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Calcium supplementation for the prevention of pre-eclampsia among low calcium intake women:

A randomized controlled trial

Trial protocol

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CALCIUM SUPPLEMENTATION FOR THE PREVENTION OF PRE-ECLAMPSIA AMONG LOW CALCIUM INTAKE WOMEN: A RANDOMIZED CONTROLLED TRIAL

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Table of contents

1 Summary

Hypertensive disorders of pregnancy (HDP) affect approximately 10% of all pregnancies. The aetiology and pathophysiology of HDP are still largely unknown and an ideal prevention and treatment strategy does not exist. Although there has been progress in perinatal survival, largely due to developments in care of small babies, HDP continue to be major contributors to maternal and perinatal morbidity and mortality, especially in developing countries. The systematic review of randomized controlled trials of calcium supplementation provides promising evidence that calcium supplementation may be effective in preventing HDP in women with low calcium intake. It is important to obtain a reliable, definitive answer to this question for both clinical and programmatic reasons.

The World Health Organization and its collaborating institutions in developing countries propose a double-blind randomized controlled trial (RCT) to evaluate the impact of calcium supplementation (1.5 g/d) started before the twentieth week of pregnancy to nulliparous women with low calcium intake, on the incidence of pre-eclampsia. The proposed trial will enrol approximately 8500 women in seven countries over a period of 18 months. The primary outcomes are the incidence of pre-eclampsia (hypertension and proteinuria) for women and preterm birth for newborns. The sample size calculation is based on a reduction in the rate of pre-eclampsia from 4% in the placebo group to 2.8% in the calcium-supplemented group (30% reduction).

The UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction (HRP) of the Department of Reproductive Health and Research (RHR) at WHO will coordinate the study in collaboration with local institutions. If the effectiveness of routine calcium supplementation in preventing pre-eclampsia is confirmed, WHO will develop and circulate recommendations for incorporation of this practice into routine antenatal care. Nutritional strategies to reach the recommended calcium intake from food sources will also be prepared and disseminated. It is expected that if the research confirms effectiveness, implementation of this low-cost intervention will contribute to the reduction of maternal and perinatal severe morbidity and mortality due to HDP in developing countries.

2 Description of the project

2.1 Introduction

Worldwide, hypertensive disorders of pregnancy (HDP) affect approximately 10% of all pregnancies. The contribution of these disorders to severe maternal and perinatal morbidity and mortality is substantial, particularly in developing countries. Pre-eclampsia, HDP's most severe presentation, is defined as high blood pressure and proteinuria occurring after the 20th week of pregnancy. Pre-eclampsia is usually diagnosed late in pregnancy complicating approximately 5% of all pregnancies. Early-onset pre-eclampsia is seen less often but it is associated with higher incidence of complications. In the advanced forms of pre-eclampsia signs of multisystem disorder are usually apparent, sometimes with the presence of generalized convulsions (eclampsia). With increasing severity the likelihood of serious maternal and perinatal morbidity increases. In the mother, these include pulmonary oedema, coagulopathy, liver dysfunction and placental abruption. Fetuses usually show signs of impaired growth and perinatal mortality is high especially in early-onset pre-eclampsia.

The aetiology and pathophysiology of pre-eclampsia are still largely unknown and there are no known effective preventive or curative measures except for delivery of the baby. The risk factors include nulliparity, previous pre-eclampsia, multiple pregnancy and underlying vascular, renal or immunological disorders. Despite knowledge of some risk factors such as parity and previous pre-eclampsia, screening tests that can be used in clinical practice for detecting women at risk of developing pre-eclampsia have not been successful.[1] This can be related to the heterogeneous nature of the disorder which also makes the effectiveness of applying a single preventive measure difficult.[2] Another possibility for the ineffectiveness of preventive interventions, which applies particularly to nutritional interventions, is that the interventions may have been tested mostly in populations with little or no deficiency to be corrected.

Nutrients (as supplements to food) are provided to populations to either increase the intake among those with a deficiency (in order to prevent or treat functional outcomes related to such a deficit), or to obtain a pharmacological, perhaps non-nutritional effect, among individuals with an adequate intake of the nutrient in question. Most of the work on calcium supplementation addressed the former with the exception of two recent trials conducted in an adequate calcium intake population in the USA and Australia.[3,4]

In the formal description of the hypothesis in 1980 [5] specific reference was made to "the causal role of calcium deficiency in the occurrence of hypertensive diseases of pregnancy." It was identified that the importance of this observation was that increasing calcium intake in populations with a deficit may reduce the incidence of pre-eclampsia. In 1988, a mechanism of action in which populations with a lower calcium intake than required during pregnancy have an increase in serum parathyroid hormone level was postulated together with a recommendation to implement a large, randomized controlled trial in a 'high risk group of primiparous young women.' [6] Since then, two such large trials have been conducted in addition to several smaller trials. Conclusions are still unclear in relation to low calcium intake populations. The present protocol is aimed at obtaining a reliable definitive answer to this promising possibility.

2.2 Systematic review of randomized controlled trials

As a follow-up of a previous systematic review, an updated review of calcium supplementation was needed to identify and to understand the sources of disparities among trials (subgroup analyses), describing patterns of treatment effect. [7,8]

Such updated, independently conducted systematic review has been completed during 1998 and updated again in 1999 in the *Cochrane Library*.[9] It includes all available trials with random allocation, in which at least 1g/day of calcium supplementation was provided (most of the trials included provided 2 g/day). The nine included studies were double-blinded, placebo-controlled trials and the methodology was in general sound in terms of allocation method including the concealment of allocation schedule, rate of exclusions after randomization and double-blinding (Table 1). Main outcomes were rates of high blood pressure (with and without proteinuria) and pre-eclampsia. A new trial conducted with a similar protocol has recently been published, and will be discussed here as well.

Inspection of the funnel plots (the plot of the relative risk from individual trials against their sample size) and the results of the individual trials in the new calcium systematic review demonstrate asymmetry of the plot and heterogeneity of results. As it has previously been shown, asymmetry in funnel plots is a predictor of the lack of agreement between several small trials and the largest trial.[8] Although the most common factor associated with asymmetric funnel plots is 'publication bias', this is unlikely to have a large role in this calcium review. The subject has been studied extensively in previous meta-analyses and most researchers working on the topic have been contacted and have offered additional data. If there are still negative trials unpublished, they are most likely to be small and of lower quality.

It was therefore decided to explore other factors (population characteristics, treatment compliance) that can be associated with differences in results. Meta-analyses should not focus on a 'Typical Relative Risk (TRR)' for all trials even using statistical strategies such as a random effects model when there are discrepancies between trials. The discrepancy in this meta-analysis between the point estimate and confidence intervals from the fixed and random effects model [10] further emphasizes the need for searching for sources of discrepancy among trials.[11]

Stratified analyses were therefore conducted by two pre-specified subgroups based on selection criteria used in individual trials: baseline dietary calcium intake (mean calcium intake in the population above or below 900 mg/day) and the risk of hypertensive disorders of pregnancy (high/low) (Tables 2 and 3). The authors of the systematic review selected this cut-off point because it represents 75% of the Recommended Dietary Allowance (RDA) for the USA of the calcium intake during pregnancy, the RDA being 1200 mg/day.

There were six trials with populations classified as low calcium intake, all with a mean calcium intake of less than 650 mg/day (mean approximately 400 mg/day). The risk of high blood pressure was reduced among women with low baseline dietary calcium (TRR= 0.49; 95% CI= 0.38 to 0.62). Among those with an adequate calcium diet the risk of high blood pressure was TRR= 0.89; 95% CI= 0.81 to 0.99. The risk of pre-eclampsia was considerably reduced in trials conducted in low calcium intake populations (TRR= 0.32; 95% Cl= 0.21 to 0.49). Among women with an adequate calcium diet, the point estimate showed a risk reduction, with the confidence interval ranging within the beneficial and harmful range; (TRR= 0.92; 95% CI= 0.75 to 1.13). Among women at high risk of hypertension, calcium

supplementation reduced the risk of high blood pressure (TRR= 0.35; 95% CI= 0.21 to 0.57) and pre-eclampsia (TRR= 0.22; 95% CI= 0.11 to 0.43). No such protective effect was seen among women at low risk of hypertension.

