

**“ObaapaVita” Vitamin A Supplementation and Maternal Mortality Trial: Randomized double-blind placebo controlled trial to evaluate the impact of vitamin A supplementation on maternal mortality in Ghana.**

## **TRIAL PROTOCOL**

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### **Funders:**

The trial was largely funded by the UK Department for International Development, with some contribution from USAID.

Vitamin A for the capsules was donated by Roche.

**Trial registration:** clinicaltrials.gov identifier NCT00211341

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## Background

Over 500,000 women die each year as a result of pregnancy and childbirth; this represents one death per minute worldwide [1]. Maternal health problems are the largest contributors to the disease burden of women of reproductive age: when grouped with perinatal complications, they are as large a contributor to the overall global disease burden as HIV, malaria and TB combined [2].

Maternal deaths occur mainly among the poor: most (99%) happen in low income countries where the lifetime risk associated with pregnancy can be as high as one death for every 22 women [1, estimate for Sub-Saharan Africa]. Conventional approaches of antenatal screening and training of traditional birth attendants have had little impact on these deaths [3]. The consensus which has recently emerged from within the maternal health community identifies three core healthcare strategies as being necessary to save women's lives: comprehensive reproductive health services; skilled care during and immediately after childbirth; and emergency obstetric care for women who develop potentially life threatening complications [4]. However, the poorest countries face "substantial obstacles" before the capacity to provide services in each of these areas becomes available [3]. Therefore, if an effective low-technology intervention to reduce pregnancy-related mortality could be found, it would be of great public health importance.

Vitamin A supplementation (VAS) of adult women may be one such intervention. Vitamin A is a fat-soluble vitamin necessary for the effective maintenance and functioning of many body tissues. Vitamin A deficiency (VAD) is an important nutritional problem in the majority of developing countries, with young children and pregnant and lactating women at greatest risk [5]. VAS has been shown to reduce all-cause mortality in children aged 6 months to 5 years by an average of 23% (95% CI 12%, 32%) [6], and vitamin A interventions now form an integral part of child nutrition and survival programmes in developing countries [7,8].

The NNIPS-2 trial in Nepal [9] suggested that this mortality benefit may extend to pregnant women, by showing that supplementation of women of reproductive age with either vitamin A or its pre-cursor (beta-carotene) reduced pregnancy-related mortality by 44% (95% CI 16%, 63%).

If this finding represents a real effect then vitamin A interventions should become an important component of maternal health initiatives. However, it would be premature to change policy or take programme action (maybe abandoning other safe motherhood interventions with proven effectiveness in favour of vitamin A) without testing efficacy in other settings. The ObaapaVitA trial aims to do this. It is the only trial of the impact of VAS on pregnancy-related mortality being conducted in Africa, where maternal mortality ratios remain amongst the highest in the world [10]. It is also the only trial which attempts to replicate the Nepal findings by supplementing all women. Another trial of VAS was conducted in Bangladesh (JIVITA-1) [11], but where supplements were only given during pregnancy. Provisional results suggest no effect of VAS on pregnancy-related mortality (RR 1.15, 95% CI 0.78, 1.81).

Around three quarters of maternal deaths are due to five main direct complications, namely haemorrhage, sepsis, hypertensive diseases of pregnancy, obstructed labour and unsafe abortion [12]. There are plausible biological mechanisms through which VAS may reduce maternal deaths, by impacting on one or more of these causes: it may reduce haemorrhage

through an effect on placentation [13,14] or blood coagulation [15,16]; sepsis through its anti-infective properties [17,18]; or abortion complications through a combination of these factors. VAS may also reduce iron-deficiency anaemia, by improving iron transport or absorption [19-21], and thus may potentially act through this mechanism. However, there is little empirical evidence to support any of these mechanisms [22].

This paper provides a detailed summary of the design of the ObaapaVitA trial. The overall aim of this trial is to evaluate whether weekly vitamin A supplementation of women of reproductive age in Ghana improves their survival or that of their babies.

## **Methods**

### **Setting**

The ObaapaVitA trial is part of a long-term collaboration between the London School of Hygiene & Tropical Medicine and the Ghana Health Service Kintampo Health Research Centre (KHRC). It is based at KHRC and involves all women aged 15 to 45 years living in seven contiguous districts in the Brong Ahafo region of Ghana: Kintampo North, Kintampo South, Wenchi, Tain, Techiman, Nkoranza North, and Nkoranza South. The trial area has a total population of about 600,000 and lies within the forest-savannah transitional ecological zone where vitamin A rich food sources are less available than in the forest regions to the south of the country. Data from a previous study in the area suggest a VAD problem of public health significance [23]; 15% of 89 primigravid pregnant women were found to be moderately deficient (plasma retinol  $<20\mu\text{g}/\text{dl}$ ) and 50% were mildly vitamin A deficient (plasma retinol  $<30\mu\text{g}/\text{dl}$ ). National estimates show that maternal mortality was high in Ghana at the start of the trial and during its conduct; WHO estimated that the maternal mortality ratio (MMR) for Ghana was 540 deaths per 100,000 live births in 2000 (range of uncertainty 140, 1000) [24], and WHO/UNICEF/UNFPA estimated it at 560 in 2005 (range of uncertainty 200, 1,300) [1].

