The effect of calcium intake on body weight in pregnant women from South Africa, Zimbabwe and Argentina participating in the Calcium and Pre-eclampsia trial.

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Thesis presented for the degree of Doctor of Philosophy in Nutrition, University of Cape Town, February 11th, 2019.

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Abstract

Introduction: The prevalence of overweight and obesity is increasing worldwide. It has been estimated that every kilogram of weight gain during adulthood represents a 3% to 6% risk increased of cardiovascular disease. There are some studies showing an inverse relationship between calcium intake and body weight. Overweight and obese women are advised to lose weight before conception, however the evidence on how to achieve this is scarce. No studies have investigated the effect of calcium supplementation on weight management before conception or during pregnancy.

Aims and objectives: The overarching purpose of this project was to provide information and enrich the body of evidence of the effect of calcium intake on body weight. The first aim was to evaluate the effect of calcium intake on body weight of fertile or pregnant women; secondly to investigate the pre-pregnancy weight status, weight gain during pregnancy and adequacy of dietary intake of pregnant women participating in the Calcium and Pre-eclampsia (CAP) trial. The third aim was to perform a systematic review of studies evaluating the effect of calcium intake on body weight.

I was part of the core research team throughout the CAP trial duration and also lead the nutritional component. The trial sample size included 540 pregnant women recruited between 2012 and 2017 in South Africa, Zimbabwe and Argentina. Women were randomized pre-pregnancy to receive 500 mg of elemental calcium or placebo until 20 weeks´ gestation, whereafter they received 1500 mg. Weight was measured pre-pregnancy and at 8, 20 and 32 weeks' gestation. Diet was assessed at 20 weeks´ gestation. Ethical approval was obtained from appropriate national and institutional ethical review bodies as applicable for each study site.

Results: There was a high proportion of women who started their pregnancy overweight or obese (73.7% in South Africa and 60.2% in Zimbabwe). Most women had an inadequate intake of micronutrients at 20 weeks pregnancy. For the most basic micronutrients like iron, calcium, folate and zinc, the percentage of women with intakes below requirements was above 90%.

Although there was no effect of calcium supplementation on body weight in the sample of the CAP trial, the calcium group had a no statistically significant smaller increase in body weight during pregnancy especially in those who were obese at the start of the trial. The systematic review shows a small but statistical effect of calcium supplementation in body weight (Mean Difference (MD) -0.33 kg, 95% CI -0.57 to -0.09); (p=0.007); 819 participants; 15 studies) and in BMI (MD -0.17, 95% CI -0.21 to -0.13); $p < 0.00001$; 695 participants; 10 studies).

Conclusion: We found a high prevalence of obesity found together with the micronutrient inadequacy which show a very poor nutritional status of women who have the possibility of getting pregnant again. This needs to be addressed so that maternal and perinatal outcomes are improved.

There is a need to implement nutritional counselling preconceptionally to these women before they fall pregnant. The results of this thesis show a no statistically significant smaller increase in body weight in women supplemented with calcium, opening a promising area of research for weight management including the study of the mechanisms involved. Before making clinical recommendations further studies are needed with higher sample size to have the power to detect clinically significant effects.

Acknowledgements

I extend sincere gratitude to the following individuals and institutions:

To supervisor, Dr Janetta Harbron and co-supervisors Ana Pilar Betran Lazaga and Jose Belizán for their unwavering support and academic guidance.

To the research team that participated in the CAP trial and to the patients who participated in the CAP trial study.

Site PIs at the CAP trial sites: Professor Justus Hofmeyr, Professor Susan Fawcus, Dr Stephen Munjanja, Dr. Rossana Chalah, Dr. Hugo Krupitzki and Dr Javier Schvartzman.

To the Cochrane Metabolic and Endocrine Disorders group.

To Luz Gibbons, Agustin Ciapponi, Maria Luisa Cafferata, Fernando Althabe, Mario Rovere, Nicole Minckas, Fabrizio Lombardo, Martin Di Marco, Juan Martin Librandi, Jose Gonzalez, Cintia White, Sabrina Molina, Iris Romero, Ignacio Gonzalez, Surya Perez.

To Alicia Carriquiry.

To Laura Lopez.

Research funding: The WHO trial A65750: "Long term calcium supplementation in women at high risk of pre-eclampsia: A randomized, placebo, controlled trial" was funded by the Department of Reproductive Health and Research of the World Health Organization in Argentina and by the Bill and Melinda Gates Foundation in South Africa and Zimbabwe.

The Argentine Fund for Horizontal Cooperation of the Ministry of Foreign Affairs funded specific on-site nutrition activities in South Africa, and WHO has committed funding the same in Zimbabwe.

Content List

List of Tables and Figures

List of abbreviations

ADM: Admission

AI: Adequate Intake

ALEA: Aleatorisation online software

BMI: Body Mass Index

CAP: Calcium and Pre-eclampsia Study

CHD: Coronary Heart Disease

CI: Confidence Interval

CMED: Cochrane Metabolic and Endocrine Disorders

CONSORT: Consolidated Standards of Reporting Trials

COVIDENCE: Systematic Review System

DRI: Dietary Reference Intake

DPV: During Pregnancy visit

EAR: Estimated Average Requirement

EER: Estimated Energy Requirement

GDT: Guideline Development Tool

GRADE: Grading of Recommendations, Assessment, Development and Evaluations

HDL: High Density Lipoproteins

HRP: Human Research Program

ICTRP: International Clinical Trials Registry Platform

ID: Identifier

IOM: Institute of Medicine

ISU: Iowa State University

LDL: Low Density Lipoproteins

LILACS: The Latin American and Caribbean Center on Health Sciences Information

LMICs: Low and Middle-income Countries

MD: Mean Difference

OR: Odds Ratio

PC-SIDE: Software for Intake Distribution Estimation

PPV: Pre Pregnancy visit

PE: Pre-eclampsia

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyse

RCT: Randomised controlled trial

RDA: Recommended Dietary Allowance

RevMan: Review Manager

RR: Risk ratio

SANHANES-1: National Health and Nutrition Examination Survey

SAMRC: South African Medical Research Council

SMD: Standadrised Mean Difference

WHO: World Health Organization

Chapter 1 - Introduction

1.1 Introduction and Motivation

Poor nutrition is one of the main contributors to burden of disease.[1,2] Nutrient intake before and during pregnancy, and weight gain during pregnancy are the main influencing factors to pregnancy outcomes and early child health.[3–7] It is well known that women who start a pregnancy in optimal nutrition status have lower risk of developing complications such as pre-eclampsia, gestational diabetes mellitus, gestational hypertension, depression, foetal macrosomia, stillbirth, preterm birth, birth by caesarean section and infant mortality.[8–13] Furthermore, high maternal body mass index (BMI) has been associated with delayed breastfeeding, post-natal weight retention and in women with gestational diabetes, a higher risk of developing chronic diseases later in life.[12]

Excessive weight gain during pregnancy and failure to get back to pre-pregnancy body weight also bear consequences later in life, as it increases the risk of obesity and cardiovascular disease.[14] Weight gain during pregnancy seems to be a better predictor of later obesity than pre-pregnancy BMI.[15,16] Interest in pre-conceptional interventions to reduce risk factors during pregnancy is growing, although their effectiveness on pregnancy outcomes is less certain.[17]

Inter-pregnancy interval is also an important moderating factor, as it may influence maternal availability of nutrients, especially in those populations with existing micronutrient deficiencies.[18]

In many low and middle-income countries (LMICs) undernutrition and overnutrition coexist in the same population.[19] Obesity rates are increasing while micronutrient deficiencies still persist, particularly in the most vulnerable groups such as women and children.[20] Inter-pregnancy interval has been shown to be an important moderating factor of nutritional status in pregnant women and pregnancy outcomes, as it may influence maternal availability of nutrients, especially in those populations with existing micronutrient deficiencies.[18] Furthermore, excessive weight gain during pregnancy and failure to return to pre-pregnancy body weight also has consequences later in life, as it increases the risk of obesity and cardiovascular disease.[14]

Interest in pre-conceptional interventions to reduce risk factors for complications during pregnancy is growing, although their effectiveness on pregnancy outcomes is less certain.[17] While many treatment options exists for obesity, with varying degree of effectiveness, weight loss in obese women during pregnancy is not recommended. Calcium intake has been receiving interest due to its role in the management of blood pressure during pregnancy and weight in adults. A systematic review of dietary intake of pregnant women carried out in 2004 indicates a low calcium intake in African and Latin American populations.[21] The study reported a median intake of 481 mg a day for Argentina and 567 mg a day for South Africa, although the number of women assessed was very few and no data were reported for Zimbabwe. It is thus likely that calcium intake of pregnant women in LMICs is

much lower than recommended daily calcium intake during pregnancy, which is around 1000 to 1300 mg a day.[22] Furthermore, for the prevention of pre-eclampsia, the WHO guidelines state that pregnant women at high risk of pre-eclampsia, including those who previously had pre-eclampsia and those from areas of low calcium intake, should take 2000 mg of calcium a day from 20 weeks´ gestation.[1] This recommendation was based on a systematic review of thirteen randomised trials which included 15730 women and showed that 2000 mg of calcium supplementation starting at 20 weeks of pregnancy resulted in the risk of pre-eclampsia being reduced by more than half when compared to a placebo (Relative Risk (RR) 0.45 95%, Confidence Interval (CI) 0.31-0.65).[23]

Davies et al. 2000 indicated that a decline in calcium intake is associated with an increase in population weight gain, even in people of normal weight.[24] A systematic review of the effect of calcium supplementation on weight management indicated a small reduction in body weight, however the included studies had a short intervention period of around 6 months and the effect during pregnancy was not assessed. [25] This evidence is questionable despite being by all randomised controlled trials because the risk of bias was not properly described. Although the individual clinical relevance of this hypothetically small weight reduction hypothetically proposed for women at childbearing age seems marginal, at a population level it could help to prevent the observed obesity global trends. [26]

The WHO Calcium and Pre-eclampsia (CAP) trial is a double blind randomised control trial that was carried out in South Africa, Zimbabwe and Argentina. The aim of the trial was to determine whether calcium supplementation before conception and during the first half of pregnancy reduces the incidence of recurrent pre-eclampsia more effectively than supplementation starting at 20 weeks, which is the current WHO recommendation. [27] In the CAP trial, non-pregnant women with a history of pre-eclampsia or eclampsia in their most recent pregnancy and with the possibility to become pregnant again were invited to participate. Eligible women were randomised to receive calcium or placebo, from admission to the trial, before pregnancy. Blinded supplementation continued pre-pregnancy and up until the 20th weeks of pregnancy if they become pregnant. After the 20th week of pregnancy, participants received calcium supplements in compliance with WHO guidelines on pregnancy supplementation.[1,28] The WHO CAP double blind randomised control trial gave an unique opportunity to assess the effect of calcium supplementation on the weight of women of fertile age and in pregnant women using data from a well-designed and monitored on-going trial. [27]

The current thesis uses data of the CAP trial to evaluate the effect of calcium on the weight of non-pregnant women and then separately in those women who became pregnant. Also, taking into account the scarcity of information related to dietary intakes in pregnant women in South Africa and Zimbabwe, a description of the dietary intake status of the CAP trial participants is justified. Lastly,

building on the flaws of the previous systematic review that was conducted in 2011[25] which showed a small effect of calcium supplementation on weight, I proposed to update the evidence by performing a good quality systematic review and meta-analysis of studies on the effect of calcium supplementation on weight in different population groups. This review was important as it incorporated new studies published after the last review in 2011, as well as population groups that were not included before, such as pregnant women.

Evaluating the effect of calcium intake on weight is essential to better understand the impact that a population-based strategy could have on pregnant women for the prevention of overweight and obesity and their consequences.

1.2 Aim and Objectives

The overarching purpose of this project was to provide information and enrich the body of evidence of the effect of calcium intake on body weight through three specific aims.

Aim 1: To evaluate the effect of calcium intake on body weight of fertile or pregnant women with a history of pre-eclampsia or eclampsia participating in the WHO Calcium and Pre-eclampsia (CAP) trial: nested study of the CAP trial RCT.

Aim 2: To investigate the pre-pregnancy weight status, weight gain during pregnancy and the adequacy of dietary intake of pregnant women in the CAP trial.

Aim 3: To perform a systematic review of studies evaluating the effect of calcium intake on body weight and summarise the evidence available.

Objectives related to aim 1 - CAP trial nested-study:

- Primary objective 1.1: To assess the effect of calcium supplementation on body weight of women in reproductive age.

- Primary objective 1.2: To assess the effect of calcium supplementation on women body weight gain during pregnancy and on the birth weight of newborns.

Secondary Objectives:

- To estimate energy intake during the second trimester of pregnancy.
- To compare the energy intake of women assigned to calcium and placebo groups. This will allow for the exploration of a potential mechanism of action for the effect of calcium supplementation on body weight.
- To estimate mean daily calcium intake during the second trimester of pregnancy.
- To explore calcium and energy intakes by age and parity.
- To assess the effect of total calcium intake (supplementation plus food) on maternal body weight during pregnancy.
- To estimate the proportion of women participating in the CAP trial during their second trimester of pregnancy with usual dietary intakes of calcium below 800 mg of calcium per day. Estimates were analysed separately for Argentina and African countries. To attain this objective, a

subsample of women enrolled in the CAP trial underwent a second dietary recall upon reaching 20 weeks of pregnancy.

- To assess the effect of calcium supplementation on maternal body weight according to dietary calcium intake levels.
- To assess the effect of calcium supplementation on weight gain during pregnancy according to Body Mass Index (BMI) groups at the start of the pregnancy.

Objectives related to aim 2 – dietary intake adequacy during the second trimester - CAP Trial.

- To assess and describe preconception weight according to WHO BMI classification.
- To assess and describe weight gain during pregnancy.
- To assess and describe the dietary intake of energy, macronutrients and micronutrients.
- To compare actual energy intake with current dietary standards for pregnant women e.g. the estimated energy requirements (EERs).
- To compare the actual intakes of carbohydrates, proteins, vitamins and minerals with current dietary standards for pregnant women e.g. estimated average requirements (EARs) and adequate intakes (AIs).
- To calculate and describe the macronutrient distribution as a percentage of total energy intake;
- To compare the macronutrient distribution as a percentage of total energy intake with the recommended macronutrient distribution ranges.

Objectives related to aim 3 - Systematic review and meta-analysis:

Primary objective 3.1: To evaluate the effect of calcium supplementation on body weight in individuals of different ages.

Systematic reviews are the best approach available to describe the evidence of health interventions. I proposed the title “Calcium supplementation for weight reduction” to the Cochrane Metabolic and Endocrine Disorders Group and it was consequently accepted and registered in September 2015 (Cochrane Registered Title number: T150806)

CAP trial nested-study

Hypothesis

- **Hypothesis 1:** Daily calcium supplementation with 500 mg of elemental calcium reduces the body weight of women of reproductive age.

- The hypothesis was only postulated for Aim 1 and on the effect of calcium on the body weight of non-pregnant women, as it was not expected that pregnant women would lose weight. For pregnant women the objective was to evaluate safety by comparing maternal body weight gain and birth weight of the baby between calcium and placebo groups to explore if there was any difference.

1.3 Contribution of candidate

I conceptualized and coordinated the nutritional component of the CAP trial in the three countries where it was performed (South Africa, Zimbabwe and Argentina). Within this role I conceptualized the protocol for my PhD thesis. I designed the 24-hour questionnaires, as well as trained and supervised the personnel for data collection and data management in the three countries of interest. I was also in charge of reviewing all data and managing queries and data quality. For this I travelled to the sites in each country annually. Finally, I performed the data analysis and interpretation. I was a member of the Steering Committee of the CAP trial which allowed me to use the data immediately after the trial was closed (See ANNEXURE A). I am also part of the Argentinean Cochrane Centre that provided the support to perform the systematic reviews. I proposed and wrote all protocols analyzed in this thesis and performed the analysis with the support of my supervisors. I had written agreement with the WHO study coordinator and co-supervisor Dr. Ana Pilar Betrán Lazaga, to implement this project proposal.

1.4 Definition of terms

Usual dietary calcium intake: Calcium intake distribution adjusted by the within person variability calculated from 2 non-consecutive 24 hour recalls from each individual of the subsample.[29]

Pre-eclampsia: pregnancy-specific syndrome was diagnosed when a pregnant woman had increased blood pressure and proteinuria after 20 weeks of pregnancy.[30] Gestational hypertension (diastolic BP >90 mmHg on two occasions 4 hours apart, or >110mmHg once, and/or systolic BP >140 mmHg on two occasions 4 hours apart, or >160mmHg once, after 20 weeks' gestation) and gestational proteinuria (2+ or more on urine dipstick, or >300mg/24 hours, or >500mg/L or urinary protein/creatinine ratio >0.034g/mmol, after 20 weeks' gestation.

Body Mass Index: index of weight-for-height defined as the weight in kilograms divided by the square of the height in metres (kg/m^2).[32]

Birth weight: body weight of the baby at birth as measured by each clinic.

1.5 Outline of thesis

Chapter Two of this thesis contains a review of literature covering the current state of overweight and obesity and their effects on health, the existent evidence of the relationship between calcium intake and body weight, the global status of calcium intake and finally a description of the use of the 24-hour recall methodology. Chapter Three focuses on the methods used throughout the sub-studies. Chapter Four includes the article "*Effect of calcium supplementation on body weight of women in reproductive age*" and further analysis to respond to aim 1: *To evaluate the effect of calcium intake on body weight of fertile or pregnant women with a history of pre-eclampsia or eclampsia participating in the WHO Calcium and Pre-eclampsia (CAP) trial*. Chapter Five includes the article "*Pre-pregnancy weight status, weight gain during pregnancy and adequacy of dietary intake of pregnant women in the CAP trial*" and further analysis to respond to aim 2: *To investigate the pre-pregnancy weight status, weight gain during pregnancy and the adequacy of dietary intake of pregnant women in the CAP trial*. Chapter Six includes a systematic review evaluating the effect of calcium intake on body weight and summarising the evidence available. The final chapter summarises these findings, discusses the potential implications, and suggests future lines of study related to the topic.

Due to the structure followed for this thesis it is inevitable that there will be overlap in content. This is particularly conspicuous in the methodology chapter and the chapters that contains articles, where the methods are more concisely described as per requirements for a journal publication.

Chapter 2 - Literature Review

2.1 Introduction

In this literature review I describe the overweight and obesity trends globally. Thereafter I focused on the literature available for South Africa, Zimbabwe and Argentina as women from these countries where included in the CAP trial. This review also provides a description of the literature available of the dietary intake of women in these countries. Furthermore, the review gives a broad overview of the causes, consequences and treatment of obesity especially during pregnancy. Finally this chapter provides a more detailed description of the role of calcium in body weight, as it was the background information required to formulate the aims of this thesis.

2.2 Overweight and Obesity

The prevalence of overweight and obesity is increasing worldwide and in different age groups.[33–35] According to the World Health Organization (WHO), the prevalence of obesity doubled between 1980 and 2008 and it is increasing more rapidly in lower-middle income countries.[36] There is also evidence that obesity is increasing more than overweight rates. In the adult population, obesity is more prevalent in women than in men. Between 23% and 29% of women are obese in the European, the Eastern Mediterranean and American Regions.[37] Obesity is also a problem in adolescents. A systematic review of 23 studies reporting national data shows that in 21 countries the prevalence of adolescent overweight and obesity was higher than 20%.[38] The prevalence of overweight and obesity in children has shown a remarkable increase over the last decades, representing a public health challenge as they tend to track into adult life. [39,40]

The South African National Health and Nutrition Examination Survey (SANHANES-1) performed in 2012 reports a prevalence of overweight and obesity of 24.8% and 39.2% for females and 20.1% and 10.6% for males, respectively.[41] More recently, the 2016 South African Demographic and Health Survey shows a slightly higher prevalence of overweight and obesity of 68% in women where one in five women was considered severely obese.[42] On the other hand, the prevalence of overweight or obesity in men was 31%, less than half of that of women. The overweight rate in children was 13% percent according to their weight-for-height measurements.

The Argentina National Health and Nutrition Survey performed in 2004 shows a prevalence of 24,9% overweight and 19,4% obesity in females, while no data were collected for men. The National Health and Nutrition Survey has not been repeated since then. However, in 2013 a National

Non-Communicable Disease Survey of Risk Factors was carried out.[43] While this survey included a general population aged 18 years or older living in cities with more than 5.000 inhabitants and has probabilistic sampling; weight was reported rather than measured. There were 32.365 people interviewed on the telephone with a response rate of 70.7%. The overweight prevalence was 43.3% for women and 31.3% for men and the prevalence of obesity was 22.9% for women and 18.8% for men. It seems that the obesity and overweight figures are increasing in Argentina, however it should be borne in mind that the National Health and Nutrition Survey used direct measurements of weight and height while the National Non-Communicable Disease Survey of Risk Factors used self-reported measurements. Recently a review of 21 studies and including 18.749 women showed some evidence that in women of reproductive age, self-reported weight and height differs slightly from direct measures.[44] The review reported an underestimation of weight by -0.94 kg (95% CI -1.17 to -0.71 kg) in the overall sample and an overestimation of height by 0.36 cm (95% CI 0.20-0.51), values that are not considered acceptable for clinical purposes. Considering however the extremely high prevalence of overweight and obesity, the data can be used to give an overall trend of the situation. Even more, taking into account the differences between self-reported weight and height shown in the review, where women under report weight and over report height, the prevalence's shown in the National Non-Communicable Disease Survey of Risk Factors for women in Argentina could be even higher.

Data from Zimbabwe comes from the Demographic Health Survey funded by the United States Agency for International Development and shows that the prevalence of overweight and obesity for women has increased from 23% in 1994 (17.3% overweight and 5.7% obesity) to 35% in 2015.[45–47] On the other hand the prevalence of overweight and obesity for men has increased in much lower rates, from 9% in 2010 to 12% in 2015, although for men there is no earlier data. For both women and men, the overweight and obesity prevalence in Zimbabweans increased with age, wealth and education. Also, those living in urban areas had a higher overweight or obesity prevalence than those living in rural areas, 46% and 28% respectively for women and 21% and 7% respectively for men.[45,48]

According to the Non-Communicable Disease Risk Factor Collaboration that holds data on obesity prevalence of 200 countries, South African women are ranked 23rd for obesity and 16th for severe obesity, whereas South African men are ranked 112th for obesity and 65th for severe obesity. [49] On the other hand women in Argentina are ranked 56th for obesity and 54th for severe obesity, whereas for men, Argentina is ranked 28th for obesity and 29th for severe obesity. Zimbabwe is ranked 88th for women obesity and 81^{rst} for severe obesity, whereas for men, Zimbabwe is ranked 158th for both obesity and severe obesity. [49] Overweight and obesity are more prevalent in women than in men in South Africa and Zimbabwe and the opposite in Argentina. Also some literature reports that obesity has more deleterious effect in women than in men.[50] A report evaluating the impact of obesity states

that an obese man is 5 times more likely to develop type 2 diabetes and 2.5 times more likely to develop high blood pressure than a healthy weight man, whereas compared to a healthy weight woman, an obese woman is thirteen times more likely to develop type 2 diabetes four times more likely to develop high blood pressure.[51]

Obesity represents a major and challenging public health problem as it increases the risk of developing high blood pressure, insulin resistance, heart disease, diabetes, osteoarthritis, sleep apnea and in pregnant women increases the risk of adverse pregnancy outcomes such as macrosomia, gestational hypertension and gestational diabetes.[9–11,36,46] It has been estimated that every kilogram of weight gain during adulthood increases the risk of cardiovascular disease by 3.1 to 5.7% and for pregnant women every 5 to 7 kg/m^2 increase in BMI doubles the risk of pre-eclampsia. [8,52] A systematic review of the economic burden of obesity worldwide estimated that those who are obese have 30% greater medical costs compared to normal weight individuals. [53]

Although the information on overweight and obesity rates specifically during pregnancy is scarce in South Africa, Zimbabwe and Argentina, there is evidence suggesting that a great number of women are reaching pregnancy with excess weight. A recent study in Johannesburg showed that 55% of the 538 participants presented excessive weight gain during pregnancy.[54] In Argentina the National Nutritional Survey of 2005 shows that 19.7% of pregnant women were overweight and 24.4 were obese.[55]

2.3 Causes and treatment of obesity

Obesity is the excessive accumulation of fat mass in the body, however obesity aetiology is complex as it is influenced by environmental and social factors, genetics, epigenetics as well as prenatal and early life influences.[56] Environmental and social factors are very important as they impact on diet and physical. This is evident by the rapid increase of obesity that cannot be due to genetics only. This is also shown by the nutritional transition with the rapid increase of obesity in urban areas where people have more access to energy-dense foods and fewer opportunities to maintain physical activity.[19,56] Although obesity is the accumulation of fat mass in the body, there are many physiological mechanisms put in place that impair easy weight reduction by merely restricting energy intake.[57] Consequently, there is no single treatment for obesity and a broad approach taking into account many risk factors should be implemented. Pregnant women are advised to have a healthy diet and to keep physically active during pregnancy, however it is recognised that most of the research on healthy

eating and exercise comes from high income countries, and less evidence is available on low and middle income countries.[4]

Despite being recognised by WHO as a disease in 1948, it was not until the year 2000 that obesity started being accepted as one of the main health risk factors.[58] Just recently countries started to recognise obesity as a disease, which is crucial for the broad approach it requires.[59]

2.4 Weight gain during pregnancy

In order to improve pregnancy outcomes, overweight and obese women are advised to lose weight before conception, however there is lack of evidence on how to manage their weight during pregnancy. [60,61] as dieting during pregnancy may increase the risk of ketosis that is harmful for the foetus, it is recommended that women not lose weight but rather gain weight at a reduced rate. The current weight gain recommendations for pregnancy specify that women classified as normal weight are advised to gain from 11.5 to 16 kg; those classified as overweight are advised to gain from 7 to 11.5 kg and those classified as obese are advised to gain from 5 to 9 kg.[62] The WHO Guidelines also report that women receiving counselling on diet and/or exercise are less likely to experience excess weight gain during pregnancy.

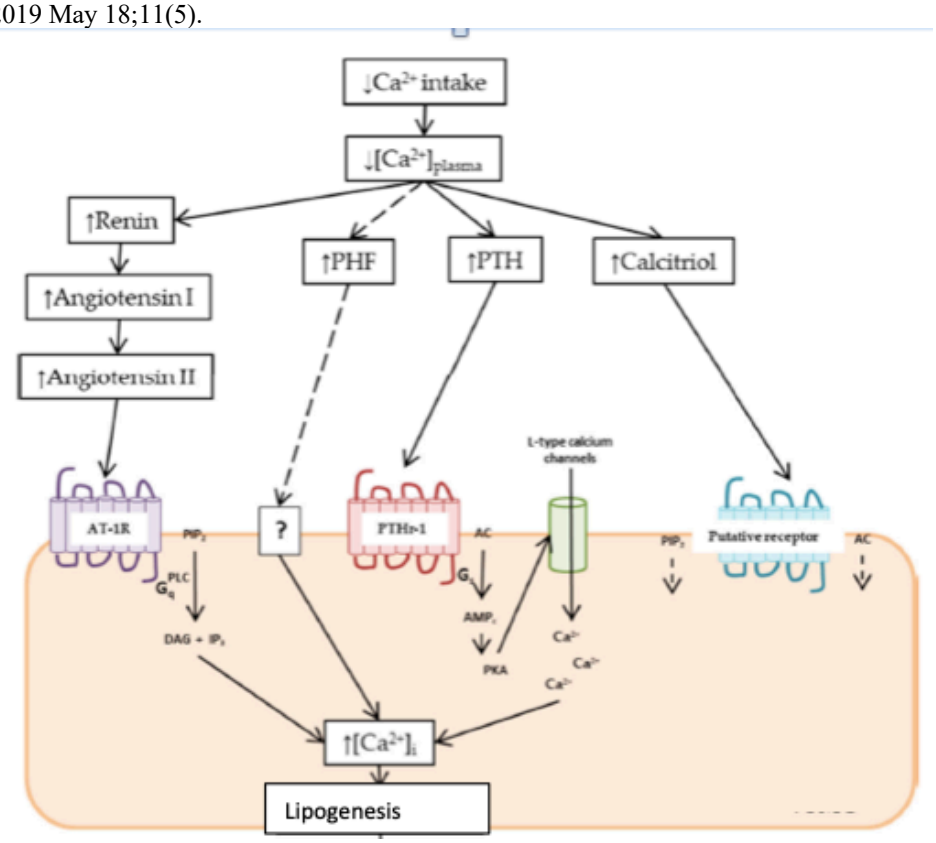
2.5 Calcium intake and body weight, postulated mechanisms

There are some studies showing an inverse relationship between calcium intake and body weight. [63] A systematic review in 2011, that included seven studies with 794 overweight or obese participants, showed that calcium supplementation compared to placebo produced a mean body weight-loss of 0.74 kg (CI -1.00 to -0.48).[25] Six of the included studies had a duration of six months with a dose of 1000 mg of elemental calcium a day and one study had a duration of 24 months with a dose of 1500 mg of elemental calcium a day.

Three mechanisms in which calcium could affect body weight have been postulated. The first one is linked to the regulation of the parathyroid hormone that is required to maintain specific calcium concentrations in extracellular fluids (See Figure 2.1).[64,65] Serum calcium is tightly regulated and small reductions stimulate parathyroid hormone and 1-25 vitamin D secretion to increase calcium resorption from the bones, kidneys as well as absorption in the intestine. However, higher levels of

parathyroid hormone and 1-25 vitamin D also stimulate calcium influx into different cell types, including the adipocyte. [63]In the adipocyte, this increase of intracellular calcium stimulates fatty acid synthase and consequent lipogenesis, and could thus lead to weight gain.[64] Low calcium diets have also been linked to insulin resistance and high blood pressure through similar collateral effects. [66,67] Likewise, higher levels of parathyroid hormone and 1-25 vitamin D increase intracellular calcium uptake into the vascular smooth muscle cell and consequently increase muscle reactivity and peripheral vascular resistance which contribute to a higher blood pressure. [68,69] In summary, hormones like parathyroid hormone, that are released to compensate for low serum calcium levels could increase of blood pressure and body weight as collateral effects.[66]

Figure 2-1: Cellular effects of a low calcium diet. From Mechanisms Involved in the Relationship between Low Calcium Intake and High Blood Pressure. Villa-Etchegoyen C, Lombarte M, Matamoros N, Belizán JM, Cormick G. Nutrients. 2019 May 18;11(5).



