased by 58% ($p<0.01$).

Fortification levels of the oil reaching households were below the SNI recommended standard, with marked fluctuations over the months. Further research is necessary to determine whether this was due to blending of fortified oil with non-fortified oil in the lower portions of the distribution system, or because of degradation of retinyl palmitate in the fortified product. As Wilmar only has a "majority" market share, it must be assumed that both factors play a role. While cooking oil is distributed directly from the factory in Palembang to the major distributor in both districts, it is less clear how it moves through the distribution network down to small village stalls. Wholesalers at below-district level will often buy on the spot market, and mix oils purchased from different suppliers. In addition, in Tasikmalaya the flow of fortified cooking oil was intermittent between December 2011 and February 2012, with cooking oil occasionally being completely unfortified, following problems with customs clearance of the premix. This issue should be largely resolved after fortification becomes mandatory and enacted (following a 1-year grace period) from 2015 onwards. The government's intent to also issue a mandatory law for packaging of all cooking oil in the market should help further.

The fluctuations in vitamin A levels of oil sampled from households in this study also underscore the importance of adequate QA/QC and enforcement to ensure uniform compliance to the SNI once fortification becomes mandatory. KFI, with support from GAIN and BioAnalyt, works with the national food and drugs authority, BPOM, to improve QA/QC capacity and standardization of analyses and results reporting across certified laboratories (including private, university, and government-owned labs) across Indonesia. BPOM should collect and analyze samples periodically, using I-Check, and HPLC for cross-validation. A next step may be to set up a national reference laboratory, and agree on procedures of analysis, and reporting of results. As part of a longer-term sustainable fortification strategy, Indonesia may wish to establish a national institution to build capacity for monitoring and evaluation of food fortification programs, including vitamin A in cooking oil. Quality assurance should also include ensuring low peroxide levels in oil, which critically determines the stability of vitamin A during storage [Laillou et al 2012]. Per Indonesia's 2012 SNI, peroxide levels are limited to a maximum of 10 meq O_2/kg [BSNi 2012]. In a study conducted in 2011, peroxide values in unbranded palm cooking oil from Java and Sumatera averaged 2.9 meq/kg across 56 samples from oil producers, and 2.55 meq/kg among 53 samples among oil distributors [Andarwulan & Martianto, 2012].

Improvements in serum retinol concentrations were above expectation, especially given the below-SNI-recommended levels of VA in oil at point-of-purchase and in households. This trend – as well as the decline in cohorts' morbidity – probably reflects an effect of the fortified oil, but also an important shift in socio-economic status of study households (Table 11). Because the increase in wealth was equally seen in the cohort groups (children 5-9 years and women 15-29 years) as in cross-sectional groups, it must partially reflect a secular trend during the study year; however data quality checks also noted that the endline survey had sampled some respondents from households not meeting the poor-household criterion, as well as some Posyandu cadres – unlike in sampling protocol and baseline sample. Ongoing socio-economic improvement and associated health effects are apparent in Indonesia nation-wide. These trends were apparently even more prominent in the disadvantaged study area, where the study year concurred with special government and presidential efforts to stimulate economic growth and mitigate poverty, creating a special office to move industry into Tasikmalaya, the district with the worst health status in West Java, during the study year.

Regression results support the positive contribution of vitamin A intake from oil to improving serum retinol concentration among children 6-59 months and 5-9 years. This effect was strongest in those children and villages with lowest serum retinol concentration at baseline. Due to large fluctuations within villages and individuals in serum retinol measurements (and possibly in determinants, such as oil consumption and retinol levels in oil), effects of RNI from oil were significant only when analyzed at the level of village-averages.

It is remarkable that we found strong vitamin A status improvements also in children 6-59 months, most of whom were covered with twice-yearly high-dose VAC supplementation. In multivariate regressions, vitamin A intake from both oil and supplementation capsules showed independent positive effects on serum status improvement in children 6-59 months. Apparently, children get complementary benefits from both 6-monthly high-dose supplementation (which reduces mortality and prevents blindness) and daily low-dose fortification.

According to the WHO standard defining vitamin A deficiency as a severe public health problem if 20% of the population has serum retinol concentrations below 20 µg/dL [WHO & UNICEF 1996, WHO 2012], vitamin A deficiency is not a severe public health problem in Indonesia anymore. However, while appropriate for distinguishing risks of blindness and mortality, this definition may be missing important additional public health consequences of sub-optimal vitamin A status, including more subtle “anti-infective” effects, as suggested by the correlations between low vitamin A levels and higher morbidity in children 5-9 years here and elsewhere [Thornton et al. 2014]. Furthermore, the weakening of this association over the year suggests that oil fortification may effectively alleviate morbidity risks associated with sub-optimal vitamin A status. The Aceh study, one of the keystone pieces of research in the child survival revolution, found a 34% reduction in mortality, but no reduction in incidence of disease [Abdeljaber, 1991]. However, the morbidity data in the current study should be interpreted with caution, as they were based on self-reporting – and the prevalence of sub-clinical infection (indicated by serum CRP and AGP-levels) did not decrease from baseline to endline. To the extent that fortification contributed to the observed reductions in morbidity, the public health implications would be tremendous,.