For this protocol, we conducted a sensitivity analysis, excluding one trial with limitations in three of the four selected methodological indicators (Table 1).[12] There was a very small reduction in the magnitude of the protective effect of calcium supplementation: low calcium intake group: pre-eclampsia; TRR= 0.34; 95% CI= 0.22 to 0.52; high blood pressure: TRR= 0.52; 95% CI 0.41 to 0.68; high risk of hypertension group: pre-eclampsia; TRR= 0.24; 95% CI= 0.12 to 0.48; and high blood pressure: TRR= 0.45; 95% CI= 0.26 to 0.78). These results do not make any substantive change to the conclusions of the review.

In the Cochrane review meta-analysis, there was a reduction in the risk of preterm delivery among women at high risk of hypertension (TRR= 0.42; 95% CI= 0.23 to 0.78) but there was no effect in any other subgroup. References to individual trials and detailed description of their methodology can be obtained from Reference 9 of this proposal, or upon request.

Since the most recent update of this systematic review (10 April 1998), two new RCTs have been published.[4,13] In a randomized, double-blinded, placebo-controlled trial conducted in Colombia 86 women at high risk for pre-eclampsia were randomized to either 450 mg of linoleic acid/day and 600 mg calcium a day (43 women) or to placebo (43 women).[13] This trial is unlikely to be eligible for the calcium supplementation systematic review update because there were two concomitant nutritional interventions and because the calcium dose was below the minimum required by the review (1g calcium/day). In this new trial there was a clinically and statistically significant reduction in the incidence of pre-eclampsia in the calcium group (9.3%) as compared with the placebo group (37.2%) (TRR= 0.25; 95% CI= 0.09 to 0.69) and an increase in the mean gestational age at birth in the calcium group (39.3 ± 1.4 weeks) as compared to 38.2 ± 2.3 weeks in the placebo group (p=0.03).

In the second new trial conducted in Australia,[4] 456 nulliparous women were enrolled in a randomized controlled, double-blind trial that assessed the effect of 1.8 gms of oral calcium supplementation versus an oral placebo. Recruitment to the trial was stopped by the steering group without knowledge of the study outcomes after 456 women were randomized (227 were in the calcium group and 229 in the placebo group) when the funds for the study were exhausted. Among the enrolled women, the treatment with calcium reduced the risk of pre-eclampsia, (RR= 0.44, 95%Cl= 0.21 to 0.90) and the risk of pre-term birth (RR= 0.44, 95% CI= 0.21 to 0.90). The rate of severe pre-eclampsia was also lower in the calcium group, 1.8% versus 2.6% (RR= 0.67, 95% CI= 0.19 to 2.35), but pregnancy induced hypertension was not different between the groups, (RR= 0.90, 95% Cl= 0.59 to1.38). Approximately 30% of women in both groups stopped taking their trial medication during the antenatal period. At trial entry, only 30% of women had calcium intake of less than 800 mg/day a day,[4] with similar mean daily calcium intake to that of the NIH/USA trial. The latest trial will be eligible for inclusion in the systematic review in the stratum of populations with adequate baseline calcium intake and low risk for hypertension.

The results of a new meta-analysis after inclusion of the Australian trial are presented in Tables 2 and 3 in the corresponding strata (appendix 1). Overall, low-risk women supplemented with calcium had a reduced relative risk (RR= 0.79; 95% CI 0.65 to 0.94) of developing pre-eclampsia. For women with adequate calcium intake the relative risk of pre-eclampsia is 0.86 with 95% CI= 0.71 to 1.05 (Tables 2 and 3).

2.2.1 Disparities between the two largest trials

The first large trial[14] was aimed at reducing the rate of pregnancy-induced hypertension and pre-eclampsia. This trial enrolled 1167 women with a mean baseline calcium intake of 650 mg/day (approximately half of the Recommended Dietary Allowance) who were randomized to receive either a supplement with 2000 mg/day to correct this overall deficit in the total sample or a placebo. Women took on average 86% of the supplement, increased their urinary excretion of calcium and had a reduced risk of pregnancy-induced hypertension and pre-eclampsia although, in the case of the latter, the 95% confidence interval of the relative risk included unity, being compatible with a beneficial or a harmful effect. On the other hand, the trial by the National Institutes of Health (NIH), USA, studied the effect of calcium supplementation of 2000 mg/day in 4336 women without a calcium intake deficit (mean baseline intake 1130 mg/day), to achieve a pharmacological preventive effect rather than correcting a nutritional deficit.[3] Furthermore, all women in both arms received multivitamin and mineral supplements including low doses of calcium (50 mg/day). This is the only large trial conducted in a pregnant population approaching the RDA for calcium.[15] Furthermore, this trial excluded women with major risk factors for pre-eclampsia (essential hypertension, insulin-dependent diabetes mellitus, multiple pregnancies, as well as other risk factors such as pre-existing renal disease). Nulliparity was the only included risk factor for the disease. Women took an average 64% of the supplement and only 20% of them used more than 90% of the medication.[3] Treatment compliance is an issue to be considered in the interpretation of pre-eclampsia prevention trials,[16] particularly among women with a low baseline calcium intake.

Even in this population with an adequate calcium intake, there was a strong trend towards reduction in the overall risk of hypertensive disorders (RR= 0.90; 95% CI= 0.81 to 1.00), pregnancy-induced hypertension (RR= 0.88; 95% CI= 0.78 to 1.01), and severe pre-eclampsia (RR= 0.85; 95% CI= 0.58 to 1.23) with calcium supplementation.[3] Interestingly, a subgroup analysis, among 885 women with a baseline calcium intake between 582 mg and 846 mg, as in the other large trial[14] (600 mg), demonstrated a similar protective effect of calcium supplementation on pre-eclampsia (RR= 0.70 95% CI= 0.43 to 1.15). Among the 946 women with good treatment compliance (as in the 1991 trial)[14] the relative risk of pre-eclampsia was 0.76, with 95% CI= 0.47 to 1.22. The confidence interval was wide (with a possibility of detrimental effect) and the result did not reach a statistically significant level probably due to a smaller number of events in the subgroup analyses. Because actual numbers are not provided it was not possible to evaluate further these stratified data. Unfortunately, although the tablets appeared identical when individually compared, there was a noticeable difference in the intensity of the colouration of the formulations when several tablets were viewed in aggregate.[15] To remedy this situation the researchers packaged the tablets individually in opaque blister packs. In spite of the efforts to solve the problem, the possibility of assessment bias exists.