A second postulated mechanism is associated with the reduction of fatty acid absorption in the intestine. Higher calcium intakes could bind to bile acids or fatty acids impairing their absorption and decreasing available energy. [70,71] One randomised control trial found that individuals assigned to cocoa butter fortified with 900 mg of calcium daily for one week had increased excretion of fatty acids whereby total absorbable energy decreased as compared to those assigned to non-fortified cocoa. [72]

Finally a third postulated mechanism is related to appetite regulation, although this has not been much studied. One randomised controlled trial (RCT) found an increase in postprandial gastrointestinal peptides such as glucagon-like peptide-1 (GLP) and glucose-dependent insulinotropic peptide in healthy individuals after receiving calcium supplementation.[73] An increase in gastrointestinal peptides has been associated with reduced appetite. [74,75]

2.6 Other effects of calcium supplementation on health

Besides the postulated effect of calcium supplementation on weight, other evidence indicates that calcium may also reduce blood pressure, pre-eclampsia (PE) and hypercholesterolemia.

A systematic review that included 13 RCTs and 15.730 women estimated that calcium supplementation compared to placebo reduced the risk of PE by 55% (RR 0.45, 95% CI 0.31 to 0.65).[23] There is also evidence that calcium supplementation also reduces systolic blood pressure in adults by 1.14 mmHg with doses of calcium 1000 to 1500 mg a day and by 2.79 mmHg with doses of calcium equal to or over 1500 mg a day.[76] In this review it was found that calcium supplementation had the greatest effect in young adults of less than 35 years as their systolic blood pressure was reduced by 2.11 mmHg. Reduction of blood pressure is one of the mechanisms by which it is believed that calcium reduces the incidence of pre-eclampsia.

Furthermore, a systematic review on calcium supplementation and lipid metabolism reported that calcium supplementation reduced low density lipoproteins (LDL) cholesterol [-0.12 mmol/L (95% CI, -0.22 to -0.02)] and increased high density lipoproteins (HDL) cholesterol [0.05 mmol/L (95% CI, 0.00 to 0.10).[77]

There is also evidence that calcium supplementation during pregnancy has a modelling effect on the offspring. Calcium supplementation with 1.5 grams after 20 weeks´ gestation showed a 27% reduction on the risk of children of supplemented mothers when they were 12 years of age in developing dental caries.[78] Furthermore, a systematic review shows that children whose mothers received calcium supplementation had a reduction of -1.92 mm Hg (95% CI -3.14 to -0.71) in systolic blood pressure at age 1 to 9 years.[79]

Metabolic syndrome is diagnosed by the presence of multiple risk factors, such as hypertriglyceridemia, low HDL cholesterol, hypertension, essential hypertension, abnormal fasting glucose levels, and abdominal or visceral obesity.[80] Calcium may have implications in three of these

risk factors -high blood pressure, cholesterol and excess body weight- which lends support to the need for further efforts to implement strategies to improve calcium intakes.

2.7 Calcium supplementation adverse events

Calcium supplements and myocardial infarction

Calcium supplements are commonly used to prevent fracture in postmenopausal women.[81,82] In a narrative review by Bolland et. al. the use of calcium supplements in postmenopausal women was discouraged based on a previous secondary analysis of randomised controlled trials (RCTs) showing a potential increase of adverse events such cardiovascular health disease as atherosclerotic vascular disease in women from New Zealand receiving calcium supplements.[83] Despite having several methodological limitations, such as self-reported events, the dissemination of the findings had a high public health impact producing a 66% decrease in monthly prescriptions of calcium supplements in New Zealand.[84]

Subsequently, Lewis et al. published a systematic review and meta-analysis that included 18 RCTs involving 63.564 participants, all reporting cardiovascular events clinically verified by hospital records or death certificate.[85–87] Seventeen trials contributed all-cause mortality data with pooled RR of 0.96 (95% CI, 0.91-1.02; p = 0.18). Five trials contributed CHD events with pooled relative RR of 1.02 (95% confidence interval [CI], 0.96-1.09; p = 0.51). The RR for myocardial infarction was 1.08 (95% CI, 0.92-1.26; p = 0.32). The authors concluded that current evidence does not support the hypothesis that calcium supplementation with or without vitamin D increases coronary heart disease or all-cause mortality risk in elderly women.

Calcium supplements and iron intake

There has been a concern related to the effects of calcium supplements on iron absorption. There are some studies showing that calcium supplements inhibit iron absorption by 28% to 55%.[88] However, more recent evidence studying the long-term consequences of taking calcium supplements showed no effect on iron status showed.[89–94]

Calcium supplements and renal stones

The link between renal stones and calcium supplement intake is not of concern if calcium intake is maintained within the recommended levels as dietary calcium restriction is not recommended for stone formers with nephrolithiasis on the contrary diets with more than a gram of calcium a day could

be protective against stone formers.[95] It is believed that the calcium that remains in the intestine would impede the absorption of products that could produce risk of renal lithiasis such as oxalates.[96] For this reason there are studies that postulate that the intake of calcium supplements during meals would decrease the absorption of oxalates and thus decrease the formation of stones.

2.8 Calcium intake review

Calcium is the most abundant mineral in the human body. It is available in estimated quantities of 1.2 kg. Ninety-nine per cent of calcium is found as calcium hydroxyapatite in the skeletal system and is essential for the creation, rigidity and maintenance of bones.[97] The remaining one per cent is distributed between the intra- and extracellular fluids where it is involved in the majority of metabolic processes as well as in muscle contraction, nervous system transmission, enzymatic activation, and hormonal function.[66] Calcium serum levels are regulated by the parathyroid hormone, vitamin D, and calcitonin. All of these control calcium bowel absorption, its bone resorption and its renal excretion. [98]

Calcium requirements are high during all stages of life. Dietary recommendations for individuals over 19 years of age vary from 1000 mg to 1300 mg, depending on the reference guidelines.[99,100] Requirements increase in specific periods of life especially during pregnancy.[66] There is no consensus regarding the recommended intake during pregnancy. While most guidelines acknowledge the increased demand of calcium during pregnancy, some guidelines increase recommendations during pregnancy up to 1300 mg a day to achieve a positive balance while others state that metabolic adaptations during pregnancy compensate the required calcium demand. [101–105]

In most low- and middle-income countries, daily calcium intake is well below recommendations however, low intakes are also observed in certain age groups, such as adolescents, in high-income countries.[21,106–108] Whereas calcium intake seems to be below 600 mg a day in low- and middle-income countries, reports from high-income countries show that the intake is above 900 mg a day depending on age groups.[97,109] A review of studies reporting dietary intakes of pregnant women from low- and middle-income countries shows consistently low calcium intakes across Asian, African and Latin American countries. [21,108]

2.9 Sources of calcium

Calcium intake is usually associated with the intake of dairy products such as milk, yoghurt and cheese, which are rich sources of calcium. However not all population have the same food patterns and dietary intakes. Whereas dairy products represent around 14% of total dietary energy intake in developed countries, in developing counties dairy products represent only around 4% of total energy intake.[110] Some reports show that Asian countries have a higher proportion of total calcium intake from non-animal foods such as vegetables, legumes and grains, though they also have much lower calcium intake overall.[110] In the United states 72% of calcium comes from dairy products and in China only around 7% of total calcium intake comes from dairy products while 30% comes from vegetables and 17% from legumes [81,111]

Calcium supplements can also provide a great proportion of calcium requirements. Some calcium supplements that are available over the counter have upto 1000 mg of calcium per pill which represents most of the nutritional recommendations for an adult. However, the use of supplements also varies between countries. In the United States and Canada around 40 percent of the adult population reported taking calcium supplements in the last month and 70 % of older women. Supplements in these countries can increase the average calcium intakes in about 10%.[81] On the other hand in Argentina very few women reported taking calcium supplements, even during pregnancy.[55,112] Due to new technology fortified foods such as cereals and juices can also become important sources.

2.10 Dietary intake requirements during pregnancy

Dietary reference values are established to account for the needs of growth, development, functioning and maintenance of health of healthy people and as references to plan and assess diets.[82] The Estimated Average Requirements (EAR) are established for healthy individuals by sex, age and lifecycle stage. Requirements are estimated to account for the needs of half of the population they are aim at. Afterwards the Recommended Daily Allowances (RDA) are derived from the EARs and are set to account for the needs of most of the individuals in the age and life stage specific group, more specifically 97 to 98 percent of the group.[113,114] The RDAs are calculated as the EARs plus two standard deviation. Whereas the RDAs are used to guide the diet of individuals the EARs are used to evaluate the diet of populations. In the case of energy, requirements are calculated using the Estimated Energy Requirement (EER) that is the average dietary energy intake predicted to maintain energy balance in a healthy adult by age, gender, weight, height and level of physical activity. Recommended dietary intakes for pregnant women vary according to different institutions.[4,82,102–106,115,116] In South

Africa and Argentina adequacy of dietary intake is usually measured using the United States dietary reference intakes. [22,41,55,117]

Energy protein and nutrient intakes requirements and recommendations for pregnancy are shown in Table 2.1. During pregnancy, these requirements are increased to account for the extra needs of foetus and maternal tissue deposition to support pregnancy.[118] For energy, it is estimated that a woman need a total of 80,000 kcal (334720 kiloJoules (kJ)) in a full-term pregnancy, however no extra energy is required during the first trimester.[118] As such pregnant women are only advised to increase energy intakes only during the second and third trimester.

Table 2.1: Summary of Energy, protein and nutrient Dietary Reference Intakes for pregnant women according to the Institute of Medicine and National research Council of the National Academies

		EAR	RDA
Energy 2nd trimester	kjoules/day	EER*+ 1400	NA
Energy 3rd trimester	kjoules/day	EER*+ 1400	NA
Protein	g/day	50	71
Calcium	mg/d	800	1000
Iron	mg/d	22	27
Folate	µg/d	520	600
Vitamin B12	µg/d	2.2	2.6
Vitamin A	µg/d	550	770
Vitamin D	IU	400	600

* Estimated Energy Requirement (kcal/day) EER = 354 - (6.91 × age [years]) + PA × [(9.36 × weight [kg]) + (726 x height [meters])] from NAP Weight Gain During pregnancy
PA= Physical Activity
EAR= Estimated Average Requirement
RDA= Recommended Daily Allowances

The upper limit for calcium intake, the level above which there is risk of adverse events, is 1000 mg a day for 0 to 6 months,1500 mg a day for 6 to 12 months, 2500 mg a day for 1 to 8 years, 3000 mg a day for 9 to 18 years, 2500 mg a day for 19 to 51 years, and 2000 mg a day for older than 51 years. For pregnant women upper limits are 3000 mg a day for those aged 14 to 18 years and 1500 mg a day for those that are older. Despite these recommended levels, the WHO guidelines for the prevention of pre-eclampsia and antenatal care for a positive pregnancy recommend calcium supplementation dose for pregnant women from areas of low calcium intake is 1500 mg a day as the evidence shows that this reduces the risk of PE.[1,81]

2.11 Nutritional status and dietary intake of pregnant women from South Africa, Zimbabwe and Argentina

There are very few published studies related to dietary intake of pregnant women in South Africa and Zimbabwe. A PUBMED search retrieved only one can be retrieved from year 2000 onwards. [119] This was confirmed by a systematic review on dietary intake in African countries that included only one article from South Africa and none from Zimbabwe. [120] The article included in the systematic review shows the nutritional status of pregnant women in one community of Western Cape Province in South Africa revealing that more than 50% of all pregnant women in that community were below the Estimated Average Requirement (EAR) for vitamins A, D, E, and C, thiamine, riboflavin, vitamin B6, folate, calcium, magnesium, iron, and zinc. Calcium intake in this group was 362 mg a day (SD ±165). The rest of the information comes from Limpopo Province. A study in rural areas of Limpopo Province shows a high prevalence of iron, vitamin B12 and folate deficiency in 262 pregnant women 50.9%, 16.4% and 10.3% respectively. [121] A smaller study also in rural areas of Limpopo Province shows deficiencies particularly of calcium, iron, zinc, niacin, folate, and vitamins A, C, E, and B6 in a sample of 46 women under 40 years of age during their second trimester of pregnancy. [122] Information from adolescent pregnant women population from Polokwane Municipality in Limpopo Province shows high inadequate intakes of iron (98%), folate (96%) and vitamin 12 (56%).[123,124] The results from another article were excluded here as the sample only included pregnant women with high alcohol intakes and therefore not representative of the general population. [125]

In Argentina the National Nutritional Survey of 2005 shows that most women had insufficient intakes of energy (64.3%) calcium (88.5%) and iron (59.3%). Very few women reported taking iron (24.4%) or folic acid (21.2%) supplements and only 1% took calcium supplements.

In conclusion, there are not many studies on diet intake in South Africa, none in Zimbabwe, and one nationally representative study in Argentina that has not been repeated in the last 10 years. The current studies show poor nutrient intake, although there is a need for more nationally representative information including biochemical assessment.

2.12 Interventions to address inadequate nutrient intakes during pregnancy

There are three broad approaches to improve dietary intake, one is behavioural intervention that although ideal, it relies on personal habits and ability; the second one is supplementation that targets individuals and the third one is fortification that aim to improving dietary intakes of the whole

population. Recommendations to improve dietary calcium intake increasing the consumption of healthy foods and/or take supplements, have been around for many years, however, these recommendations show limited impact in low and middle-income countries (LMICs). Unfortunately, many women are contacted too late in pregnancy, find it difficult to follow the advice to improve their diet due to economical constraints, find it difficult to take supplements daily, or live in areas without access to supplements.[23] Implementing this recommendation relies on healthcare workers contacting pregnant women.

Micronutrient supplementation

Interventions, such as calcium supplementation or food fortification, have been used for many years as strategies to increase calcium intake.

The World Health Organization published in 2016 recommendations for a positive pregnancy that included micronutrients supplementation for the improvement of maternal and infant outcomes.[126] The guidelines recommend supplementation with iron and folic acid to all pregnant women, and with calcium and vitamin A to women in certain areas that have a high prevalence of deficiency.[126] In populations where calcium intake is low, the WHO recommends supplementation with 1.5–2.0 g elemental calcium/day from 20 weeks´ gestation until the end of pregnancy for the prevention of pre-eclampsia.

However, the applicability of this recommendations is of concern as supplements are frequently consumed in high-income countries; however reports show that this is an uncommon practice in low- and middle-income countries.[97] Due to the lack of timely access to health care systems, difficulties in supplement distribution, and patient non-adherence, women in LMIC's struggle to consume the recommended supplements. In our recent research trial of calcium supplementation commencing pre-conceptionally and continuing up to 20 weeks´ gestation it was shown that around only 50% of women had a good compliance (>= 80% of tablets taken) with the supplementation.[127] This indicates that even in the context of a research trial it is very difficult to achieve a good calcium intake and that strategies other than supplementation, such as fortification should be develop.

Micronutrient fortification

Fortification has been used in high-income countries for more than 80 years, yet despite this vast experience; food fortification products are not readily accessible to low socioeconomic groups.[128]

Experiences in food fortification as public health measures in order to reach a whole population exist with diverse results as not all have been properly evaluated. Mandatory food fortification has

contributed to health improvement by lowering the incidence of goitre, beriberi and pellagra. Currently more than 130 countries have mandatory fortification of salt, and around 85 have mandatory fortification of wheat flour. The WHO evaluation of food fortification nutrition interventions recommends as food fortification vehicles the use of maize flour fortified with iron and folic acid; salt fortified with iodine and iron containing powders for children in areas where prevalence of anaemia is 20% or higher.[129–135] There are successful experiences improving zinc, vitamin A, folic acid, vitamin D and calcium deficiencies at population level fortifying other staple foods. Experiences from high-income countries also show positive results. A Danish study found that calcium fortification of flour reduced the number of adults with low calcium intakes from 22% to 6 %.[136] In the UK fortification of white flour with calcium has been mandatory since 1943 and calcium fortified cereals and bread are the major contributors to calcium intake in children. [128,137] Studies looking at consumption of ready to use cereals fortified with calcium found that they help to reach adequacy of calcium intake in populations from France, Ireland, Spain and Northern Ireland. [138–140] It is also acknowledged the restoration of micronutrients naturally present in foods that are removed during industrialization processes, such as vitamin B complex in maize flour.[128] There are also experiences in drinking water fortification that include fortification of public water supplies with fluor to prevent dental cavities that have been implemented for more than 50 years in more than 25 countries, however as these are public health policies that were already established, most evidence comes from observational studies.[141,142] There are some experiences in Asia with iodized water, however as iodine has limited stability it is not always cost-effective.[128] Iron and ascorbic acid water fortification to prevent iron deficiency anaemia have been explored and although successful they are still under investigation. [143,144]

Fortification interventions need to be further evaluated and new fortification vehicles should be explored. Some of the mandatory fortification is on foods such as salt and staple foods like flour as the majority of the population eats them. Exploring fortification vehicles that are part of a healthy diet should be part of the research agenda in los and middle income countries. Stakeholders may wish to consider culturally appropriate fortification vehicles that are part of a healthy diet. There is a need to investigate on the technical feasibility of fortification vehicles culturally acceptable and then to develop and test strategies to provide the fortified food in a diversity of existing or novel distribution systems.

There is a need to explore other minerals and such as calcium that can be added to water in order to increase its nutritional values. Water is part of a healthy diet and water intake should be reinforced, so adding minerals to public drinking water that is of free access could improve some mineral intake.

Water with high calcium content is present in some mineral waters and also in sources of hard water in some populations. More recently the water retailed industry has through marketing increased the sales of different type of water. In the United States although calcium content of piped water is very low, there are several brands of bottle water with calcium content above 200 mg/l and upto 400 mg/l.[145] Increasing calcium content of water, could represent an important source of calcium to improve diet quality. Increasing the calcium content of water could become an important source of calcium that improves diet quality. Water enriched with calcium could improve calcium intake not only through direct consumption as drinking water, but also through increasing the calcium content of foods cooked in this water. This would include boiled foods, juices, and soups, among others. Calcium in water is present in the ionic form, with a high bioavailability similar to milk.[146]A review shows that the pooled data from 4 studies indicates a mean absorbability ratio for calcium in water and in milk of 1.084 ± 0.043.[147]

Calcium content of water can vary negligent to values similar to some dairy products. Data from Canada and the US show that tap water have between 8 to 135 mg of calcium per liter.[148] A study in Spain shows calcium values from 0.5 to more than 337 mg/l of water. However higher values such as 523 mg/l have been reported in Algeria.[149]

It has been estimated that in LMICs, urban areas have a high coverage to improved drinking water (92%) and 70% of the urban population has access to piped water within the household. In contrast, in rural areas, only 25% of the population has access to piped water.[150] The implementation of water fortification would need to be adapted to the situation of drinking water access of the population of interest.

Dietary reference values are established to account for the needs of growth, development, functioning and maintain health.[102] For each nutrient, requirements are calculated based on a selected health outcome, in the case of calcium, requirements are based to maintain bone health.

Other effects of calcium on health were not taken into account to establish the recommendations. In 1997 the Institute of Medicine (IOM) considered there was not enough evidence at the time to set recommendations and published adequate intakes for calcium for all life stage groups.[99,106,151] Calcium and Vitamin D recommendations were reassessed in 2010 for the US and Canadian population.[22] The report mentions that recommendations were based on the calcium content of human breast milk for infants, balance studies for ages 1–50 years, and observational and clinical trial for those older than 50 years. However this time the evidence it was also concluded that there was still not enough evidence to include other outcomes besides bone health.[152] However, since then almost

ten years have passed and more evidence on the role of calcium has emerged and thus it could be a time to update the recommendations.

In South Africa fortification of certain types of maize meal with vitamin A, thiamine, riboflavin, niacin, pyridoxine, folic acid, iron and zinc and wheat flour (excluding crushed wheat, pearled wheat, semolina, self-raising flour and flour with an ash content <0.60% db) is mandatory following the promulgation of R504 Regulations since 2003.[153] In Zimbabwe a fortification strategy was launched in 2016 for mandatory fortification of sugar with vitamin A; cooking oil with vitamin A and D; and wheat flour with vitamin A, thiamine, riboflavin, niacin, pyridoxine, vitamin B12, folic acid, iron and zinc.[154] Argentina has mandatory fortification of wheat flour with iron, folic acid, thiamine, riboflavin and niacin since 2001. The three countries have mandatory salt fortification with iodine South Africa since 1994, Zimbabwe since 1993, and Argentina since 1967. [153–155]

2.13 Dietary methodology: assessment of dietary intake during pregnancy

Different tools such as food frequency questionnaires, dietary records and 24-hour recalls, can be used to measure dietary intake. Each method has it advantages and disadvantages. Briefly, food frequency questionnaires require that the interviewed person recalls an average of all food and drinks taken in during a period of time that usually last weeks or months. They are expensive to prepare but economical to run and they require that the interview person performs several calculations. Diet records requires that the interviewed person writes all the foods and drinks just after they are consumed, so the participants need to be literate and highly motivated as the interviewed person can modified their diet so as to simplify the task. Lastly 24-hour recalls required that the interviewed person recalls all the foods and drink consumed in the previous day and as this is the methodology used in the thesis it is described in more detail below.

24-hour recall methodology

The 24-hour recall is a subjective dietary assessment tool and as any subjective method is prone to recall bias derived from the participant's experiences and perceptions.[156] However the advantages of this type of methods are that they have lower cost, are easier to use, can be adapted to different scenarios and are feasible to be used in large studies as compared to objective methods. It has been suggested that 24-hour recalls is subject to less recall bias than other subjective dietary assessment methods, such as diet histories or food frequency checklists.[157] Quantified food frequency questionnaires usually require use of generic memory and higher numeracy skills in the population interviewed to quantify average food intakes over a period of time.[158] A major advantage of the

24-recall method is that high literacy of the respondent is not required and that inter-observer differences are minimised.[156]

Within-person variance of the energy and nutrient intake

A single day, 24-hour dietary recall is a reliable methodology to assess individual intake on the day recalled with the purpose of estimating the population mean. However, as food intakes have a wide day-to-day variability, an intake result obtained on one single day is not sufficient to measure usual intake or to assess nutrient inadequacies. Extrapolating one single recall assessment to make conclusions on the usual intake would lead to a biased estimate of the fraction of the population with intakes above or below some standard.[29]

Different approaches to estimating usual intake

A conceptual definition of usual intake is the average of several daily intakes of a person or a population.[159] It has been described that the measurement of single day intake is a deviation of usual intake, and that deviation is called a measurement error.[160] However, it has been postulated that the average of several recalls would not lead to a correct measurement of usual intake.[29,159] Applying several dietary recalls to the whole population is economically impractical when the sample is large, and there is some evidence that the results are likewise biased due to participant tiredness, low quality of reported information and response burden.[161,162] In this way the first interview is believed to be more accurate that the following ones and it is shown to usually lead to higher intake estimates than the following interviews. Although FFQs tend to measure longer periods of time and have been used as a measurement of usual intake they have more systematic errors than 24 hour recalls. [29,163] Besides there is evidence that the energy intake measured with FFQ does not correlate with doubly labelled water and that for quantitative assessments FFQ performance is poor.[164,165] 24-hour recalls adjusted for person to person variability leads to distributions much more closely to biomarkers than the distributions estimated from FFQs. [29]

Usual intake has a day-to-day variability (or within person variability) and a person-to-person variability. The within person variability if measured in a restricted number of days, as it is commonly the case in research, is usually larger than the real day-t-day variability and also usually larger than the person-to-person variability, making the distribution curve having a longer tails than the real distribution.[163] When the objective is to measure the proportion of individuals below or above a certain threshold value, for example below the EAR or above the UL, if there is more variability than in reality, the proportion estimated will be unrealistically large. As explained above, it is not feasible and it also introduces bias averaging multiple recalls, however there are some statistical methods developed to mitigate this problem.

Statistical models have been developed to improve estimation of usual intakes obtained from the 24-hour recalls by adjusting the distribution by within-person variance.[128] In this way, by adjusting the distribution, these statistical models reduce the within-person variability so that the main variability left is the person-to-person (between persons) variability. The adjustment can be done with an external variance from a similar population or by repeating the dietary recall to a subsample of the study population.

Dodd et al. described and compared four approaches that have been developed to estimate usual intake.[29] The first of these methods was the National Research Council method, that is useful only for data with close to normal distributions, however it does not produce standard errors for estimated parameters, does not adjust for day of the week or interview order and it is not useful for nutrients or foods that are not frequently consumed.

The second method is the Best Power method is similar to the National Research Council method, that is also useful only for data with close to normal distributions. However, the National Research Council method produces standard errors for estimated parameters and adjusts for day of the week or interview order.

The National Research Council and the Best Power methods assume that the nutrient intake distributions are nearly normal, however dietary intakes are frequently not normally distributed as individuals can have intakes away from the mean to the right of the distribution but the same is not possible to the left of the distribution as the values cannot be negative. [29]

The third method is the Iowa State University (ISU) method that can be applied to complex surveys, can produce standard errors for estimated parameters, adjusts for day of the week or interview order, however it is not useful to assess the usual intake of nutrients or foods that are not frequently consumed. For this thesis I selected the ISU method as there is evidence it produces good estimates of usual intake distributions of most nutrients.[163,164,166] The ISU method for estimating usual intake analytically assesses and removes the within-person variation. The ISU method for estimating usual intake can correct some characteristics of dietary intake data such as within person variability in intakes, correlation of intakes reported over consecutive days, effect of day of week, recall order, nonnormality of reported intakes and survey nonresponse.[163,164] However the ISU method has some limitations as well as it assumes that a 24 hour recall is an unbiased estimate of the day measured for that individual. [29] Besides, when duplicate recalls are made in very few individuals the estimated intake of some micronutrients can be less accurate than that for macronutrients or those consumed

more frequently. Furthermore, less frequently consumed foods such as oysters and nutrients such as lycopene, beta- cryptoxanthin and alfa-carotene may require more than two 24-h recalls. [164]

The fourth and last method reviewed by Dodd et al. is the Iowa State University Food method. Its most distinguishable characteristic is that it can be used to assess the usual intake of nutrients or foods that are infrequently consumed, however similar to the ISU method, one limitation of this method is that if the repeated sample is too small the estimation of usual intake might not be accurate.

Although with different approaches, these methods can correct the errors related to within-person variability. On the other hand, reporting bias of specific foods or errors in the food composition databases are not contemplated with any of these methods and none of them allow estimating the individual usual intake of a single individual.

2.14 Concluding remarks

Overweight and obesity prevalence is high in South Africa, Zimbabwe, and Argentina. More particularly in women, obesity prevalence in women doubles the obesity prevalence in men in several countries. These statistics on obesity prevalence thus indicate that many women in SA, Zimbabwe and Argentina may enter pregnancy when already overweight and obese and this may result in severe health outcomes for both the mother and offspring. Besides, as obesity treatment is complex and is beyond individual will, women struggle to lose weight in the postpartum.

Calcium supplementation may help to achieve calcium recommendation intakes as well weight management. A systematic review of calcium and weight in 2011 shows a small reduction in body weight however there are new studies since then and this warrants to be updated. None of the included studies in the systematic review were on pregnant women. The effect of calcium supplementation on preconceptional weight and weight gain during pregnancy has never been investigated. Thus a study that investigates weight change in a large number of women that were followed from before conception and during pregnancy is essential, as it will provide novel insights on the effects of calcium supplementation on the mother and their infants.

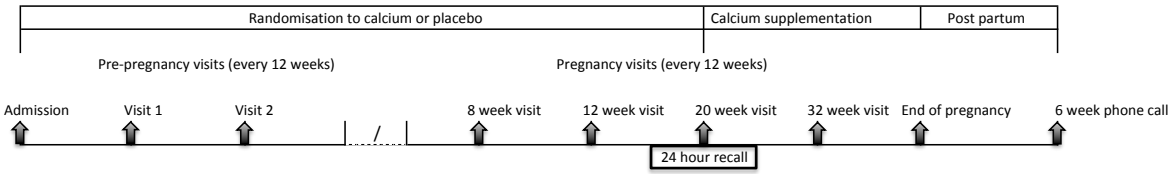
Chapter 3 - Methodology

3.2 CAP trial methodology (Aims 1 and 2)

Study design

The CAP trial was a multi-centre randomised, double-blind placebo-controlled clinical trial of women from South Africa, Zimbabwe and Argentina to assess the effect of calcium on pre-eclampsia. This nested study used the same design and population to assess the effect of calcium on body weight. Women were recruited before pregnancy and were requested to return to the clinic every 12 weeks for follow up health check-ups and resupply with study tablets. For those who became pregnant, visits were scheduled at weeks 8, 20, and 32 of gestation (Figure 3.1). Outcomes of pregnancy were recorded, as well as the mother and baby's general health status at 6 weeks after delivery to ensure safety of participating women and their babies. Weight and height were recorded at admission and weight was also recorded at 8, 20 and 32 weeks of pregnancy. Dietary assessment was conducted at 20 weeks of pregnancy (See Figure 3.1).

Figure 3.1: Study flow chart



I- Methods and Procedures

Target Population: Participants were all women recruited in the CAP trial with no other specific requirement for this proposal. CAP trial participants were parous women whose most recent pregnancy had been complicated by pre-eclampsia/eclampsia and who were intending to become pregnant, women were not eligible if they were less than 18 years old; were pregnant; were taking calcium supplementation; had chronic hypertension with proteinuria; had a history or symptoms of urolithiasis, renal disease or parathyroid disease; were not in a sexual relationship; were using long-term contraception or were not willing to give informed consent.

Women were recruited from 5 hospitals in South Africa, two hospitals in Zimbabwe and 3 hospitals in Argentina. Study sites for the CAP trial were selected from locations where populations have a known low calcium intake.[112,167,168] ha Individual calcium intake level cut-off was not included as eligibility criteria, as this was a pragmatic study looking at the effect in pregnant women. However, as part of the Steering Committee of the CAP trial, I proposed and designed the dietary assessment described below to all CAP trial pregnant women reaching 20 weeks of pregnancy with the purpose of confirming the low calcium intake.