### **Aims and objectives**

The aim of the ObaapaVitA trial is to assess whether VAS should be a component of Safe Motherhood programmes. The main objective is to evaluate the effect of weekly, low dose VAS given to all women of reproductive age on pregnancy-related mortality, and to compare this with the effect on overall mortality. Secondary objectives are: to explore the possible causal pathways through which VAS might impact on pregnancy-related mortality, by examining the effect of supplementation on a range of maternal morbidities; to evaluate the effect of weekly VAS given to all women of reproductive age on perinatal and infant mortality; and to contribute data to any future meta-analyses that explore the effect of VAS on cause-specific pregnancy-related maternal mortality.

### **Overview of trial design**

ObaapaVitA is a cluster-randomised, double-blind, placebo-controlled trial. All women of reproductive age (15 to 45 years) living in seven, predominantly rural, districts in the Brong Ahafo region in Ghana are randomised, according to their cluster of residence, to receive a weekly dose of either 25,000 IU of vitamin A or placebo. Clusters are defined on a geographical basis; a cluster consists of all women living in the compounds visited by a particular fieldworker in a particular week. Each fieldworker is responsible for four clusters (a fieldwork area or FWA), visiting one cluster per week over a 4-weekly cycle. Randomisation is blocked, so that women in two of the four clusters receive vitamin A capsules and women in the other two clusters receive placebo capsules. These capsules are

distributed at the 4-weekly home visits, during which surveillance data are also collected on pregnancies, births, deaths and migrations. Capsule distribution is supported by an extensive Information, Education and Communication (IEC) programme designed to encourage women to take their capsules each week on Sundays. The main surveillance is augmented by data collection on adherence to the intervention, socio-demographic status, maternal morbidity (using data capture at the four main district hospitals in the trial area) and cause of death (using verbal post-mortem interviews or VPMs).

Implementation of the trial was phased by district, starting in Kintampo North and South in December 2000, in Wenchi and Tain in June 2001, in Techiman in June 2002, and in Nkoranza North and South in January 2003. Capsule distribution ended in September 2008, with data collection continuing for one further four-week cycle until the end of October 2008.

### **Trial preparation and mapping**

Preparations for fieldwork in each district started with meetings with district health, political and traditional authorities, followed by community meetings to introduce the trial and answer any questions. All compounds were mapped and allocated a unique compound number; these were painted clearly on an outside wall so that they could be located easily during fieldwork. In addition, geographical coordinates were obtained for all compounds in large villages and towns and for centroids of smaller villages, using handheld GPS receivers which worked in tandem with a base receiver at KHRC. In addition, roads and other important physical features were mapped.

### **Participants & Recruitment**

All women between the ages of 15 and 45 years at the time of recruitment, who were capable of giving informed consent and who planned to live in the trial area for at least the next 3 months were eligible to participate in the trial. At the initial implementation in each district, fieldworkers visited all compounds over a 4-8 week period and explained the trial to eligible women by reading from a standard sheet with information on the trial in Twi (the most commonly spoken language in the locality). Women were given the opportunity to ask questions and an interpreter was used if the woman did not speak Twi. Women were then asked, using a standard declaration, if they consented to be registered into the trial and, if they agreed, were asked to endorse this declaration on the enrolment form using their signature or thumbprint. Enrolment continued throughout the trial; fieldworkers recruited any women who migrated into their compounds or girls who became fifteen during the course of the trial. Each woman was given a unique trial identification number, consisting of the compound number and their individual number within that compound. Once recruited, women remained in the trial for its duration regardless of their age.

### **Cluster demarcation, fieldwork areas and day groups**

Groups of contiguous compounds within the same geographical area were demarcated into clusters. These were designed to contain an initial maximum of 100-120 enrolled women, a number logistically feasible for a fieldworker to visit in a week, and allowed some room for increase over the course of the trial.

In the small villages, cluster demarcation was carried out using compound listings showing numbers of eligible women per cluster. Villages were assigned to one cluster, part of a cluster (joined with all or a number of compounds in an adjoining village), or several clusters depending on the number of women recruited and distances between villages. Then groups of

4 contiguous clusters were assigned to individual fieldworker areas (FWAs). Clusters were allocated a 4-character code comprising the district code, followed by the week group (1, 2, 3, or 4 denoting the week in the 4-weekly fieldwork cycle that the cluster will be visited by the fieldworker), and a 2-character FWA code. Within each cluster, contiguous compounds were divided into day groups (Monday, Tuesday, Wednesday or Thursday) to provide approximately equal numbers of women to be visited by the fieldworker each day. Friday was reserved for field meetings, collection of forms, and allocation of forms for the following week.

In each of the large villages and towns, computerised maps were used. First, the number of FWAs needed was calculated, based on the total number of women recruited. Groups of contiguous compounds corresponding to potential FWAs were highlighted on the screen, and ArcView Geographic Information System (GIS) software used to display the total number of eligible women in the FWAs. The FWA boundaries were adjusted until all sizes were appropriate. Next, each FWA was displayed and the exercise repeated to divide it up into 4 clusters, then each cluster was displayed to further divide into the 4 day groups.

There are a total of 272 fieldworker areas and 1086 clusters in the trial; two FWAs only contain 3 clusters (area 41 in Tain and area 29 in Nkoranza South), since they were not large enough to divide into four.