A reassuring finding is that, even in a population with such high total calcium intake (approximately 3 grams per day), there was no significant difference between groups in the rate of urolithiasis during pregnancy or neonatal hypocalcemia.[3]

2.3 Long-term effects of calcium supplementation

The possibility of intrauterine programming of blood pressure and the risk of various chronic diseases later in life has recently attracted considerable interest. This hypothesis, implicating diet, impaired maternal nutritional state, and lower birth weight,[17,18,19] suggests that fetal life is a period for programming physiological functions. This is a concept naturally extended

from the already demonstrated long-term deleterious effects of intrauterine growth retardation on postnatal physical growth, cognitive and neurological development.[20,21,22] These are issues of tremendous relevance to developing countries where a large proportion of newborns suffer from intrauterine nutritional restrictions.[23]

The effect of a nutritional intervention during pregnancy (to correct a deficit) on the blood pressure levels of the children from calcium supplemented women[24] was explored for the first time in the context of a randomized trial using the population of a large, randomized, placebo-controlled trial.[14] Children of a mean age of 7 years, whose mothers were randomly assigned during pregnancy to receive 2 g/day of elemental calcium (n=298) or placebo (n=293), were eligible for the follow-up study. Among these eligible children, 86.2% in the calcium group and 89.2% in the placebo group were evaluated at 7 years of age.[24]

Overall systolic blood pressure was lower in the calcium group, even though the confidence interval included both a reduction and an increase in the systolic blood pressure, (mean difference - 1.4 mmHg; 95% CI= - 3.2 to 0.5) than the placebo group. The effect was found predominantly among children whose body mass index at assessment was above the median for this population (mean difference in systolic blood pressure - 5.8 mmHg [-9.8 mm Hg to -1.7 mm Hg] for children with an index >17.5 and - 3.2 mmHg [-6.3 mmHg to -0.1 mmHg] for those with an index of >15.7 to 17.5). The risk of high systolic blood pressure was also lower in the calcium group than in the placebo group (RR= 0.59; 95% CI= 0.39 to 0.90) and particularly among children in the highest quartile of body mass index (RR= 0.43; 0.26 to 0.71). Although considerable debate has been conducted during the past several years on the effect of calcium intake during adulthood and blood pressure and cardiovascular diseases, very large prospective observational studies (nurse's health study; health professional's follow-up study and Iowa Women's Health Study) have shown a reduction on blood pressure, hypertension or ischaemic heart disease mortality among men, women and postmenopausal women.[25,26,27] These data add strength to the effect of calcium during pregnancy and fetal life. The exposure of high calcium intake could be more important with long-term consumption or during developmental periods.

2.4 Justification for a new trial

It seems clear that there is promising evidence of a protective effect of calcium supplementation during pregnancy on pre-eclampsia when provided to women with deficient calcium intake, and that evidence is biologically plausible. There is, however, strong support for the concept that definitive confirmation is needed in the context of an adequately sized and methodologically sound randomized controlled trial targeted specifically to a population with low calcium intake, i.e. the most likely to benefit from such an intervention. This is because most of the trials in low calcium intake populations were small and prone to exaggerate the protective effect, with the largest of them having wide confidence intervals, including one (the null hypothesis). Furthermore, as the implementation of daily calcium supplementation early in pregnancy will need substantial efforts, including early antenatal care and community organization, which are mostly not readily available in developing countries, the avoidance of implementing an intervention without compelling evidence is crucial.

The need for a definitive trial has been raised on several occasions recently. The Cochrane review concluded, *"Further randomised trials should concentrate on women at high risk of gestational hypertension, and communities with low dietary calcium intake."* [9] A commentary on the randomized controlled trials available concluded that *"Until a large trial*

of calcium supplementation in women at high risk for toxaemia or with poor dietary calcium intake is completed, calcium should not be used for primary or secondary prevention." [28] The editor of the journal where the most recent trial was reported *(Aust NZ J Obstetrics and Gynaecology)* recommended: *"Further large well-designed and appropriately-funded trials are indicated to clarify the role for calcium supplementation in pregnancy."* [4] Finally, a recent publication discussing the discrepancies between meta-analysis results and the large NIH trial written by the NIH investigators concluded *"Additional carefully controlled randomized trials are needed to establish whether calcium supplementation may indeed reduce the incidence of pre-eclampsia in healthy women at high-risk especially in the presence of low dietary calcium intake."* [29] Considering all these recent comments, we believe that there is urgency to conduct a definitive trial along the lines suggested to answer the question "Should pregnant women with low baseline calcium intake increase their routine intake starting before 20 weeks of pregnancy to reduce the incidence of pre-eclampsia?"

The WHO/HRP Maternal Health Research Strategic Review Committee and the Scientific and Technical Advisory Group (STAG) in its 1999 meeting have included this project among those of high priority and recommended its implementation.

2.5 Objective

The principal objective of the trial is to test whether increasing calcium intake among nulliparous pregnant women with low calcium diets leads to a clinically relevant reduction in the incidence of pre-eclampsia. The higher calcium intake will be achieved by means of a daily supplement.

2.6 Hypothesis

The hypothesis to be tested is that 1.5g calcium supplementation a day to low calcium intake nulliparous pregnant women reduces the risk of pre-eclampsia as compared with the placebo group.

3 Study design

The study will be a multicentre randomized, placebo-controlled, double-blind trial. The calcium supplement will be 1500 mg/day given as carbonate. This will be a pragmatic trial in which all nulliparous women presenting for antenatal care at any time before 20th week of gestation regardless of their gynaecological history or other obstetric characteristics will be eligible for the trial.

3.1 Settings

The trial will be conducted in antenatal clinics in developing countries serving a population of women with low calcium intake. The collaborating centres will be from the following countries that have expressed an interest to participate in the trial: Argentina, Colombia, Egypt, India, Peru, South Africa and Vietnam. These centres, which have experience in conducting trials, have been contacted and feasibility studies have been completed. These centres also have experience in detailed follow-up of large numbers of women. The study population in these centres should have a considerable proportion of women starting antenatal care before 20 weeks of pregnancy, an already existing routine antenatal care system and capacity to monitor all deliveries. Moreover base-line calcium intake from the

populations served by these centres (similar to the population to be recruited) will be completed before enrolment starts.

3.1.1 Routine antenatal and postnatal care

The study will not alter or interfere with any treatment or care given routinely to women in the antenatal care clinics or delivery institutions selected for the trial. Routine measures should include at least:

1. Attendance at ANC clinics starting before 20 weeks of pregnancy (up to 19 weeks and 6 days). The frequency and content of the visits will follow the standard practice of the clinic but at least one clinical visit will be carried out every four weeks to all enrolled women.

2. Monitoring standardized blood pressure measurement and routine urine screening for proteinuria at each antenatal care visit.

3. Assessment of birth weight and gestational age at delivery or shortly afterwards.

3.2 Intervention: calcium supplements

The women will be randomized to receive either a calcium supplement or a placebo from the time of enrolment, until delivery or initiation of any magnesium sulphate treatment, or the suspicion of urolithiasis. Women starting antenatal care before the twentieth week will be randomized at the first visit. Most of the women will therefore be supplemented for approximately five to six months. The diagnosis of pre-eclampsia or hypertension is not a reason for discontinuation of treatment.

The choice of calcium carbonate as the supplement was made because it is the cheapest of all other calcium supplements, making it more feasible for programme implementation in the case that the trial demonstrates its effectiveness and because all published studies and an ongoing trial in the Gambia have used this salt. Furthermore, several studies have demonstrated the absorption of calcium carbonate to be higher in comparison with other calcium salts.

There will be three chewable tablets containing 500 mg elemental calcium to be taken every day. Women will be instructed to chew tablets at meals, but at least 3 hours away from any iron supplements[30 31 32]. The control group will receive three tablets a day of identical characteristics, colour and taste as the intervention tablet prepared and packed by the same pharmaceutical manufacturer.

All women will be encouraged not to take any additional calcium supplements. For those women who need analgesics, acetaminophen, and for those needing antacids, a non-calcium antacid will be recommended.

The dose 1500 mg/day, lower than the 2000 mg/day used in previous trials was selected for the following reasons:

a) The study populations have an intake level (median < 600 mg/day) that will be raised to a level above the RDA of 1200 mg with 1500 mg/day supplementation.

b) This level of calcium intake is more likely to be achievable from dietary sources alone. In this way, if the trial shows beneficial results from supplementation, the populations will not have to depend solely on supplementation by tablets.

c) Compliance is likely to be higher with three tablets. Problems with compliance have been reported in previous trials where women expressed problems with taking 4 large tablets every day. Furthermore, the tablets that will be used in this trial will be chewable and it is anticipated that compliance will be less of a problem in this trial. Insufficient compliance does not constitute a reason for excluding a patient from the trial.