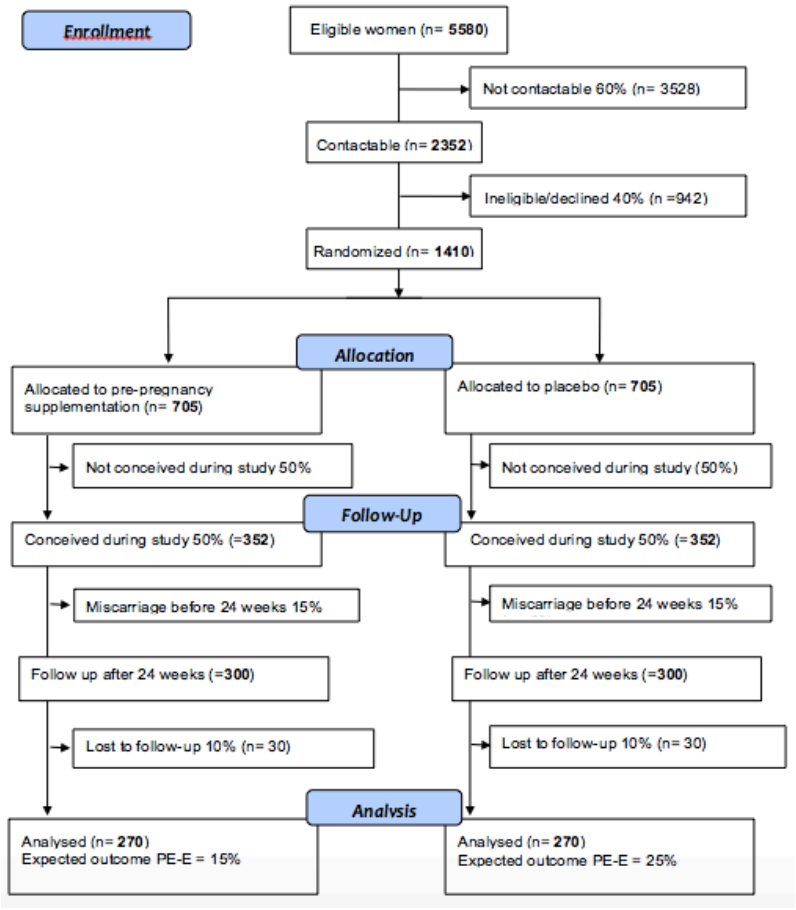
Sample Size: The sample size calculated for the CAP trial was 540 women with a pregnancy of more than 20 weeks. Figure 3.2 shows the CAP trial Consort Diagram that was designed for the CAP trial protocol to plan the participants flow and ensure reaching the desired sample size. This was the sample size available to evaluate pregnancy body weight (Objective 1.2). It was estimated that the sample size to evaluate pre-pregnancy body weight (Objective 1.1) would be slightly higher, around 600, as those having miscarriages between 8 to less than 20 weeks will only provide data for objective 1.2.

This sample size enabled to detect a difference of 1.2 kg between the calcium and placebo groups with a type 1 error of 0.05 and a power of 80%. Although smaller weight reductions are also clinically relevant at a population level, this trial was not powered to detect them. However as the proposal was to update the body of evidence performing a meta-analysis, these results added to the results of other studies and increase the sample size to detect smaller changes.

The trial started in 2011 and ended in 2017. The CAP trial was a unique study that recruited women before they become pregnant; therefore it was not possible to predict the representation of hospitals or even countries in the final sample of pregnant women.

Argentinean sites joined the trial at a later time; they had a smaller number of pregnancies, relative to the African sites. Therefore, if the number of women who fell pregnant was too small in Argentina by the time the CAP trial was closed, there was a possibility that we will not been able to perform a separate country-specific analysis.

Figure 3.2: Consort Diagram of the expected number of participants at each step of the study. This diagram was part of the CAP trial proposal



Inclusion criteria: the trial recruited women with history of pre-eclampsia or eclampsia in the immediately previous pregnancy, older than 18 years of age and not pregnant, could become pregnant again within the trial period and who were willing to give informed consent to participate. Pre-eclampsia was defined as gestational hypertension (diastolic BP >90 mmHg on two occasions 4 hours apart, or >110mmHg once, and/or systolic BP >140 mmHg on two occasions 4 hours apart, or >160mmHg once, after 20 weeks' gestation) and gestational proteinuria (2+ or more on urine dipstick, or >300mg/24 hours, or >500mg/L or urinary protein/creatinine ratio >0.034g/mmol, after 20 weeks' gestation. Eclampsia was defined as occurrence of generalized seizures in women with pre-eclampsia.

Exclusion criteria: women with chronic hypertension and proteinuria, history or symptoms of urolithiasis, renal disease of parathyroid disease, or those taking calcium supplements.

Location of the research: the study was carried out in South Africa, Zimbabwe and Argentina.

The South African sites included Groote Schuur hospital and Mowbray Maternity hospital in Cape Town; East London Hospital Complex, which comprises Frere and Cecilia Makiwane Hospitals; and Chris Hani Baragwanath Academic Hospital, Soweto, Johannesburg. Cape Town, Johannesburg and East London are all large cities situated in three different provinces in South Africa. East London has

71% of the population Black African, 35% Coloured and 14% White. Cape Town has 39% of the population Black African, 42% Coloured and 15% White. Johannesburg has 77% of the population Black African, 4% Coloured and 14% White.[169] The sites in Zimbabwe were Harare Maternity and Mbuya Nehanda Maternity Hospitals, both in Harare city. Sites in Zimbabwe and South Africa were public secondary or tertiary referral hospitals serving urban, peri–urban and rural lower-income populations who reside in formal and informal housing and do not have private medical insurance.[170] According to the Demographic survey Zimbabwe has 98.3% of its population of African origin mainly speakers of Shona and Ndebele.[171] In Argentina the sites were "Nuestra Señora de las Mercedes" Maternity Hospital in San Miguel de Tucumán that is the referral public hospital of northwest Argentina serving a lower socioeconomic population and Hospital Italiano and CEMIC (Centro de Educación Médica e Invetigación Clínica) in Buenos Aires a private hospital used by the population whose health care is provided by labour union insurance funds or the private sector.

Subject recruitment: Participants were identified retrospectively by checking hospital records of women who had pre-eclampsia or eclampsia and also prospectively by inviting women attending one of the health service facilities mentioned above. Women were then contacted by telephone to inform them about the trial and to enquire whether they were interested in learning more about it. Women who express interest were invited to attend a special pre-pregnancy clinic at the hospital where they are offered routine pre-pregnancy screening and counselling, given detailed information about the project, and invited to participate. Those interested were asked about exclusion criteria, and if there were no obvious exclusion criteria present, they were invited to attend the trial clinic for further discussion of the trial and to confirm inclusion criteria. Only when women had signed the informed consent, evaluation of blood pressure, proteinuria and pregnancy test were performed. Soon after eligibility was confirmed women were then randomised. Teams trained for the CAP trial were involved in the recruitment and follow up of participants.

Randomisation

The random allocation sequence was created using computer-generated random numbers in balanced blocks of variable size, stratified by site. Treatment allocation was centralised and participants and personnel were blinded to treatment groups, decreasing the potential risk of bias. Non-pregnant women enrolled in the trial were allocated to the next available sequentially numbered pack provided by an online randomisation system (ALEA). The ALEA software contained the numbered packs of calcium or placebo available at each site. Tablets were packed in 12-week treatment bottles. The ALEA also provided the appropriate follow-up pack at each 12-week visit to ensure continuation of the same treatment without unblinding while participants remained randomised (See Figure 3.1).

Randomisation lasted until 20 weeks´ gestation, from that point all women received unblinded calcium tablets until delivery.

Intervention:

Study participants were assigned to receive:

- Calcium group: 1 tablet of 500 mg of elemental calcium a day in the form of calcium carbonate.
- Control group: 1 placebo tablet a day, identical to the calcium tablet.

Participants were advised to take one tablet a day at least 2 hours before or after meals or other tablets, no further info or dietary advice was given.

Justification of the calcium dose

A 500 mg dose was decided on based on the information from the study populations suggesting that the average daily calcium intake from the diet was around 400 to 600 mg. [27,172] An extra 500 mg a day would thus allow reaching dietary calcium recommendations for these populations. [21] Furthermore, 500 mg of calcium is the maximum level that could be included with food fortification. This is important as the intervention of the trial is supplementation before pregnancy and the recommendation to supplement all women of fertile age would not be feasible and consequently a fortification strategy would be more appropriate.

Participants received a health check-up by a member of the research team every 12 weeks where they were provided with a new bottle of supplements with each visit. At each visit they were requested to bring the old bottle of supplements to monitor compliance. The returned supplements were counted and registered in the study forms. In those cases where the woman did not bring the bottle, they were phoned later on to assess the number of supplements taken.

In order to maintain women and study personnel blinded to the intervention group, bottles were identified with a number and assigned using a web-based randomisation system.

Outcomes:

1. Body weight in kg, at admission and at 8, 20 and 32 weeks during pregnancy.
2. Body Mass Index in kg/m^2.
3. Birth weight in grams.
4. Maternal energy intake at 20 weeks of pregnancy in kcal per day.
5. Maternal calcium intake at 20 weeks of pregnancy in mg per day.

6. Maternal protein, carbohydrates, fibre, sugars and fat intake in grams per day.
7. Maternal iron, magnesium and zinc intake in mg per day
8. Maternal folate, vitamin D, vitamin A intake in mcg per day.

Measurement, assessments and tests

Information on the women's weight, height, pregnancy status, dietary information including supplement intake, baby´s birth weight and the assigned intervention have been obtained from the data collected in the CAP trial.

Anthropometric measurements:

Body weight (in kilograms) and height (in centimetres) were recorded at admission and weight was also recorded at 8, 20 and 32 weeks of pregnancy. Measurements were taken by each hospital's research team and recorded in the questionnaires designed for the study. Body weight was measured using a digital scale to the nearest 0.1 kg. Measurements were taken in light clothing and women were asked to remove their shoes. Height was measured to the nearest 1 centimetre using a stadiometer while the participant was not wearing shoes. Scales and stadiometers were those provided by each hospital but they remained the same throughout the study.

Body Mass Index (BMI) was calculated for each women at admission and at 8 weeks of pregnancy (*surrogate of preconceptional weight*) as weight (kg)/ height (m^2). Women were classified prepregnancy according to the WHO BMI standards for adults, defined as underweight (BMI < 18.5), normal (18.5 ≤ BMI < 25), overweight (25 ≤ BMI < 30), or obese (BMI ≥ 30). [173]

Birth weight (in grams) was taken by each hospital team and recorded in the questionnaires designed for the study. Birth weight was measured to the nearest 1 gram. Baby scales were those provided by each hospital and remained the same throughout the study.

To evaluate the effect of calcium on the body weight of non-pregnant women of fertile age (objective 1.1) we analysed the difference in the body weight collected at admission from the non-pregnant women and the body weight collected at 8 weeks of pregnancy. The weight at 8 weeks of pregnancy was used as a surrogate of preconceptional body weight as evidence from a cross-sectional study of a 1000 women showed that there is no significant change in mean maternal body weight or BMI in the first trimester. [174] Although the time period between admission to the study until evaluation at 8 weeks of pregnancy varied for each women, this was a randomised controlled trial and it was expected that the average supplementation time for the calcium and placebo groups are the same.

The effect of calcium supplementation on body weight gain during pregnancy was evaluated using the weight difference between week 8 to 20 weeks as all women received calcium supplementation from 20 weeks until the end of pregnancy. At 32 weeks of pregnancy it was evaluated if the difference found at 20 weeks persisted afterwards when both groups receive calcium supplementation. The newborns' birth weight and maternal body weight was compared between both groups to evaluate the safety of the intervention.

Dietary intake assessment:

A 24-hour multiple pass dietary recall method was used to ascertain dietary intake. Dietary data was obtained from personal interviews with all pregnant women enrolled in the CAP trial during their scheduled trial visit at 20 weeks pregnancy. A sub-sample of women was assessed at a second time point during a scheduled pregnancy visit, in order to estimate usual intake.

The recall took the form of a guided interview lasting approximately 20 minutes and involved recording food intake of the previous day on a questionnaire. The questionnaire was adapted from a triple pass 24-hour recall developed by the Kings College and pilot tested in South Africa and Argentina.[29,117] The Dietary assessment Education Kit (DAEK) was used to assist with portion size estimation. [175]

CAP trial study staff at the clinics interviewed participants. All women were asked to enumerate all foods, drinks and dietary supplements taken on the previous day. Afterwards, detailed information of each item was collected to identify the correct portion size and food composition for each item. Portion size estimation was done using household measurements and comparison with photographic material. At the end of the interview, all items were reviewed in chronological order, prompting for additional foods or drinks consumed.

To minimize possible communication problems, we used the food photographic material to show the pictures or portion size of the specific food item. Translators assisted with Xhosa and Zulu speakers in South Africa, while all participants in Zimbabwe were fluent in English and in Argentina, all interviews and questionnaires were in Spanish.

Statistical models have been developed to improve estimation of usual intakes obtained from 24-hour recalls by adjusting the distribution by within-individual variance. [128] In this way, by adjusting the distribution, these statistical models reduce the within-person variability so that the main variability left is the person-to-person (between person) one. The adjustment can be done with an external variance from a similar population or by repeating the dietary recall to a subsample of the population. As there are no published within-person variances for pregnant women in South Africa, Zimbabwe

or Argentina, I proposed obtaining this information from a subsample of the population. For this thesis I selected the Iowa State University (ISU) method as there is evidence it produces good estimates of usual intake distributions of most nutrients.[163,164,166]

The Iowa State University method for estimating usual intake analytically assesses and removes the within-person variation. The method assumes that a 24 our recall is an unbiased estimate of the day measured for that individual. [29]

The method includes four steps:

1. It adjusts the nutrient intake distribution data to the day of the week the first nutritional assessment was performed and to the sequence order of interviews. This adjustment is done using the least squares method to minimized the errors that day of the week and order of the interview bring to the dietary intake data.
2. Then the distribution of each nutrient is power transformed to make it a normal distribution. Exponents from 1 to 10 are tested in the power transformation and then mapped into the normal scale via a cubic spline transformation. The Anderson-Darling test is used to detect departures from normality, if the p-value is less than 0.05 the hypothesis of normality is rejected.
3. Next compute the usual intake of nutrients for each individual calculating each individual own daily intake variance between the first and the second interviews in the subsample that have repeated measures. This method contemplates that individual variances can be different between subjects.
4. The last step is an inverse mean back-transformation to produce a distribution of usual intakes in the original scale.[166]

The recommendations of the Iowa State University method for calculation of the population variance require that at least 50 women have a second dietary assessment to be able to assess within person variance, otherwise the estimates might not be accurate. [176] Following this, the subsample for second recall were all pregnant women recruited in Argentina and 50 pregnant women from South Africa and Zimbabwe. In Africa, the attempt was made to perform the assessment in at least 50 consecutive women. However, if a woman missed the follow-up visit, the next woman coming in was recruited. The ISU method has some constrains, when duplicate recalls are made in very few individuals the estimated intake of some micronutrients can be less accurate than that for macronutrients or those consumed more frequently.

Other variables

The following variables will also be collected for the purposes of this proposal:

1. Intervention group: calcium or placebo. This information was available when the study finished, data unblinded and database prepared for analysis.
2. Time of exposure to the intervention: days from admission date (Admission questionnaire, question 1) to end of intervention period (20-week questionnaire, question 1).
3. Compliance: was calculated with the number of supplements returned at each visit (proportion taken of expected tablet intake). This information was available in all follow-up visit questionnaires.
4. Age of the woman at admission: this was calculated subtracting admission date (Admission questionnaire) minus birthdate.
5. Age of the woman at pregnancy: this was calculated subtracting pregnancy date (8-week visit questionnaire, question 1) minus birthdate.
6. Parity: Total number of deliveries of gestations of >24 weeks irrespectively of the outcome (Admission questionnaire, question 3).
7. Inter-pregnancy interval (for those that become pregnant during the study): Interval since previous pregnancy (Admission questionnaire, question 4).
8. Months since last birth: for the analysis of non-pregnant women it was calculated as the date of the end of the last pregnancy reported at admission of the study, minus admission date. For the analysis of women it was calculated as the date of the end of the last pregnancy reported at admission of the study minus date at 20 weeks of pregnancy.
9. Blood Pressure: systolic and diastolic; in mm Hg: defined as the lowest of two blood pressure measurements taken three minutes apart.
10. Pre-eclampsia in current pregnancy: yes or no (Delivery questionnaire, question 22).
11. Eclampsia in current pregnancy: yes or no (Delivery questionnaire, question 23).
12. Country of recruitment: South Africa, Zimbabwe, Argentina. Participant screening numbers allow identifying recruitment site and country.

Data Collection procedures and quality assurance

Data Collection

Weight and height were recorded at admission and weight was also recorded at 8, 20 and 32 weeks of pregnancy. Energy and calcium intake were assessed by a 24-hour recall at the clinic during the trial visit at 20 weeks pregnancy. If the visit was missed, effort was made to trace women to perform the recall at a visit as close as possible to the 20-week of pregnancy (See Figure 1.1).

Intervention compliance was measured by counting returned tablets at each visit that was scheduled every 12 weeks before pregnancy and at 8, 20 and 32 weeks during pregnancy.

Data collected at the admission and follow up visits were recorded in standardized questionnaires that were developed in English and then tested and translated into Spanish for the participants recruited in Argentina. Adverse events were recorded on a separate form.

Copies of the English version of the admission, pre-pregnancy and pregnancy visits, delivery and adverse events forms can be found in Annexure 1. However, all forms were also available in Spanish.

Data quality assurance

Data were collected in carbon copy data recording sheets. Double data entry was performed in *OpenClinica*, which allows for detection of inconsistencies and the tracking of changes. The *OpenClinica* system is an open-source web-based software, protected and reliable, totally compliant with Good Clinical Practice (GCP) and regulatory guidelines as well as the HRP/WHO Standard Operating Procedures (SOPs) for managing clinical trials. These procedures have been used in previous international multicentre trials sponsored or coordinated by WHO and proven to be efficient.

The study was coordinated by the Department of Reproductive Health and Research of the WHO, which performed site supervisions of the data collection and maintained the database.

Fieldworkers

Interviewers were trained by the PhD candidate to complete 24-hour recalls, dietary assessment techniques and portion estimation using a booklet of pictures with locally relevant portion sizes.[175] After each recall, interviewers were asked to complete a question to be able to assess whether it was very difficult to take the 24-hour recall due to communication problems that can impair the validity of the information reported (See Data Collection Forms: Dietary assessment questionnaire).

Participants were prompted to report any supplement containing calcium in addition to the study tablets. Type and frequency of additional dietary supplement products were recorded. This information was used to later review supplement intake during pregnancy and whether the supplement product taken contained calcium. The established supplemented amount was added to the daily calcium intake calculated from the rest of the participant´s diet. Women participating in the study were advice not to take any other type of calcium supplement including those issued by the hospital.

The 24-hour recall data from the interviews were entered into a food classification software by the local CAP trial staff at each site. African data were entered using the South African Food Finder III

developed by the South African Medical Research Council that uses South African Food composition tables and Food Quantities Manuals as no such data exists for Zimbabwe.[177] In Zimbabwe we used the same food composition database as in South Africa, which includes fortified foods by South African law 2003, but not in Zimbabwe, this may produce that the prevalences of inadequate intake in Zimbabwe are even higher than those reported for vitamin A, thiamine, riboflavin, niacin, pyridoxine, folic acid, iron and zinc. In Argentina, data were entered into an excel spreadsheet for later analysis in SPSS. Data from and Argentina were calculated using the Argentinean Ministry of Health chemical composition table (SARA). All data were stored in computers used for the CAP trial that had restricted access under lock and key for the duration of the study. Daily intakes of energy, macronutrients and micronutrients were calculated.

After data entry of the 24-hour recall, the information from each interview was sent to me and I was responsible for reviewing the information. I then performed the analysis to calculate the calcium intake of the interviewee. Afterwards I confirmed the results to the sites in order to ensure the quality of the data collected. Final values were then entered in *OpenClinica* for each site. At least twice a year I travelled to each site to supervise the information collected and ensure data quality.

Statistical method and analysis:

Intention-to-treat analysis was used and the results were reported according to the CONSORT guidelines. Baseline characteristics were compared to confirm effectiveness of the randomisation process. Participant lost to follow-up was compared between groups to detect any imbalances.

Numerical data were tested for normality using skewness and kurtosis z-values, the Shapiro-Wilk test p-value and visual outputs from histograms, Normal Q-Q plots and box plots. Numerical data with a normal distribution were described using mean (±SD) and those with non-normal distribution using median (IQR). All categorical data were described using percentages.

The principal outcome of the study was change in body weight.

Analysis for objective 1.1: *Effect of calcium supplementation on body weight of women on fertile age (body weight at 8 weeks used as surrogate of preconceptional weight).*

Analysis for objective 1.2: *To assess the effect of calcium supplementation on women body weight gain during pregnancy and newborn birth weight.*

Change of body weight from admission to 8 weeks of pregnancy (objective 1.1) and from 8 weeks to 20 weeks of pregnancy (end of randomisation period) (objective 1.2) was calculated for each woman and compared between the calcium and placebo groups.

If there were differences at 20 weeks of pregnancy, and in order to evaluate if differences found at 20 weeks persist at 32 weeks when both groups received calcium supplementation, the change of body weight from 20 weeks to 32 weeks of pregnancy (all women were receiving calcium) was calculated for each woman.

In all cases the mean difference (MD) of change was compared between the calcium and placebo groups using a T-test. If the distribution of the difference in body weight was not normal the median of the differences were compared using a Wilcoxon test.

The mean birth weight was be compared between the calcium and placebo groups.

If there were imbalances in baseline characteristic for important prognostic factors, the effect on weight was analysed performing a linear regression, adjusting for baseline covariates (age, parity, time of exposure, BMI).

Dietary assessment analyses:

Data analysis to calculate nutrient intake: each nutrient distribution was tested for normality and skewed variables were logged transformed. As nutrient intakes are usually right-skewed, this methodology requires a power transformation to make the intake distribution look as close to normal as possible. Means and standard deviations were analysed using descriptive statistics in SPSS.

Data analysis to calculate usual nutrient intake: The usual intake population distribution was calculated using the Iowa State University methodology to estimate population variance. After testing the distribution for normality, a further step consists of adjusting the distribution to within-person and between-person variability. The within-person variability was calculated using the information obtained from the repeated interview to the subsample of at least 50 subjects of the population. The variance component was estimated using the Software for Intake Distribution Estimation (PC-SIDE, version 1.0, 2003; Department of Statistics, Iowa State University, Ames).[176]

Descriptive measures of calcium intake (mean, standard deviation) and within- and between-person variance component were calculated. Afterwards, the proportion of individuals with calcium intake below 900 mg a day was calculated separately for Argentina and Africa.

The EAR cut-point was used to assess adequacy of intake of macro and micronutrients. The percentage women with an intake below the EAR was calculated for each nutrient and compared between the intervention and control groups using Chi-square tests.

All data were cleaned and analysed using SPSS version 22. For all statistical analyses a p-value <0.05

indicated statistical significance.

Ethical considerations

The WHO protocol A65750: "Long term calcium supplementation in women at high risk of pre-eclampsia: A randomised, placebo, controlled trial" which also includes the assessment of calcium intake was approved by the Scientific and Ethical Review Group of the UNDP/UNFPA/UNICEF/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction at the Department of Reproductive Health and Research of WHO, and the WHO Research Ethics Review Committee, Geneva, Switzerland.[27] The trial sites also have approvals from their local IRBs. Approval has been obtained from the Faculty of Health Sciences, Human Research Ethics Committee (HREC) of University of Cape Town (Ref no 457/2010, Site Principal Investigator: Dr. Sue Fawcus). Permission to conduct the study have been obtained at each site including Mowbray Maternity Hospital (MMH). This nested study was approved by the UCT HREC (Ref no. 884/216).

Protection of Human Subjects

Human subjects' involvement and characteristics: Women who meet the inclusion criteria for the CAP clinical trial were recruited from the selected hospitals. Women received close, personalized follow-up before and during pregnancy, and had telephone access to the research medical officer. They were referred promptly for any pregnancy complications, which occur. Their surveillance during pregnancy was more closely supervised than in the routine antenatal clinic. They also received calcium supplementation from 20 weeks of pregnancy as currently recommended by WHO guidelines.

Source of material: The study data was collected from interviews with the women. All data collected was obtained specifically for research purposes.

Potential risks: It was very unlikely that serious side-effects of the calcium supplementation could occur as the intervention dose of this trial was low -500 mg a day -compared to previous studies that provided 2000 mg a day. Any adverse events and serious adverse events whether they were thought to be related to the trial intervention or not, were recorded by the CAP trial researchers on special forms and then submitted to Data Safety and Monitoring Board (DSMB) and the relevant ethics committees.

A Data Safety and Monitoring Board (DSMB) with no direct involvement in the trial was appointed. The role of DSMB was to deal with any ethical issues that could have arisen while the trial was in progress. Interim analyses were undertaken and the decision on if the trial continued was discussed

at the yearly Steering Committee meetings. Reporting and handling of adverse events was in accordance with GCP guidelines.

A part from this there was very little risk to the subjects in this study. The interview might have caused psychological discomfort, but timing of the interview was selected to suit subjects' preferences. The interviewers were field workers who had regular contact with the participants for follow-up visits, and had, due to the nature of the study, developed trust with the participants. In addition, the interviewers were specifically trained to minimise the discomfort.

Before enrolment, all participants received an explanation of the study including objectives, the reason why they were invited, the randomisation process and the time that the activities of the study required. It was also highlighted that participation was voluntary and if they accepted they were free to withdraw from the study at any time. All participants were provided with a copy of the informed consent form and those willing to join the study were asked to sign.

3.2 Systematic review methodology (Aim 3)

This systematic review and meta-analysis of randomised control trials had the objective of evaluating the effect of calcium, as supplements or food fortification compared to placebo or none fortified food, on body weight. The systematic review followed the Cochrane methodology.

All randomised controlled trials reporting the effect of calcium supplementation on overweight and obese individuals including pregnant participants with duration of at least 2 months were included. For adults, overweight was classified according to WHO standards as BMI equal or higher than 25 to less than 30 and obese as BMI equal or higher than 30.[178] For children and adolescents validated classifications for overweight or obese children or adolescents such as the WHO child growth standards for 0 to 60 months, WHO growth references for school aged children and adolescents using BMI for age, the International Obesity Task Force child BMI cut offs that are derived from BMI centiles at 18 years, and BMI z scores were accepted. [179,180]

The primary outcomes were body weight, health related quality of life as evaluated by a validated instrument such as the Centre for Disease Control and Prevention health-related quality of life questionnaire and adverse events as hypercalcaemia, hypercalciuria, nephrolithiasis, coronary heart disease, secondary hyperparathyroidism, anaemia, gastrointestinal symptoms. Secondary outcomes were defined as body mass index (BMI) and waist circumference.

I developed the search strategy with the assistance of the Cochrane group and it was then translated to include Medline, the Cochrane Central Register of Controlled Trials, LILACS, EMBASED,

clinicaltrials.gov and World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) Search Portal (http://apps.who.int/trialsearch/).

I screened the title and abstract of all references retrieved by the search strategy and another three researchers independently did the second screening. I obtained from PubMed all full texts for those articles satisfying the inclusion criteria. Disagreements were resolved with a third opinion. I extracted all full texts and co-supervisor Janetta Harbron performed the second extraction independently using COVIDENCE in a predesigned form. Both extractions were compared and disagreements were discussed and resolved. I listed those trials registered in clinicaltrials.gov and searched for publications. If the trials were not finished I listed them separately. Risks of bias were also assessed independently using the same system. Risks of bias were defined by Cochrane 'Risk of bias' assessment tool and judged as low, high, or unclear risk.[181,182]

I expressed dichotomous data as odds ratio (OR) or risk ratio (RR) with 95% confidence interval (CI) and calculated mean differences or standardised mean differences (SMDs) for those trials using different measurement scales and 95% CIs for continuous outcome measures. Effect estimates were calculated from p values, t statistics or other available statistics.

Heterogeneity was identified by visually inspecting the forest plots and by using a standard Chi^2 test with a significance level of $\alpha = 0.1$ and I^2 statistic, which quantifies inconsistency across trials to assess the impact of heterogeneity on the meta-analysis; where an I^2 statistic $\geq 75\%$ indicates a considerable level of heterogeneity.[182–184]

I included the pre-specified subgroup analysis: menopausal status: pre- and post-menopausal women, age: children, adults, older adults, gender, BMI: obese, overweight, physical activity, calcium supplementation, baseline energy intake: restricted energy intake or not, pregnancy status and type of diet as co-intervention.

Chapter 4 : Article 1

Effect of calcium supplementation on body weight of women in reproductive age (Primary objective 1.1)

This article describes the effect of a daily calcium supplementation of 500 mg on body weight of women before and during pregnancy.

4.1. Introduction

Obesity represents a major and challenging public health problem as it increases the risk of developing high blood pressure, insulin resistance, heart disease, diabetes, osteoarthritis and sleep apnea.[9,36] During pregnancy, obesity increases the risk of adverse pregnancy outcomes.[11,185] A review shows that obesity before and during pregnancy increases the risks of gestational diabetes and hypertension, mental ill health before and after pregnancy, caesarean section, preterm birth, large for gestational age, miscarriage, stillbirth and foetal death.[10] It has been estimated that a 5 to 7 kg/m^2 higher pre-pregnancy Body Mass Index (BMI) doubles the risk of pre-eclampsia.[8,52] Even an increase of 1 or 2 BMI units between pregnancies increases the risk of hypertension and gestational diabetes.[61]

Several studies have investigated the modulating effect of calcium intake in body weight.[25,63,186] In animal models changes in lipid metabolism with calcium supplementation have been illustrated.[187,188] Sun and Zemel (2004) reported that after induced weight loss, obese mice on a diet low in calcium experienced increased lipogenesis and rapid body weight regain whereas calcium supplementation inhibited the weight regain.[189] In humans a systematic review in 2011, that included seven studies with 794 overweight or obese participants, showed that calcium supplementation compared to placebo produced significantly larger mean body weight loss of 0.74 kg (CI -1.00 to -0.48).[25] Six of the included studies had a duration of six months with a dose of 1000 mg of elemental calcium per day and one a duration of 24 months with a dose of 1500 mg of elemental calcium per day.