### **Intervention**

The intervention consists of a single, weekly, oral dose of 25,000 IU (7500µg) of vitamin A in soybean oil in a dark red opaque soft gel capsule or an identical-looking placebo capsule containing soybean oil only. The dose of vitamin A was selected to deliver the recommended dietary allowance to women in the intervention arm, whilst also being safe during pregnancy [25]. Women are given 4 capsules during each 4-weekly fieldworker visit, and instructed to take one on each Sunday until the next visit. The capsules are manufactured by Accucaps Industries Limited, Canada; the vitamin A was donated by Roche. The women are given vials to safely store the capsules, containing cotton wool to absorb humidity in the rainy season.

Adherence to the capsules is encouraged through a wide-ranging IEC programme which has developed and adapted throughout the life of the trial. Formative research was conducted before the trial began to inform the content of the programme, the findings of which have been published previously [26]. The ongoing activities of the IEC programme include: educating trial women to take their capsule on the same day (Sunday) to foster social support, reduce forgetfulness and allow reminding activities to be focused on a single day; reminder announcements on radio, in the community (using loudspeaker vans and drum beaters) and in churches and mosques; discussions via radio programmes and community meetings initially to introduce the trial and later to address any concerns about the capsules; distribution of posters to all compounds with pictorial reminders and a calendar for women to mark when they have taken their capsule; a book of frequently asked questions (FAQs) with answers, given to traditional healers, traditional birth attendants, drug sellers and health workers in the trial area as well as all fieldworkers; and a “message of the month” which fieldworkers deliver to women during their 4-weekly visits. Fieldworker reports and focus groups with community members have been conducted regularly to identify new adherence issues and possible solutions.

### **Randomisation**

Randomisation of clusters is blocked, so that women in two of the clusters within a fieldworker area receive vitamin A capsules and women in the other two receive placebo capsules. This scheme has several advantages. First, a fieldworker only ever has one capsule jar in their possession, containing the capsules to be given to the women they're scheduled to visit that week; this avoids any chance of a fieldworker giving a woman an incorrect type of capsule. Second, all women living in a compound receive the same type of capsule; this avoids any risk of them taking the wrong type of capsule if the vials get mixed up. Third, since FWAs cover geographically small areas, blocking by FWA is equivalent to geographic matching of vitamin A and control groups.

A computer-generated randomisation list was prepared by a statistician who is a member of the Data Monitoring and Ethics Committee (DMEC) but is not involved in the day-to-day running of the trial. The list was given directly by him to the capsule manufacturers (Accucaps Industries Limited, Canada), who produced clearly labelled capsule jars for each cluster for each week of the trial. No trial personnel have access to the randomisation list or to any information that would allow them to deduce or change the allocation of clusters.

### **Blinding**

All trial subjects and personnel (including those distributing capsules and collecting, processing and analysing data) are blinded to the assignment of the intervention for the duration of the trial.

### **Data collection**

Capsules are distributed by fieldworkers (FWs) at 4-weekly home visits, during which surveillance data are also collected on pregnancies, births and vital events such as deaths and migrations. This 4-weekly surveillance is augmented by a number of additional data collection activities, all performed by specially-trained supervisors under the supervision of senior field staff (head of fieldwork, co-ordinators or site leaders) and the trial epidemiologist and/or director.

***Four-weekly home visits:*** Each week, fieldworkers receive an up-to-date listing for their cluster, showing, the enrolled women within these compounds, and their pregnancy status. A "Month" form is completed for each woman, "Month (Standard)" if she was not pregnant at the last visit, and "Month (Pregnant)" if she was. Both include questions on whether she was present, if not whether she had died, any morbidity requiring treatment outside the home or hospitalisation, her pregnancy status, specific pregnancy-related morbidity (if she is pregnant), the number of capsules taken last month, the number of any capsules left in her vial and the number given out. The number of capsules will be four unless the woman reported that she was about to travel and would not be at home for the next visit; in this case the FW is authorised to give her extra capsules up to a maximum of 12. In addition, there is a scheduled 4-week fieldwork break each year over Christmas; eight rather than 4 capsules are therefore distributed at the last visit before Christmas. The "Month (Standard)" form includes additional questions concerning the duration of the pregnancy to be completed for women newly identified as pregnant, while the "Month (Pregnant)" form include an additional question on the outcome of the pregnancy, completed if the woman is no longer pregnant.

Other forms are completed as required during the 4-weekly visit. A "Profile" form collecting socio-demographic information is completed as soon as a woman reports that she is pregnant. A birth results in a whole series of forms: a "Birth" form collecting data on pregnancy,

delivery, the baby (or babies) and newborn care practices is completed as soon as the birth is reported; a “Postpartum” form collecting morbidity information is completed during the next visit; “Infant” form(s) are also completed at this next visit collecting data on the status, breastfeeding practices and morbidity of the baby(ies) born; subsequent “Infant” forms completed at all following visits until the baby reaches 12 months of age, or dies.

**Verbal post-mortems (VPMs):** VPMs are carried out for all deaths in trial women and their infants, to ascertain information that will allow for the classification of the cause of death, and in women, identification of whether the death was pregnancy-related. A supervisor visits the household of the deceased and interviews a close friend, relative or care-taker about the circumstances surrounding the death, including an open history, and specific questions on symptoms. The ideal respondent is the person who most looked after the deceased during their final illness at home or in the hospital, and not a specified relative. The second choice is someone who lived in the same household and knew about the illness but did not look after the deceased. If the ‘ideal’ respondent is not present when they first visit, supervisors are told to revisit the household at least 3 times before opting for the next most appropriate respondent. They are also encouraged to allow more than one respondent to participate in the interview, since additional respondents may give complementary and useful additional information.