The correlation observed between serum retinol and RBP concentrations (**Annex 7**) was similar to earlier studies elsewhere in Indonesia [Semba 2002; de Pee & Dary 2002], but lower than in recent studies in other countries, where both values were determined by the same laboratory [de Pee & Dary 2002; Danneskiold-Samsøe et al. 2013]. This may point to less than optimal precision in retinol and/or RBP measurements in the study, an interpretation that would be consistent with the lack of correlation in individuals’ retinol and RBP concentrations between baseline and endline (not shown). Low precision in retinol measurements further explains why regression models with individual-level retinol as outcome failed to establish meaningful retinol concentration predictors, apart from a strong ‘regression to the mean’ effect.

Among infants 6-11 months, stunting was only half as prevalent at endline compared to baseline. Such a very large decrease over a single year is unexpected and certainly multi-causal. Vitamin A has been shown to improve linear growth in severely VA-deficient children [Hadi, 2000], but at the relatively low VAD prevalence in the study an increased (own and/or maternal) VA intake and status are unlikely to contribute to stunting reduction. More likely, the unexpected differences may be an artefact of poor quality of height and length measurements, especially in the baseline survey, as suggested by the more than typical variation and outliers in HAZ [Mei & Grummer-Strawn 2007]. Length measurement is difficult in infants below 12 months. Socio-economic differences between baseline and endline surveys are also likely to have contributed, but it remains unclear to which extent these differences reflect an ongoing secular trend, and/or differences between the two survey samples.

Both baseline (20%) and endline (9%) stunting prevalences were far below those measured on Java in recent nationally representative surveys (Riskesdas 2007, 2010 and 2013, and SEANUTS [Sandjaja 2013]) – and this contrast remains unexplained, especially given the study’s purposive

sampling of poor villages and households. The striking correlation between poor vitamin A status and lower HAZ at baseline points to shared (poverty-related) risk factors. And the disappearance of this Vitamin A/HAZ association at endline indicates that oil fortification succeeded in reducing vitamin A deficiency especially in the neediest, worst-growing children.

We observed no significant improvements in average hemoglobin concentrations (except for a slight significant improvement in children 5-9 years), and no significant decreases in anemia prevalence. In comparison, earlier studies including in Indonesia in the mid-1980s and 1990s found positive effects of improved vitamin A status (through supplementation) on hemoglobin and anemia risk, against a background of much higher prevalences of both anemia [Suharno 1993, Semba 2002, Tanumihardjo 2004] and VAD. Quite likely, nation-wide fortification of wheat flour with iron since the early 2000s has contributed to reducing anemia in Indonesia, although this has never been formally evaluated.

The other iron markers measured did not yield a consistent pattern of improved iron status, as would have been expected from a trial of successful VAC distribution for Moroccan school children with high vitamin A deficiency and high anemia prevalence [Zimmermann et al 2006].

V.1. Limitations of the study

Being framed by the nature of a private-sector market-based intervention, the design of the study did not include a control group. Therefore results cannot conclusively be interpreted as demonstrating impact of the oil fortification, although analyses of RNI contributions and multivariate regressions of vitamin A status improvements adjusting for likely confounders are highly supportive of such an impact.

Consumption of oil and (animal) foods and their vitamin A RNI contributions were based on a single 24-hour dietary recall. These were done through a short questionnaire, that had not been designed and conducted optimally, and that was administered by under-trained enumerators lacking skills in standardized dietary recall methods, and without the aid of pictures of portion sizes that would have facilitated the dietary recall. Food consumption outside the home was not recorded. The dietary data were subsequently analyzed against an outdated national food composition table. The dietary recalls generally recorded a lower animal-source food intake (as well as overall protein, energy and fat intake) at endline compared to baseline, for all groups except children 12-59 months (**Annex 9**). Expenditures on food remained stable and it is probable that this apparent contradiction in part reflects increased food prices. In addition, the data collection and quality control procedures during baseline and endline were sufficiently different to warrant caution in interpretation of the dietary intake data. For lactating mothers, total energy intake according to the dietary recall at endline was an implausibly low <1200 kCal, suggesting that overall intake and RNI contributions from food, and possibly oil, may have been under-estimated. Conversely, there is a suspicion that animal food intake for young children (12-59 months) may have been over-reported by mothers – which may explain the high (43-64%) RNI intakes from animal foods in these groups.

Breast milk analyses confirmed improved, high vitamin A concentration in breast milk in the endline survey after fortification started (**Figure 4**). A validation of these results had been planned with Dr. Sherry Tanumihardjo, University of Winconsin USA, but got cancelled due to suspected damage to the breast milk samples during their shipment from Jakarta to Wisconsin. Because of uncertainties in the accuracy of vitamin A concentrations measured in breast milk (at Bogor University), and since the study did not measure volumes of breast milk consumed by children, we refrained from quantifying vitamin A intake and RNI contributions for infants from breast milk.