Three mechanisms in which calcium could affect body weight have been postulated. The first one is linked to the regulation of the parathyroid hormone that is required to maintain specific calcium concentrations in extracellular fluids.[64,65] Low calcium intakes stimulate parathyroid hormone and 1-25 vitamin D secretion to increase calcium resorption from the bones, kidneys as well as absorption in the intestine. However, higher levels of parathyroid hormone and 1-25 vitamin D also stimulate calcium influx into different cell types, including the adipocyte. [65] In the adipocyte, this increase of intracellular calcium stimulates fatty acid synthase and lipogenesis. [64] Low calcium diets have also been linked to insulin resistance and high blood pressure through similar collateral effects. [66]

Likewise, higher levels of parathyroid hormone and 1-25 vitamin D increase intracellular calcium in the vascular smooth muscle cells and consequently increase muscle reactivity, peripheral vascular resistance and thus higher blood pressure. [68,69] In this way, hormones that are released to compensate for low serum calcium levels could produce as collateral effects an increase of blood pressure and lipogenesis. [66]

A second postulated mechanism is associated to the reduction of fatty acid absorption in the intestine.[190–192] [192] This mechanism is linked to the fact that calcium absorption is inefficient, only 15 to 58% of calcium is absorbed, thus a large proportion of calcium remains in the intestine.[192,193] The calcium remaining in the intestine has the effect to bind and form soaps with different substances. This is the case of calcium binding to oxalate and decreasing stone formation.[194] Besides this effect, higher calcium intakes could provide more calcium remaining in the intestine and binding to bile acids or to fatty acids impairing their absorption and decreasing available energy. [70,71,190,195] This same mechanisms was proposed for the effect of calcium on improvement of lipid profile and decrease in cardiovascular disease.[192] However, as some of the evidence comes from dairy products, it is still controversial if the effects seen are due to calcium or to other component of dairy products.[190]

Finally, the third and with least evidence mechanism postulated to explain the relationship between calcium intake and body weight is the effect on appetite.[190,191] Higher calcium intakes have been linked to increase of glucagon-like peptide-1 that reduces appetite. [73] On the other hand Tordoff described a different pathway.[196] Some minerals such as calcium, magnesium and sodium produce a phenomenon to crave for foods rich in these minerals. As calcium is present in energy containing foods these appetite for calcium would increase energy intake and increase weight.[190]

In order to improve pregnancy outcomes, overweight and obese women are advised to lose weight before conception, however the evidence on how to achieve this is scarce.[61] Also, for overweight or obese pregnant women there is lack of evidence on how to manage their weight during pregnancy.[60,61] Until more robust evidence is available recommendations are still to gain weight, although at a reduced rate, as dieting during pregnancy may increase the risk of ketosis that is harmful for the foetus. According to the US Institute of Medicine guidelines women that are overweight should aim to gain 7 to 11.5 kg and women who are obese should aim to gain 5 to 9 kg during the entire pregnancy.[197] With the current high overweight and obesity prevalence among women of child-bearing age and low calcium intakes in several low and middle income countries, calcium supplementation might aid weight management before and during pregnancy.[107,198]

The Calcium And Pre-eclampsia (CAP) trial was a randomised controlled trial aimed to test the effect of calcium supplementation commencing before pregnancy and up to 20 weeks' gestation on the incidence of pre-eclampsia in women with history of pre-eclampsia.[168] The aim of this sub-study is to evaluate the effect of calcium supplementation on body weight in women of fertile age with emphasis in overweight and obesity before they conceive and the effect of the supplementation once they became pregnant.

4.2. Methods

The protocol of the multi-centre CAP trial has been previously published.[168] The trial included women from South Africa, Zimbabwe and Argentina.

Participants

Women were eligible if they had pre-eclampsia or eclampsia in their most recent pregnancy, if they were in a sexual relationship, not pregnant, not using contraception and if they provided informed consent. Pre-eclampsia was defined as a diastolic BP >90 mmHg on two occasions 4 hours apart, or >110mmHg once, and/or a systolic BP >140 mmHg on two occasions 4 hours apart, or >160mmHg once and 2+ or more on a urine dipstick, or a urinary protein excretion of >300mg/24 hours, or >500mg/L or urinary protein/creatinine ratio >0.034g/mmol, after 20 weeks' gestation or diagnosed by the attending clinicians. Exclusion criteria included: less than 18 years of age; chronic hypertension as reported by the women with persistent proteinuria confirmed with a dipstick at admission; calcium supplement intake; and history or symptoms of urolithiasis, renal disease or parathyroid disease. Women were eligible if they had hypertension with no proteinuria at admission. Women were recruited from five hospitals in South Africa (one hospital in Cape Town, one in Stellenbosch one in Johannesburg, and two in East London), two hospitals from Zimbabwe (located in Harare) and three hospitals in Argentina (one in Tucuman, one in the city of Buenos Aires and one in the province of Buenos Aires). Study sites for the CAP trial were selected from locations where populations have a known low calcium intake. [21,117,172]Individual calcium intake level cut-off was not included as eligibility criteria, as this was a pragmatic study looking at the effect in pregnant women.

Intervention

Women of fertile age were recruited before pregnancy and randomised until 20 weeks´ gestation to receive a calcium supplement containing 500 mg of elemental calcium as calcium carbonate per day or placebo identical in shape, colour and taste to the calcium tablet. Women were asked to chew the tablet during the day, 2 hours before or after taking food or iron supplements. From 20 weeks´

gestation until delivery all women received three tablets of 500 mg of elemental calcium per day (thus 1500 mg per day) in accordance with WHO recommendations. [1]Women did not receive any other dietary advise from the team throughout the study period.

Before pregnancy, all women were asked to return every 12 weeks where they were provided with a new bottle of supplements. Women who became pregnant were also asked to return every 12 weeks during their pregnancy study visits at 8, 20 and 32 weeks´ gestation.

Sample size

The sample size for the CAP trial was a total of 540 women with a pregnancy of more than 20 weeks. This was the expected sample size available to evaluate change in body weight at 32 weeks´ gestation. It was also estimated that the sample size to evaluate pre-pregnancy body weight would be slightly higher than the one to evaluate weight at 20 or 32 weeks, as those having miscarriages, quit the trial or have their weight not registered after 8 weeks will only provide pre-pregnancy data for the analysis. The expected sample size at 8 weeks´ gestation was 704 women and at 20 weeks´ gestation 600 women.

With any of these sample sizes we planned be able to detect a difference of 1.2 kg between the calcium and placebo groups with a type 1 error of 0.05 and a power of 80%. Although smaller weight reductions are also clinically relevant at a population level, this trial was not powered to detect them. However the results could contribute to improve the evidence if combined with other studies in a meta-analysis in order to increase the sample size and detect the existence of smaller changes.

Randomisation

Sequence generation

The sequence was generated using computer-generated random numbers in a ratio of 1:1 and in balanced blocks of variable size, stratified by site.

Allocation concealment

Calcium and placebo tablets given throughout the trial were packed in identical bottles containing 84 tablets each. Before and until 8 weeks´ pregnancy, women were given one bottle of their allocated

treatment at each visit. At the 20 and 32 weeks´ pregnancy visits women were given three bottles of calcium tablets. Allocation was done using an online service that provided the next available treatment for each new participant. During follow up visits the system ensured continuation of the treatment allocated at admission without revealing group allocation.

Blinding

Participants as well as investigators and outcome assessors were blinded to group allocation.

Implementation

Participants were identified retrospectively through reviewing hospital records of women who had pre-eclampsia or eclampsia. Women were then contacted by telephone to inform them about the trial and to enquire whether they are interested in learning more about it. Women were also contacted prospectively by inviting those attending one of the participating sites. Women who expressed interest were then invited to attend a special pre-pregnancy clinic, at the hospital where they were offered routine pre-pregnancy screening and counselling. At the special pre-pregnancy clinic they received detailed information about the project during an individual consult with the site researcher. Those interested were asked about exclusion criteria, and if there were no obvious exclusion criteria present, they were invited to participate in the trial. Only when women have signed the informed consent form, evaluation of blood pressure, proteinuria and pregnancy test were performed to confirm eligibility criteria before admission and randomisation. Teams trained for the CAP trial were involved in the recruitment and follow up of participants. Participants were admitted from 12th of July 2011 to 8th of September 2016.

4.3. Methods for this sub-study

Participants

For the analysis of this sub-study our sample included all participants of the CAP trial that had body weight registered at 8 weeks of pregnancy, n=457 (230 allocated to calcium and 227 allocated to placebo).

Measurement

Data collected at admission and used for the analysis of this sub-study included maternal age, height and weight, number of previous pregnancies and date of birth of last pregnancy complicated with pre-eclampsia and country where women lived. For women who became pregnant, body weight was measured during pregnancy at 8, 20 and 32 weeks´ gestation during the planned visits. Compliance to the study supplements was measured at admission and at each visit. Participants were asked to

bring the study bottle to each visit and the supplements left were recorded. In those cases where the woman did not bring the bottle, they were phoned later on to assess the number of supplements taken. Compliance was calculated as a percentage of the number of used supplements of the total number of supplements that should have been taken.

Research nurses specially trained for the CAP trial assessed all anthropometric and clinical assessment at admission and during follow up visits at each participating site.

Body weight was measured to the nearest 0.1 kg, women were measured in light clothing and they were asked to remove their shoes. Height was measured to the nearest 0.1 centimetre using a stadiometer while the participant was not wearing shoes. Scales and stadiometers were those provided by each hospital and remained the same throughout the study. The CAP trial Manual of Operations and the Standard Operating Procedures (SOPs) provided clear instructions on how women should be weighed and measured.

Pre-pregnancy BMI was calculated as weight (kg) divided by the square of the body height (meters) using measurements recorded at admission. Women were classified according to the WHO BMI standards for adults, defined as underweight (BMI < 18.5), normal (18.5 ≤ BMI < 25), overweight (BMI ≤ 25 and <30), or obese (BMI ≥ 30). [173]

Dietary energy and calcium intakes were measured at 20 weeks´ gestation using a triple pass 24-hour recall adapted from the method developed by Nelson et. al. and before the first CAP trial interview it was pilot tested in Argentina, South Africa and Zimbabwe. [117,156]

Birthweight of infants was recorded from clinical records as women were only followed until 32 weeks´ pregnancy.

Statistical analysis

Baseline data were compared between the calcium and placebo groups for those randomised, became pregnant and had weight measurements at 8 weeks´ gestation. We used an intention-to-treat (ITT) approach for this analysis as recommended by the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Continuous variables were described with means and standard deviations and categorical variables with percentages. To evaluate the effect of calcium on body weight in women of fertile age we analysed the information collected from admission to 8 weeks´ gestation as we did not have further weight measurements after admission and before pregnancy. We used weight at 8 weeks´ gestation

as a surrogate of preconceptional body weight. Evidence from a cross-sectional study of 1000 women shows there is no significant change in mean maternal body weight or BMI in the first trimester. [174]

We evaluated the effect of calcium supplementation on weight at 20 weeks´ gestation in women that started calcium supplementation preconceptionally using the difference between weight at admission and weight at 20 weeks´ gestation as all women stopped being randomised to calcium or placebo at 20 weeks. All women received calcium supplementation from 20 weeks´ gestation until the end of pregnancy; however we also evaluated if any difference was found at 32 weeks´ gestation. To compare continuous variables between intervention and control group a t-test was used and to compare categorical values a chi^2 test was used.

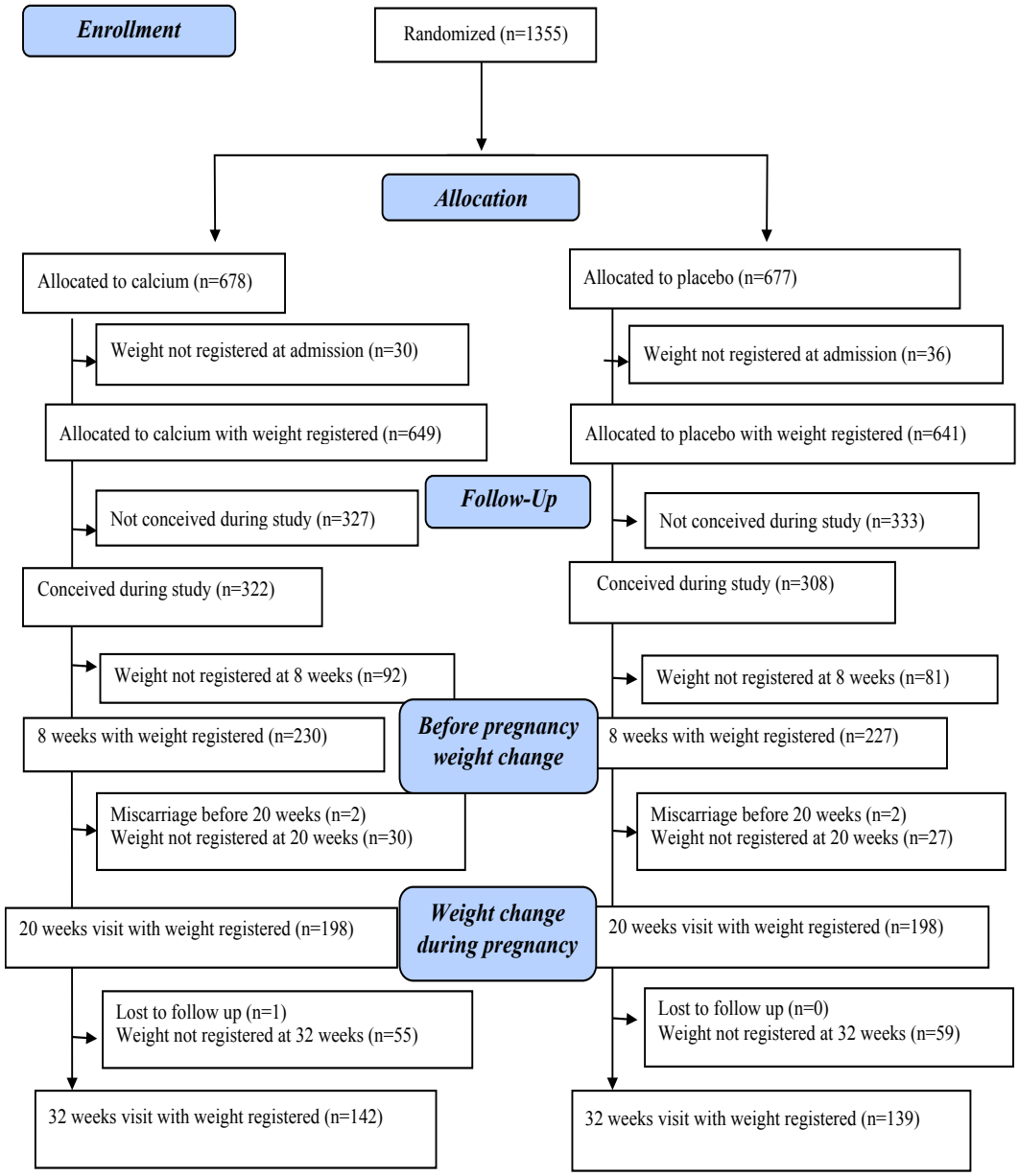
We then performed the same analysis of the effect of calcium supplementation at 8, 20 and 32 weeks´ gestation for the following three BMI classification groups: underweight or normal, overweight and obese. To explore the effect in those that complied with the intervention the analysis for those that complied with 80% of the treatment was done separately.

Finally, as the time between admission and pregnancy was different for each woman we evaluated the effect of calcium supplementation from admission to 8 weeks´ gestation by months of supplementation. We selected the cut off points reviewing the literature and decided to have three groups from 1) 2 to less than 6 months, 2) from 6 to less than 12 months and 3) more than 12 months, as there are studies showing effect with duration between 2 and 6 months however there are no studies with duration of less than 2 months and very few of more than 6 months. A p-value of 0.05 was used to define significant differences.

4.4. Results

A total of 1355 women were admitted to the study and randomised to receive calcium supplementation (n= 678) or placebo (n=677) (See figure 4.1). Of those, 630 women conceived during the study with 322 in the calcium group and 308 in the placebo and of those 230 allocated to calcium and 227 allocated to placebo had information on body weight at 8 weeks´ gestation; 198 allocated to calcium and 198 allocated to placebo had information on body weight at 20 weeks´ gestation and 142 allocated to calcium and 139 allocated to placebo had information on body weight at 32 weeks´ gestation. None of the participants were taken off calcium or placebo groups.

Figure 4.1: Women for the analysis of the effect of calcium on weight



Baseline characteristics of randomised women, for those who became pregnant in the CAP trial and those included in this sub study are illustrated in table 4.1. We did not find any significant difference at baseline in any of the variables between those allocated to calcium or placebo in any of the groups nor any difference between groups (Table 4.1).

Table 4.1: Comparison of baseline characteristics of women included in the CAP trial study and women included in this study. Mean values with standard deviations (SD)

	BASELINE ALL IN CAP TRIAL							BASELINE IN THIS SUB-STUDY						
	PLACEBO			CALCIUM			p value*	PLACEBO			CALCIUM			p value
	N	Mean	SD	N	Mean	SD		N	Mean	SD	N	Mean	SD	
Maternal Age	677	30.4	5.9	678	30.2	5.8	0.518	227	29.0	5.0	230	29.7	5.4	0.145
Parity	677	2.0	1.1	678	1.9	1.1	0.240	227	1.9	1.1	230	2.0	1.1	0.246
Weight at ADM** (kg)	641	76.9	18.8	649	76.2	18.5	0.497	227	74.0	16.3	230	75.8	16.8	0.266
Height at ADM (cm)	613	160.0	6.7	617	160.2	6.3	0.489	215	159.9	6.4	221	160.7	6.4	0.16
BMI*** at ADM	608	30.1	6.9	610	29.7	7.1	0.347	215	28.9	6.2	221	29.3	6.6	0.530
Months since last birth with PE§	648	24.6	36.3	645	22.1	29.7	0.181	212	20.2	26.2	224	20.9	27.3	0.789

* Differences were tested using a t-test and a p value of 0.05
** ADM= Admission
*** BMI= Body Mass Index
§ PE= pre-eclampsia

The average duration of receiving calcium or placebo from admission at baseline to 8 weeks´ gestation was 11.3 (SD± 8.8) months and 11.2 (SD± 9.3) months for those allocated to the calcium and placebo groups respectively. The body weight of all women increased during this period, however those allocated to placebo had a mean increase in body weight of 1.5 (SD ±6.1) kg whereas those allocated to calcium had a mean increase of 1.1 (SD ±5.5) kg although there was no statistical difference (Table 4.2). There was no difference between calcium and placebo group in body weight change from admission to body weight at 20 or 32 weeks´ gestation (Table 4.2). From admission to 20 weeks women allocated to placebo had an increase in weight of 4.0 kg (SD ±7.0) and those allocated to calcium 3.9 kg (SD ±6.0) (p=0.811). From admission to 32 weeks´ gestation the weight of women allocated to placebo increased by 8.3 kg (SD ±7.3) and those allocated to calcium 7.7 kg (SD ±6.6) (p=0.457).

Table 4.2: Weight change between admission and 8, 20 and 32 weeks´ gestation by baseline Body Mass Index (BMI)

	PLACEBO		CALCIUM		
	n	Mean difference (SD)	n	Mean difference (SD)	p value*
Weight change at 8 weeks´ gestation	227	1.5 (6.1)	230	1.1 (5.5)	0.408
BMI** at ADM*** < 25	72	0.8 (4.2)	70	1.5 (4.4)	0.334
25<=BMI at ADM<30	72	1.7 (5.3)	65	0.8 (4.0)	0.261
BMI at ADM>=30	83	2.0 (7.9)	95	0.9 (7.0)	0.330
Weight change at 20 weeks´ gestation	198	4.0 (7.0)	198	3.9 (6.0)	0.811
BMI at ADM < 25	62	4.6 (5.7)	60	4.5 4.7)	0.916
25<=BMI at ADM<30	63	3.9 (6.6)	53	3.4 (4.4)	0.628
BMI at ADM>=30	73	3.7 (8.2)	85	3.7 (7.6)	1.000
Weight change at 32 weeks´ gestation	139	8.3 (7.3)	142	7.7 (6.6)	0.457
BMI at ADM < 25	44	8.2 (5.4)	42	9.2 (5.5)	0.398
25<=BMI at ADM<30	50	8.2 (6.6)	37	8.1 (5.0)	0.936
BMI at ADM>=30	45	8.6 (9.4)	63	6.5 (7.9)	0.225

* Differences were tested using a t-test and a p value of 0.05
** BMI= Body Mass Index
*** ADM= Admission

Table 4.2 also shows change in body weight from admission to 8, 20 and 32 weeks´ gestation by baseline BMI categories. We found that at 8 and 32 weeks´ gestation women who started the trial with BMI equal or higher than 30, had a 1 kg (p=0.330) and 2.1 kg (p=0.225) higher increase in body weight respectively if they received placebo as compared to calcium however none of these differences were statistically significant. On the contrary, we found that women who started the trial with a normal BMI, had a 0.7 kg (p=0.334) and 1.0 kg (p=0.398) higher increase in body weight at 8 and 32 weeks' gestation respectively if they received calcium compared to placebo, however none of these differences were statistically significant.

Dietary energy and calcium intake was only measured at 20 weeks´ gestation. At this point the average total daily energy intake was 1815.2 kcal (SD ±802.8). There was no significant difference (p=0.902) in energy intake between women assigned to calcium (1812.8 (SD ±795) kcal) or to placebo (1824.2 (SD ±822) kcal). There was no difference in calcium intake from the diet between the groups, those allocated to calcium (n=143) had a mean dietary calcium intake of 418.9 (SD ±249.2) mg whereas those allocated to placebo (n=153) had a mean dietary calcium intake of 435.7(SD ±348.9) mg.[199]

We found that women of different age or parity had similar energy, however calcium intakes were lower in older women. Those aged between 20 to less than 35 years had a mean energy intake of 1859.6 kcal (SD ±810) and those aged 35 or more had a mean intake of 1620.1 kcal (SD ±775.6) (p=0.046). Those women with 1, 2 or 3 or more previous birth had a mean energy intake of 1938.9 kcal (SD ±917.8), 1789.5 kcal (SD ±714.9) and 1675.0 kcal (SD ±729.7) respectively (p=0.069). Those aged between 20 to less than 35 years had a mean calcium intake of 432.9 mg (SD ±325) and those aged 35 or more had a mean intake of 427.8 mg (SD ±308.2) (p=0.922). Those women with 1, 2 or 3 or more previous birth had a mean calcium intake of 429.7 mg (SD ±331.4), 437.8 mg (SD ±345.6) and 425.1 mg (SD ±274.4) respectively (p=0.825).

There was no difference in birth weight between those babies born to mothers randomised to calcium 2670 grams (SD ±1021) and those to placebo 2686 grams (SD ±846) (p=0.897).

Table 4.3 shows compliance of women measured at 8, 20 and 32 weeks´ gestation. We did not find any difference in compliance between calcium and placebo groups at any point. Only around 50 to 60 % of women had a compliance of 80% or more with the study supplements. We ran the analysis taking into account only those that had complied with 80% of the supplements or more and found no differences between the groups (Table 4.4).

Table 4.3: Participants compliance of 80% or more of the study supplements

	PLACEBO			CALCIUM			
Compliance (>80% tablets taken)	N	n	%	N	n	%	p value*
From last PPV[§] up to before 8 weeks	227	131	57.7	230	122	53	0.363
From last PPV up to 20 weeks	198	117	59.1	198	118	59.6	1
From last PPV up to 32 weeks	139	72	51.8	142	79	55.6	0.629

*Differences were tested using a chi2 test and a p value of 0.05

[§]PPV = Pre-pregnancy visit

Table 4.4: Weight change between admission and 8, 20 and 32 weeks´ gestation by baseline Body Mass Index (BMI) in those that complied with 80% or more

	PLACEBO		CALCIUM		
	n	Mean difference (SD)	n	Mean difference (SD)	p value*
Weight change at 8 weeks´ gestation	131	1.1 (4.4)	122	1.4 (5.4)	0.578
BMI** at ADM*** < 25	44	1.1 (4.4)	35	1.5 (4.8)	0.688
25<=BMI at ADM<30	42	1.7 (4.1)	39	1.1 (3.2)	0.476
BMI at ADM>=30	45	0.5 (4.7)	48	1.6 (7.0)	0.374
Weight change at 20 weeks´ gestation	117	3.4 (5.7)	118	3.6 (5.6)	0.867
BMI** at ADM*** < 25	35	4.5 (6.4)	39	4.1 (4.3)	0.774
25<=BMI at ADM<30	41	3.4 (5.3)	29	3.2 (4.2)	0.839
BMI at ADM>=30	41	2.5 (5.1)	50	3.3 (7.1)	0.554
Weight change at 32 weeks´ gestation	72	8.4 (7.8)	79	7.7 (5.6)	0.558
BMI at ADM < 25	22	6.6 (5.1)	24	9.2 (3.8)	0.065
25<=BMI at ADM<30	27	9.0 (4.9)	20	8.8 (4.3)	0.906
BMI at ADM>=30	23	9.4 (11.7)	35	6.2 (6.8)	0.190

* Differences were tested using a t-test and a p value of 0.05;
** BMI= Body Mass Index;
*** ADM= Admission

Finally we analysed the effect of calcium supplementation on body weight by time in the study between admission to pregnancy and we did not find any difference between the calcium and placebo groups. (Table 4.5)

Table 4.5: Weight change in kilograms from admission to 8 weeks´ gestation by months of supplementation

	PLACEBO			CALCIUM			
	n	Mean difference	SD	n	Mean difference	SD	p value*
Weight change at 8 weeks´ gestation							
Time in study < 2 months	6	1.2	2.6	5	-0.2	2.7	0.418
Time in study 2 to 6 months	81	0.6	3.9	77	0.3	4.2	0.635
Time in study 6 to 12 months	55	1.3	3.7	72	1.3	5.7	0.957
Time in study >=12	85	2.5	8.6	76	1.7	6.6	0.511

* Differences were tested using a t-test and a p value of 0.05

4.5. Discussion

In this study we evaluated the effect of a low dose of calcium supplementation on body weight change starting preconceptionally and up to 8, 20 and 32 weeks´ gestation in women with high risk of pre-eclampsia. To our knowledge this is the first study that investigated the effect of calcium supplementation on weight change in pregnant women. We did not find an effect of calcium supplementation in reducing 1.2 kg or more of body weight in women with high risk of pre-eclampsia, however we observed that the calcium group had a smaller increase in body weight at 8, 20 and 32 weeks' gestation although none of these differences were statistically significant. Sub-group analyses indicated that obese women receiving calcium had a lower increase in body weight than those who received placebo, however normal weight women receiving calcium had a higher increase in weight than those receiving placebo. Although these differences were not statistically significant, the finding for normal weight women was unexpected.

We were not able to demonstrate that calcium supplementation decreases body weight as has been shown in animal models; it could be that in humans there are other more influencing factors affecting body weight.[200] Furthermore, our study was not restricted to women who were overweight or obese as were the basic studies and the systematic review that showed that calcium supplementation decreases body weight.[25] Although the mean difference in body weight between treatment arms was not statistically significant, the difference is in line with the effect shown in a systematic review in overweight and obese women. [25] It is also possible that the Calcium dose of 500 mg of elemental calcium a day was too low to influence weight as the systematic review included many studies with doses of 1000 mg of elemental calcium or more a day.[25] It is also likely that the time period that women received supplementation was not sufficient for a large number of women in our sample, as 37% of the women had a supplementation time less than 6 months. The previous systematic reviewed indicated that the minimum time needed to have a statistical effect on body weight is 6 months. [25] It should be noted that there were no differences in weight change between the treatment groups for different time periods of calcium supplementation. Lastly, it must be borne in mind that we conducted this investigation during pregnancy, where other physiological or hormonal mechanisms may influence the results. For example, intestinal calcium absorption is generally low, around 25%, however during pregnancy intestinal calcium absorption doubles or triples to meet foetal requirements. [201] This adaptation might have some influence in calcium metabolism.

One of the potential mechanisms of action for the effect of calcium supplementation on body weight was through the effect of calcium in appetite.[65] However, the fact that the energy intake at 20 weeks´ gestation was not different between the calcium and placebo groups may indicate that calcium supplementation with 500 mg a day has no effect on the total energy intake of women. There was also no difference in dietary calcium intake at 20 weeks´ gestation between the groups suggesting that women randomised to calcium truly had a higher calcium intake.

The study showed no difference in birthweight between those babies born to mothers randomised to calcium or placebo. This finding precludes the possibility that maternal calcium intake could have a role on foetal growth.

Strengths

This was a randomised controlled trial with standardised procedures used in all sites. Women in this study were followed up regularly and weight and height were measured by the same research team before and during pregnancy.

Limitations

It is important to note that the findings of this study is only applicable to women with a risk of pre-eclampsia and not generalizable to all pregnant women. It has been shown that, obesity, hypertension and endothelial dysfunction are risk factors of PE. In this way, women in the CAP trial might have had an increase occurrence of these diseases compared to the general population and therefore different physiological changes. [202,203] Besides women with limited access to hospital delivery are not represented in this study.

Besides the small sample size another limitation was that we had only the admission measurement of body weight before pregnancy for each participant and thus we had to use body weight measured at 8 weeks as a proxy of pre-pregnancy body weight.

Although the time period between admission to the study until evaluation at 8 weeks´ gestation varied for each woman, the average supplementation time for the calcium and placebo groups were similar and time-period on supplementation did not influence the results.

Other reasons why we did not find any effect could be that the study lacked power to detect differences smaller than 1.2 kg, this can introduce a type II error where there is not power to

detect a true effect. ~~and that~~ Besides, in this study compliance was not optimal as only 50 to 60 % of women took 80% or more of the assigned supplements.