3.3 Participants

3.3.1 Eligibility criteria

Pregnant women attending antenatal care in the participating centres will be eligible if the following criteria are met:

1. Gestational age <20 weeks

2. Nulliparous

3. Willing and able to give consent. (Informed consent for minors will be obtained following country specific age limits)

Randomization will be conducted during the first ANC visit before 20 weeks of gestation. It was decided to include all nulliparous women regardless of their gynaecological history or early pregnancy complications (such as threatened abortion or hyperemesis gravidarum) to cover a high-risk group most readily identifiable. Although there are other risk factors such as positive family history and pre-eclampsia in a previous pregnancy, the screening process to identify these cases is likely to be cumbersome and will be difficult to implement given the diverse populations included in the trial.

3.3.2 Exclusion criteria

Women will be excluded under the following conditions:

- History of urolithiasis or symptoms suggestive of urolithiasis or any renal disease such as haematuria, flank pain, etc.
- Parathyroid disease.
- Blood pressure ≥ 140mmHg systolic or ≥ 90mmHg diastolic, receiving treatment or had history of hypertension.
- Taking diuretics or any digoxin treatment.
- Plan to deliver in a health facility outside the study area or at home.
- Taking phenytoin or tetracyclines.

3.4 Outcome variables

Outcome variables are classified as primary or secondary. The primary outcomes of the study are selected on the bases of the following: they can be affected by the intervention, represent an important clinical outcome, are recorded routinely by treatment blinded observer and occur frequently to provide sufficient statistical power. The primary maternal outcome of the trial is pre-eclampsia. The definitions for trial endpoints are described in detail in appendix 7.

Hypertension is defined as blood pressure greater than or equal to 140 mmHg systolic and/or 90 mmHg diastolic occurring in two occasions at least four hours to a week apart after the 20^{th} week of pregnancy. Diastolic blood pressure will be measured at 5^{th} Korotkoff sound, which is the disappearance of the sounds. Proteinuria is defined if protein in urine is ≥ 300 mg in 24 hours urine specimen or corresponding level of 2+ or more on dipstick. Preeclampsia is defined as hypertension associated with proteinuria. Severe pre-eclampsia is defined as 160 mmHg systolic and/or 110 mmHg diastolic or greater on two occasions at least 4 hours apart or a single BP reading of 160 mmHg and/or 110 mmHg or greater if it required treatment with an antihypertensive and at least 2+ proteinuria.

For women directly admitted to a hospital without being referred from an antenatal clinic with a diagnosis of pre-eclampsia, the highest recorded blood pressure measurement will be extracted from medical records and diagnosis of high blood pressure will be based on it.

Maternal secondary outcomes are: Early onset pre-eclampsia (pre-eclampsia starting before 32 weeks of gestation), pregnancy induced hypertension (PIH), eclampsia and placental abruption.

The primary neonatal outcome is pre-term birth (<37weeks of gestation). Neonatal secondary outcomes are low birth weight (birth weight <2500 grams), spontaneous pre-term delivery and medically indicated pre-term delivery, admission to newborn intensive care unit for more than 2 days, fetal and neonatal mortality, and perinatal mortality.

A random sample of medical records of delivered patients with and without pre-eclampsia will be photocopied and reviewed by a team of independent investigators to evaluate the agreements between study definitions and clinical diagnoses.

3.5 Methods

3.5.1 Sample size and power considerations

The total minimum sample size was determined to be 8500 women, with half allocated to receive calcium supplements and the other half to receive placebo tablets. This sample size is sufficient to obtain 80% power to detect a 30% reduction in the rate of pre-eclampsia in the calcium group (2.8%), as compared with the placebo group rate of 4%. This rate in the placebo group is based on data obtained from one of the populations considered for the study, with HRP/RHR/WHO supported population based data collection system. This rate is at the lower end of the range from other candidate centres and gives the sample size calculation a conservative approach. An even more conservative estimation is a reduction in the rate of pre-eclampsia from 3.5% in the placebo to 2.45% in the calcium group, which requires a total of 9000 women. A total of 4500 women will be sufficient to show a reduction from 4% to 2.40% or a RR of 0.60. This latter sample size will be used as the milestone for the first interim analysis.

3.5.2 Randomization

Simple randomization will be conducted independently for each study site by the HRP/RHR statistical unit at WHO headquarters and provided to the pharmaceutical manufacturer.

The random allocation sequence will be generated using computer-generated random numbers. Randomization will be to the two arms of the trial and stratified by country. Blocking with randomly varying groups of 6-8 will be used to restrict randomization within the strata (country) (SAS Software, Copyright© 1989, 1994 SAS Institute, Inc., N.C., USA).

Random allocation technique consists of allocating consecutively numbered treatment boxes for each woman, including in each box seven independent bottles, each of them with tablets for four weeks of treatment. Each bottle contains 100 tablets . Therefore each subject will have seven bottles with the same randomization number. Treatment boxes will be kept at the clinic. Bottles will be provided consecutively as needed every month after randomization. When the woman comes for antenatal care, she will return the used bottle from the previous month and will be given the next month's bottle from her box. She will return the bottle after 4 weeks, regardless of whether she finished the tablets or not. Each bottle is numbered from 1 to 7 within the box and should be used sequentially.

3.6 Clinical measures

At each visit and at delivery, clinical data will be collected and recorded in forms designed for the study.

3.6.1 Blood pressure

Blood pressure measurements will be standardized. The equipment must be serviced locally before the initiation of the study. The subject should be at rest, seated, for 5 minutes; the cuff should be placed on the right arm at the level of the heart before the measurements. Two blood pressure measurements of systolic and diastolic will be taken using a standard sphygmomanometer at 3-minute intervals. Leave the cuff deflated on the subject's arm and wait for 3 minutes to take the second measurement. Diastolic blood pressure will be measured at the 5th Korotkoff sound, which is the disappearance of the sounds.[33]

The standard sphygmomanometer will be used because its calibration and use are well established, it is considered the gold standard against which new devices have to be tested [34], and allows the measurement of the true systolic, diastolic, and pulse pressures. [35] The alternative to the use of standard sphygmomanometer would be to rely on automated devices. However, it has been observed that many automated devices were inaccurate in measuring

blood pressure. [36] Furthermore, the cost of a high quality automatic sphygmomanometer could be as high as USD 1000 making it prohibitive for use in this trial. Therefore, it is felt that the use of a standard sphygmomanometer is justified within the context of a pragmatic, double-blind, large trial. At every centre, the trial coordinator will train staff on their abilities to measure blood pressure according to guidelines contained in the trial document: *A practical guide on how to measure blood pressure and test for proteinuria*. The trial coordinator will be trained before the initiation of recruitment. Retraining sessions will be carried out every three months. At monthly intervals, staff will be tested for reliability of blood pressure measurements. Test-retest procedures will be performed using a double stethoscope and results of the measurements will be recorded on appropriate forms to calculate agreement between examiners.

3.6.2 Urine analysis

Midstream urine will be collected routinely, according to guidelines contained in the trial document: *A practical guide on how to measure blood pressure and test for proteinuria*, from all women receiving ANC visits, on admission in labour or before elective caesarean section to detect the presence of protein. Measurements of urinary protein will be carried out in urine samples without any visible blood contamination. For subjects with vaginal discharge samples will be taken after carefully cleaning of the introitus. In subjects with pre-labour rupture of membranes the presence of proteinuria should be verified by sterile catheter. Dipstick for qualitative urine analysis will be used in all ANC visits and at admission in the labour ward throughout the study.

3.6.3 Anthropometry

Anthropometry (weight and height) of the mother during the first antenatal visit, and birth weight of her baby will be routinely collected. The methodology will be standardized and careful quality control of equipment instituted. Women wearing light clothes will be weighed on a calibrated scale. Height will be measured without shoes.