All VPMs are reviewed by two experienced doctors, who independently code the likely cause of death and whether it was pregnancy-related. If the two doctors disagree on either of these, the form is reviewed by a third doctor; a consensus coding is accepted when at least two of the three coders agree. If there was no consensus on cause of death, but complete agreement on the pregnancy status, then the 3 coders discussed the VPM form to see whether they could agree a cause. If all three coders differed in their ascertainment of pregnancy status, the form was reviewed by an experienced obstetrician (fourth coder), who assigned pregnancy status and cause of death.

**Hospital data capture:** Field supervisors are based at the four main district hospitals (in Kintampo, Nkoranza, Techiman and Wenchi) in the trial area to capture data on pregnancy-related morbidity. They complete a “Hospital” form for any pregnant, delivering or postpartum trial women admitted; this contains questions on the hospital diagnosis, the delivery (if applicable) and information on clinical signs and management. This started at the beginning of 2004.

**Adherence monitoring:** “Profile” and “Adherence” forms are completed for a random sub-sample of 40 women per week; the sub-sample is chosen to comprise 10 women who should have been visited the previous week and therefore have 3 capsules remaining in their vial (having taken one on Sunday), 10 women who should have 2 capsules, 10 who should have 1 capsule and 10 who should have no capsules remaining. The latter group are always visited before their current week’s visit is due. Selected women are interviewed by specially trained IEC supervisors about their socio-demographic characteristics (“Profile” form) and their adherence to the capsules, their exposure to reminding activities and any perceived side effects (“Adherence” form) . The number of capsules remaining in their vial is also checked and recorded. These data are monitored to ensure adherence rates remain high, and that there are no unforeseen side effects or concerns being reported. Any problems are investigated, and solutions are designed and implemented on an ongoing basis by the trial management and IEC teams.

***Pregnancy migration surveys:*** Since 2005, surveys have been carried out to try and ascertain pregnancy outcomes and survival status of pregnant women who migrated out of the trial area during their pregnancy, by interviewing neighbours and family members who were still in contact with them or who had news of them. These surveys have been conducted to check that the loss to follow-up of pregnant women due to migration has not led to any bias in the findings.

***Serum retinol sub-study:*** Blood samples will be taken by trained phlebotomists from a random sample of 400 pregnant women (200 from the vitamin A arm and 200 from the placebo arm), and 400 non-pregnant women (200 from each arm) to compare mean serum retinol concentrations and proportions vitamin A deficient between women taking vitamin A and placebo capsules.

### **Outcome measures**

***Pregnancy-related mortality:*** This is expressed as the number of pregnancy-related deaths per 100,000 pregnancies and includes all deaths occurring during pregnancy, at delivery or up to 42 days postpartum, irrespective of the cause [27]. Pregnancy related mortality (which includes all deaths during pregnancy or the 42 days postpartum) was chosen as the outcome instead of maternal mortality (which excludes co-incident deaths in this period) because it was comparable to the Nepal trial, and because it was operationally the easiest to define in the absence of good clinical diagnoses and post mortems. Deaths among trial women will be classified as pregnancy-related if the monthly surveillance or hospital data capture records indicate that the woman was currently or recently pregnant, or had recently delivered, or if the history collected in the verbal post mortem (VPM) indicates a current or recent pregnancy. Pregnancies are recognised pregnancies reported to the interviewers.

***All-cause female mortality:*** This is expressed as the number of deaths per 1000 women years of follow-up and includes all deaths among women under active surveillance in the trial at the time of death.

***Maternal morbidity:*** A serious maternal morbidity is defined as a hospitalisation occurring during pregnancy or up to 42 days postpartum, and will be expressed as the percentage of pregnancies resulting in an admission with one of the following twelve diagnoses: severe pre-eclampsia; eclampsia; obstructed labour; emergency Caesarean section; instrumental delivery; puerperal sepsis; spontaneous abortion; clinically significant malaria; clinically significant anaemia; ante-partum haemorrhage; post-partum haemorrhage; and shock.

***Perinatal and infant mortality:*** Several indicators will be compared between the two treatment groups:

- stillbirth rate (based on babies born dead at 6 months gestation or later, and expressed per 1000 live and still births);
- early (deaths in the first 7 days of life), late (deaths from days 8 to 28) and overall neonatal mortality rates, all expressed per 1000 live births;
- early infant (deaths from 0 to 5 months), late infant (deaths from 6 to 12 months) and total infant mortality rates, expressed per 1000 infant-years of follow-up;
- perinatal mortality rate (combining stillbirths and early neonatal deaths and expressed per 1000 live and still births);



- successful pregnancies (a pregnancy resulting in a child and mother surviving to the end of the neonatal period).

### **Sample size**

Sample size calculations indicated that data on 82,000 pregnancies would give 90% power to detect a 33% reduction in pregnancy-related mortality (and 76,000 pregnancies an 80% power to detect a 30% reduction) from a baseline of 450 deaths per 100,000 pregnancies, at the 5% significance level allowing for a 10% design effect; this is likely to be an overestimate of the level of clustering since maternal mortality is a rare event and the cluster sizes are small. It was estimated that these data would be available if the trial continued until October 2005. However, data from the early years of the trial suggested lower fertility and maternal mortality rates than originally anticipated; the DMEC carried out conditional power calculations in 2003 and recommended that the trial be extended until October 2008. Funding for this was secured. The DMEC have examined accrual rates annually since that time, and considered them satisfactory.