A further limitation is the fact that we did not use birthweight percentiles when comparing the effect of calcium supplementation during pregnancy on the effect of birthweight. [204]

4.6. Conclusion

This study shows that it is feasible to recruit women preconceptionally from low and middle countries and to follow them up throughout pregnancy.

We found that a low dose of calcium supplementation of 500 mg of elemental calcium a day had no statistically significant effect on body weight in women preconceptionally and during pregnancy. The sample size of the study allowed power to detect at 8 weeks´ gestation a difference of 1.2kg or higher between the calcium and placebo groups. In this way a clinically significant reduction smaller than 1.2 kg could have been missed due to a type II error where there is not power to detect a true effect.[205,206] Although the results were not statistically significant the effect found was in the same direction of previous results. Even though the individual clinical relevance of a small weight reduction has been questioned, at a population level it could help to prevent the observed obesity global trends. [186]

Although the results from this study do not support any implication for practice, it is of interest to report this type of study so as to build on the evidence. Future studies with more power are desirable to assess if those randomised to calcium had higher body weight increment if their BMI was less than 25 at the start of the intervention and a lower body weight increment if their weight was BMI 30 or more at the start of the intervention.

Chapter 5 : Article 2

Pre-pregnancy weight status, weight gain during pregnancy and adequacy of dietary intake of pregnant women in the CAP trial

This chapter reports the pre-pregnancy weight status, weight gain during pregnancy and adequacy of dietary intake of pregnant women in the CAP trial that is Aim 2 of this thesis. Part of the information for this aim was published in open access in the journal BMC Pregnancy and childbirth where I describe the anthropometry and dietary intake of women participating in the CAP trial during the second trimester of pregnancy.

"I confirm that I have applied for permission by the University of Cape Town's Doctoral Degrees Board to include the following publication(s) in my thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publication(s):

Cormick G, Betrán AP, Harbron J, Dannemann Purnat T, Parker C, Hall D, Seuc AH, Roberts JM, Belizán JM, Hofmeyr GJ; Calcium and Pre-eclampsia Study Group. Are women with history of pre-eclampsia starting a new pregnancy in good nutritional status in South Africa and Zimbabwe? BMC Pregnancy Childbirth. 2018 Jun 15;18(1):236.

5.1. Abstract

Background: Maternal nutritional status before and during pregnancy is an important contributor to pregnancy outcomes and early child health. The aim of this study was to describe the preconceptional nutritional status and dietary intake during pregnancy in high-risk women from South Africa and Zimbabwe.

Methods: This is a prospective observational study, nested to the CAP trial. Anthropometric measurements before and during pregnancy and dietary intake using 24-h recall during pregnancy were assessed. The Intake Distribution Estimation software (PC-SIDE) was used to evaluate nutrient intake adequacy taking the Estimated Average Requirement (EAR) as a cut-off point.

Results: Three hundred twelve women who had pre-eclampsia in their last pregnancy and delivered in hospitals from South Africa and Zimbabwe were assessed. 73.7 and 60.2% women in South Africa and Zimbabwe, respectively started their pregnancy with BMI above normal (BMI ≥ 25) whereas the prevalence of underweight was virtually non-existent.

The majority of women had inadequate intakes of micronutrients. Considering only food and beverage intake, none of the micronutrients measured achieved the estimated average requirement. Around 60% of pregnant women reported taking folic acid or iron supplements in South Africa, but almost none did so in Zimbabwe.

Conclusion: We found a high prevalence of overweight and obesity and high micronutrient intake inadequacy in pregnant women who had the previous pregnancy complicated with pre-eclampsia. The obesity figures and micronutrient inadequacy are issues of concern that need to be addressed. Pregnant women have regular contacts with the health system; these opportunities could be used to improve diet and nutrition.

Trial registration: PACTR201105000267371. Registered 06 December 2010.

Keywords: Nutrient intake, Weight, Pregnancy, Supplement, Obesity, BMI

5.2. Introduction

Nutrition status of women before and during pregnancy is one of the main contributors to pregnancy outcomes and early child health.[3] In many low and middle-income countries undernutrition and overnutrition coexist in the same population.[19] Obesity is increasing while micronutrient deficiencies still persist, particularly in the most vulnerable groups such as women and children.[20] Consequently, women start pregnancy with higher risks to develop complications such as pre-eclampsia, gestational diabetes mellitus, gestational hypertension, depression, foetal macrosomia, stillbirth, preterm birth, birth by caesarean section and infant mortality.[8–13] In addition, high maternal body mass index (BMI) has also been associated with delayed breastfeeding, weight retention and in women with gestational diabetes, a higher risk of developing chronic diseases.[8] Inter-pregnancy interval is also an important factor that may influence maternal availability of nutrients, especially in those populations with existing micronutrient deficiencies.[18]

Interest in pre-conceptional interventions to reduce risk factors during pregnancy is growing, although their effectiveness on pregnancy outcomes is less certain.[17] Current WHO Guidelines on antenatal care recommend supplementation with iron and folic acid to all pregnant women, and with calcium and vitamin A to women in specific areas with a high prevalence of deficiency.[126] The WHO guidelines also recommend supplementation with 1.5–2.0 g elemental calcium/day from 20 weeks´ gestation until the end of pregnancy for the prevention of

pre-eclampsia in those areas where calcium intake is low. The WHO Guidelines also report that women receiving counselling on diet and/or exercise are less likely to experience excess weight gain during pregnancy, although the evidence on the impact of other pregnancy outcomes is less certain.[126]

In South Africa, the National Health and Nutrition Examination Survey (SANHANES-1) reported a prevalence of overweight (BMI ≥ 25 kg/m2) and obesity (BMI ≥ 30) in women of 24.8% and 39.2%, respectively in 2012.[41] Data from Zimbabwe in 2000 shows a prevalence of overweight and obesity in women of 17.4% and 5.7% respectively.[46] However information on overweight and obesity rates as well as dietary intake during pregnancy is scarce in these countries. In South Africa, high levels of micronutrient inadequacy have been reported in a sample of alcohol and non-alcohol consumer pregnant women in their second half of pregnancy from Cape Town as well as women with gestational diabetes mellitus from Cape Town.[125,207] Nutrient and supplement intake information would be important to better plan and tailor interventions to improve pregnancy outcomes.[18]

We conducted a randomised controlled trial to evaluate the effect of pre-pregnancy calcium supplementation on the incidence of recurrent pre-eclampsia (Calcium and Pre-eclampsia: CAP trial).[167] This was a multi-country trial conducted in South Africa, Zimbabwe and Argentina. This manuscript presents the results of a sub-analysis of the CAP trial with the aim of describing the nutritional status of women from South Africa and Zimbabwe that became pregnant during the CAP trial. More specifically, we aimed to describe levels of overweight and obesity before and during pregnancy, and the adequacy of macronutrient and micronutrient intake during pregnancy.

5.3. Participants and Methods

This was a nested prospective observational study of women from South Africa and Zimbabwe recruited in the CAP trial.[168] The CAP trial was a multi-centre randomised, double-blind placebo-controlled clinical trial with the objective to determine whether calcium supplementation before conception and during the first half of pregnancy reduces the incidence of recurrent pre-eclampsia more effectively than supplementation starting at 20 weeks, which is the current WHO recommendation. In the CAP trial, non-pregnant women with history of pre-eclampsia or eclampsia in their most recent pregnancy were invited to participate as they are at higher risk of developing pre-eclampsia in subsequent pregnancies. Once admitted in the trial, participants

were required to attend study sites every 12 weeks for follow up until pregnancy occurred. Pregnant women were followed up throughout their pregnancy and trial visits were scheduled at 8, 20 and 32 weeks´ gestation. Eligible women were randomised to receive either 500 mg of elemental calcium daily or placebo from recruitment and blinded supplementation continued while participants were non-pregnant or until 20 weeks' gestation. From 20 weeks' gestation, all participants received calcium supplements in compliance with WHO guidelines.[1] The CAP trial started in 2011 and recruitment was completed in September 2016.

Settings and study population

Participants were recruited from government secondary or tertiary urban referral hospitals with large obstetric units serving urban and rural populations. The maternity and obstetric units included in the CAP trial were located in Cape Town (1), East London (2) and Johannesburg (1) in South Africa; and in Harare (2), Zimbabwe. Women were eligible for the CAP trial if they had pre-eclampsia or eclampsia in their most recent pregnancy, if they were not pregnant but in a sexual relationship, not using contraception and if they gave informed consent. For admission we reviewed the participant clinical records and accepted the clinical evaluation of pre-eclampsia or eclampsia reported there. Exclusion criteria included: less than 18 years of age; chronic hypertension with persistent proteinuria; calcium supplement intake; and history or symptoms of urolithiasis, renal disease or parathyroid disease.[168] For a complete list of eligible criteria please refer to the published protocol.[168] In this analysis, we included women recruited in the CAP trial who became pregnant and reached 20 weeks´ gestation between March 2013 to March 2016.

Anthropometric assessment and clinical data collection

Variables used for this sub-study included: age, height, pre-pregnancy weight, number of previous pregnancies and date of birth of last pregnancy complicated with pre-eclampsia. This data were collected at admission and for those that became pregnant, weight was also measured during pregnancy at 8, 20 and 32 weeks´ gestation throughout the planned trial visits. Research nurses specially trained for the CAP trial assessed all anthropometric, clinical and dietary variables at admission and during follow up visits at each participating site.

Body weight was measured to the nearest 0.1 kg in light clothing and without shoes. Height was measured to the nearest 1 centimetre using a stadiometer while the participant was not wearing shoes. Scales and stadiometers were those provided by each hospital and remained the same throughout the study. The Manual of Operations and the Standard Operating Procedures (SOPs) provided clear instructions on how women should be weighted and measured.

Pre-pregnancy BMI was calculated as weight (kg) divided by the square of the body height (m) using measurements recorded at admission. Women were classified according to the WHO BMI standards for adults, defined as underweight (BMI < 18.5), normal (18.5 ≤ BMI < 25), overweight (BMI ≥ 25), or obese (BMI ≥ 30).[173]

Gestational weight gain was calculated by subtracting the weight at 8 weeks´ gestation from the weight at 32 weeks´ gestation, since the participant's weight at delivery was not assessed.

Dietary assessment

The dietary intake of participants was assessed at 20-weeks' gestation using a triple pass 24-hour dietary recall adapted from the method developed by Nelson et. al. Before the CAP trial started it was piloted in South Africa and Zimbabwe.[156] The 24-hour recall is a guided interview to assess food intake of the previous day. CAP trial research nurses were trained in-site in March 2013 to administer the triple pass 24-hour recalls and to use the Dietary Assessment Education Kit (DAEK) to assist with the portion size estimation.[175] Xhosa and Zulu translators were trained at the sites that required them.

Reported food intakes from the 24-hour recall were entered and analysed using the Food Finder III computer program, provided by the South African Medical Research Council (SAMRC) to obtain daily energy and nutrient intakes for each participant. If properly conducted, a single day 24-hour dietary recall is a reliable method to assess individual intake on one day and can be used to estimate a population mean.[29] However, as food and nutrient intakes have a wide day-to-day variability, data obtained from one single day is not sufficient to describe the usual intake or to assess the proportion of individuals with intakes below certain thresholds (e.g. below requirements). Statistical models have been developed to better estimate usual nutrient intakes in a population by adjusting for within-individual intake variability.[128] These statistical models reduce the within person variability adjusting the distribution so that the main variability left is the person-to-person one. We used the Iowa State University (ISU) method that produce standard errors for estimated parameters, adjusts for day of the week or interview order. The ISU method as there is evidence it produces good estimates of usual intake distributions of most nutrients, however it is not useful to assess the usual intake of nutrients or foods that are not frequently consumed.[163,164,166] Therefore, in order to estimate the proportion of women with intakes below requirements we used the Intake Distribution Estimation software (PC-SIDE, version 1.0, 2003; Department of Statistics, Iowa State University, Ames) that applies the ISU model and requires

a sample of at least 50 dietary assessments that are repeated on a non-consecutive day to the first assessment.[71,176] The ISU method for estimating usual intake can correct some characteristics of dietary intake data such as within person variability in intakes, correlation of intakes reported over consecutive days, effect of day of week, recall order, nonnormality of reported intakes and survey nonresponse.[163,164] However the ISU method has some constrains, when duplicate recalls are made in very few individuals the estimated intake of some micronutrients can be less accurate than that for macronutrients or those consumed more frequently. Besides, less frequently consumed foods such as oysters and nutrients such as lycopene, beta- cryptoxanthin and alfa-carotene may require more than two 24-h recalls. [164]

For these purposes, a second 24-hour dietary recall assessment was administered in a subsample of women on a non-consecutive day after the first 24-hour recall. Energy and nutrient intake distributions from the single 24-hour recall were thus adjusted by within-person variance obtained from the second 24-hour recall assessment and by interview weekday to estimate usual nutrient intake and to calculate the proportion of women with intakes below requirements.

The Estimated Average Requirement (EAR) of carbohydrates and each micronutrient as recommended by the Institute of Medicine (IOM) for pregnant women was used as the cut-off point to assess adequacy of nutrient intake.[208] The Estimated Energy Requirement (EER) for pregnant women during the second trimester was calculated for each participant using the age, weight and height at admission and according to the Dietary Reference Intake (DRI) formula for adult women.[62] As data of physical activity was not collected the value for sedentary lifestyle was used conservatively. Energy intake obtained from the first 24-hour recall assessment was divided by the EER then normalized using PC-SIDE.[159] The 80% of EER during pregnancy suggested by Goldberg was used to calculate the plausibility of energy intake.[209] Protein adequacy was calculated using the EAR of 0.88 grams per kg of body weight, using weight at 20 weeks´ gestation [62]

A specific questionnaire was also included to investigate supplement intake during pregnancy. Women were asked about frequency and dose of the supplements and medicines. Trial supplementation was not computed in the dietary assessment of this sub-study as it was the intervention being tested and not, otherwise, part of the diet of this group of women.

5.4.Statistical Analysis

Categorical values were described using percentages and numerical variables using means and standard deviations (SD). Statistical data analyses were performed using the SPSS 23.0 software

package (IBM, New York, NY, USA). Dietary intake variables were log transformed and tested for normality using the Anderson-Darling statistical test using the PC-SIDE software. The software performs three steps: adjustments for weekday; transformation to normality using power transformation; and estimation of within-person variance using an error measurement model.

5.5. Ethics

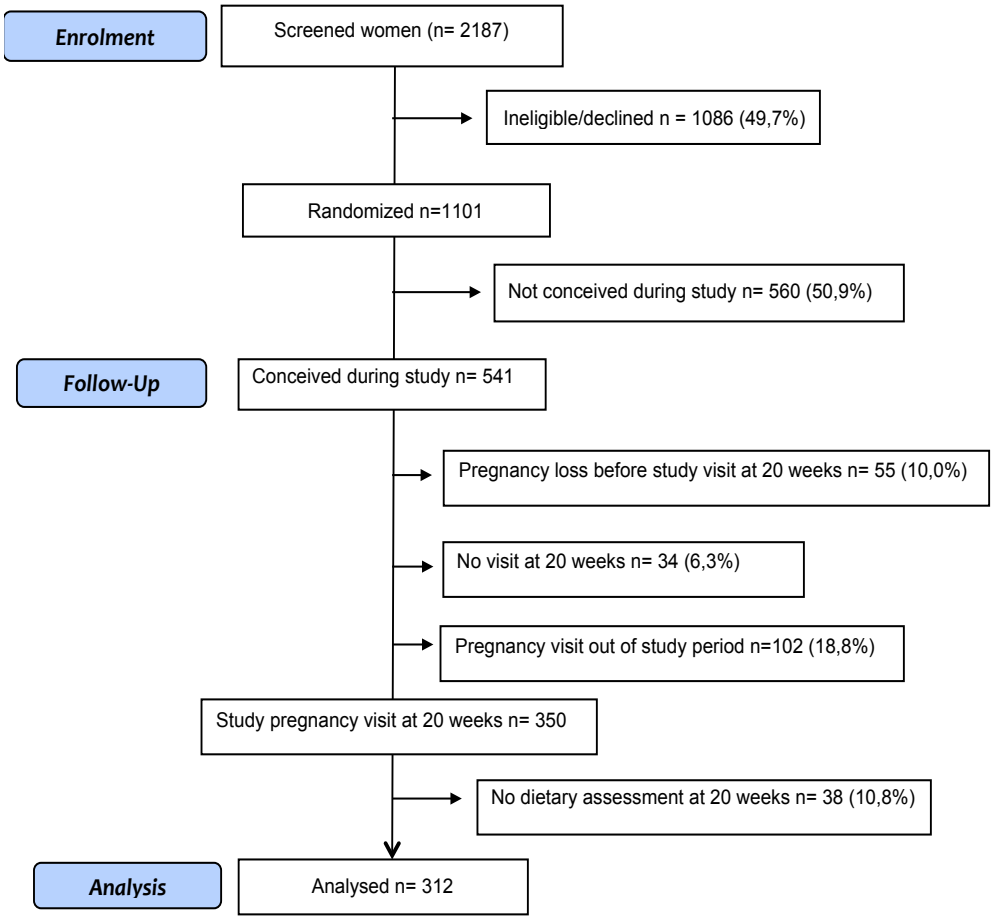
Ethical approval was obtained from appropriate national and institutional ethics review bodies as applicable for each study site, and all participants provided informed written consent. The study was approved by the Research Project Review Panel of the UNDP/UNFPA/ UNICEF/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction at the Department of Reproductive Health and Research of WHO, and the WHO Research Ethics Review Committee, Geneva, Switzerland.

Data management procedures were compliant with good clinical practice (GCP).[210]

5.6. Results

A total of 2187 women were screened in South Africa and Zimbabwe during the sub-study period (March 2013 to March 2016) and 1101 (50.3%) were eligible and accepted to participate (Figure 5.1). Of the 1101 participants randomised, 541 (49.1%) became pregnant of whom, 55 (10.0%) had a miscarriage or a pregnancy termination before 20 weeks´ gestation, 34 did not attend to the pregnancy visit at 20 weeks' gestation, and 102 (18.8%) completed the visits at 20 weeks´ gestation outside the sub-study period. A total of 350 women were eligible for this sub-study, however 38 (10.8%) missed the dietary assessment interview. Thus, we present the results of 312 women that completed the dietary assessment at the 20 weeks´ gestation visit. Of these women 224 (71.8%) were from South Africa and 88 (28.2%) from Zimbabwe. Repeated dietary assessments were obtained from 107 (34.3%) women, 79 from South Africa and 28 from Zimbabwe.

Figure 5.1: Flow chart



At the time of the assessment, women from South Africa had been in the study an average of 12.5 (SD ±7.4) months and women from Zimbabwe 13.1 (SD ±7.4) months. Their inter-pregnancy interval was 24.5 (SD ± 22.5) months in South Africa and 30.3 (SD ±23.7) months in Zimbabwe (Table 5.1).

Table 5.1: Participant Characteristics

	All		South Africa		Zimbabwe	
	n= 312	%	n=224	%	n=88	%
Age (years)						
Less than 20	1	0.3	0	0	1	1.1
20 to less than 35	255	81.7	182	81.3	73	83
35 and older	56	17.9	42	18.8	14	17.9
Parity						
1	121	38.8	93	41.5	28	31.8
2	111	35.6	79	35.3	32	36.4
3 or more	79	25.3	51	22.8	28	31.8
Missing	1	0.3	1	0.4	0	0
Months in study from admission to 20 weeks´ gestation						
Mean (sd)	312	12.5 (7.4)	224	13.1 (7.4)	88	11.0 (7.4)
Less than 6 month	94	30.1	60	26.8	34	38.6
6 to less than 12 month	88	28.2	59	26.3	29	33.0
12 to less than 24 month	97	31.1	79	35.3	18	20.5
24 or more month	32	10.3	25	11.2	7	8.0
Missing	1	0.3	1	0.4	0	0
Months since last birth with PE to 20 weeks´ gestation						
Mean (sd)	312	24.5 (22.5)	224	30.3 (23.7)	88	27.4 (19.0)
Less than 6 month	9	2.9	7	3.1	2	2.3
6 to less than 12 month	54	17.3	39	17.4	15	17.0
12 to less than 24 month	94	30.1	62	27.7	32	36.4
24 or more month	135	43.3	102	45.5	33	37.5
Missing	20	6.4	14	6.3	6	6.8
Anthropometric variables						
Height at admission - mean (sd)	286	160.3 (6.4)	202	159.9 (6.4)	84	161.1 (6.4)
Weight at admission - mean (sd)	302	76.1 (17.4)	217	78.9 (18.2)	85	69.0 (12.6)
Weight at week 8 of gestation - mean (sd)	251	77.1 (16.9)	173	80.2 (17.7)	78	70.2 (12.4)
Weight at week 20 of gestation - mean (sd)	300	80.2 (17.5)	215	83.5 (18.1)	85	71.9 (12.6)
Weight at week 32 of gestation - mean (sd)	224	84.0 (17.0)	158	88.2 (17.5)	66	74.0 (10.1)
Mean BMI at admission - mean (sd)	283	29.6 (6.3)	201	30.7 (6.6)	82	26.8 (4.5)
Body Mass Index at admission						
Underweight (BMI < 18.5 kg/m2)	3	1.0	2	0.9	1	1.1
Normal (18.5 BMI < 25 kg/m2)	62	19.9	34	15.2	28	31.8
Overweight (25 BMI < 30 kg/m2)	95	30.4	62	27.7	33	37.5
Obesity I (30 BMI 35 kg/m2)	75	24.0	58	25.9	17	19.3
Obesity II (35 BMI 40 kg/m2)	32	10.3	30	13.4	2	2.3
Obesity III (BMI >40 kg/m2)	16	5.1	15	6.7	1	1.1
Missing	29	9.3	23	10.3	6	6.8

Clinical characteristics

At recruitment, the mean age of women in this sub-study was 29.2 years (SD ± 5.2) in South Africa and 29.3 (SD ± 4.8) in Zimbabwe. Parity was three or more in about 25% of the women (22.8% and 31.8% in South Africa and Zimbabwe, respectively). The mean height was 159.9 cm (SD ±6.4) in South Africa and 161.1 cm (SD ±6.4) in Zimbabwe; and the mean weight before pregnancy was 78.9 kg (SD ±18.2) in South Africa and 69.0 kg (SD ±12.6) in Zimbabwe. The prevalence of overweight was 27.7% in South Africa and 37.5% in Zimbabwe while the prevalence of any degree of obesity was 46.0% in South Africa and 22.7% in Zimbabwe (Table 5.1). In total, 73.7% and 60.2% women in South Africa and Zimbabwe, respectively entered the trial with BMI above normal. On the other hand, the prevalence of underweight was virtually non-existent in both countries.

Gestational Weight Gain

We found that women who were initially classified according to their BMI as normal weight had gained from 8 to 32 weeks' gestation an average 8.9 kg (SD ± 4.4) in South Africa and 7.4 kg (SD ± 3.3) in Zimbabwe. Those classified as overweight had gained 7.8 kg (SD ± 4.5) in South Africa and 5.8 kg (SD ±3.8) in Zimbabwe and those classified as obese had gained 5.9 kg (SD ± 6.2) in South Africa and 3.1 kg (SD ± 4.1) in Zimbabwe.

Diet

Macronutrients

The average total daily energy intake was 1765.6 kcal (SD ±346.6) in South Africa and 1827.9 (SD ±303.9) in Zimbabwe. Average daily carbohydrate, fat and protein intakes were 230.8 (SD ± 57.5) grams, 59.1 (SD ± 6.4) grams and 54.7 (SD ±7.8) grams in South Africa and 213.6 (SD ± 22.3) grams, 76.4 (SD ± 24.8) grams and 52.9 (SD ± 25.8) grams in Zimbabwe, respectively (Table 2). Most (54.4%) of total energy intake came from carbohydrates, 27.8% from fats and 12.6% from proteins in South Africa while the percentages in Zimbabwe were 48.0%, 36.4% and 11.3% respectively.

Average daily intake of total sugars was 45.6 (SD ± 40.9) grams in South Africa and 34.5 (SD ±26.0) grams in Zimbabwe representing 10.6% and 7.5% of the total energy intake. The majority of women in both countries had lower than recommended protein intake (71.1% for South Africa

and 98.3% for Zimbabwe) and a higher intake of carbohydrates (2.9% for South Africa and 0% for Zimbabwe) (Table 5.2).

Table 5.2: Usual intake from foods and beverages, excluding supplements, estimated using repeated 24-hour recalls in 312 women and repeated in a sub-sample of 107 women, and percentage of women with usual intake below Estimated Average Requirement (EAR)

Estimated Daily Usual Intake *	South Africa n= 224	Zimbabwe n=88	EAR¶	% women with intakes below EAR	
				South Africa	Zimbabwe
Energy (kcal)	1765.6 (346.6)	1827.9 (303.9)	NA**	NA	NA
EER Estimated Energy Requirement (%)	0.88 (0.20)	0.93 (0.14)	0.8	37.7	19.1
Protein (g)	54.7 (7.8)	52.9 (25.8) §	NA	NA	NA
Protein (g/kg)	0.82 (0.43)	0.79 (0.04)	0.88	71.1	98.3
Carbohydrates (g)	230.8 (57.5)	213.6 (22.3)	135	02.9	0.00
Total Sugars (g)	45.4 (19.4)	35.4 (10.7)	NA	NA	NA
Fats (g)	59.1 (6.4)	76.4 (24.8)	NA	NA	NA
Calcium (mg)	441.0 (97.7)	360.5 (171.4)	800	99.9	97.6
Iron (mg)	9.9 (5.3)	7.9 (1.7)	22	96.9	100.0
Folate (mcg)	253.2 (69.1)	240.6 (46.2)	520	99.9	100.0
Mg (mg)	239.6 (51.1)	262.5 (38.8)	290	83.9	77.3
Zn (mg)	7.3 (1.7)	6.4 (1.6)	9.5	90.4	95.7
Se (mcg)	37.9 (4.3)	37.5 (11.8)	49	99.1	83.7
Riboflavin (mg)	1.3 (0.6)	0.8 (0.5)	1.2	54.0	84.0
Niacin (mg)	13.0 (3.8)	13.0 (3.7)	14	63.8	64.9
Vitamin C (mg)	82.4 (45.1)	109.80 (41.8)	70	46.6	15.6
Vitamin E (mg)	10.1 (3.8)	26.4 (9.1)	12	73.5	03.7

¶ EAR: Estimated average requirement

* Usual intake was obtained using the IOWA methodology.

** NA (Not Applicable)

§ This value represents the mean of the first interview.

Micronutrients

Adjusted usual nutrient intakes (from food and beverages, excluding supplements) are presented in Table 5.2. Micronutrient inadequacy was highly prevalent in both countries. Almost all women in South Africa had inadequate intakes of folate (99.9%), calcium (99.9%), iron (96.9%) and selenium (99.1%), while the majority also had inadequate intakes of magnesium (96.9%), zinc (90.4%), niacin (63.8%) and vitamin E (73.5%). More than half of the women had inadequate intakes of riboflavin (54.0%) while less than half had inadequate intake of vitamin C (46.6%).

All women in Zimbabwe had inadequate dietary intakes of iron and folate (100.0%); the majority also had inadequate intakes of calcium (97.6%), magnesium (77.3%), zinc (95.7%), selenium (83.7%) and riboflavin (84.0%). The intake of vitamin C (15.6%) and E (3.7%) was however, adequate in the majority of women from Zimbabwe. (Table 5.2)

Supplements

In South Africa, 62.9% of women (141) reported taking 5 mg of folic acid supplements, 57.1 % (128) reported taking iron supplements with doses ranging between 75 to 400 mg; 24.1% (54) reported taking vitamin C with doses ranging between 100 to 250 mg daily and 9.8% (22) reported taking vitamin B complex. At 20 weeks of pregnancy, women reported taking these supplements for a mean period of 2.1 to 2.7 months. Other supplements reported include calcium gluconate, magnesium sulphate and copper sulphate. Most of the supplements were provided by the hospital. On the other hand, a total of 29 (12.9%) women reported taking multivitamins for a mean period of 1 to 2 months, which are not provided by the hospitals.

In Zimbabwe, only one woman reported taking iron supplements during pregnancy. No other types of supplements intake were reported what so ever.

5.7.Discussion

This study shows that a high proportion of women whose previous pregnancy was complicated by pre-eclampsia in hospitals from South Africa and Zimbabwe started their subsequent pregnancy overweight or obese (73.7% in South Africa and 60.2% in Zimbabwe). In fact, obesity affected about 1 in 4 women in Zimbabwe, and as many as 1 in 2 women in South Africa were obese. Furthermore, at 20 weeks´ gestation more than 90% of these women had intakes of micronutrients, like iron, calcium, folate and zinc below requirements.

Overweight and obesity problems have already been reported in these countries. The prevalence of overweight or obese women found in our study is higher than the 64% that has been reported for South Africa by the SANHANES-1 and the 54.9% and 25% that the WHO Global Database on Body Mass Index reports for South Africa in 2004 and Zimbabwe in 2006 respectively, but in line with other studies conducted in South Africa that reported 69%.[31,211,212] The fact that we only included women who had a previous pregnancy complicated with pre-eclampsia could contribute to the higher overweight or obesity prevalence in our study population.[46,168] A link between obesity and hypertensive disorders of pregnancy has been reported in the literature. A systematic review concluded that for every 5 to 7 kg/m^2 increase in BMI, the risk of developing

pre-eclampsia doubles which confirms the relevance and critical importance of developing and implementing special efforts to control the BMI of these women before they become pregnant.[213]

We found that women with normal BMIs' at 8 weeks´ gestation, compared to those with higher BMI, gained more weight at 32 weeks, which is in accordance to recommendations.[197] However, as we only assessed weight up to 32 weeks´ gestation and most gestational weight gain occurs after 20 weeks´ gestation, we would expect that many of these women would exceed the Institute of Medicine recommendations. In our study, women classified as normal weight gained from 8 to 32 weeks' gestation an average 8.9 kg (SD ± 4.4) in South Africa and 7.4 kg (SD ± 3.3) in Zimbabwe of the 11.5 to 16 kg recommended for this group. Those classified as overweight gained 7.8 kg (SD ± 4.5) in South Africa and 5.8 kg (SD ±3.8) in Zimbabwe of the 7 to 11.5 kg recommended for this group and those classified as obese gained 5.9 kg (SD ± 6.2) in South Africa and 3.1 kg (SD ± 4.1) in Zimbabwe of the 5 to 9 kg recommended for this group.[4] Programmes and interventions to reduce obesity before pregnancy and control weight gain during pregnancy would be advisable in view of the findings of this analysis.