3.7 Assessment of nutrient intake

The major reasons for documenting nutrient intake in previous studies were to determine the amount of calcium obtained from sources other than the study tablets and the quantities of other nutrients that may affect calcium absorption or retention. The nutrient intake assessment was often made by 24 hr recalls conducted at randomisation and at 32-33 weeks gestation. In the present study the populations have been selected based on existing knowledge of their calcium intake. However a formal calcium intake assessment will be done in a random sample of pregnant nulliparous women at their first antenatal visits before 20 weeks of gestation in the study clinics to ensure that these institutions currently serve a low-calcium intake population. It has been decided a priori that the population cut-off level for median calcium intake will be 600 mg/day. Centres serving a population with a higher median calcium intake will not be eligible for the trial. Following this population assessment before the trial commences, no further individual intake evaluation of enrolled women will be undertaken. This is because of the pragmatic nature of the trial, which is aimed at testing the effectiveness of the supplement in populations with general low calcium intake. The conclusion and recommendations, if the results support the need for extra calcium, will not call for an individual screening for low calcium intake before supplementation.

4 Ethical aspects

This study is designed to determine whether an increase in calcium intake during the second half of pregnancy would benefit mothers in regions where dietary calcium intakes are substantially below recommended levels. Pre-eclampsia, pregnancy-induced hypertension and eclampsia are serious health problems for women in most developing countries. Two issues merit further discussion under ethical aspects.

4.1 Calcium supplements

Calcium carbonate tablets are commonly used as an antacid preparation and there are no specific contraindications for its use in pregnant women. Concern has been expressed that high levels of calcium supplementation could lead to renal stone formation due to hypercalciuria.[37] However, no problems associated with calcium supplementation have been reported in intervention studies of pregnant women using calcium carbonate in doses 1000-2000 mg/day.[38] T[REFNOTA]he dietary calcium intakes of women in these studies were often moderate-high, resulting in very high total intakes, often exceeding 3000 mg/day. The dietary calcium intakes of women considered for this trial are low, and total intakes of individuals in the calcium group of this study are unlikely to exceed 2000 mg/day. In addition, in a recent study of calcium requirements of lactating women in the Gambia, despite calcium supplementation with 1000 mg/day, only moderately elevated urinary calcium outputs were observed and these were significantly lower than those of unsupplemented British women (Jarjou and Prentice, unpublished). The association between high calcium intakes and stone formation has been questioned by a study, which showed an inverse relationship between the incidence of stones and calcium intakes.[39] It would, therefore, appear that the likelihood of an increased risk of renal calculus occurring in the study subjects is remote but the situation will be monitored closely throughout the study.

4.2 Participation of women

The women will be recruited at ANC clinics. It will be emphasised that enrolment in the study is voluntary, that she can withdraw at any time from all or part of the study, and that any decision she takes in this respect will have no bearing on the medical care she and her family receive. The study will be explained verbally, according to the information sheet and consent will be recorded with a signature or thumbprint.

Enrolment will be at the discretion of the staff responsible for each mother. No treatment of any kind will be withheld from a mother because of her participation in the study and mothers who develop hypertension or pre-eclampsia will be given appropriate therapy.

Informed consent for possible enrolment will be obtained antenatally following routine practices in the participating hospitals and a staff of the trial will inform women about consent for participation. Depending on local requirements, verbal consent may be considered adequate to recruit women. The consent form has been translated into local languages as necessary. The WHO approved consent form is presented in Appendix 4. Participants will not be named in any reports arising from the trial.

5 Follow-up

The study primary endpoints will be met when:

- The woman has delivered.
- Pre-eclampsia or eclampsia is diagnosed (in this case the woman should continue receiving supplements). These women must be followed up until delivery according to the study protocol.

Long-term follow-ups may be undertaken depending on their economic feasibility in subgroups of women but are not planned at this stage.

5.1 Assessment of compliance

At each monthly antenatal care visit following enrolment, the bottle which was given to the woman at the previous visit will be collected and a new bottle for the next 4 weeks will be provided. All bottles should be returned to the subject's treatment box. Every time a bottle is returned, the number of tablets left should be counted and recorded in the Subject Treatment Record form (STR). At the end of the follow-up of each subject, the total number of tablets given and returned will be calculated and recorded in the End of the Study and Delivery form (DEL).

6 Quality control procedures

6.1 Before recruitment

1. Previous RCTs conducted by some of the selected centres on this subject have assisted in optimising the procedures of the trial.

2. The survey among participating centres on baseline calcium intake of the served pregnant population will assist in determining the baseline calcium intake estimates of the study population.

3. All centres piloted the trial procedures, including the use of data collection forms.

4. Both the protocol and the trial report will include requirements laid out in the CONSORT statement.

5. Each centre has provided the Trial Coordination Unit in HRP/RHR/WHO, Geneva with their recruitment target based on their pre-trial obstetric statistics.

6.2 During recruitment

1. Completed data collection forms will be returned to WHO in Geneva monthly; data checking and entry will be continuous. All data will be double entered, cleaned and queries checked immediately with the local investigators. Prompt return of data collection forms and speedy clarification of queries will facilitate verification of data.

2. Double-blinding will reduce biases in the monitoring of women and assessment of outcomes.

3. Randomization will take place when the woman's name and hospital number is written on the next treatment box and in the subject number list. If a box is not used for whatever reason, it will be returned to WHO unopened with only the study number on it. The used boxes and bottles will be kept in the centres until the WHO site visitor has checked them.

4. Good Clinical Practice (GCP)[40] procedures will be followed.

5. A random sample of boxes will be sent for content testing to ascertain whether the content matches coding. Similarly, a random sample of tablets will be analysed to check the calcium and placebo contents. This will be conducted by the manufacturer without the involvement of the trial unit in Geneva.

6. The storage conditions will be monitored by dataloggers which record the temperature and humidity ensuring that the trial medications are kept under optimal conditions.

7. The following procedures will be adopted to standardize outcome assessment:

a) Training sessions will be conducted according to specific methods and adiuovisual material developed by professional trainers in blood pressure measurements (Shared Care Inc., California) to ensure that blood pressure measurements will be standardized through all centres. Before the initiation of the trial, specialized trainers from Shared Care will standardize and certify in blood pressure measurements all the principal investigators and the field coordinators from each centres during a training session to be held at the WHO headquarters in Geneva.

b) Intra- and inter-observer errors will be evaluated monthly during the trial using a double stethoscope according to a standard methodology developed by Shared Care Inc.

c) Staff will undergo regular retraining sessions for blood pressure measurement every three months using the audiovisual training material developed by Shared Care Inc.

6.3 After recruitment

Data will be analysed and reported as intention-to-treat, stratification for effect modifiers will be determined a priori and the draft analysis plan follows the dummy tables enclosed. (Appendix 2).

7 Follow-up procedures

The main requirement from collaborating centres will be that women are followed from the first ANC visit before 20 weeks until delivery. The trial medications should be discontinued only if the subject refuses to take the medication, when parenteral magnesium sulphate therapy is initiated, and in case of suspicion of urolithiasis.

8 Data management and analysis

8.1 Data management

Data will be collected prospectively by the researchers at the local collaborating centre and forms will be sent to the UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction (HRP) of the Department of Reproductive Health and Research at WHO headquarters in Geneva monthly following the standard data management procedures of HRP/RHR.

The researchers will record side effects. Although it is very unlikely that serious adverse events will occur, these will be recorded in special forms and will be returned by the local investigators to the Trial Coordination Unit (HRP/RHR/WHO in Geneva) by facsimile within 24 hours of the event. These procedures have been used in multiple multicentre[REFNOTA] trials and provent to be very efficient and compliant with the Good Clinical Practice (GCP) principles and data management.[40] Furthermore the investigator will report all adverse events in a timely and correct manner to the local authorities according to local national law. The WHO coordinating unit will share this information with Nycomed Pharma.

Data entry will be done centrally in Geneva.