### **Fieldwork organisation**

Weekly team meetings for all fieldworkers are held at the district offices, during which fieldworkers provide a report of their work (including the number of events captured and the proportion of women absent) and discuss any relevant issues. After the meeting, fieldworkers submit their completed forms and capsule jars from the preceding week, and receive the listings, forms and capsule jars for the following week of work. A fieldworker will thus only ever have in their possession the capsule jar for the cluster, trial month and week number for the current week of work. All submitted forms are checked by field office staff for errors or omissions and a note of all events is made for comparison with future listings.

All fieldworkers have completed senior secondary school and can speak, read and write in English and Twi. They must pass an entrance examination at KHRC, perform well during a 4-week training programme with continuous assessment, and agree to be resident in the area in which they work. The training programme covers an introduction to work in the Centre and basic research methods, the trial design and how the fieldwork is organised, interviewing and communication skills, instruction on individual data collection forms, and provisions for ongoing support and supervision. It is run by the research team at the centre, and comprises lectures, small group discussions, test interviews and field practice using the fieldwork manual, which contains detailed descriptions of all the fieldwork procedures and copies of the forms. Fieldworkers are supported in the field by supervisors, who receive additional training. A supervisor is responsible for a team of 10-15 fieldworkers allowing them to visit each one in the field at least once every two weeks, to observe the fieldworker at work and provide feedback. Supervisors also re-visit compounds on their own to conduct random blind re-interviews and to verify events reported by fieldworkers.

### **Data management**

Approximately 30,000 completed data collection forms are received from the field at the KHRC computer centre every week. These are logged into the filing office in batches, before being logged out and passed to the data entry clerks for entry into the database management system setup using Visual FoxPro. All forms are double-entered and are then subject to extensive range and consistency checks, including consistency checks within individual forms, between forms, and between new data and information already held in the databases. Queries are detailed in a weekly problem form report which is sent to the trial statistician or

director for investigation and resolution. A memo is sent to the field asking for information to be verified or checked on any queries that cannot be resolved using the forms and database or. Once all checks have been completed and queries resolved, the weekly data is appended to relational database tables containing all trial data. It is also used to update master tables on women concerning their pregnancy status and on both women and infants concerning any deaths or migration out of the trial area. The updated database tables are then used to produce paper listings which are distributed to fieldworkers to guide their next week's visits. The turnaround time from completing a form in the field to the information being on the database is 3 weeks. Trial progress statistics are produced each week and monitored by the trial statistician; they include data on the numbers of women enrolled in each district and the number of pregnancies, births and deaths. The ObaapaVitA database is kept on a dedicated server, which is automatically backed-up every 24 hours. Weekly back-ups are made (on cd-rom or dvd) and kept off-site in Kintampo; monthly backups are sent to LSHTM.

### **Analysis of main outcomes**

***Participant flow & comparability of treatment arms:*** All analyses will be conducted according to a pre-established analysis plan, agreed by the DMEC. The flow of participants through enrolment, allocation to intervention, follow-up and analysis will be documented, and loss to follow-up described. Socio-demographic and socio-economic characteristics of women in the vitamin A and placebo arms will be compared separately for pregnant women, and for the random sample of women selected for adherence monitoring, to examine for adequate randomisation.

***Vitamin A status of trial women:*** Mean serum retinol concentrations and the percentage of women vitamin A deficient will be presented separately for pregnant and non-pregnant women in the two treatment arms. The levels in the placebo arm will document the levels of deficiency in the trial population, while comparison of the treatment arms will assess the extent to which small weekly doses have changed vitamin A status.

***Migration within the trial and change of treatment arms:*** Several women will have moved compounds and therefore between clusters during the course of the trial; when they move, they will receive the same treatment as other women in their new cluster. It is expected that on average, half of the moves will result in no change in treatment arm, a quarter will result in a change from vitamin A to placebo and a quarter in a change from placebo to vitamin A. The implications of such migration within the trial for its analysis and interpretation will be the basis of a separate paper. The approach is summarised below:

As vitamin A stores require some time to become replete or depleted, it is necessary to consider four periods when women are first recruited the trial or when they move between clusters within the trial: lag, run-in, carry-over and wash-out periods:

- ***Lag:*** the period that a woman must be on vitamin A before it is considered likely to have its full impact on the outcome. During this period, events should not contribute to the analysis.
- ***Run in:*** this is a shorter period at the start of the lag period during which vitamin A supplementation is likely to have little or no impact on the outcome.
- ***Carry over:*** the period after stopping vitamin A supplementation when the effect of the intervention will be little reduced. During this period, women that change from vitamin A to placebo could continue to contribute data on events to the vitamin A group.

- **Wash out:** this is longer than the carry-over period. It is the period after stopping vitamin A supplementation by which any effect will have worn off.

The lag and run-in periods apply to women when they are first recruited into the trial, or to women that move from the placebo to vitamin A treatment group. The carry-over and wash-out periods apply to women who move from the vitamin A to placebo treatment group. In order to ensure balanced lengths of follow-up in each treatment group, the same inclusion/exclusion rules should be applied whichever direction the switch between treatment groups happens. This therefore implies that equal values be used for: the lag and wash-out periods; and the run-in and carry-over periods. Since scientific data to determine lengths of these periods are not available, we consulted vitamin A experts in order to build a consensus. On the basis of responses received, and in consultation with the DMEC, it was decided to use: 2 months for the run-in and carry-over periods; and 6 months for the lag and wash-out periods. Thus women will be considered as belonging to their pre-move treatment arm for a period of 2 months after changing treatment arm (run-in and carry-over). They will then be considered eligible to contribute to the new treatment arm only after another 4 months has elapsed (a total of 6 months post-change; lag and wash-out).