The energy intake we report is similar to those reported for women in the US National Health and Nutrition Survey in 2010-2011 where the prevalence of overweight and obesity are also higher than 70% [212,214] According to Goldberg, if the estimated usual intake is below 80% of the estimated average requirement for a person, this would imply underreporting. In our study this would imply 37.7% of underreporting in South Africa and 19.1% in Zimbabwe.[209] Fat intake as a percentage of total energy was within the recommended range of 20 to 35% of total energy in South Africa (27.8%), but slightly higher than recommended in Zimbabwe (36.4%). The high fat intake in Zimbabwe was due to a higher intake of polyunsaturated fats. In both countries, carbohydrates and protein intake as a percentage of total energy were within the recommended ranges of 46-65% and 10-35% respectively.[208] However, the intake of sugar in South Africa was slightly above the recommended maximum of 10% of total energy. Considering recommended grams of macronutrients, the women from both countries had mostly adequate total grams of carbohydrate intakes, but the majority had inadequate grams per kilograms of protein intake. It is thus important that interventions should focus on increasing the intake of affordable protein sources and decreasing sugar intake.

The prevalence of inadequate micronutrient intake from food sources was high in both countries. For the most basic micronutrients like iron, calcium, folate and zinc, the percentage of women below requirements was above 90% in both countries. It is likely that underreporting of dietary intake by almost 40% of the women in South Africa may have contributed to the high prevalence

of inadequate intake. However, it must also be borne in mind that the consumption of energy-dense foods such as sugar was higher than recommended and underreporting of energy-dense foods would not have affected micronutrient intake. Furthermore, similar to our results recent publications have also reported high prevalence of inadequate micronutrient intake in pregnant women that are not treated for other medical conditions and pregnant women with gestational diabetes mellitus attending public health care facilities in Cape Town. [125,207]

The most common supplements taken in South Africa were folic acid, ferrous sulphate, and vitamin C, all issued by the hospital. There is a policy in South Africa to supplement pregnant women with 5 mg of folic acid and 200 mg of ferrous sulphate (equivalent to 40 mg elemental iron) daily, which allowed those women taking the supplements to reach the recommendations.[215] The elemental iron supplementation provided by this policy is in accordance with the WHO guidelines for antenatal care, however folic acid supplementation is more than 10 times the recommended amount.[4] On the other hand, vitamin C is not recommended, as there is no evidence of an impact on birth outcomes. In contrast, only one woman in Zimbabwe reported taking supplements and this may be due to the fact that there is no policy to provide supplements during pregnancy in Zimbabwe and very few women bought commercial supplements. The inadequate intakes are worrying as iron and folic acid supplementation are known to prevent anaemia, puerperal sepsis, low birth weight and preterm birth; folate to prevent neural tube defects, calcium supplementation in areas with low calcium intake in order to reduce risk of pre-eclampsia; vitamin A in deficient areas to prevent night blindness. Regarding the diet a higher intake of vitamin E is usually related to higher intakes of oils and fats whereas vitamin C indicates an increased intake of fruits and vegetables.[216,217]

Strengths

Strengths of this study include the standardised procedures used throughout the trial in all sites. Women in this study had close follow up from the same research team before and during pregnancy.

The 24-hour recall dietary assessment method used is subject to less recall bias than other dietary assessment methods, such as diet histories or food frequency checklists.[157] Major advantages of using 24-hour recalls are that high literacy of the respondent is not required and that inter-observer differences are minimised.[156] On the other hand, food frequency questionnaires usually

require use of generic memory and higher numeracy skills in the population interviewed to quantify average food intakes over a period of time.[158]

Limitations

A main limitation in this sub-study is the limited generalizability of the findings as the study population were women with high risk of pre-eclampsia and they were women who had a previous hospital delivery. It has been shown that, obesity, hypertension and endothelial dysfunction are risk factors of PE. In this way, women in the CAP trial might have had an increase occurrence of these diseases compared to the general population and therefore different physiological changes. [202,203] Besides women with limited access to hospital delivery are not represented in this study.

We did not use the same scales to measure weight across sites, as weight was not the main outcome of this trial. However, women were assessed with the same scale throughout the study at each participating site.

We did not use any technique to corroborate energy intake, however under-reporting has been described in the literature in women especially in those with high BMI.[218]

Food data from both countries was analysed using the SAMRC-Food composition database as there is not a local food composition database in Zimbabwe. There were a few cases where foods reported during the assessment were not found in the database, and they were added as the most similar item in terms of macronutrients and calcium content. Nevertheless, this was only in a few cases and we believe it cannot affect the main results of this sub-study. Besides, in Zimbabwe we used the same food composition database than in South Africa, which includes fortified foods by South African law 2003, this may have the prevalences of inadequate intake in Zimbabwe even higher for vitamin A, thiamine, riboflavin, niacin, pyridoxine, folic acid, iron and zinc. Although not having a database for Zimbabwe might be a limitation, it prevents showing differences that are related to errors of the food composition tables rather than of the nutrient intake.[219]

The IOWA methodology to estimate usual intake requires at least 50 repeated interviews. We obtained repeated 24-hour recall for 107 women, however we obtained fewer than 50 repeated interviews for Zimbabwe so the estimation might not be as accurate as for South Africa. One of the limitation of the methodology used to estimate usual intake is that when duplicate recalls are

made in very few individuals the estimated intake of some micronutrients can be less accurate than that for macronutrients or those consumed more frequently.

5.8. Conclusion

We found a high prevalence of overweight and obesity and high prevalence of inadequate intakes of protein and micronutrients in pregnant women who had a previous pregnancy complicated with pre-eclampsia. Although this group is not representative of the general population of pregnant woman, taking into account the increasing prevalence of overweight and obesity worldwide among young age groups, the obesity and micronutrient inadequacy figures reported in this study are issues of concern that need to be addressed so that maternal and perinatal outcomes are improved.[33,34] Noticing the differences found in both countries regarding BMI and nutrient intake it would be interesting to explore the reasons behind as it could help to tackle the problem.

Supplement intakes during pregnancy seem to be essential for these groups of women to achieve requirements of key micronutrients. Policies should be reinforced and reviewed according to the most recent evidence. Pregnancy is a period when women may have more regular contacts with the health system and, if health care services were integrated for mothers and babies, these regular contacts could be maintained after delivery to improve maternal health.[220] These opportunities could be used to deliver dietary and nutritional interventions to high-risk women to improve the outcomes of future pregnancies.[221] Furthermore, taking into account that women with a history of pre-eclampsia are at higher risk of developing cardiovascular disease later in life, pregnancy and postnatal periods could be an ideal time for preventing future health complication from a young age.

5.9. Further analysis to comply with the PhD proposal

Preconceptional weight according to WHO BMI classification

Preconceptionally women participating in the CAP trial had a mean height of 160.3 cm (SD 6.4), a mean weight of 76.1 kg (SD 17.4) and a mean BMI of 29.6 (6.3) (See table 5.1). According to BMI WHO classification only 1 % of women were underweight and only 19.9% were normal weight. The vast majority of women were overweight 30.4% or obese 48.7% (Table 5.1).

Women in South Africa had a higher mean weight and BMI than in Zimbabwe (p=0.001 for both weight and BMI) however the height and age were similar.

Weight gain during pregnancy

Mean weight at 8, 20 and 32 weeks was 77.1 (16.9), 80.2 (17.5) and 84.0 (17.0) respectively, however women in South Africa had a mean weight of approximately 10 kg more than in Zimbabwe throughout pregnancy (Table 5.2).

Chapter 6 : Article 3

Calcium supplementation for weight reduction.

6.1.Introduction

Description of the condition

The prevalence of overweight and obesity is increasing worldwide across different age groups.[33,34] According to the World Health Organization (WHO), the prevalence of obesity doubled between 1980 and 2008 and it is increasing more rapidly in lower- and middle-income countries.[36] In the adult population, obesity is currently more prevalent in women than in men; between 23% and 29% of women are obese in the European, Eastern Mediterranean and American regions.[37] Obesity can lead to high blood pressure, heart disease, stroke, diabetes and insulin resistance.[36] A systematic review of 23 trials reporting national data on adolescent obesity shows that in 21 countries the prevalence of overweight and obesity was higher than 20%.[38] The prevalence of overweight and obesity in children has shown a remarkable increase over recent decades, representing a public health challenge as this prevalence tends to track into adult life.[39,40] A systematic review of the economic burden of obesity worldwide estimated that compared to normal weight individuals, those who are obese have 30% greater medical costs.[53] Moreover, it has been estimated that every kilogram of weight gain during adulthood increases the risk of cardiovascular disease by 3.1% to 5.7%.[52]

Description of the intervention

Calcium is the most abundant mineral in the human body. It is available in estimated quantities of 1.2 kg. Ninety-nine per cent of calcium is found as calcium hydroxyapatite in the skeletal system and is essential for this system's creation, rigidity and maintenance.[97] The remaining one per cent is distributed between the intra- and extracellular fluids where it is involved in the majority of the metabolic processes as well as in muscle contraction, nervous system transmission, enzymatic activation, and hormonal function.[147] Calcium metabolism acts over the 0.1% located in the extracellular fluid. Calcium serum levels are regulated by the parathyroid hormone, vitamin D, and calcitonin. All of these control calcium bowel absorption, its bone resorption and renal excretion.[222]

Calcium requirements are high during all stages of life.[147] Dietary recommendations for individuals over 19 years of age vary from 1000 mg to 1300 mg, depending on the reference guidelines.[22,100]

In most low- and middle-income countries, daily calcium intake is well below recommendations; however, low intakes are also observed in special age groups, such as adolescents, in high-income countries.[106] Whereas calcium intake seems to be below 600 mg a day in low- and middle-income countries, reports from high-income countries show that the intake is above 900 mg a day depending on age groups.[97] A review of studies reporting dietary intakes of pregnant women from low- and middle-income countries shows consistently low calcium intakes across Asian, African and Latin American countries.[108]

Interventions, such as calcium supplementation or food fortification, have been used for many years as strategies to increase calcium intake. Calcium supplementation is currently recommended by the WHO during pregnancy for the improvement of maternal and infant outcomes.[1] Calcium supplements are frequently consumed in high-income countries; however reports show that this is an uncommon practice in low- and middle-income countries.[97]

There is some evidence of an inverse relationship between calcium intake and body weight.[63] A systematic review found that among overweight or obese individuals, calcium supplementation compared to placebo produced a mean body weight loss of 0.7 kg (95% confidence interval (CI) -1 to -0.5).[25] Six of the included trials had a duration of six months with a dose of 1000 mg of elemental calcium per day while one trial had a duration of 24 months with a dose of 1500 mg of elemental calcium per day. The clinical relevance of this reduction has been questioned. However, at a population level, a small effect could help prevent the observed global trends.[186]

Adverse effects of the intervention

According to the Institute of Medicine, calcium intake upper limits are between 2000 mg to 3000 mg daily depending on the age group.[22]

The following adverse events have been described for high calcium intakes:

Cardiovascular diseases

Several trials have shown an inverse association between calcium intake and blood pressure or hypertension.[223] However, a secondary analysis of a trial designed to assess the effect of calcium supplementation on bone mineral density among postmenopausal women described a higher risk of self-reported myocardial infarction among those who received calcium supplements.[83] The results of this secondary analysis have been questioned, as the change in risk was no longer

significant when the analysis was limited to data verified by hospital records.[224] A recent review concluded that there is no firm evidence that calcium supplementation increases coronary heart disease or all-cause mortality risks in elderly women.[85] The review highlights that self-reported myocardial infarction should not be used as the primary outcome in randomised controlled trials (RCTs) of calcium supplementation, as it can be confused with gastrointestinal symptoms.

Gastrointestinal symptoms

A review showed an increased rate of self-reported gastrointestinal events in participants receiving calcium compared with placebo (Risk Ratio (RR) 1.43 (1.28 to1.59).[86] The gastrointestinal events reported were acute abdominal pain, indigestion and constipation. However, no relationship to the calcium salt formulation or dose was reported.

Nephrolithiasis

There is some controversy as to whether increasing calcium intake reduces or increases the risk of kidney stone formation. One proposed explanation is that the effect depends on the basal dietary calcium intake. Calcium in the intestine binds to potential stone formation factors such as oxalates, which restrict its absorption and reduce the risk of stone formation.[147] However, after this binding is saturated, higher calcium intakes do not produce further benefits. One RCT of calcium supplementation of 1000 mg a day combined with vitamin D showed an increased risk of kidney stones in the intervention group, however intakes in this group were higher than calcium recommended intakes as baseline mean calcium intake was 1148 mg ± 654 mg a day.[225] Another RCT evaluating men with a history of kidney stones, allocated to receive either a high or low calcium diet, showed a 50% decrease in the recurrence of kidney stone formation.[226]

Calcium supplements and iron absorption

There have been concerns related to the effects of calcium supplements on iron absorption based on short term studies reporting that calcium supplements inhibit iron absorption by 28% to 55% depending on the dose, type of salt used, time of supplementation and if the food contained hem or non-hem iron.[88] However, evidence shows no effect on iron status of prolonged calcium supplementation taken at the same time or separate of meals.[89–94] A study of infants supplemented with calcium glycerophosphate or placebo found no difference in iron status at 4 and 9 months, mean change in serum ferritin was -24.5 to -46.6 μg/L in both intervention and placebo groups respectively.[227] Another study that supplemented adolescent girls with 1 g of calcium citrate

malate/day for 4 years did not find difference in iron status; serum ferritin average concentrations in the supplemented group at baseline and years 1, 2, 3, and 4 were 29.1 ± 1.3, 31.1 ± 1.5, 31.1 ± 1.6, 30.6 ± 2.0, and 29.6 ± 1.9 μg/L, respectively; and in the placebo group, concentrations were 29.3 ± 1.4, 33.8 ± 1.7, 32.3 ± 1.4, 30.9 ± 1.5, and 29.5 ± 1.6 μg/L, respectively (p = 0.88, 0.23, 0.56, 0.88, and 0.96 for baseline and years 1, 2, 3, and 4, respectively).[228] Similarly, another study of 113 adolescent girls supplemented with 500 mg of calcium/day as calcium carbonate found no differences on iron status markers. At one year hemoglobin was 136 g/L in the supplemented group and 134 g/L in the placebo group (p=0.31); ferritin was 25.4 μg/L in the supplemented group and 26.1 μg/L in the placebo group (p = 0.73).[93]

A study that supplemented post-partum women with 500 mg of calcium/day as calcium carbonate found that those supplemented had a mean serum ferritin of 28.4 mg/L and 27.5 mg/L in the placebo group (p > 0.5).[92] A study in 24 healthy individuals also showed that supplementation with 1200 mg of calcium/day, as calcium carbonate had no significant effect on hemoglobin or hematocrit at 6 months. Mean hemoglobin at 6 months was 136 g/L in the calcium group compared to 139 ± 4 in the control group and 0.416 ± 0.013 in the calcium group compared to 0.424 ± 0.009 in the control group.[229]

With this evidence it has also been recommended that pregnant women take calcium supplements together with iron and folic acid to improve adherence.[230] This recommendation is in line with that of Molgaard et al. where public health recommendations should be based on long term studies and not in short term studies that are mainly to study the mechanisms. [93]

How the intervention might work

Three mechanisms by which calcium could affect body weight have been postulated. The first is linked to the regulation of the parathyroid hormone that is required to maintain calcium concentrations in extracellular fluids.[64,65] Serum calcium is tightly regulated and small reductions stimulate parathyroid hormone and 1-25 vitamin D secretion to produce an increase of calcium resorption from the bones, reabsorption from the kidneys, and absorption in the intestine. However, high levels of parathyroid hormone and 1-25 vitamin D also stimulate calcium influx into different cell types.[63] In the adipocyte, this increase of intracellular calcium stimulates fatty acid synthetase and lipogenesis.[65] Low calcium diets have also been linked to insulin resistance and high blood pressure through similar collateral effects of increased parathyroid levels.[147] A second postulated mechanism is related to appetite regulation. Higher calcium intakes have been linked to an increase of glucagon-like peptide-1 that reduces appetite.[73] A third mechanism is associated with the reduction of fatty acid absorption in the intestine. Higher calcium intakes

could bind to bile acids or to fatty acids impairing their absorption and decreasing available energy.[70,71]

Why it is important to do this review

A decline in calcium intake has been observed with an increase in population weight gain.[24,190,192] On a population level, a small decrease in body weight could help reverse the trend of increased weight gain. Two systematic reviews on the effect of calcium supplementation on weight have been conducted in 2006 and 2011. The first systematic review found that calcium supplementation compared to placebo reduces weight by 0.7 kg (95% CI 0.5 to 1.25) in overweight or obese people, however of the seven included studies five did not assess quality of evidence.[25] The other systematic review reported that calcium supplementation compared to placebo reduces weight by 0.4 kg (CI 0.3 to 1.1) in the general population, however studies where participants had a calcium intake of less than 300 mg of calcium a day or where published before 2002 were excluded.[231] These reviews had a comprehensive search, however the trials had a duration of at least 6 months and did not included pregnant women. Several trials have been published since these reviews, at least two studies with a shorter intervention period. Besides, the Cochrane library of systematic reviews recommends that reviews are updated every 2 years.[232] In our review, we included trials with overweight and obese individuals.

6.2. Objectives

To assess the effects of calcium supplementation for weight reduction in overweight or obese people. All randomised controlled trials reporting the effect of calcium supplementation on overweight and obese individuals including pregnant participants with duration of at least 2 months were included.

6.3. Methods

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCTs) assessing the effect of calcium compared with placebo or control in which the intervention had a minimum duration of two months as specified in the review protocol.[233]

Types of participants

We included overweight or obese participants of any age or gender. We also included pregnant women.

We classified participants as being overweight or obese using the body mass index (BMI), which is a person's weight divided by the square of the person's height (kg/m^2).

Diagnostic criteria for overweight and obesity

Adults: overweight BMI ≥ 25 to < 29.9, obesity BMI >30.[173]

Children and adolescents: we accepted validated classifications for overweight or obese children or adolescents such as the World Health Organization (WHO) child growth standards for 0 to 60 months, WHO growth references for school aged children and adolescents using BMI for age [16,179], the International Obesity Task Force child BMI cut offs that are derived from BMI centiles at 18 years, and BMI z-scores.

Types of interventions

We investigated the following comparisons of intervention versus control/comparator.

Intervention

(a) Oral calcium supplementation

(b) Calcium food or beverage fortification

Comparator

- Placebo compared with (a) or (b)
- Non-calcium fortified food or beverage compared with (b)

Calcium fortification could include salt of calcium carbonate, sulphate, citrate, citrate malate, chloride, hydroxyapatite, phosphate, acetate, lactate, glycerophosphate, gluconate, oxide or hydroxide. Calcium content in these salts varies from 9% to 70%.[128]

Concomitant interventions had to be the same in both the intervention and comparator groups to establish fair comparisons.

Minimum duration of intervention

We only considered RCTs in which the intervention had a minimum duration of two months.

Minimum duration of follow-up

We did not specify a minimum duration for follow up.

Specific exclusion criteria

We excluded trials of participants with chronic illnesses that affect calcium absorption or metabolism, such as lactose intolerance, inflammatory bowel disease (Crohn's disease, ulcerative colitis) or bariatric surgery patients.[234]

Types of outcome measures

We did not exclude trials because one or several of our primary or secondary outcome measures were not reported in the publication. In case none of our primary or secondary outcomes was reported, we excluded the trial.

Primary outcomes

- Body weight.
- Health-related quality of life.
- Adverse events.

Secondary outcomes

- Anthropometric measures other than body weight.
- All-cause mortality.
- Morbidity.
- Socioeconomic effects.

Method of outcome measurement

- Body weight (kg) measured at month 2, 6, 12 or more.
- Health-related quality of life: evaluated by a validated instrument such as the Center for Disease Control and Prevention health-related quality of life questionnaire and measured

at month 2, 6, 12 or more. Health-related quality of life refers to a multi-dimensional concept that includes domains related to physical, mental, emotional, and social functioning. It focuses on the impact health status has on quality of life rather than direct measures of population health or causes of death and morbidity, which is included in the secondary outcome.

- Adverse events: defined as total incidence of adverse events occurring at any time after initiation of the intervention. Specific adverse events will be specified as incidence of:
    - Hypercalcaemia: defined as the proportion of participants who have a serum calcium level above the upper limit of 10 mg/dL.
    - Hypercalciuria: defined as the proportion of participants who have a 24-hour urine collection of calcium > 250 mg in women and > 300 mg in men [235]or > 4 mg/kg for men and women.[236]
    - Nephrolithiasis: defined as the proportion of participants who experience a kidney stone clinically or radiologically.
    - Coronary heart disease (CHD): including myocardial infarction, angina pectoris and acute coronary syndrome, and chronic CHD verified by clinical review, hospital record, or death certificate.
    - Secondary hyperparathyroidism: assessed by parathyroid hormone levels above the upper limit of 65 pg/mL.[237]
    - Anaemia: measured by serum haemoglobin levels below 110 g/L in children 6 to 59 months of age and pregnant women; 115 g/L in children 5 to 11 years of age; 120 g/L in children 12 to 14 years of age and non-pregnant women and 130 g/L in men.[238]
    - Gastrointestinal symptoms: defined as the proportion of participants who experience constipation, anorexia, nausea, vomiting, or epigastric pain.
- Anthropometric measures other than body weight: defined as body mass index (BMI) and waist circumference at month 2, 6, 12 or more.
- All-cause mortality: defined as death from any cause, occurring at any time after initiation of the intervention.
- Morbidity: defined as diabetes, CHD or stroke diagnosed at any time after initiation of the intervention.
- Socioeconomic effects: such as direct costs defined as admission/readmission rates, average length of stay, visits to general practitioner, accident/emergency visits,

medication consumption at month 2, 6, 12 or more; indirect costs: defined as resources lost due to illness by the participant or their family member at month 2, 6, 12 or more.

Timing of outcome measurement

For body weight, health-related quality of life, anthropometric measures other than body weight and socioeconomic effects at month 2, 6, 12 or more. For all other outcomes measures: any time after participants were randomised to the intervention/comparator groups.

Search methods for identification of studies

Electronic searches

We searched the following sources from inception of each database to the specified date and placed no restrictions on the language of publication.

- Cochrane Central Register of Controlled Trials (CENTRAL)
- MEDLINE
- Embase.
- LILACS
- ClinicalTrials.gov
- World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) Search Portal (http://apps.who.int/trialsearch/).

We continuously applied a MEDLINE (Ovid SP) email alert service established by the Cochrane Metabolic and Endocrine Disorders (CMED) to identify newly published trials using the same search strategy as described for MEDLINE (Table 6.1). After supplying the final review draft for editorial approval, the CMED Group performed a complete updated search on all databases available at the editorial office and send the results to the review authors. Should we identified new trials for inclusion, we would have evaluated these, incorporate the findings into our review, and resubmitted another Cochrane Review draft.[239]

Table 6.1: Electronic Search Strategies

Cochrane Central Register of Controlled Trials (Cochrane Register of Studies Online)

1. MESH DESCRIPTOR Calcium Compounds EXPLODE ALL TREES
2. MESH DESCRIPTOR Calcium
3. calcium:TI,AB,KY
4. #1 OR #2 OR #3

5. MESH DESCRIPTOR Obesity EXPLODE ALL TREES
6. MESH DESCRIPTOR Weight Loss
7. MESH DESCRIPTOR Overweight
8. (obes* or overweight):TI,AB,KY
9. (weight ADJ (reduction? or loss?? or control or management)):TI,AB,KY
10. body weight:TI
11. body mass index:TI
12. BMI:TI
13. #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12
14. #4 AND #13

MEDLINE (Ovid SP)

1. exp Calcium Compounds/
2. Calcium/
3. calcium.tw.
4. or/1-3
5. exp Obesity/
6. Weight Loss/
7. Overweight/
8. (obes* or overweight).tw.
9. (weight adj (reduction? or loss?? or control or management)).tw.
10. body weight.ti.
11. body mass index.ti.
12. BMI.ti.
13. or/5-12
14. 4 and 13

[15-25: Cochrane Handbook 2008 RCT filter - sensitivity max. version]

15. randomized controlled trial.pt.
16. controlled clinical trial.pt.
17. randomi?ed.ab.
18. placebo.ab.
19. drug therapy.fs.
20. randomly.ab.
21. trial.ab.
22. groups.ab.
23. or/15-22
24. exp animals/ not humans/
25. 23 not 24
26. 14 and 25

[27: Wong 2006a – systematic reviews filter – Spec version]

27. cochrane database of systematic reviews.jn. or search*.tw. or meta analysis.pt. or medline.tw. or systematic review.tw.
28. 14 and 27
29. 26 or 28

Embase (Ovid SP)

1. calcium.tw.
2. exp obesity/
3. weight reduction/
4. (obes* or overweight).tw.
5. (weight adj (reduction? or loss?? or control or management)).tw.
6. body weight.ti.
7. body mass index.ti.
8. BMI.ti.
9. or/2-8
10. 1 and 9

[11: Wong 2006b "sound treatment studies" filter - SDSSGS version]

11. random*.tw. or clinical trial*.mp. or exp treatment outcome/
12. 10 and 11

LILACS (iAHx)

(MH:"Calcium Compounds" OR MH:"Calcium" OR calcium OR calcio) AND (MH:"Obesity" OR MH:"Weight Loss" OR MH:"Overweight" OR obes$ OR overweight OR sobrepeso OR "weight reduction" OR "weight loss" OR "weight control" OR "weight management" OR peso OR masa OR massa OR IMC)
+ Filter "Controlled Clinical Trial"

International Clinical Trials Registry Platform (ICTRP) Search Portal (Standard search)

overweight* AND calcium OR
obes* AND calcium OR
weight reduction AND calcium OR
weight loss AND calcium OR
weight control AND calcium OR
weight management AND calcium

ClinicalTrials.gov (Advanced search)

Search Terms: obese OR obesity OR overweight OR "weight loss" OR "weight reduction" OR "weight control" OR "weight management"
Interventions: calcium

If we detected additional relevant keywords during any electronic or other searches, we would have modified the electronic search strategies to incorporate these terms and documented the changes.

Searching other resources

We tried to identify other potentially eligible trials or ancillary publications by searching the reference lists of included trials, systematic reviews, meta-analyses and health technology assessment reports. In addition we contacted authors of included trials to identify additional information on the retrieved trials and, if further trials exist, trials that we may have missed.

Data collection and analysis

Selection of studies

Two review authors (Gabriela Cormick and Janetta Harbron) independently scanned the abstract, title, or both, of every record we retrieved in the literature searches, to determine which trials we should assess further. We obtained the full-text of all potentially relevant records. We resolved any disagreements through consensus or by recourse to a third reviewer. If we could not resolve a disagreement, we planned to categorise the trial as a 'study awaiting classification' and we planned to contact the trial authors for clarification. We presented an adapted Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram to shows the process of trial selection.[240]

Data extraction and management

For trials that fulfilled the inclusion criteria, two review authors (Gabriela Cormick and Janetta Harbron) independently extracted key participant and intervention characteristics. We reported data on efficacy outcomes and adverse events using standard data extraction sheets from the CMED Group. We resolved any disagreements by discussion or, if required, by consultation with a third reviewer.

We emailed all authors of included trials to enquire whether they were willing to answer questions regarding their trials. We thereafter sought relevant missing information on the trial from the primary author(s) of the article, if required.

Dealing with duplicate and companion publications

In the event of duplicate publications, companion documents or multiple reports of a primary trial, we maximised the information yield by collating all available data and used the most complete dataset aggregated across all known publications. We listed duplicate publications, companion documents, multiple reports of a primary trial and trial documents of included trials (such as trial registry information) as secondary references under the study identifier (ID) of the included trial. Furthermore, we also listed duplicate publications, companion documents, multiple reports of a trial and trial documents of excluded trials (such as trial registry information) as secondary references under the study ID of the excluded trial.

Data from clinical trial registers

In case data of included trials are available as study results in clinical trial registers such as ClinicalTrials.gov or similar sources, we made full use of this information and extract data. If there was also a full publication of the trial, we collated and critically appraised all available data. If an included trial was marked as a completed study in a clinical trial register but no additional information (study results, publication or both) is available, we added the references of the trial.

Assessment of risk of bias in included studies

Two review authors (Gabriela Cormick and Janetta Harbron) independently assessed the risk of bias of each included trial. We resolved any disagreements by consensus, or by consultation with a third reviewer. In cases of disagreement, we consulted the rest of the group and made a judgement based on consensus. If adequate information was not available from trial authors, trial protocols or both we contacted trial authors for missing data on 'Risk of bias' items.

We used the Cochrane 'Risk of bias' assessment tool assigning assessments of low, high or unclear risk of bias.[181,241] We evaluated individual bias items as described in the *Cochrane Handbook for Systematic Reviews of Interventions* according to the criteria and associated categorisations contained therein.[181,241]

Summary assessment of risk of bias

We presented a 'Risk of bias' graph and a 'Risk of bias' summary Figure 6.1.

We distinguished between self-reported, investigator-assessed and adjudicated outcome measures.

We considered the following outcomes as self-reported.

- Adverse events
- Health-related quality of life
- Body weigh as measured by participants
- Anthropometric measures other than body weight
- Socioeconomic effects

We considered the following outcomes as investigator-assessed.

- Body weight
- Adverse events
- Anthropometric measures other than body weight
- All-cause mortality
- Morbidity
- Socioeconomic effects

Risk of bias for a trial across outcomes: some risk of bias domains like selection bias (sequence generation and allocation sequence concealment) affect the risk of bias across all outcome measures in a trial. In case of high risk of selection bias, all endpoints investigated in the associated trial were marked as 'high' risk. Otherwise, we did not perform a summary assessment of the risk of bias across all outcomes for a trial.