8.2 Protocol compliance

The main factor determining protocol compliance will be appropriate timing of enrolling the patients early in pregnancy to allow for sufficient exposure to the treatment and follow-up during gestation. Loss of patients or missing ANC visits will reduce treatment compliance as well as outcome detection.

8.3 Unblinding

The need for unblinding should be extremely rare as the trial intervention is not associated with severe side effects and it will not delay or prevent standard management of the patient in the case of a complication such as renal calculus. If the woman develops pre-eclampsia or eclampsia, she will be treated according to routine treatment protocols regardless of the supplementation status. If, however, unblinding is needed for any reason the trial coordinator in Geneva will be contacted to reveal the treatment.

8.4 Data processing

Data entry and checking will be continuous and queries will be followed vigorously to ensure that clarification will be without delay.

8.5 Analysis plan

An analysis plan has been finalised before recruitment starts. A draft layout of the proposed analysis plan is presented in Appendix 2 in dummy tables.

Principal analyses will be on an intention-to-treat principle with comparisons made between calcium and placebo for primary and secondary outcomes. Stratification will be made for

time gestational age of entry into the trial by each woman and by baseline calcium intake level of populations served by the hospitals. These hospitals will be classified as serving a population of very low (< 200mg), low (200-400mg), or medium (400-599mg) calcium intake before initiation of the trial. An exploratory analysis will be conducted for the hypothesis that the effect will be larger among women who started treatment before 16th week of pregnancy and among populations with the lowest baseline calcium intake.

8.6 Data Safety and Monitoring Committee

A Data Safety and Monitoring Committee (DSMC) with no direct involvement in the trial will be appointed. Professor Jack Moodley of South Africa will chair it. The role of DSMC will be to deal with any ethical issues that may arise while the trial is in progress, and to scrutinise an interim analysis. Reporting and handling of adverse events will be in accordance with the GCP guidelines.

The DSMC will be asked to give advice regarding the trial if they have proof beyond doubt of an important advantage or disadvantage for one of the treatment groups, and they consider that the results are likely to affect clinical practice. Based on previous trials it seems unlikely that a major disadvantage will be apparent.

An interim analysis is going to be suggested to the DSMC to be conducted after the completion of the first 4500 subjects. If the difference between the proportions of occurrence of pre-eclampsia in the two groups is significant in a two-sided test at the level α=0.01 then the trial will be stopped prematurely. The adjustment for repeated testing at the final analysis will then be negligible, leaving a significance level of practically α=0.05.[41,42]

9 Duration of the project.

It is anticipated that the whole project can be completed in approximately three years if the time scale of appendix 5 is adhered to. The recruitment will begin in November 2001.

10 Project management

The trial coordination and management, including data management and analysis, will be by the Special Programme of Research, Development and Research Training in Human Reproduction (HRP) of the Department of Reproductive Health and Research at WHO headquarters. Dr José Villar, Dr Metin Gülmezoglu, and Dr Merialdi will be the trial coordinators in Geneva, assisted by other staff of HRP/RHR as required (Trial Coordination Unit). The trial management will include coordination and execution of the following activities which require administrative and clinical research input.

10.1 Preparation for the trial

10.1.1 Coordination activities

- Site visits before recruitment: standardisation of trial procedures; reception, handling and storing of trial materials; possible recruitment rate.
- Discussion of logistics of treatment administration with collaborators.
- Establish communication procedures between the centres and Geneva.
- Designation of committees: Steering Committee, Data Safety and Monitoring Committee (DSMC).
- Finalise protocol.
- Submit proposal to ethics committees.
- Organization of interim and final collaborators meetings.

10.1.2 Trial materials

- Preparation of data collection forms and consent forms.
- Preparation of Manual of Operations and other trial manuals.
- Purchase of the boxes for the treatment bottles and their contents.
- Decide on mailing procedure and size of packages containing packs of trial materials.
- Send packages to centres.

10.1.3 Data processing and system preparation

- Randomization of subjects (HRS/HRP/RHR, Geneva).
- Feasibility study (HRS/HRP/RHR, Geneva).
- System set-up for data entry and validation (HRS/HRP/RHR, Geneva).
- System set-up for production of monitoring reports (HRS/HRP/RHR, Geneva).

10.1.4 Statistical issues

- Trial size determination.
- Preparation of dummy tables.
- Definition of monitoring reports to be produced.

10.2 Conduct of the trial

10.2.1 Coordination activities

- Trial staff during the course of the trial will conduct at least two site visits to each centre to monitor trial progress. The first is expected after 3 months of recruitment.
- Communication with local investigators to monitor trial progress.
- Communication with the data monitoring and steering committees of the trial.
- Weekly meetings of the Trial Coordination Unit and data management group (HRS/HRP/RHR, Geneva).
- Data management and statistical analysis.

- Data entry.
- Data validation and production of queries based on a pre-set of cut off points for data verification.
- Update of the master file using batches of new data or corrections coming from validation checks and/or answers to queries.
- Correspondence between HRS/HRP/RHR and the centres related to queries.
- Monitoring reports: recruitment, adverse events, loss to follow-up, completeness of data for main outcomes.
- Statistical analysis: interim analyses, final analysis.

10.2.2 Administrative

- Preparation of a trial newsletter.
- Assistance with the organization of trial-related meetings including travel arrangements.
- Maintaining a mailing list of trial contacts (collaborators, trial steering and data monitoring committee members).
- Posting, photocopying, faxing.

11 Links with other projects

There will be eight centres in seven countries. All of these, Argentina, Colombia, Egypt, India, Peru, South Africa and Vietnam, are experienced in calcium supplementation trials or in other WHO multicentre collaborations. Argentina has also participated in the Collaborative Eclampsia Trial, the Misoprostol trial and the Antenatal Care trial, also funded by WHO. Egypt, South Africa and Vietnam participated in the WHO Misoprostol trial.

This trial is linked to the Maternal Health Research Programme of HRP/RHR for the prevention of leading causes of maternal mortality which has been approved by the Maternal Health Research Strategic Group and the Scientific and Technical Advisory Group (STAG) of HRP/RHR. Furthermore, this trial is linked to another smaller RCT being conducted in the Gambia by the University of Cambridge and the UK MRC exploring in a detailed manner the mechanisms by which high calcium intake to calcium deficient women can influence blood pressure during pregnancy. The endpoints in this latter trial are physiological measurements and blood pressure and the trial is not designed to test the hypothesis of prevention of pre-eclampsia.

12 Main problems anticipated

Most anticipated problems have been resolved during the preparation of the protocol based on the experience of previous trials. The main challenge remains, however, the follow-up of large numbers of women enrolling early in pregnancy in developing country settings.

13 Expected outcomes of the study

13.1 Clinical

It will be established definitely whether calcium supplementation fulfils the requirements of a practical and effective preventive strategy for pre-eclampsia among low calcium intake nulliparous women.

13.2 Public health

If calcium is found to be effective, it will be possible to introduce it for use as a prophylactic for pre-eclampsia to all nulliparous pregnant women in populations with low calcium intake. Evidence of its subsequent effectiveness in reducing eclampsia and maternal mortality will have major public health implications in developing countries reproductive health status.

14 Dissemination

The following routes of dissemination will ensure the widest possible distribution:

a) Publication of major findings in a mainstream journal.

b) Publication of major findings in national journals by local collaborators.

c) Reporting of trial findings in WHO publications such as *Progress in Human Reproduction Research* and *Safe Motherhood* newsletters, and others.

d) The systematic review of the topic will be updated for *The Cochrane Database of Systematic Reviews*.

e) The practical implications of the trial results can be incorporated within a short time into other WHO activities such as *The WHO Reproductive Health Library*, the *Mother and Baby Package, Essential Care Practice Guides in Pregnancy and Newborn Care,* Safe Motherhood workshops, etc. which would have effects on training and implementation of findings of the trial at country level.