**Intention-to-treat analyses:** The primary analysis for each main outcome will be intention-to-treat, where intention to treat is defined by a woman's cluster of residence, and which may therefore change during the course of the trial if she moves to a different cluster within the trial area. These analyses will exclude the first 6 months data following recruitment, and the 6 months data following any change of treatment arms, since it is considered that this is the minimum lag-period before which small weekly doses of vitamin A could be considered to achieve their maximum effect. In addition a modified "intention to treat" analysis will be carried out for pregnancy-related mortality, in which the analysis will be restricted to women who were seen by their fieldworker at least 3 times in the 6 months before the end of the pregnancy, in order to maximise the likelihood of detecting the true effect of vitamin A supplementation.

**"Pure" intention-to-treat analyses:** In addition "pure" intention-to-treat analyses will be conducted, in which women will be excluded as soon as they change treatment arms. Data from the first 6 months of recruitment will be excluded as described above.

**Sensitivity analyses:** A series of sensitivity analyses are planned to test the robustness of the findings including: (i) repeating both intention-to-treat and "pure" intention-to-treat analyses without any exclusions, and with different values for the "run-in" and "lag" periods; and analyses using different definitions of pregnancy-related mortality, such as using a 90-day cut-off for deaths post-partum instead of 42 days.

**Statistical methods:** All analyses will account for the cluster-randomised design. Random-effects logistic regression will be used for the analyses of outcomes where the denominator is pregnancies or births, except where model estimates are found to be unreliable; in such cases, generalized estimating equations (GEE) will be used instead. Random-effects Poisson regression models will be used for outcomes where the denominator is person-years. Mean serum retinol concentrations will be compared using a *t* test, or mean log concentrations if the distributions are found to be skewed. The proportions with serum retinol concentrations of < 30µg/dl (mild deficiency) and < 20µg/dl (moderate deficiency) will be compared using  $\chi^2$  tests.

### **Additional analyses**

Data collected provide the opportunity to explore a range of other research questions. Four main areas of work are currently planned: pregnancy-related mortality and morbidity; perinatal and infant mortality; inequities in outcome, access to key preventive interventions and use of services; and methodological issues. Analyses examining the impact of early infant feeding practices on all-cause and infection-specific mortality, and on mortality in low birth weight infants have already been published [28-30].

### **Trial monitoring**

The Trial Steering Committee (TSC) has 12 external members, chosen to facilitate dissemination and uptake of any findings within Ghana as well as to provide technical support. Membership comprises several key policy makers from the Ghana Health Service ( former Director of Medical Services; Deputy Director General; director, Health Research Unit; deputy director, Public and Family Health, Reproductive Health Unit; Coordinator, National Vitamin A Programme; former Head, Nutrition Unit; Regional Director of Health Services, Brong Ahafo Region), together with: a WHO national programme officer (maternal and child health); a UNICEF programme officer (nutrition); a Ghanaian consultant obstetrician with clinical and research experience; a lecturer in Statistics and Technical Demography, University of Ghana, Legon; and a Canadian professor with technical expertise in statistical methods, clinical trials and health services research. It is also attended by representatives from the Department for International Development, UK Government (the main funders), the principle investigators and members of the trial management team. The Data Monitoring and Ethics Committee (DMEC) has six members. The Chair is a professor of epidemiology and biostatistics with a special interest in the design and analysis of cluster randomised trials. The other members are leading experts in epidemiology and medical statistics, obstetrics, perinatal epidemiology, and community medicine, based in the UK and Ghana.

Both committees meet annually to examine trial conduct and advise the trial management team. The DMEC also perform annual blinded safety analysis; data are not examined in detail unless a one tailed P-value of  $<0.01$  is detected for an increase in any mortality outcome in the vitamin A arm. In June 2006, they conducted a full interim analysis to examine for differences between treatment groups in pregnancy-related maternal mortality, all-cause female mortality, neonatal mortality and successful pregnancies. The analyses were only partially unblinded; the data were examined by treatment group but the identities of the two groups were not revealed. In addition, the DMEC monitor the vitamin A content of the capsules twice yearly. Two batches of capsules from two clusters (one vitamin A, one placebo), randomly selected for each analysis by the statistician who produced the randomisation list, are analysed. The first batch contains capsules that have not yet been distributed in the field, to ensure that capsules production is correct; the second batch contains capsules returned from the field, to ensure that vitamin A activity remains acceptable even after capsules have been stored by women in their homes for a month.

### **Ethical approval**

The trial protocol was reviewed and approved by the ethics committees of the Ghana Health Services and the London School of Hygiene and Tropical Medicine. It is registered with clinicaltrials.gov (identifier NCT00211341). Full informed consent was obtained from all trial participants as described above. A paper has been published describing their understanding of the trial and examining the factors associated with this knowledge [31].

## **Discussion**

This trial will be the first to examine the impact of VAS on pregnancy-related mortality in Africa, and the first to compare the impact on pregnancy-related mortality with an impact on adult female mortality. It will provide findings that will be generalizable to other rural African populations and, along with the results of the trials in Nepal and Bangladesh, it will provide data to allow policy makers to assess whether vitamin A interventions should become a key component of maternal health initiatives.