Results of the multivariate regressions should be interpreted with the following additional *caveats* in mind:

- The RNI contributions from oil and from animal foods were the best possible estimates based on available data, but probably imprecise. The vitamin A content of cooking oil used in the regression analysis was based on all oil samples available (both freshly analyzed and those

analyzed 6-18 months after collection). Part of the variation in oil RNIs among households and villages thus reflects varying storage times among oil samples, and this study artefact / non-differential mis-classification may have diluted the apparent relation between RNI contribution and improvement in vitamin A status. The validity and precision of i-Check measurements of vitamin A in oil, compared to HPLC as the gold standard, remains under active investigation by the SEAFAST laboratory, in preparation for the use of i-Check for nation-wide program QA/QC, which was beyond the scope of the current study.

- RNI contributions from oil were extrapolated from selected households (from which a child 5-9 years or woman 15-29 years participated in the study cohort), via village-averages, to non-sampled households.
- The focus on the village as unit of analysis effectively reduced random noise in both the independent and dependent variables, notably the fluctuation in serum retinol values within and among individuals that likely reflects physiological variation as well as measurement imprecision. However, analysis with 24 villages gave only 23 degrees of freedom and thus, the regression analyses had only limited statistical power. This may explain why only one or a few predictor variables at a time came out as statistically significant. In any model, for example RNI from oil *or* baseline serum retinol concentration, but not both – and no effect of socio-economic variables entered the model. Nevertheless, the village-level analysis showed a consistent positive effect of RNI from oil (though not always significant) and good overall statistical fit (R^2 between 40-75%), whereas regressions at individual level all gave unacceptably poor fit – likely due to measurement error in both the key predictor variable RNI contribution from oil (oil consumption, and oil VA levels) and in the outcome variable serum retinol concentration.

Due to these limitations, we cannot exclude that in reality there may be stronger effects of vitamin A intake from oil (and of socio-economic determinants) than the regressions were able to detect.

Finally, based on its sampling design the population evaluated was poorer than Indonesia's nation-wide average, but similar in terms of oil purchase, storage, cooking and consumption behaviours, as well as in overall diet {Martianto et al., 2005;Martianto et al. 2009;Indonesia Badan Pusat Statistik Direktorat Diseminasi Statistik, 2010;Andarwulan & Martianto, 2012}. Recent other surveys found either higher vitamin A deficiency prevalence (pre-school children on Central Java {Pangaribuan et al, 2003}; school children in Makassar, 2008 {Martianto et al, 2009}) or lower prevalence (pre-school children in Semarang {Kartasurya et al, 2012}, and a national survey of pre-school and school children in 2011 {Sandjaja et al, 2013}). Future evaluations of the pending mandatory oil program should tell if the magnitude of Vitamin A status improvements shown here can be replicated at national scale.

Results should be interpreted against Indonesia's background of partial fortification with vitamin A of dry skim milk powder (mandatory, since mid-1990s) and about half of wheat flour noodles (voluntarily, since the early 2000s) {Semba et al, 2011}. Since our vitamin A intake analysis used Indonesia's latest official food composition table, from 1974 i.e. well before milk powder and noodle fortification starated, we may have underestimated vitamin A intake from foods.

V.2. Next steps

Further analyses that may strengthen the study's interpretation and programmatic implications include:

- determinants of infant vitamin A status at the level of **mother-infant pairs**, including **RNI contributions from breast milk** (although the study did not record breast milk consumption volumes);
- **cohort morbidity using the full dataset of a year of bi-monthly reporting**, instead of the 8 monthly summaries presented in this report;

- **Vitamin A intake from oil and non-oil foods estimated from household expenditures** instead of dietary consumption. These would need to address known quality and interpretation challenges in expenditure data, most importantly expenditure being at household level not individual level, and high-end outliers in oil purchase from traders of frittered foods (to exclude). While mothers tend to remember with a great deal of precision the expenditures for food within the last week, many staples are purchased (or bartered) on a multiple-monthly basis, leaving the available single data point of expenditure data (one at baseline, one at endline) inevitably imprecise.
- Alternative multivariate models of determinants of serum vitamin A improvement:
  - focusing socio-economic covariates (household wealth etc.) on *baseline-to-endline shifts* instead of on their value at endline;
  - Possibly considering **RBP or a combined retinol/RBP rank as possibly more precise vitamin A status indicator**, than retinol alone.
- Oil RNI contributions and **VA status improvements by wealth quintile** (or other socio-economic disaggregation), to assess to what extent fortification is an equitable, pro-poor strategy.

VI. CONCLUSIONS AND PROGRAMMING IMPLICATIONS

In conclusion, this pilot project shows the possibilities of large-scale oil fortification to improve poor people's vitamin A intake and status. This impact notably includes infants 6-11 months, who do not themselves consume much oil yet, but appear to benefit via maternal consumption during pregnancy and/or breastfeeding. The study furthermore highlighted striking associations between poor vitamin A status and morbidity in children 5-9 years and non-lactating women, which weakened from baseline to endline as morbidity fell most in those children and women with poorest vitamin A status at baseline.