Risk of bias for each outcome within a trial and across domains: we assessed the risk of bias for each outcome measure including all of the entries relevant to that outcome, i.e. both trial-level

entries and outcome-specific entries. 'Low' risk of bias is defined as low risk of bias for all key domains, 'unclear' risk of bias as unclear risk of bias for one or more key domains and 'high' risk of bias as high risk of bias for one or more key domains.

Risk of bias for an outcome across trials and across domains: these are our main summary assessments that were incorporated in our judgements about the quality of evidence in the 'Summary of findings' tables. 'Low' risk of bias is defined as most information coming from trials at low risk of bias, 'unclear' risk of bias as most information coming from trials at low or unclear risk of bias and 'high' risk of bias as a sufficient proportion of information coming from trials at high risk of bias.

Measures of treatment effect

Dichotomous data

When at least two trials are available for a comparison and a given outcome, we expressed dichotomous data as odds ratio (OR) or risk ratio (RR) with 95% confidence interval (CI).

Continuous data

We calculate mean differences (when trials use the same measure) or standardised mean differences (SMDs) (when trials use different measurement scales) and 95% CIs for continuous outcome measures. When necessary, we calculated effect estimates from P values, t statistics or other available statistics. For those studies, which provide only change scores, we performed separate analyses to those studies, which provide only final values. We combined both values using the generic inverse variance method.[181]

Dealing with missing data

If possible, we obtained missing data from trial authors and carefully evaluated important numerical data such as screened, randomly-assigned participants as well as intention-to-treat, and as-treated and per-protocol populations. We investigated attrition rates (e.g. drop-outs, losses to follow-up, withdrawals), and we critically appraised issues concerning missing data and imputation methods (e.g. last observation carried forward).

In trials where the standard deviation (SD) of the outcome was not available at follow-up, or cannot be recreated, we standardised by the average of the pooled baseline SD from those trials in which this information was reported.

Where means and SDs for outcomes have not been reported and we did not received the needed information from trial authors, we imputed these values by estimating the mean and variance from the median, range, and the size of the sample.[242]

We investigated the impact of imputation on meta-analyses by performing sensitivity analyses, and we reported per outcome which trials were included with imputed SDs.

Assessment of heterogeneity

We identified heterogeneity (inconsistency) by visually inspecting the forest plots and by using a standard Chi² test with a significance level of $\alpha = 0.1$. In view of the low power of this test, we also considered the I^2 statistic, which quantifies inconsistency across trials to assess the impact of heterogeneity on the meta-analysis; where an I^2 statistic $\geq 75\%$ indicates a considerable level of heterogeneity.[182–184]

When we found heterogeneity, we would have attempted to determine possible reasons for it by examining individual trial and subgroup characteristics.

Assessment of reporting biases

As we included 10 or more trials investigating a particular outcome, we used funnel plots to assess small-trial effects. Several explanations may account for funnel plot asymmetry, including true heterogeneity of effect with respect to trial size, poor methodological design (and hence bias of small trials) and publication bias. We therefore interpreted results carefully.[243]

Data synthesis

We planned to undertake (or display) a meta-analysis only if participants, interventions, comparisons and outcomes are judged to be sufficiently similar to ensure an answer that is clinically meaningful. Unless good evidence shows homogeneous effects across trials, we primarily summarised low risk of bias data using a random-effects model.[244] We interpreted random-effects meta-analyses with due consideration to the whole distribution of effects, ideally by presenting a prediction interval.[245,246] A prediction interval needs at least four trials to be

calculated and specifies a predicted range for the true treatment effect in an individual trial.[247] For rare events such as event rates below 1% we used Peto's odds ratio method, provided that there was no substantial imbalance between intervention and comparator group sizes and intervention effects are not exceptionally large. In addition, we also performed statistical analyses according to the statistical guidelines presented in the *Cochrane Handbook for Systematic Reviews of Interventions*.[248]

Subgroup analysis and investigation of heterogeneity

We expected the following characteristics to introduce clinical heterogeneity, and planned to carry out the following subgroup analyses with investigation of interactions.

- Menopausal status: pre- and post-menopausal women.
- Age: children, adults, older adults.
- Sex.
- BMI: obese, overweight.
- Physical activity: sedentary or active.
- Calcium supplementation: low dose ≤ 500 mg, moderate dose 500 to 1000 mg, high dose ≥ 1000 mg.
- Baseline energy intake: restricted energy intake or not.
- Pregnancy status.
- Type of diet as co-intervention.

Sensitivity analysis

When applicable we planned to perform sensitivity analyses to explore the influence of the following factors on effect sizes:

- Published trials.
- Taking into account risk of bias, as specified in the Assessment of risk of bias in included studies section.
- Very long or large trials to establish the extent to which they dominate the results.

Certainty of evidence

We included a 'Checklist to aid consistency and reproducibility of GRADE assessments' (Table 6.3), to help with standardisation of the 'Summary of findings' (Table 6.4).[249] We presented

results for the outcomes as described in the Types of outcome measures section above. If meta-analysis was not possible, we presented the results in a narrative format in the 'Summary of findings' table. We justified all decisions to downgrade the quality of trials using footnotes, and we made comments to aid the reader's understanding of the Cochrane Review where necessary.

We presented the overall certainty of evidence for each outcome specified under Types of outcome measures: 'Summary of findings' (Table 6.4) according to the GRADE approach, which takes into account issues related not only to internal validity (risk of bias, inconsistency, imprecision, publication bias) but also to external validity, such as directness of results. Two review authors independently rated the certainty of evidence for each outcome. Differences in assessment were solved by discussion or consultation with a third researcher.

Summary of findings table

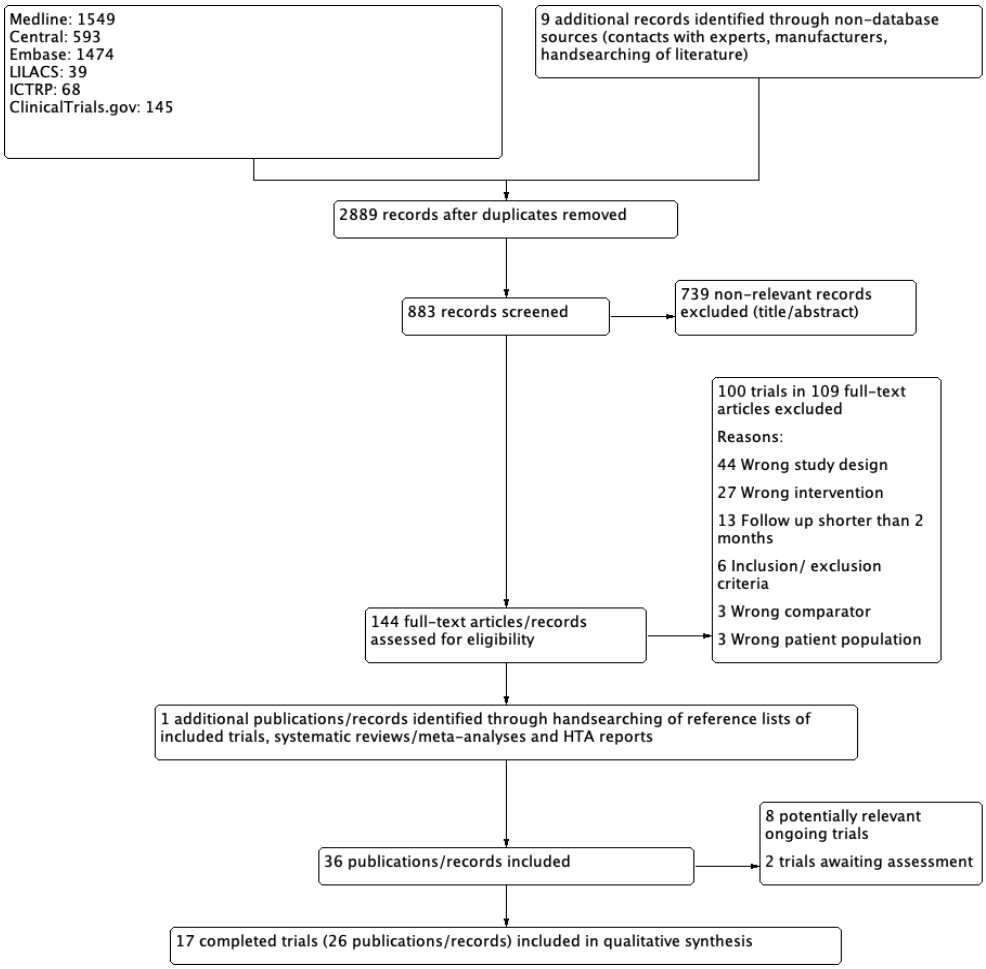
We presented a summary of the evidence in a 'Summary of findings' (Table 6.4). This provided key information about the best estimate of the magnitude of the effect, in relative terms and as absolute differences, for each relevant comparison of alternative management strategies, numbers of participants and trials addressing each important outcome and a rating of overall confidence in effect estimates for each outcome. We created the 'Summary of findings' table based on the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions* ([250]) using Review Manager (RevMan 5.3) table editor. [251] We will report the following outcomes, listed according to priority.

- Body weight
- Health-related quality of life
- Adverse events
- All-cause mortality
- Morbidity
- Socioeconomic effects.

6.4. Results

The search strategy generated 883 hits after duplicates were removed. After screening of titles and abstracts 144 papers remained to evaluate for formal inclusion and exclusion criteria. Seventeen randomised controlled trials (RCTs) reported in 26 articles fulfilled the inclusion criteria and were included in the review (See Figure 6.1). We also identified eight ongoing trials.

Figure 6.1: Prisma Diagram Flow of Screened and included studies



We identified two main comparisons: fifteen trials compared calcium versus placebo (Asemi 2015; Li 2010; Menon 2009; Palacios 2009; Ricci 1998; Shalileh 2010; Shapses 2001; Shapses 2004; Shidfar 2011; Tabesh 2015; Wang 2009; Wagner 2007; Yanovski 2009; Zemel 2004; Zemel 2009) [252–264] [265] and two trials compared a high and a low dose of calcium supplementation, participants in the controlled group received calcium and placebo in order to achieve a lower dose (Riedt 2005; Riedt 2007).[266,267] None of the trails included assessed food fortification.

Six trials had no co-intervention (Asemi 2015; Li 2010; Palacios 2009; Shidfar 2011; Wang 2009; Yanovski 2009)[252–254,258,263], eleven trials had a weight reduction co-intervention - two trials had diet counselling (Ricci 1998; Shapses 2001)[259,261], five trials a 500 kcal/day restriction diet (Menon 2009; Shalileh 2010; Shapses 2004; Zemel 2004; Zemel 2009) [255–257,260,262], one trial had a 500 kcal restriction diet plus physical activity (Wagner 2007) [268], two trials a diet of no

more than 1200 to 1500 kcal a day (Riedt 2005; Riedt 2007) [266,267] and one had vitamin D as co-intervention (Tabesh 2015)[264].

Overview of trial populations

1255 participants were randomised in total, with 961 participants finishing their respective study, 76.2% of participants finishing the trial in the intervention and 77.0% in the controlled groups. Individual sample size ranging from 15 (Menon 2009) to 335 (Yanovski 2009) [268].

Trial design

All seventeen RCTs had a parallel and a superiority design. Only one study was performed in four different sites (Zemel 2009[256]), three studies reporting having one site (Wagner 2007; Wang 2009; Yanovski 2009[254,265,268]) and for the rest of the studies the information was missing.

Four of seventeen trials were double-blinded for participants and personnel (Li 2010, Palacios 2009, Tabesh 2015; Wang 2009[253,258,264,269]) and thirteen did not report blinding for participants and personnel. Two of seventeen trials (Palacios 2009, Wang 2009; Yanovski 2009[254,258]) reported blinding for outcome assessors.

Trials were published from year 1998 to year 2015. Only eight trials reported the run-in period, and for these trials the earliest recruitment started during the year 2000 (Riedt 2005)[266] and the latest recruitment finished in 2014 (Shapses 2004[262]) (See Table 6.2).

The duration of the trial intervention ranged between 8 weeks to 24 months. None of the studies assessed the effect after finishing the intervention; therefore the follow-up period was the same as the intervention period. None of the trials reported anticipated termination of the trial.

Settings

Out of the seventeen included trials, nine were performed in the USA (Ricci 1998; Riedt 2005; Riedt 2007; Shapses 2001; Shapses 2004; Wagner 2007; Yanovski 2009; Zemel 2004; Zemel 2009) [65,254,259,261,262,266–268,270], four in Iran (Asemi 2015; Shalileh 2010; Shidfar 2011; Tabesh 2015) [252,260,263,264], one in Brasil (Menon 2009) [257], two in China (Li 2010; Wang 2009) [253,265] and one in Puerto Rico (Palacios 2009)[258].

Table 6.2: Characteristics of included studies

Trial ID	Intervention(s) and comparator(s) (in mg of elemental calcium)	Trial Duration	Country	Age (mean years (SD))	Body weight (mean kg (SD))	BMI (mean kg/m² (SD))
Tabesh 2015	I: 1000 mg as calcium carbonate	8 weeks	Iran	53.7 (5.7)	76.1 (8.8)	30.4 (4.2)
	C: placebo			51 (6.1)	77.4 (10.5)	30.3 (3.8)
Asemi 2015	I: 1000 mg as calcium carbonate	8 weeks	Iran	25 (6.7)	71.5 (11.9)	28.3 (4.7)
	C: placebo			24.3 (5.2)	71.9 (13.8)	27.5 (5.2)
Shidfar 2011	I: 1250 mg as calcium carbonate	8 weeks	Iran	35.1 (4.8)	81.4 (7.7)	27.5 (1.6)
	C: placebo			33.8 (4.8)	85.6 (8)	27.9 (1.8)
Shalileh 2010	I: 1000 mg as calcium carbonate	24 weeks	Iran	36.6 (7.8)	77.7 (16.9)	29.7 (3.9)
	C: placebo			36.6 (8)	76.3 (8.2)	29.8 (3.3)
Li 2010	I: 162 mg as calcium carbonate	26 weeks	China	41.6 (9)	77.5 (8)	30.5 (2.5)
	C: placebo			41.2 (6.8)	80.8 (9.7)	31.1 (2.7)
Menon 2009	I: 400 mg as calcium carbonate	3 months	Brasil	—	80 (1)	34 (5)
	C: placebo			—	87 (9)	34 (4)
Palacios 2009	I: 600 mg as calcium carbonate	21 weeks	Puerto Rico	35.3 (2.2)	115.2 (5.6)	38.5 (1.9)
	C: placebo	—		39.5 (2.2)	104.9 (4.5)	36.7 (1.6)
Yanovski 2009	I: 1500 mg as calcium carbonate	24 months	USA	38.9 (10.5)	94.5 (20.5)	33.2 (6.8)
	C: placebo			38.7 (10.4)	94 (20.5)	33.6 (6.8)
Zemel 2009	I: 900 mg as calcium carbonate	12 weeks	USA	26.2 (4.8)	82.7 (14.8)	29.9 (2.6)
	C: placebo			25.3 (4.9)	80.1 (12.4)	29.4 (2.7)
Wagner 2007	I1: 800 mg as calcium phosphate	12 weeks	USA	41.6 (1.6)	196.7 (8.9)	33.4 (1.4)
	I2: 800 mg as calcium lactate	—		40.2 (1.8)	196.2 (5.8)	33.3 (0.7)
	C: placebo	—		36 (2.2)	190.7 (11.3)	32.4 (1.5)
Riedt 2007	I: 1000 mg as calcium citrate	6 months	USA	38 (6.4)	73.2 (4.4)	27.7 (2.1)
	C: 200 mg as calcium citrate			38 (6.4)	71.4 (6.7)	27.7 (2.1)
Riedt 2005	I: 1000 mg as calcium citrate	6 months	USA	61.3 (6.7)	71.3 (6.4)	26.9 (2.1)
	C: 200 mg as calcium citrate			61.6 (6.4)	73.8 (6.9)	27.2 (1.7)
Zemel 2004	I: 800 mg as calcium carbonate	24 weeks	USA	46 (8)	99.8 (4.5)	35 (4.1)
	C: placebo			46 (8)	103.1 (6.1)	35 (4.1)
Shapses 2004	I: 1000 mg as calcium citrate malate or calcium citrate	25 weeks	USA	PostM 61.6 (8.6) / PreM 40.4 (5.4)	PostM 84.1 (9.4) / PreM 93.7 (13.6)	PostM 32.1 (3.5) / PreM 33.9 (3.9)
	C: placebo			PostM 57 (8.2) / PreM 41.5 (6.8)	PostM 89.4 (10.3) / PreM 93.5 (14.3)	PostM 32.8 (4.2) / PreM 34.7 (5.9)
Shapses 2001	I: 1000 mg as calcium citrate malate	6 months	USA	40.4 (5.8)	94.9 (14.9)	34 (3.9)
	C: placebo			40.4 (5.8)	93.8 (13)	34.5 (3.4)
Ricci 1998	I: 1000 mg as calcium citrate malate	6 months	USA	58.3 (9.1)	88.5 (12.9)	33.2 (4.6)
	C: placebo			58.3 (9.1)	88.0 (9.9)	32.9 (4.5)

I= intervention. C= comparison, PostM= postmenopausal , PreM premenopausal

Participants

A total of 369 participants were from low- and middle-income countries from Latin America and Asia and 879 were from the USA. There were no studies from Africa, Europe or Oceania. No ethnic groups were identified in the studies.

All seventeen studies included adults and older adults participants ranging from 18 to 80 years, however one trial did not specify the age of participants (Menon 2009)[257]. Ten trials included only women (Asemi 2015; Li 2010; Menon 2009; Ricci 1998; Riedt 2005; Riedt 2007; Shapses 2001; Shapses 2004; Wagner 2007; Wang 2009)[252,253,257,259,261,262,265–268], six trials included men and women (Palacios 2009; Shalileh 2010; Tabesh 2015; Yanovski 2009; Zemel 2004; Zemel 2009)[65,254,258,260,264,270] and one included only men (Shidfar 2011)[263]. Of the ten trials including only women; three were specifically in premenopausal women (Riedt 2007; Shapses 2001; Wagner 2007) [261,267,268] two were specifically about postmenopausal women (Ricci 1998; Riedt 2005) [259,266]; two in pre and postmenopausal women (Shapses 2004; Wang 2009)[262,265] and the remaining three did not specify (Asemi 2015; Li 2010; Menon 2009)[252,253,257].

Four of seventeen trials reported co morbidities. One included women with polycystic ovarian syndrome and who were vitamin D deficient (Asemi 2015)[252], one calcium stone formers (Menon 2009)[257], one included type 2 diabetic subjects (Tabesh 2015)[264] and one study included participants with increased risk for cardiovascular disease (Wang 2009)[265]. Major exclusion criteria of participants in the included trials were taking supplements or any medication for a chronic disease, having cardiovascular or renal disease, having a recent weight change.

Diagnosis

All participants included in the trial were classified as overweight or obese. Three trials included overweight participants with BMI between 25 to less than 30 kg/m^2.

Interventions

One of seventeen trials reported studying the participants before start of the trial.[256]

All interventions were supplements taken orally. Daily dose of elemental calcium ranged from 0.162 to 1.5 grams. Most frequent dose was 1 gram/day that was used in eight out of sixteen studies.

Out of the 17 included studies, eleven studies used calcium carbonate as the intervention salt (Asemi 2015; Menon 2009; Palacios 2009; Shalileh 2010; Shapses 2001; Shidfar 2011; Tabesh

2015; Wang 2009; Yanovski 2009; Zemel 2004; Zemel 2009)[252,254,270,256–258,260,261,263–265], two used citrate (Riedt 2005; Riedt 2007)[266,267], one calcium citrate or calcium citrate malate (Shapses 2004)[262], one study calcium citrate malate (Ricci 1998)[259], one study used calcium lactate and calcium phosphate (Wagner 2007)[268]. In one trial (Li 2010)[253] it was not specified the salt used.

All seventeen trials used placebo. However, two trials compared a high and a low dose of calcium supplementation, to achieve a lower dose participants in the controlled group received , one with calcium and the other one with placebo (Riedt 2005; Riedt 2007). [266,267]

Outcomes

Eight of seventeen trials explicitly stated a primary outcome in the publication (Asemi 2015; Li 2010; Menon 2009; Palacios 2009; Shalileh 2010, Shapses 2004; Wang 2009; Yanovski 2009)[252–254,257,258,260,262,265]. Most commonly defined primary outcomes in publications were body weight and fat mass (Li 2010; Menon 2009; Palacios 2009; Shapses 2004; Yanovski 2009)[253,254,257,258,262].

Only three trials had the protocol registered (Asemi 2015; Tabesh 2015; Yanovski 2009)[252,254,264]. Two trials (Asemi 2015; Yanovski 2009)[254,271] were registered before starting recruitment and reported primary outcomes stated in the protocol. One trial was registered days after starting recruitment and their stated main outcome in the publication is their secondary outcome of their published protocol.

Primary outcomes

Fifteen of seventeen trials provided body weight as endpoint measurement (Li 2010; Menon 2009; Palacios 2009; Ricci 1998; Riedt 2005; Riedt 2007; Shalileh 2010; Shapses 2004; Tabesh 2015; Wagner 2007; Yanovski 2009; Zemel 2004; Zemel 2009)[253–260,262,264,266–268]. One trial provided BMI that is a secondary outcome in our systematic review (Shidfar 2011)[263]. None of the trials reported health-related quality of life or adverse events as endpoints.

One study did not report any of the primary outcomes of this review (Wang 2009)[265].

Secondary outcomes

Three trails reported waist circumference (Asemi 2015; Menon 2009; Tabesh 2015)[252,257,264]. Five trials reported BMI (Asemi 2015; Menon 2009; Shapses 2001; Tabesh 2015; Yanovski 2009)[252,254,257,261,264].

None of the trials investigated all-cause mortality, morbidity/complications or socioeconomic effects.

Excluded studies

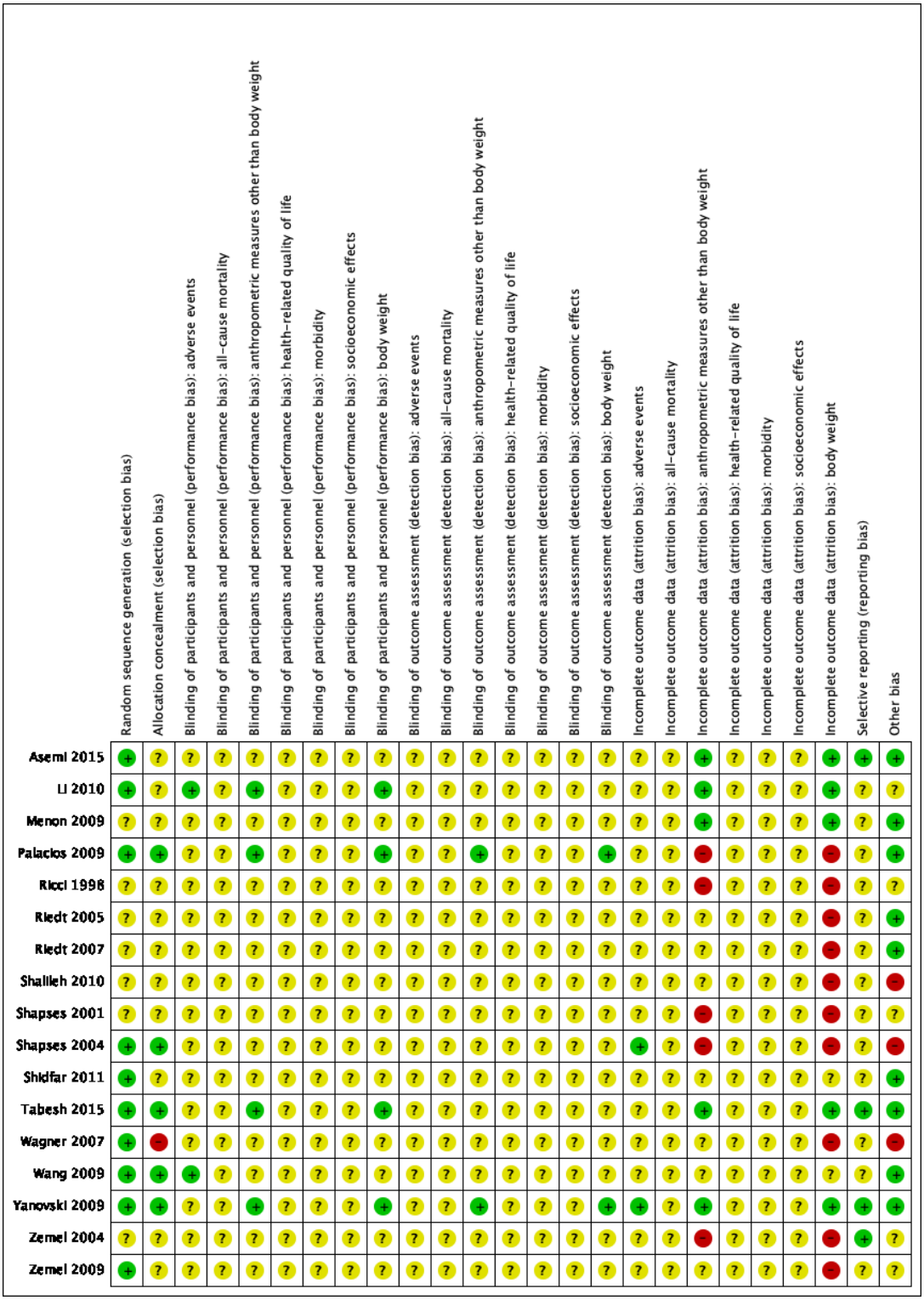
After evaluation of the full publication, we excluded 109 full-text articles. The main reasons for exclusion were design not being an RCT, a wrong intervention or duration of less than 2 months.

Risk of bias in included studies

Each trial was assessed for the following seven domains: (1) random sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) incomplete outcome data, (6) selective reporting and (7) other bias (See Figure 6.2 and Figure 6.3).

No studies were judged to have a low risk of bias in all seven domains. One study reported to have low risk of bias for six domains (Yanovski 2009)[254] and another study reported to have low risk of bias for 5 domains (Palacios 2009[258]). Two studies were judged to have a low risk of bias in four domains (Asemi 2015, Li 2010)[252,253]. One study was judged to have a low risk of bias in three domains (Tabesh 2015)[264]. Four studies were judged to have a low risk of bias in two domains (Shidfar 2011, Menon 2009, Shapses 2004, Zemel 2004)[257,262,263,270]. Two studies were judged to have a low risk of bias in one domain: Riedt 2005, Riedt 2007.[266,267] Five studies had no domain judged as low risk of bias (Ricci 1998, Shalileh 2010, Shapses 2001, Wagner 2007, Zemel 2009)[256,259–261,268].

Figure 6.2: Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



For Palacios 2009, much information was gathered from correspondence with the author.[258]

For the following domains, the studies were assessed both on a study level and an endpoint level (primary outcome) individually: blinding of participants and personnel, blinding of outcome

assessment and incomplete outcome data. Wang 2009 was only assessed on study level, as it did not report any primary or secondary outcomes for this review.[265] The rest of the sixteen studies were assessed for the primary outcome, body weight. The following five studies Asemi 2015; Menon 2009; Shapses 2001; Tabesh 2015; Yanovski 2009 were assessed for the outcome BMI (secondary outcome: anthropometric measures other than body weight for this review).[252,254,257,261,264]

Allocation (selection bias)

The following six studies were judged to have a low risk of selection bias by adequately describing the generation of random sequence: Asemi 2015, Li 2010, Shapses 2004, Shidfar 2011, Wang 2009; Yanovski 2009.[252–254,262,263,265] The following eleven studies were judged to have an unclear risk of bias due to insufficient information: Menon 2009, Palacios 2009, Ricci 1998, Riedt 2005, Riedt 2007, Shalileh 2010, Shapses 2001, Tabesh 2015, Wagner 2007, Zemel 2004 and Zemel 2009.[189,256–261,264,266–268]

Blinding (performance bias and detection bias)

Only four of seventeen trials reported being double-blinded for participants and personnel (Li 2010, Palacios 2009, Tabesh 2015, Yanovski 2009)[253,254,258,264]. Only two of seventeen trials (Palacios 2009, Yanovski 2009)[254,258] reported blinding for outcome assessors.

Incomplete outcome data (attrition bias)

Percentage of attrition rates (range) are described in all trials with losses to follow-up; three of seventeen trials reported intention-to-treat analysis (Asemi 2015; Yanovski 2009; Zemel 2009)[252,254,256], one of seventeen trials had no loss to follow-up (Menon 2009[257]). Seven of seventeen trials (Asemi 2015; Li 2010; Palacios 2009; Ricci 1998; Riedt 2007; Shalileh 2010; Shidfar 2011)[252,253,258–260,263,267] had loss to follow-up and a detailed description of reasons for participants' withdrawals.

Ten of seventeen trials (Palacios 2009; Ricci 1998; Riedt 2005; Riedt 2007; Shalileh 2010; Shapses 2001; Shapses 2004; Wagner 2007; Zemel 2004; Zemel 2009)[255,256,258–262,266–268] had attrition rates with possible impact on body weight and BMI outcomes, specially Ricci 1998; Riedt 2005 that excluded women of the analysis for not losing weight.[259,266]

Selective reporting (reporting bias)

Only three trials had the protocol registered (Asemi 2015; Tabesh 2015; Yanovski 2009)[252,254,264]. Two trials (Asemi 2015; Yanovski 2009)[252,254] were registered before staring recruitment and reported primary outcomes stated in the protocol. One trial was registered days after starting recruitment and the stated main outcome in the publication the secondary outcome of the published protocol.

Other potential sources of bias

Three studies reported that baseline characteristics including anthropometric measurements and gender were different between allocation groups (Shalileh 2010; Shapses 2004; Wagner 2007)[260,262,268] and three studies did not report this baseline measurements for which they were classified as unclear risk (Ricci 1998; Shapses 2001; Zemel 2009)[256,259,261].