15 Authorship for publications

The Steering Committee has agreed to adopt the so-called "modified conventional form" to be used for the publications of the trial. This form includes attribution to the investigators that were primarily responsible for the trial followed by the name of the corporate research group: e.g. Ann A. Meyeis, Henry C. Brown, ..., for the WHO Calcium Supplementation during Pregnancy Trial Research Group.

The list of investigators primarily responsible for the study will be prepared by the Steering Committee and should include only those actively involved in the preparation, organization, implementation of the trial as well as data analysis and manuscript preparation.

The order of authorship in individual papers will be decided by the Group. Credit will be given to the other participants of the trial such as associated staff at participating centers and key committee membership and will be listed at the end of the manuscript. Acknowledgements will be expressed in a footnote to the title or in a section following the credits. A WHO/HRP/RHR certificate of collaboration will be given to doctors, midwives, nurses and local staff who contributed to the trial but whose names do not appear in the main papers. This strategy has been used in previous trials and is highly appreciated by collaborators.

There will be a Publications Sub-Committee of the Trial Steering Committee. This Sub-Committee will be responsible for:

- preparing a list of tentative publications
- reviewing all papers sent for publication
- reviewing and authorization of secondary analysis proposed
- certifying authorship of individual papers .

16 TRIAL COMMITTEES

16.1 Local investigators and institutions

The following investigators and institutions are considered as possible sites:

- Mrs Liana Campodonico, CREP, Rosario, Argentina
- Mrs María Ximena Rojas, University of Javeriana, Bogota, Colombia
- Dr Hany Abdel-Aleem, Assiut University, Assiut, Egypt
- Dr Manorama Purwar, Clinical Epidemiology Unit, Nagpur, India
- Dr Matthews Mathai, Christian Medical College & Vellore Hospital, Vellore, India
- Dr. Nelly Zavaleta, Instituto de Investigacion Nutricional, Lima, Peru.
- Dr Justus Hofmeyr, University of the Witwatersrand, Johannesburg, South Africa
- Dr Nguyen Thi Nhu Ngoc, Hungvuong Hospital, Ho Chi Minh City, Viet Nam

16.2 Steering Committee

The Steering Committee will be composed of the following:

- Local investigators
- Trial coordination Unit (José Villar, Metin Gülmezoglu, Mario Merialdi, Guillermo Carroli, Gilda Piaggio)
- Dr Ann Prentice, MRC Human Nutrition Research, Cambridge, UK
- Dr Marshall D. Lindheimer (Chairperson) Department of Obstetrics and Gynaecology The University of Chicago, Illinois, USA

16.3 Data and Safety Monitoring Committee

The Data and Safety Monitoring Committee will be composed of the following:

Professor Jack Moodley, Department of Obstetrics and Gynaecology, University of Natal Medical School, South Africa. (Chairperson)

Dr. Elizabeth Thom, The George Washington University, Washington D.C., USA

Professor Michel Vallotton, University of Geneva, Geneva, Switzerland

Minutes of Data and Safety Monitoring Committee will be distributed for Clinical Pharmacovigilance.

17 APPENDICES

APPENDIX 1: Systematic review of calcium supplementation

APPENDIX 2: Dummy tables for analyses

APPENDIX 3: Flow chart for women during the trial period

APPENDIX 4: Consent form

APPENDIX 5: Timeline for the trial

APPENDIX 6: CONSORT STATEMENT points in the protocol

APPENDIX 7: Endpoint definitions

APPENDIX 1: SYSTEMATIC REVIEW OF CALCIUM SUPPLEMENTATION

Table 1. Indicators of methodological quality of randomized trials included in the Systematic Review of calcium supplementation during pregnancy

Trial	Generation of random schedule	Concealment of allocation schedule	Double-Blinding	Exclusions after Randomization (%)
CPEP 1997	Computer generated	Yes	Yes	Ca = 5.8 Placebo = 5.3
L. Jaramillo 1997	Random number table	Yes	Yes	Ca = 6.7 Placebo = 3.6
Purwar 1996	Computer generated	Yes	Yes	Ca = 5.8 Placebo = 5.1
S. Ramos 1994	Computer generated	Yes	Yes	Ca = 12.1 Placebo = 0
Belizán 1991	Computer generated	Yes	Yes	Ca = 2.4 Placebo = 2.2
L. Jaramillo 1990	Unclear	Unclear	Yes	large unexplained discrepancies between groups
Villar 1990	Computer generated	Yes	Yes	Ca = 5.2 Placebo = 7.3
L. Jaramillo 1989	Random number table	Unclear	Yes	Ca = 10.9 Placebo = 15.6
Villar 1987	Computer generated	Yes	Yes	All 52 women randomized included in analysis
Crowther 1999	Computer generated	Yes	Yes	All 456 women randomized had data on primary outcomes

APPENDIX 1
Table 2. Effect of routine calcium supplementation in pregnancy on high blood pressure

	Number of trials	**Calcium n/N**	**Control n/N**	**Typical RR (95% CI)**
Low-risk	6	611/3146	732/3161	0.84 (0.76, 0.92)
High-risk	3	15/141	54/156	0.35 (0.21, 0.57)
Adequate calcium diet (≥ 900 mg/day)	4	547/2505	614/2517	0.90 (0.81, 0.99)
Low calcium diet (< 900 mg/day)	5	79/782	172/800	0.49 (0.38. 0.62)

RR = Relative Risk; CI = Confidence Interval
High blood pressure with or without proteinuria

APPENDIX 1

Table 3. Effect of routine calcium supplementation in pregnancy on pre-eclampsia

	Number of trials	**Calcium n/N**	**Control n/N**	**Typical RR (95% CI)**
Low-risk	6	188/3146	240/3161	0.79 (0.65, 0.94)
High-risk	4	8/266	47/291	0.22 (0.11, 0.43)
Adequate calcium (≥ 900 mg/day)	4	169/2505	174/2288	0.86 (0.71, 1.05)
Low calcium diet (900 mg/day)	6	27/907	90/935	0.32 (0.21. 0.49)

RR = Relative Risk; CI = Confidence Interval

APPENDIX 2: DUMMY TABLES FOR ANALYSES

Table 4: Recruitment rates by centre

	Argentina	Colombia	Egypt	India	Peru	South Africa	Vietnam
Calcium							
Placebo							
Total							

Table 5: Characteristics of women at trial entry

	Calcium		Placebo	
	N	%	N	%
Age (mean, SD)				
Age < 17 years				
Education level (≤ primary school)				
Gestational age at first visit (mean SD)				
Randomized before 16 weeks' gestation				

Table 6: Compliance with allocated treatment

Compliance	Calcium	Placebo
Mean		
SD		
Interquartile range		
Compliance index (mean tab.s taken/ideal number)		

Table 7: Trial outcomes: Mother

	Calcium		Placebo	
	N	%	n	%
Primary outcomes				
Pre-eclampsia				
Secondary outcomes				
Pregnancy Induced Hypertension (PIH)				
Severe pre-eclampsia				
Eclampsia				
Pre-term delivery				
Mean gestational age at delivery (SD)				
Hospital stay more than 7 days				

► Stratified analyses will be conducted for randomization before 16 weeks and 20 weeks, and calcium intake