Study findings should be interpreted in the light of the design without a control group and several data quality issues. The health improvements were unexpectedly large in view of the below-standard levels of vitamin A in oil reaching households. They likely reflect a contribution from fortified oil, as well as significant socio-economic developments in the study area, and possibly sampling and methodological differences between the surveys.

Meanwhile, findings suggest that there is much to be gained from improving vitamin A status in poor Indonesian communities, with benefits possibly extending into reduced child morbidity, even if Indonesia no longer meets the WHO criteria for having a significant vitamin A public health problem [WHO 2012].

To optimize the impact of the oil fortification program now launched nation-wide, adequate QA/QC and enactment will be essential to assure universal industry compliance with fortification standards. Comprehensive monitoring and evaluation should include availability and consumption of fortified oil, and ideally health effects, through follow-up surveys in the study area and other sentinel sites. Health impact evaluation might consider using RBP concentration as a cheaper indicator of vitamin A status than serum retinol concentration.

Indonesia is a leading supplier of cooking oil for neighboring countries, which have a similarly high vitamin A deficiency burden and universal consumption of palm oil as the major source of fat. The program therefore has tremendous potential to reduce vitamin A deficiency and its associated disease and death burden throughout Asia and beyond, in a highly cost-effective way.

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VIII. ANNEXES

ANNEX 1. Conversion factors for vitamin A

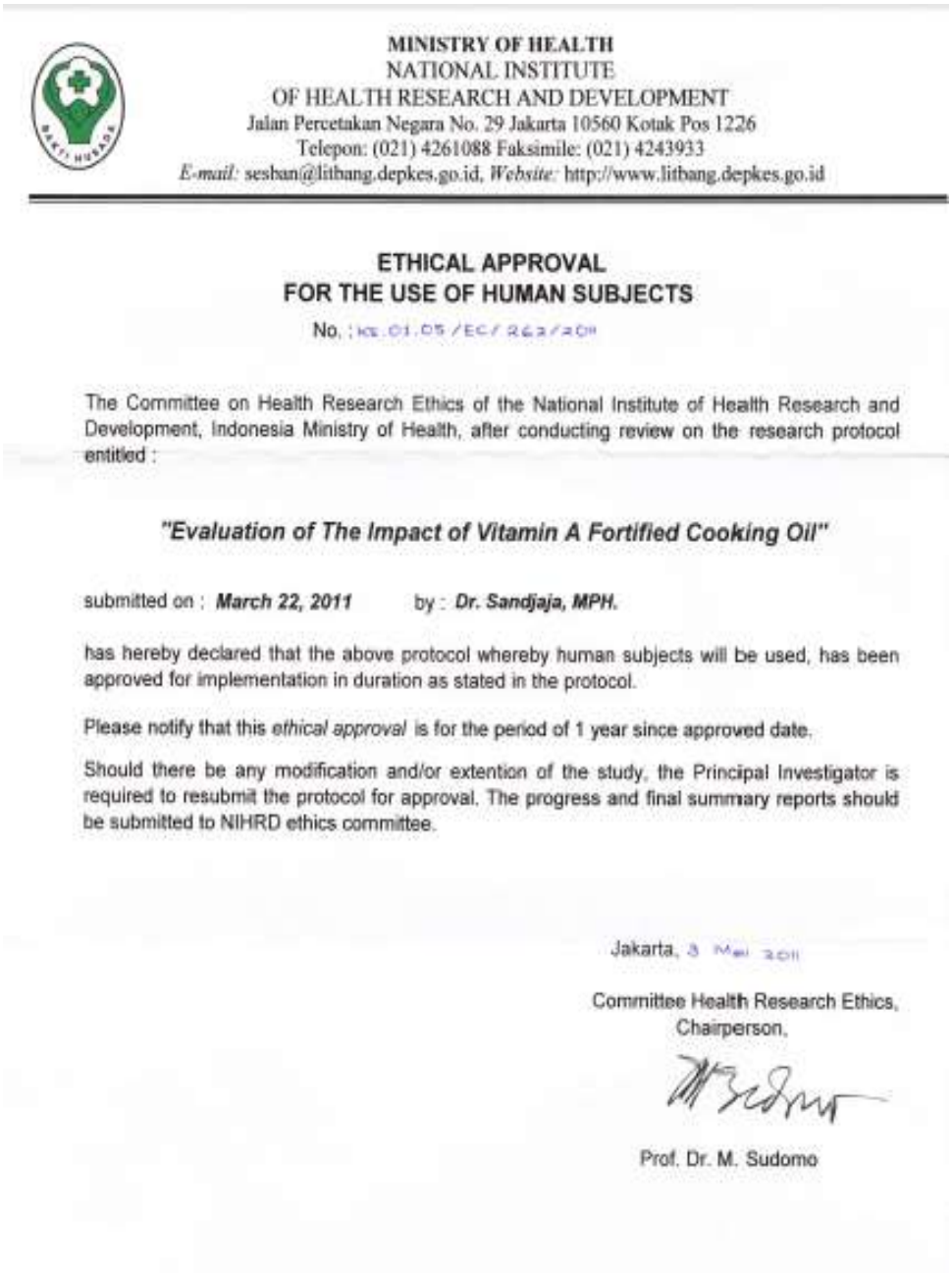
1 IU Retinol Equivalent (RE, in serum or breastmilk) = 0.3 microgram Retinol Equivalent (RE)
1 IU retinyl palmitate (in oil) = 0.55 mg retinyl palmitate

ANNEX 2. Questionnaires and Informed consent forms

in Dropbox, folder *Impact study KFI VitA Oil fortification > Questionnaire and InformedConsentForms*. Access via Mr. Ifrad (ifrad.dds@gmail.com).