Because vitamin A offers the potential of a completely different new strategy to add to the armamentarium of safe motherhood programmes, we rank establishing the effectiveness of vitamin A in preventing pregnancy-related mortality as one of the most important research questions to be answered for maternal health. Unlike the proposed alternatives which depend on strengthening the health system, vitamin A is a technology that may be effective in primary prevention. It is safe, inexpensive, and could potentially be delivered through a variety of means; it is also more comparable to the tools characterizing successful public health initiatives such as family planning, immunization, and childhood vitamin A supplementation. Equally important, however, is to verify whether the impact of vitamin A shown in the NNIPS-2 trial [9] is real: if not, there is the danger that other interventions with proven effectiveness could be abandoned in favour of vitamin A. There is still little direct evidence of a biologically plausible mechanism for the effect reported in the Nepal trial and it is important for this to be verified.

If the trial shows a positive effect, there is little doubt that attempts should be made to enhance the vitamin A status of women in vitamin A deficient areas. We recognise that the approach adopted in the trial (namely 4-weekly visits by field workers supported by an extensive IEC strategy) is unlikely to be feasible or cost effective on a programmatic level. However, our aim is to establish a proof-of-principle with respect to regular low dose VAS, and data from the trial will provide considerable information on the feasibility of self-administration of supplements as well on the most effective strategies to promote adherence. If vitamin A is effective, distribution mechanisms to be explored would include social marketing of capsules, food fortification, and possibly even technological developments such as implants or patches. The likelihood of finding a successful distribution mechanism is considerable given that there is already an international vitamin A initiative, and both UN agencies (UNICEF) and donors (USAID) have put considerable effort into vitamin A supplementation in children and have a good track record for implementing these programmes [32,33]. There are also many food fortification initiatives, for example work by the World Bank on iodine supplementation which has explored and identified many of the problems and solutions involved in food fortification, as well as several initiatives exploring the fortification of common food products with vitamin A, including cooking oil, flour, sugar and monosodium glutamate [34,35]. Moreover, as there is considerable policy and programmatic interest in VAS for children, it is likely that such interest can be broadened to encompass supplementation for women.

It is increasingly recognised that poverty not only increases the risk of ill health, but that ill health in turn plays a major role in creating and perpetuating poverty [36]. Analyses of service-use by poverty quintiles shows the gap between the richest and poorest 20% within countries is widest for maternity care (and professional delivery care in particular) compared to other public health interventions such as childhood immunisation services or treatment of

childhood illnesses [37]. A community-based intervention such as vitamin A is likely to address the needs of the very poorest women, as these are the individuals least likely to have access to professional birth attendants and emergency obstetric care, and evaluating the potential of vitamin A supplementation to reduce pregnancy-related mortality in these communities is therefore of high priority.

## References

1. WHO. *Maternal Mortality in 2005: estimates developed by WHO, UNICEF, UNFPA, and the World Bank*. WHO: Geneva; 2007.
2. Mathers CD, Bernard C, Moesgaard Iburg K, Inoue M, Ma Fat D, Shibuya K, Stein C, Tomijima N, Xu H. *Global burden of disease in 2002: data sources, methods and results*. WHO: Geneva; 2003.
3. Campbell OM, Graham WJ; Lancet Maternal Survival Series steering group. **Strategies for reducing maternal mortality: getting on with what works**. *Lancet* 2006; **368**(9543): 1284-99.
4. Starrs A. **Delivering for women**. *Lancet* 2007; **370**(9595): 1285-7.
5. West KP Jr. **Extent of vitamin A deficiency amongst preschool children and women of reproductive age**. *J Nutr* 2002; **132**(Suppl 9): 2857-66.
6. Beaton GH, Martorell R, Aronson KJ, Edmonston B, McCabe G, Ross AC, Harvey B. *Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries*. ACC/SCN Nutrition Policy Discussion Paper No. 13, pp1-120. WHO: Geneva; 1993.
7. Bhutta ZA, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, Haider BA, Kirkwood B, Morris SS, Sachdev HP, Shekar M; Maternal and Child Undernutrition Study Group. **What works? Interventions for maternal and child undernutrition and survival**. *Lancet* 2008; **371**(9610): 417-40.
8. UNICEF. *Tracking progress in maternal, newborn and child survival: The 2008 report*. UNICEF: New York; 2008
9. West KP Jr, Katz J, Khattry SK, LeClerq SC, Pradhan EK, Shrestha SR, Connor PB, Dali SM, Christian P, Pokhrel RP, Sommer A; The NNIPS-2 Study Group. **Double blind, cluster randomised trial of low dose supplementation with vitamin A or beta carotene on mortality related to pregnancy in Nepal**. *BMJ* 1999; **318**(7183): 570-5.
10. Ronsmans C, Graham WJ; Lancet Maternal Survival Series steering group. **Maternal mortality: who, when, where, and why**. *Lancet* 2006; **368**(9542): 1189-200.
11. Christian P, West KP Jr, Labrique A, Klemm R, Rashid M, Shamim AA, Katz J, Wu L, Sommer A. **Effects of maternal vitamin A or beta-carotene supplementation on maternal and infant mortality in rural Bangladesh: the JIVITA-1 trial**. In *Proceedings of the Micronutrient Forum: 16-18 April 2007; Turkey*. IVACG: 2007; 129.

12. Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look P. **WHO systematic review of causes of maternal deaths.** *Lancet* 2006; **367**(9516): 1066-74.
13. Smeland S, Bjercknes T, Malaba L, Eskild W, Norum KR, Blomhoff R. **Tissue distribution of the receptor for plasma retinol-binding protein.** *Biochem J* 1995; **305**(2): 419-24.
14. Dimenstein R, Trugo NM, Donangelo CM, Trugo LC, Anastácio AS. **Effect of subadequate maternal vitamin-A status on placental transfer of retinol and beta-carotene to the human fetus.** *Biol Neonate* 1996; **69**(4): 230-4.
15. Horie S, Kizaki K, Ishii H, Kazama M. **Retinoic acid stimulates expression of thrombomodulin, a cell surface anticoagulant glycoprotein, on human endothelial cells. Differences between up-regulation of thrombomodulin by retinoic acid and cyclic AMP.** *Biochem J* 1992; **281**(1): 149-54.
16. Shibakura M, Koyama T, Saito T, Shudo K, Miyasaka N, Kamiyama R, Hirose S. **Anticoagulant effects of synthetic retinoids mediated via different receptors on human leukemia and umbilical vein endothelial cells.** *Blood* 1997; **90**(4): 1545-51.
17. Semba RD. **The role of vitamin A and related retinoids in immune function.** *Nutr Rev* 1998; **56**(1 Pt 2): S38-48.
18. Cox SE, Arthur P, Kirkwood BR, Yeboah-Antwi K, Riley EM. **Vitamin A supplementation increases ratios of proinflammatory to anti-inflammatory cytokine responses in pregnancy and lactation.** *Clin Exp Immunol* 2006; **144**(3): 392-400.
19. Vijayalakshmi P, Lakshmi RN. **Effect of vitamin A supplementation on serum levels of these nutrients among expectant mothers.** *Indian J Nutr Dietet* 1983; **20**: 149-52.
20. Suharno D, West CE, Muhilal, Karyadi D, Hautvast JG. **Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia.** *Lancet* 1993; **342**(8883): 1325-8.
21. García-Casal MN, Layrisse M, Solano L, Barón MA, Arguello F, Llovera D, Ramírez J, Leets I, Tropper E. **Vitamin A and beta-carotene can improve nonheme iron absorption from rice, wheat and corn by humans.** *J Nutr* 1998; **128**(3): 646-50.
22. Faisel H, Pittrof R. **Vitamin A and causes of maternal mortality: association and biological plausibility.** *Public Health Nutr* 2000; **3**(3): 321-7.
23. Cox SE, Staalsoe T, Arthur P, Bulmer JN, Tagbor H, Hviid L, Frost C, Riley EM, Kirkwood BR. **Maternal vitamin A supplementation and immunity to malaria in pregnancy in Ghanaian primigravids.** *Trop Med Int Health* 2005; **10**(12): 1286-97.
24. WHO. *Maternal Mortality in 2000: estimates developed by WHO, UNICEF, and UNFPA.* WHO: Geneva; 2004.

25. IVACG Statement: *Safe doses of vitamin A during pregnancy and lactation*. IVACG: Washington; 1998.
26. Hill Z, Kirkwood B, Kendall C, Adjei E, Arthur P, Agyemang CT. **Factors that affect the adoption and maintenance of weekly vitamin A supplementation among women in Ghana**. *Public Health Nutr*. 2007; **10**(8): 827-33.
27. WHO. *International statistical classification of diseases and related health problems*. Tenth Revision. WHO: Geneva; 1992.
28. Edmond KM, Zandoh C, Quigley MA, Amenga-Etego S, Owusu-Agyei S, Kirkwood BR. **Delayed breastfeeding initiation increases risk of neonatal mortality**. *Pediatrics* 2006; **117**(3): 380-6.
29. Edmond KM, Kirkwood BR, Amenga-Etego S, Owusu-Agyei S, Hurt LS. **Effect of early infant feeding practices on infection-specific neonatal mortality: an investigation of the causal links with observational data from rural Ghana**. *Am J Clin Nutr* 2007; **86**(4):1126-31.
30. Edmond KM, Kirkwood BR, Tawiah CA, Owusu Agyei S. **Impact of early infant feeding practices on mortality in low birth weight infants from rural Ghana**. *J Perinatol* 2008; **28**(6):438-44.
31. Hill Z, Tawiah-Agyemang C, Odei-Danso S, Kirkwood B. **Informed consent in Ghana: what do participants really understand?** *J Med Ethics*. 2008; **34**(1): 48-53.
32. Houston R. *Why they work: An analysis of three successful public health interventions. Vitamin A supplementation programs in Ghana, Nepal, and Zambia*. The MOST project: Arlington; 2003.
33. UNICEF. *Vitamin A supplementation: A decade of progress*. UNICEF: New York, 2007.
34. The World Bank. *World Bank enriching lives: Overcoming vitamin and mineral malnutrition in developing countries*. The World Bank: Washington DC, 1994.
35. Hetzel BS. **Eliminating iodine deficiency disorders – the role of the International Council in the global partnership**. *Bull World Health Organ* 2002; **80**(5): 410-12.
36. Gwatkin DR. **How much would poor people gain from faster progress towards the Millennium Development Goals for health?** *Lancet* 2005; **365**(9461): 813-7.
37. Houweling TA, Ronsmans C, Campbell OM, Kunst AE. **Huge poor-rich inequalities in maternity care: an international comparative study of maternity and child care in developing countries**. *Bull World Health Organ* 2007; **85**(10): 745-54.