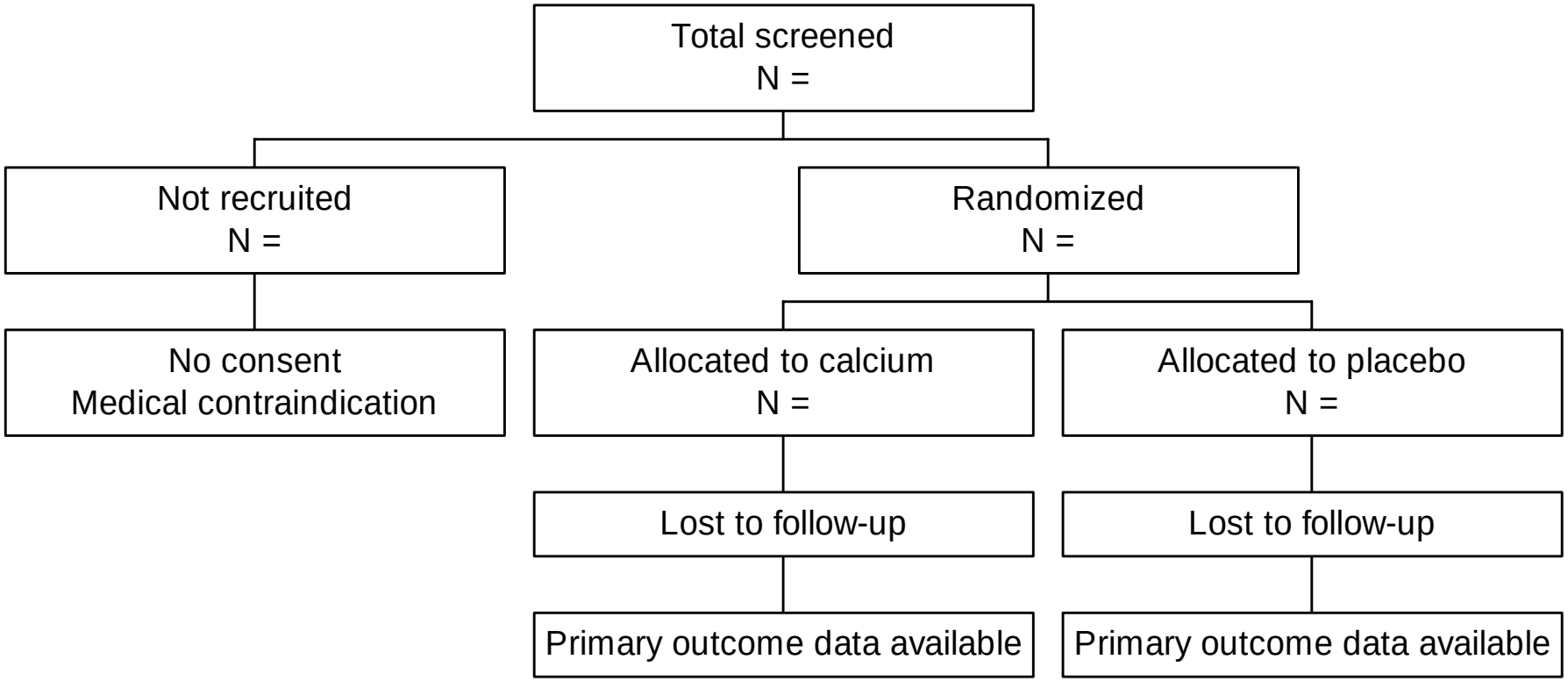
Table 8: Trial outcomes: Baby

	Calcium		Placebo	
	N	%	n	%
Preterm birth				
Low birth weight				
Mean birth weight (SD)				
Stillbirth				
Neonatal death				
Perinatal death				
Admission to NICU				

Table 9: Side effects

	Calcium		Placebo	
	N	%	n	%
Any side-effect				

APPENDIX 3: FLOW CHART OF WOMEN APPROACHED DURING THE TRIAL



APPENDIX 4: SAMPLE CONSENT FORM

To be read to each prospective participant in her own language. One copy of signed form to be given to participant.

Purpose of the study

We are interested in finding out whether your health and the health of your unborn baby can be improved by giving you tablets containing calcium to eat while you are pregnant. This work will benefit women around the world, as it will help us to understand what foods women need to eat while they are pregnant. We are asking you to take part in a study in which you will be given three tablets to chew every day until your baby is born. Half of the women in the study will be given calcium tablets. Calcium is an ingredient in foods such as milk that you may normally eat. The other women in the study will be given tablets which are very similar in appearance but which do not contain calcium. The tablets will be kept in the clinic, and we will ask you to visit it once a month to collect them and you should bring back unused tablets. The effect of calcium on your blood pressure and health, and the health and growth of your baby will be measured at the clinic and at the time of delivery.

There is no known risk of taking the calcium tablets and the amount of calcium is equal to that which is in 1.5 litres of milk.

Your involvement in this study is entirely voluntary and if you decide not to take part, it will not affect the medical treatment you or your family gets in any way. If you decide to join the study, you are free to leave the study at any time without explanation. All information you provide or that is taken from your medical records will be treated under complete confidentiality.

Consent

This explanation of the study was given by ……..

Date ……..

Clinic ……..

Name of participant ……..

I agree to take part in this study and understand that my participation is voluntary and will not affect my medical treatment in any way and all information will be kept confidential.

Signature ……..Date .....

Signature of Witness …… Date .....

APPENDIX 5: TIMELINE FOR THE TRIAL

	Oct 99	Jan 2000	Feb 2000	Mar 2000	Apr 2000	May 2000	Jun 2000	Jul 2000	Aug 2000	Sep 2000	Oct 2000	Nov 2000	Dec 2000	Nov 2001	→	May 2003
Protocol review and approval																
SERG																
Trial materials																
Site visits																
Piloting trial procedures																
Recruitment and follow-up until delivery																
SC / PI mtg																
Trial newsletter																
DSMC meeting																
Calcium survey																

APPENDIX 6: CONSORT STATEMENT REQUIREMENTS

Heading	Description	Section	Page
Title	Study identified as a randomized trial	Cover page	
Abstract (summary)	Structured abstract		1
Introduction	Goals, hypotheses	2.6, 2.6	7
Methods			
Protocol	Study population	3.3	9
	Intervention	3.2	8
	Outcomes	3.4	10
	Sample size	3.5.1	11
	Rationale and methods for analysis	3.5.2	11
	Stopping rules	8.6	19
Assignment			
	Unit of randomization	3.5.2	11
	Methods used	3.5.2.	11
	Allocation concealment and timing of assignment	3.5.2.	11
	Separating generator from executor	3.5.2.	11
Blinding	Mechanism	3.5.2-8.3	11;18
Results	Dummy tables	Appendix 2	29-31

APPENDIX 7: ENDPOINT DEFINITIONS

These definitions are based on a recent report of Canadian Hypertension Society Consensus Conference with some modifications, mainly on terminology.[43]

Study endpoint	Definition
Pre-eclampsia	Hypertension: Blood pressure greater than or equal to 140 and/or 90 mmHg occurring in two occasions at least four hours to a week apart after the 20th week of pregnancy. Diastolic Blood pressure will be measured at the 5th Korotkoff sound, which is the disappearance of the sounds. AND Proteinuria: ≥ 300 mg in 24 hours urine specimen or corresponding level of 2+ or more on dipstick.
Severe pre-eclampsia	Is defined as 160 and/or 110 mm Hg or greater on two occasions at least 4 hours apart or a single BP reading of 160 and/or 110 mm Hg or greater if it required treatment with an antihypertensive and at least 2+ proteinuria.
Early onset pre-eclampsia	Pre-eclampsia starting before 32 weeks of gestation.
Pregnancy-induced hypertension	Hypertension: Blood pressure greater than or equal to 140 and/or 90 mmHg occurring in two occasions at least four hours to a week apart after the twentieth week of pregnancy without proteinuria. Diastolic Blood pressure will be measured at the 5th Korotkoff sound, which is the disappearance of the sounds.
Eclampsia	Occurrence of seizures (convulsions) in association with pre-eclampsia. Note that the criteria for a diagnosis of pre-eclampsia may not always be apparent. Unless the woman is a known epileptic, convulsions (grand-mal type) during pregnancy should be regarded as eclampsia even if proteinuria and high blood pressure are not apparent at the time of convulsions.
Other endpoints	Preterm delivery: < 37 weeks of gestation Low birth weight: < 2500 grams

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