ANNEX 3. Ethical approval letters

NIHRD Ethical Review Committee approval letter for the baseline study number KE.01.05/EC/262/2011 dated May 3, 2011:



MINISTRY OF HEALTH
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ETHICAL APPROVAL
FOR THE USE OF HUMAN SUBJECTS

No. : KE.01.05/EC/262/2011

The Committee on Health Research Ethics of the National Institute of Health Research and Development, Indonesia Ministry of Health, after conducting review on the research protocol entitled :

"Evaluation of The Impact of Vitamin A Fortified Cooking Oil"

submitted on : ***March 22, 2011*** by : ***Dr. Sandjaja, MPH.***

has hereby declared that the above protocol whereby human subjects will be used, has been approved for implementation in duration as stated in the protocol.

Please notify that this *ethical approval* is for the period of 1 year since approved date.

Should there be any modification and/or extention of the study, the Principal Investigator is required to resubmit the protocol for approval. The progress and final summary reports should be submitted to NIHRD ethics committee.

Jakarta, 3 Mei 2011

Committee Health Research Ethics,
Chairperson,

Prof. Dr. M. Sudomo

Ethical approval letter from the Ministry of Health Research Division Ethical Review Committee for the endline survey in 2012 (Number KE.01.05/EC/409/2012 dated May 16, 2012):

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NATIONAL INSTITUTE
OF HEALTH RESEARCH AND DEVELOPMENT
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ETHICAL APPROVAL
FOR THE USE OF HUMAN SUBJECTS

No. : KE.01.05/EC/409/2012

The Committee on Health Research Ethics of the National Institute of Health Research and Development, Indonesia Ministry of Health, after conducting review on the research protocol entitled :

"Evaluation of The Impact of Vitamin A Fortified Cooking Oil (Year 2 : Endline)"

submitted on : ***April 23, 2012*** by : ***Sandjaja, MPH., Dr.PH.***

has hereby declared that the above protocol whereby human subjects will be used, has been approved for implementation in duration as stated in the protocol.

Please note that this *ethical approval* is for the period of 1 year since approved date.

Should there be any modification and/or extention of the study, the Principal Investigator is required to resubmit the protocol for approval. The progress and final summary reports should be submitted to NIHRD ethics committee.

Jakarta, May 16, 2012

Committee of Health Research Ethics,
Chairperson,

Prof. Dr. M. Sudomo

ANNEX 4. Selected study areas in Tasikmalaya and Ciamis districts

Tasikmalaya				Ciamis			
Sub-district		Village		Sub-district		Village	
1.	Manonjaya	1.	Kamulyan	1.	Banjarsari	1.	Ciherang
		2.	Margahayu			2.	Sindangasih
		3.	Margaluyu			3.	Sukasari
4.	Rajapolah	4.	Dawagung	2.	Cipaku	3.	Selacai
		5.	Manggungjaya			4.	Selamanik
		6.	Manggungsari			5.	Bunisari
6.	Sukaraja	7.	Janggala	3.	Sindangkasih	7.	Sukasenang
		8.	Sukapura			8.	Gunung Cupu
		9.	Margalaksana			9.	Sindangkasih
4.	Tanjungjaya	10.	Tanjungjaya	4.	Ciamis	10.	Sindangrasa
		11.	Cibalanarik			11.	Maleber
		12.	Cintajaya			12.	Ciamis

ANNEX 5. Eligibility Criteria

a) Women of early reproductive age:
   - 15-29 years of age, female
   - Not pregnant and not lactating (clinical examination, for vital fetus signs)
   - Consenting
   - No counter-indications for blood sampling (e.g. hemophilia)
   - No known severe disease (chronic or acute)
   - No severe anemia (< 7 g/dL)

b) Lactating women:
   - 15-49 years of age, female
   - Not pregnant (clinical examination, for vital fetus signs)
   - Lactating, with a high reported frequency of breastfeeding
   - Consenting
   - No counter-indications for blood sampling (e.g. hemophilia)
   - No known severe disease (chronic or acute)
   - No severe anemia (< 7 g/dL)

c) Breastfed infants 6-11 months of age:
   - Be of correct age
   - Being breastfed by the lactating woman included in the study
   - No counter-indications for blood sampling (e.g. hemophilia)
   - No known severe disease (chronic or acute)
   - No severe anemia (< 7 g/dL)
   - No severe underweight (< -3.0 Z-score)

d) Young children 12-23 months of age:
   - Be of correct age
   - No counter-indications for blood sampling (e.g. hemophilia)
   - No known severe disease (chronic or acute)
   - No severe anemia (< 7 g/dL)
   - No severe underweight (< -3.0 Z-score WHZ)
   - No severe overweight (> 3.0 Z score WHZ)

e) Preschool age children 24-59 months of age:
   - Be of correct age
   - No counter-indications for blood sampling (e.g. hemophilia)
   - No known severe disease (chronic or acute)
   - No severe anemia (< 7 g/dL)
   - No severe underweight (< -3.0 Z-score WHZ)
   - No severe overweight (> 3.0 Z WHZ)

f) School age children 5-9 years of age:
   - Be of correct age
   - No counter-indications for blood sampling (e.g. hemophilia)
   - No known severe disease (chronic or acute)
   - No severe anemia (< 7 g/dL)
   - No severe overweight (> 3.0 Z WHZ).

ANNEX 6. Serum retinol, RBP and ferritin concentrations with and without sub-clinical inflammation

Table A-1. Serum retinol (µg/dL), RBP (µmol/L) and ferritin (µg/L) in subjects with and without SCI, pooled between baseline- and endline surveys.

Age group	Variable	Including subjects with SCI		Excluding subjects with SCI		p-value
		N	Mean±SD	N	Mean±SD	
Infants 6-11m	Retinol	142	27.3±10.7	482	32.9± 12.6	<0.001
	RBP	142	0.9±0.2	482	1.03±0.3	<0.001
	Ferritin	142	40.2±33.9	482	26.7±28.2	<0.001
Children 12-23m	Retinol	124	28.9±12.4	328	35.1±12.0	<0.001
	RBP	124	0.9±0.2	328	1.1±0.2	<0.001
	Ferritin	124	35.3±40.0	328	19.1±17.78	<0.001
Children 24-59m	Retinol	118	30.2±11.2	337	38.2±12.9	<0.001
	RBP	118	1.0±0.2	337	1.1±0.2	<0.001
	Ferritin	118	48.7±39.6	337	32.4±21.4	<0.001
Children 5-9y	Retinol	59	33.6±13.9	280	35.7±11.2	0.212
	RBP	59	1.0±0.2	280	1.1±0.2	0.025
	Ferritin	57	77.7±46.7	280	50.0±29.8	<0.001
Lactating mothers	Retinol	145	27.7±11.1	484	33.0±12.6	<0.001
	RBP	145	0.9±0.2	484	1.0±0.3	<0.001
	Ferritin	145	39.8±33.7	484	26.7±28.1	<0.001
Non-lactating women 15-29y	Retinol	92	43.9±16.2	247	45.7±18.2	0.415
	RBP	92	2.3±2.6	247	1.8±1.6	0.035
	Ferritin	92	72.7±49.8	246	54.6±39.9	0.001

Table A-2. Mean serum retinol concentration (µg/dL), with and without adjustment for SCI

Age group	(n)	Unadjusted mean±SD	Adjusted mean±SD	p-value
Infants 6-11 m	Baseline (n=303)	29.8±12.3	30.7±12.6	<0.001
	Endline (n=321)	33.4±12.2	34.5±12.2	<0.001
Children 12-23 m	Baseline (n=227)	33.0±14.0	34.0±14.5	<0.001
	Endline (n=226)	33.8±10.5	35.0±10.5	<0.001
Children 24-59 m	Baseline (n=223)	35.1±14.0	36.0±14.1	<0.001
	Endline (n=232)	37.1±11.9	38.3±11.7	<0.001
Children 5-9y	Baseline (175)	33.9±12.7	34.7±12.9	<0.001
	Endline (170)	37.4±11.6	39.2±12.3	<0.001
Lactating mothers	Baseline (n=303)	29.8±12.3	30.7±12.6	<0.001
	Endline (n=326)	33.6±12.3	34.7±12.4	<0.001
Non-lactating women 15-29y	Baseline (170)	41.6±18.9	42.7±19.2	<0.001
	Endline (169)	48.8±15.7	50.9±16.5	<0.001

Table A-3. Mean serum RBP concentration (µmol/L), with and without adjustment for SCI

Age group	(n)	Unadjusted mean±SD	Adjusted mean±SD	p-value
Infants 6-11m	Baseline(n=303)	0.95±0.2	0.98±0.2	<0.001
	Endline (n=321)	1.04±0.3	1.08±0.3	<0.001
Children 12-23m	Baseline (n=227)	0.99±0.3	1.03±0.3	<0.001
	Endline (n=225)	1.05±0.3	1.09±0.3	<0.001
Children 24-59m	Baseline (n=223)	1.07±0.3	1.10±0.3	<0.001
	Endline (n=232)	1.12±0.3	1.16±0.2	<0.001
Children 5-9y	Baseline (175)	1.03±0.2	1.05±0.2	<0.001
	Endline (154)	1.11±0.3	1.13±0.3	<0.001
Lactating mothers	Baseline (n=303)	0.95±0.2	0.98±0.2	<0.001
	Endline (n=326)	1.05±0.3	1.09±0.3	<0.001
Non-lactating women 15-29y	Baseline (170)	2.33±2.6	2.41±2.8	<0.001
	Endline(169)	1.49±0.5	1.56±0.6	<0.001

Table A-4. Mean serum ferritin concentration (µg/L), with and without adjustment for SCI

Age group	(n)	Unadjusted mean±SD	Adjusted mean±SD	p-value
Infants 6-11 m	Baseline (n=303)	28.4±29.9	25.3±25.4	<0.001
	Endline (n=321)	31.0±30.3	27.6±27.5	<0.001
Children 12-23 m	Baseline (n=227)	23.6±26.0	20.2±20.7	<0.001
	Endline (n=226)	23.5±27.6	20.1±19.5	<0.001
Children 24-59 m	Baseline (n=223)	35.2±30.2	31.5±22.8	<0.001
	Endline (n=232)	38.0±26.1	33.6±22.7	<0.001
Children 5-9y	Baseline (173)	50.4±29.6	46.5±25.4	<0.001
	Endline (164)	59.2±39.1	54.8±33.5	<0.001
Lactating mothers	Baseline (n=303)	28.4±29.9	25.3±25.4	<0.001
	Endline (n=326)	31.0±30.1	27.6±27.4	<0.001
Non-lactating women 15-29y	Baseline (170)	53.1±37.2	49.3±35.1	<0.001
	Endline (168)	66.0±48.3	58.2±41.3	<0.001

ANNEX 7. Serum Retinol-RBP correlation

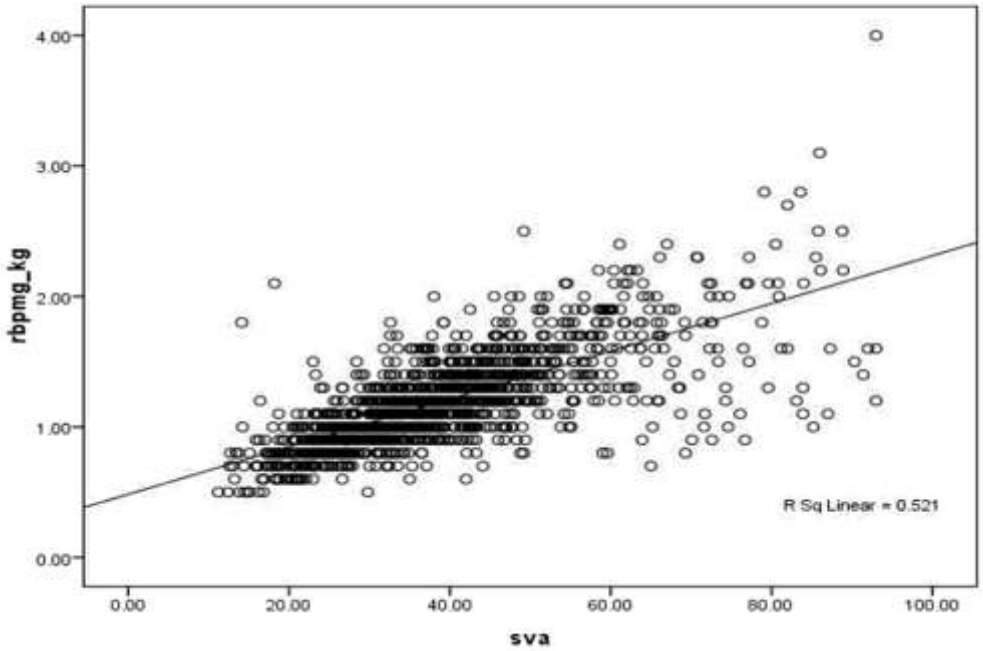

Figure A-1. Correlation between serum RBP and retinol concentrations (pooled data for all groups, from baseline survey, without adjustment for CRP/AGP)

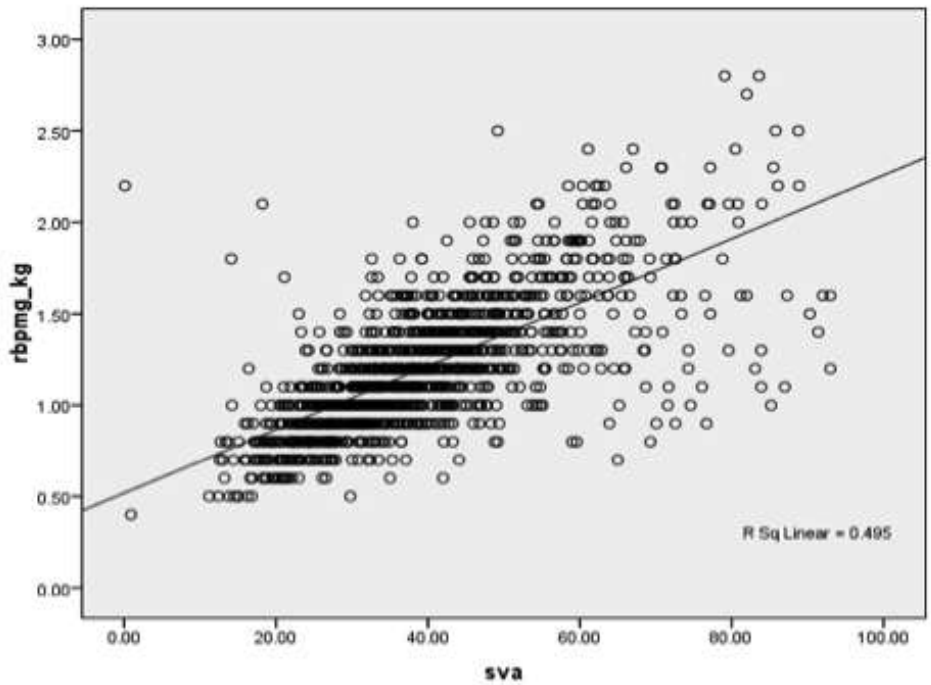

Figure A-2. Correlation between serum RBP and retinol concentrations (pooled data for all groups, at endline, without adjustment for CRP/AGP)

ANNEX 8. Retinol-binding protein results, baseline and endline

Table A-5. Retinol-binding protein (RBP) (µmol/L) at baseline and endline (adjusted for sub-clinical inflammation)

Group	Baseline		Endline		mean diff [95% CI]	% diff	Sig. (2-tailed)
	n	Mean±SD	n	Mean±SD			
Infants 6–11m	303	0.98±0.2	321	1.08±0.3	0.09 [0.05-0.14]	9.7	<0.001
Children 12-23m	227	1.03±0.3	225	1.09±0.3	0.06 [0.01-0.10]	5.4	0.022
Children 24–59m	223	1.10±0.3	232	1.16±0.2	0.05 [0.01-0.01]	4.9	0.021
Children 5-9y	154	1.05±0.2	154	1.13±0.3	0.09 [0.04-0.14]	8.4	<0.001
Lactating Mothers	303	0.98±0.2	326	1.09±0.3	0.1 [0.06-0.15]	10.6	<0.001
Non-lactating women 15-29y	168	2.39±2.7	168	1.56±0.6	0.83 [0.4-1.26]	34.8	<0.001

Like serum retinol, retinol binding protein (RBP) concentration improved from baseline to endline in all groups (**Table A-5**).

Serum retinol and RBP concentrations correlated positively and significant (r^2=0.52 at baseline and 0.495 at endline, without CRP/AGP-based adjustment). Despite the overall increase in mean concentrations of both biomarkers, their relationship was similar across the two cross-sectional surveys.

ANNEX 9. Intake of energy, protein, fat and animal foods at baseline and endline survey, from single 24-hour dietary recall.

Energy Total Intake (Cal/d)	**Baseline**			**Endline**			**Sig. (2-tailed)**
Group	n	**Mean**	SD	n	**Mean**	SD	
6 - 11 Months	255	**305**	231.05	340	**239**	154.07	.000
12 -23 Months	209	**663**	422.63	237	**582**	335.37	.025
24 - 59 Months	207	**966**	428.53	243	**843**	302.13	.000
Lactating Mothers	273	**1311**	568.11	346	**1104**	359.56	.000
School age Children	175	**953**	447.75	175	**941**	333.25	.736
Non-lactating women 15-29 years	160	**1048**	423.26	160	**996**	303.24	.174
Total Protein Intake (g/d)							
Group	n	**Mean**	SD	n	**Mean**	SD	
6 - 11 Months	255	**10.20**	8.62	340	**7.19**	5.59	.038
12 -23 Months	209	**21.81**	15.30	237	**18.65**	13.60	.021
24 - 59 Months	207	**29.65**	14.72	243	**27.08**	11.45	.038
Lactating Mothers	273	**38.78**	17.81	346	**33.01**	12.19	.000
School age Children	175	**29.09**	14.37	175	**28.34**	11.07	.524
Non-lactating women 15-29 years	160	**31.77**	15.28	160	**29.81**	11.90	.138
Animal Protein Intake (g/d)							
Group	n	**Mean**	SD	n	**Mean**	SD	
6 - 11 Months	255	**5.90**	6.59	340	**3.45**	4.16	.000
12 -23 Months	209	**21.81**	15.30	237	**18.65**	13.60	.182
24 - 59 Months	207	**14.67**	11.62	243	**14.30**	9.44	.713
Lactating Mothers	273	**13.87**	11.18	346	**11.45**	8.32	.002
School age Children	175	**14.05**	11.07	175	**12.18**	7.71	.047
Non-lactating women 15-29 years	160	**14.30**	11.90	160	**11.45**	8.53	.005
Fat Intake (g/d)							
Group	n	**Mean**	SD	n	**Mean**	SD	
6 - 11 Months	255	**8.51**	9.42	340	**5.81**	5.65	.019
12 -23 Months	209	**25.53**	20.12	237	**22.02**	14.44	.034
24 - 59 Months	207	**36.71**	19.81	243	**32.91**	14.45	.019
Lactating Mothers	273	**44.02**	26.59	346	**36.14**	17.42	.000
School age Children	175	**36.58**	21.50	175	**33.37**	14.80	.068
Non-lactating women 15-29 years	160	**39.00**	19.56	160	**34.59**	16.76	.030