Figure 6.3: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

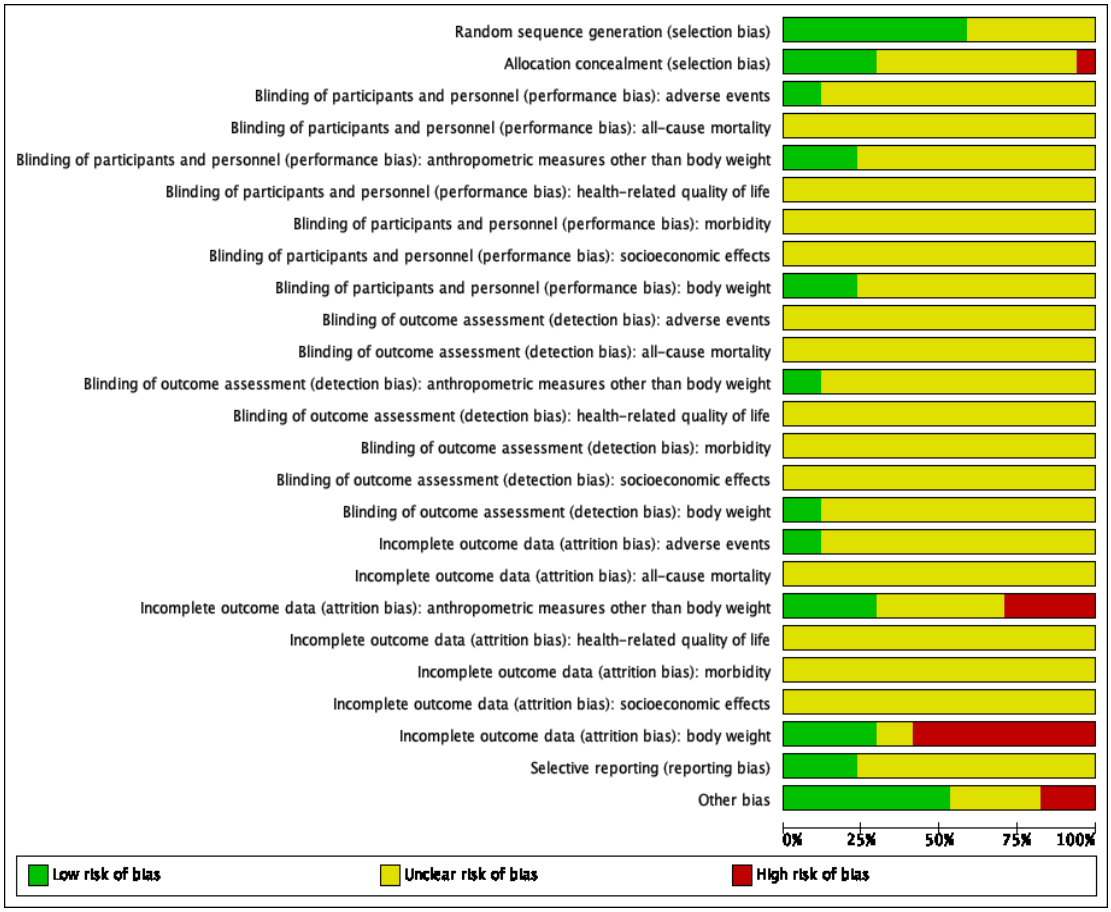


Table 6.3: Checklist to aid consistency and reproducibility of GRADE assessments

	Body weight	
Trial limitations (risk of bias)[a]	Was random sequence generation used (i.e. no potential for selection bias)?	Unclear
	Was allocation concealment used (i.e. no potential for selection bias)?	Unclear
	Was there blinding of participants and personnel (i.e. no potential for performance bias) or outcome not likely to be influenced by lack of blinding?	Unclear
	Was there blinding of outcome assessment (i.e. no potential for detection bias) or was outcome measurement not likely to be influenced by lack of blinding?	Unclear
	Was an objective outcome used?	Yes
	Were more than 80% of participants enrolled in trials included in the analysis (i.e. no potential reporting bias)?[e]	No (↓)
	Were data reported consistently for the outcome of interest (i.e. no potential selective reporting)?	Yes
	No other biases reported (i.e. no potential of other bias)?	No (↓)
	Did the trials end up as scheduled (i.e. not stopped early)?	Yes
Inconsistency[b]	Point estimates did not vary widely?	Yes
	To what extent did confidence intervals overlap (substantial: all confidence intervals overlap at least one of the included studies point estimate; some: confidence intervals overlap but not all overlap at least one point estimate; no: at least one outlier: where the confidence interval of some of the studies do not overlap with those of most included studies)?	Substantial
	Was the direction of effect consistent?	Yes
	What was the magnitude of statistical heterogeneity (as measured by I^2) - low (I^2<40%), moderate (I^2 40%-60%), high I^2>60%)?	Low
	Was the test for heterogeneity statistically significant (0.1)?	No
Indirectness	Were the populations in included studies applicable to the decision context?	Yes
	Were the interventions in the included studies applicable to the decision context?	Yes
	Was the included outcome not a surrogate outcome?	Yes
	Was the outcome timeframe sufficient?	Sufficient
	Were the conclusions based on direct comparisons?	Yes
Imprecision[c]	Was the confidence interval for the pooled estimate not consistent with benefit and harm?	Yes
	What is the magnitude of the median sample size (high: 300 participants, intermediate: 100-300 participants, low: <100 participants)?[e]	Low (↓)
	What was the magnitude of the number of included studies (large: >10 studies, moderate: 5-10 studies, small: <5 studies)?[e]	Large
	Was the outcome a common event (e.g. occurs more than 1/100)?	N/A
Publication bias[d]	Was a comprehensive search conducted?	Yes
	Was grey literature searched?	No (↓)
	Were no restrictions applied to study selection on the basis of language?	Yes
	There was no industry influence on studies included in the review?	Yes
	There was no evidence of funnel plot asymmetry?	Yes
	There was no discrepancy in findings between published and unpublished trials?	Yes

[a] Questions on risk of bias are answered in relation to the majority of the aggregated evidence in the meta-analysis rather than to individual trials
[b] Questions on inconsistency are primarily based on visual assessment of forest plots and the statistical quantification of heterogeneity based on I^2
[c] When judging the width of the confidence interval it is recommended to use a clinical decision threshold to assess whether the imprecision is clinically meaningful
[d] Questions address comprehensiveness of the search strategy, industry influence, funnel plot asymmetry and discrepancies between published and unpublished trials
[e] Depends on the context of the systematic review area
(↓): key item for potential downgrading the quality of the evidence (GRADE) as shown in the footnotes of the 'Summary of findings' table(s); GRADE: Grading of Recommendations Assessment, Development and Evaluation; N/A: not applicable

The quality of the evidence is Moderate as shown analysed by the GRADE methodology and shown in the Summary of findings table (Table 6.4). There was a downgrade as one of the four points, quality for methodological limitations, was unclear for some items in several studies (Figure 6.2 and 6.3). However the results of the meta-analysis were consistent, the confidence

intervals were precise, the body weight and BMI outcomes were directly measured and there was no evidence of publication bias (Figure 6.4 to Figure 6.13).

Table 6.4: Summary of findings table

Outcomes	Assumed risk Control	Corresponding risk Calcium	Relative effect (95% CI)	No of participants (trials)	Quality of the evidence (GRADE)
Body weight (kg) Follow-up: 8 weeks to 24 months	The mean body weight change ranged across control groups from -8.8 to 7.0 kg	The mean body weight change ranged across intervention groups from -9.0 to 5.5 kg	MD -0.33 kg in the intervention group; 95% CI -0.57 to -0.09	705 (11)	⊕⊕⊕⊖[1] Moderate
Health-related quality of life	No trials reported health-related quality of life				
Adverse events	One trial reported foot surgery in a participant who received placebo. [254]	One trial reported "2 women that withdrew the study for health problems.[252] 3 women discontinued the study "2 for constipation and one for cancer".[262]			⊕⊖⊖⊖ **very low**
All-cause mortality	No trials reported all-cause mortality				
Morbidity	No trials reported morbidity				
Socioeconomic effects	No trials reported socioeconomic effects				

The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **MD:** mean difference; **RR:** risk ratio; BMI: body mass index

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Downgraded due to serious methodological limitations as most risk of bias domains were uncertain.

Effects of interventions

The summary of the results are presented in Table 6.4: Summary of findings and Table 6.3 consistency and reproducibility of GRADE assessments.

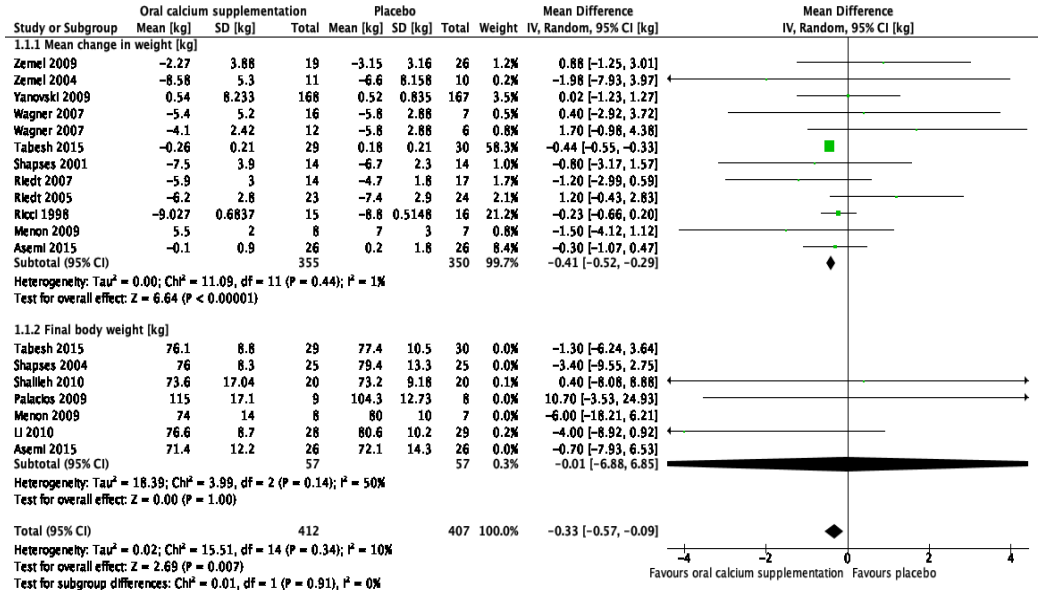
Primary outcomes

Body weight

Fifteen trials (Asemi 2015; Li 2010; Menon 2009; Palacios 2009; Ricci 1998; Riedt 2005; Riedt 2007; Shalileh 2010; Shapses 2001; Shapses 2004; Tabesh 2015; Wagner 2007; Yanovski 2009; Zemel 2004; Zemel 2009)[189,252–254,256,257,259–262,264,266–268] provided data for body weight.

Including all studies in a random-effects meta-analyses, calcium supplementation compared to placebo reduces body weight (Mean Difference (MD) -0.33 kg, 95% CI -0.57 to -0.09); ($p < 0.007$) with no considerable heterogeneity detected between the studies; $I^2 = 10\%$; 819 participants; 15 studies) (Figure 6.4).

Figure 6.4: Oral calcium supplementation versus placebo: Primary Outcome: Body weight in kg. Random effect



We found no studies assessing beverage fortification versus placebo or beverage fortification versus non-calcium fortified food.

Health-related quality of life

None of the included trials reported health related quality of life.

Adverse events

None of the included trials reported adverse events.

Secondary outcomes

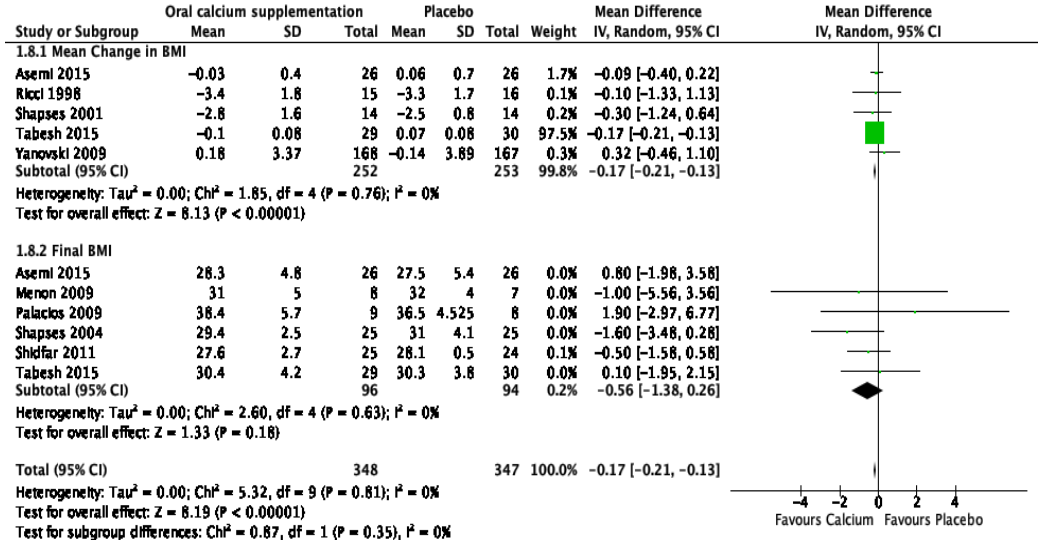
Anthropometric measures other than body weight

BMI

Nine trials (Asemi 2015; Menon 2009; Palacios 2009; Ricci 1998; Shapses 2001; Shapses 2004; Shidfar 2011; Tabesh 2015; Yanovski 2009)[252,254,257–259,261–264] provided data for BMI. Including all studies in a random-effects meta-analyses, calcium supplementation compared to placebo

reduces BMI (MD -0.17, 95% CI -0.21 to -0.13); p < 0.00001 with no heterogeneity detected between the studies; I^2 = 0%; 695 participants; 10 studies).

Figure 6.6: Oral calcium supplementation versus placebo: Outcome: Body Mass Index (BMI). Random effect



All-cause mortality

None of the included trials reported this outcome.

Morbidity

None of the included trials reported this outcome.

Socioeconomic effects

None of the included trials reported this outcome.

Calcium food or beverage fortification versus placebo

We did not include any study measuring food or beverage fortification vs placebo.

Calcium food or beverage fortification versus non-calcium fortified food or beverage

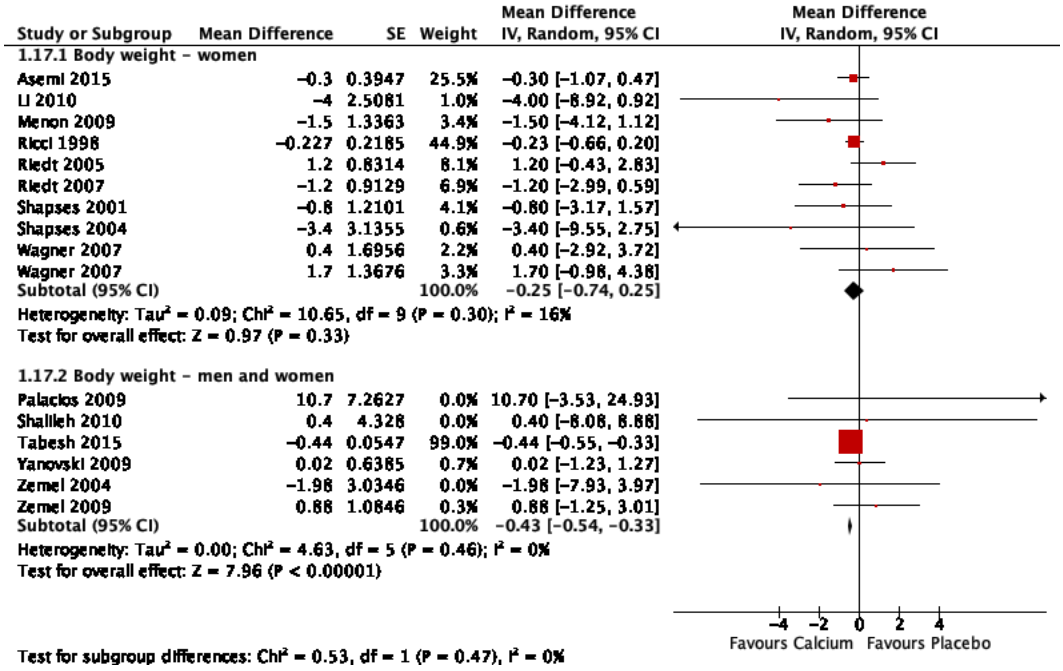
We did not include any study measuring food or beverage fortification vs none-fortified food or beverage.

Subgroup analyses

We did not perform pregnancy status or age subgroup analyses because there were not enough studies to estimate effects.

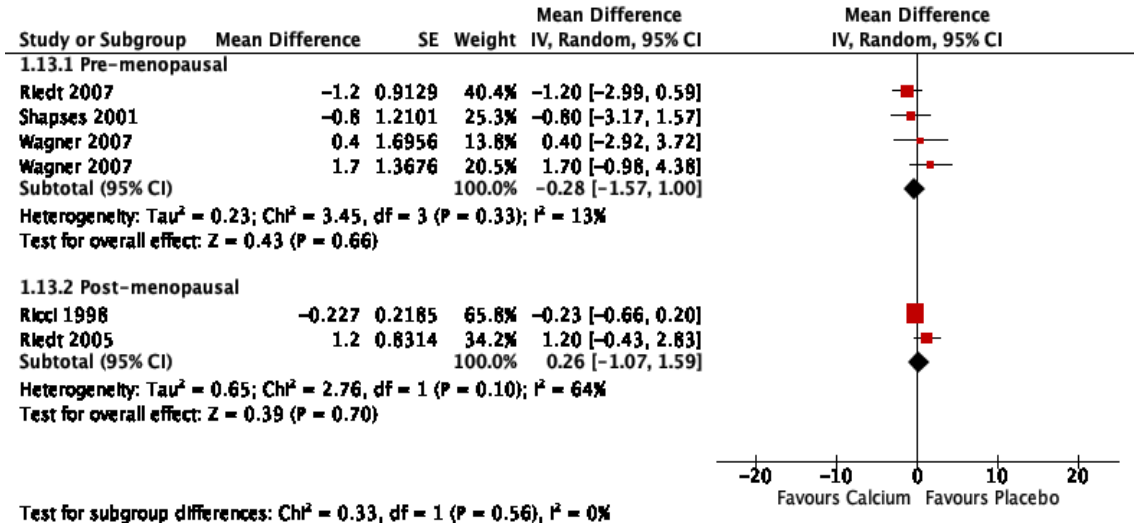
- Gender: Nine trials included only women (Asemi 2015; Li 2010; Menon 2009; Ricci 1998; Riedt 2005; Riedt 2007; Shapses 2001; Shapses 2004; Wagner 2007[252,253,257,259,261,262,266–268]), six trials included men and women (Palacios 2009; Shalileh 2010; Tabesh 2015; Yanovski 2009; Zemel 2004; Zemel 2009[254–256,258,260,264]) and one included only men, however the study only reported BMI (Shidfar 2011[263]). The studies including men and women showed a reduction of calcium supplementation compared to placebo in body weight of (MD -0.43 kg, 95% CI -0.54 to -0.33), however the evidence including only women was no strong (MD -0.25 kg, 95% CI -0.74 to 0.25).

Figure 6.7: Oral calcium supplementation versus placebo: Outcome: Body weight by gender. Random effect



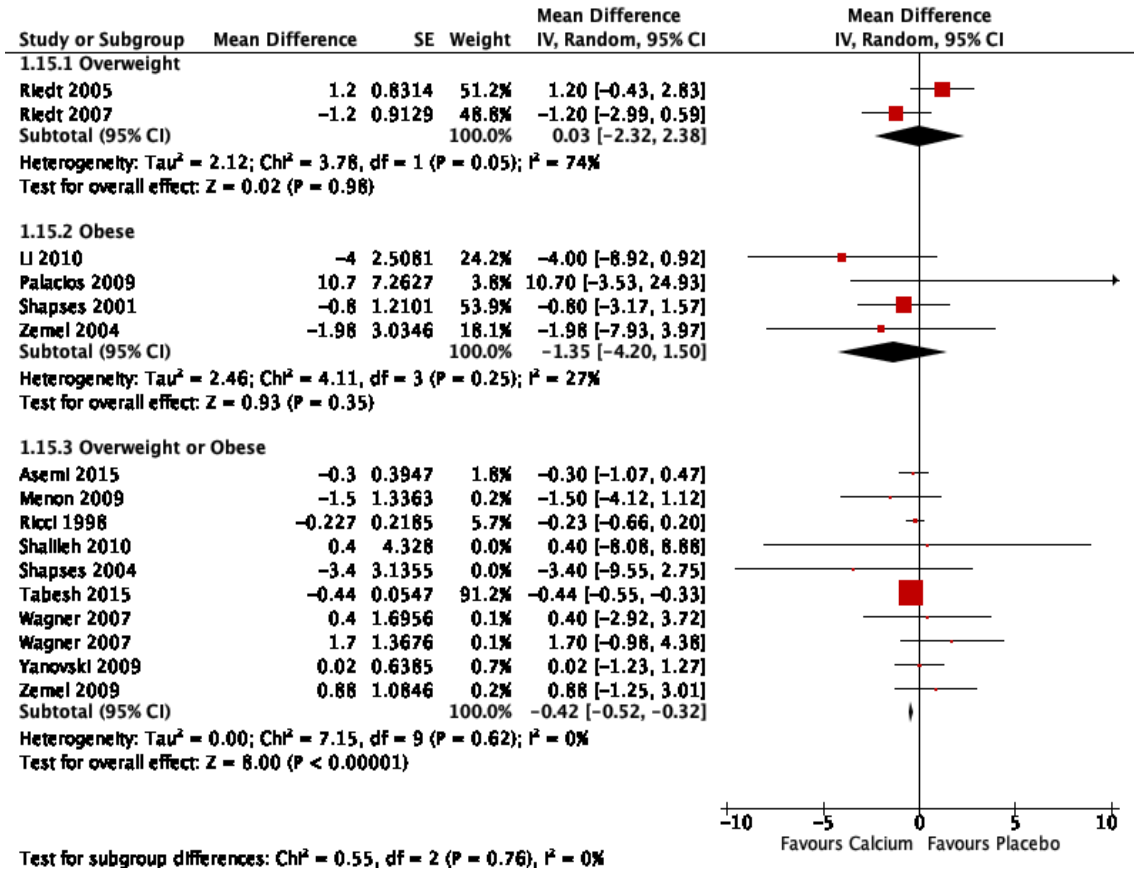
- Menopausal status: pre- and post-menopausal women. Eleven trials included only women; three were specifically in premenopausal women (Riedt 2007; Shapses 2001; Wagner 2007)[261,267,268] two were specifically about postmenopausal women (Ricci 1998; Riedt 2005)[259,266]; one in pre and postmenopausal women (Shapses 2004)[262] and the remaining four did not specify (Asemi 2015; Li 2010; Menon 2009; Palacios 2009)[253,257,258,271]. The meta-analysis of only those studies reporting post-menopausal status shows no effect of calcium supplementation on body weight (MD 0.26, 95% CI -1.07 to 1.59) p = 0.70, nor if these studies are analysed by pre-menopausal women (MD -0.28, 95% CI -1.57 to 1.00) p = 0.66).

Figure 6.8: Oral calcium supplementation versus placebo: Outcome: Body weight by menopausal status



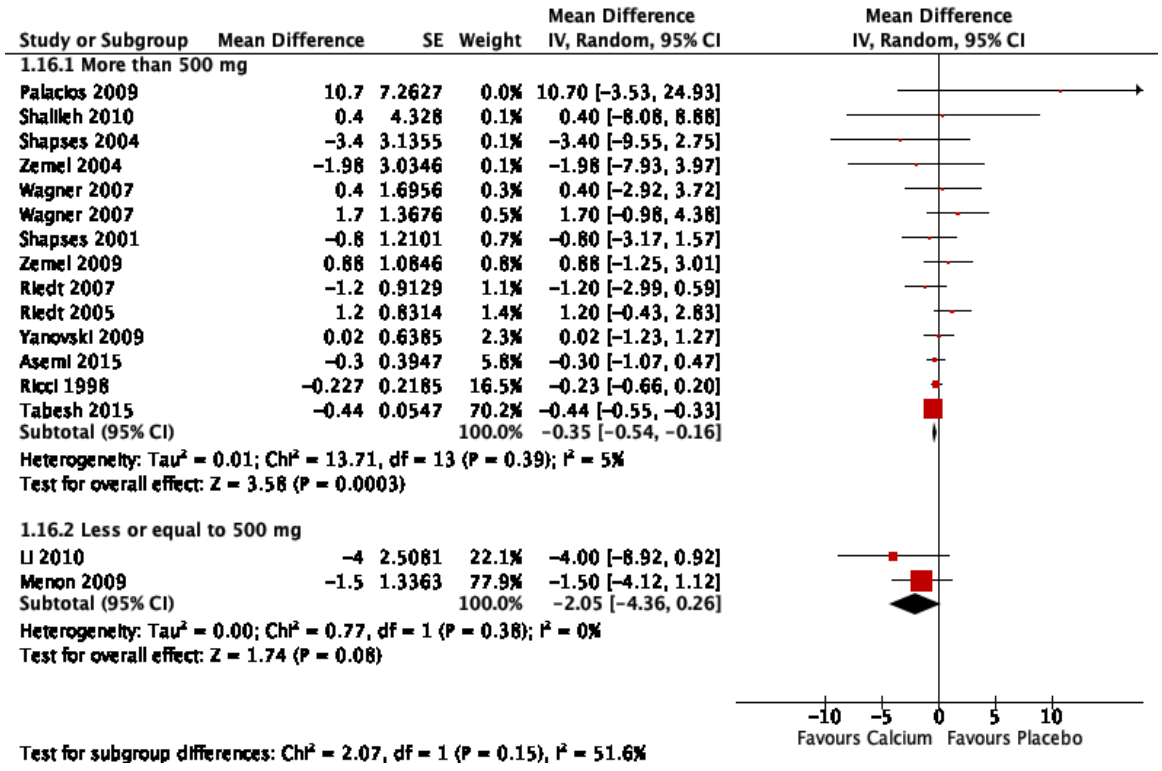
- Basal BMI: Three trials included only overweight participants (Riedt 2005; Riedt 2007; Shidfar 2011)[263,266,267] and four trials only included obese participants; (Palacios 2009; Li 2010; Shapses 2001; Zemel 2004)[253,255,258,261]. Nine trails included participants that were overweight or obese (Asemi 2015; Menon 2009; Ricci 1998; Shapses 2004; Wagner 2007; Shalileh 2010; Tabesh 2015; Yanovski 2009; Zemel 2009)[252,254,256,257,259,260,262,264,268]. There is no effect of calcium supplementation in the meta-analysis of those trials including only overweight (MD 0.03, 95% CI -2.32 to 2.38) p = 0.98 or obese subjects (MD -1.35, 95% CI -4.20 to 1.50) p = 0.35.

Figure 6.9: Oral calcium supplementation versus placebo: Outcome: Body weight by BMI status



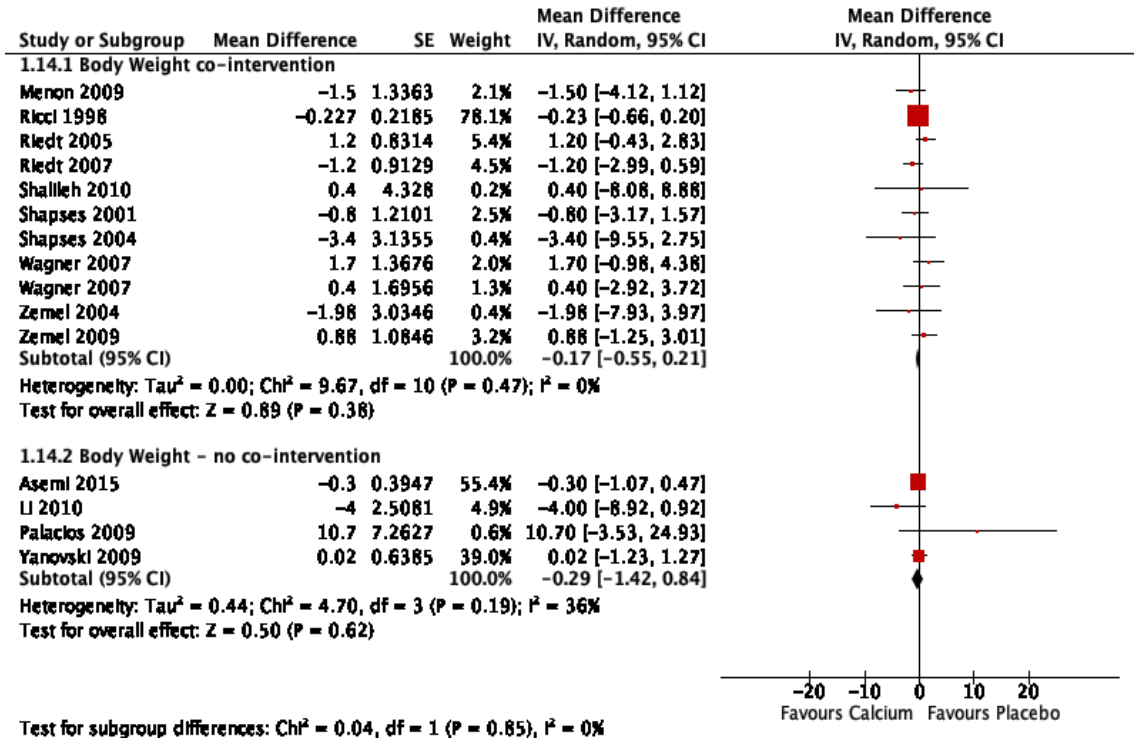
- Calcium supplementation: low dose ≤ 500 mg, moderate dose 500 to 1000 mg, high dose ≥ 1000 mg (Figure 6.10). Most frequent dose was 1 gram/day that was used in eight out of sixteen studies (Asemi 2015; Ricci 1998; Riedt 2005; Riedt 2007; Shalileh 2010; Shapses 2001; Shapses 2004; Tabesh 2015)[252,259–262,264,266,267]. Two studies used higher doses, one 1.25 grams/day (Shidfar 2011)[263] and one 1.5 grams/day (Yanovski 2009)[254]. Two trials used 0.8 grams/day (Wagner 2007; Zemel 2004)[268,270]; one 0.9 grams/day (Zemel 2009)[256] and one 0.6 grams/day (Palacios 2009[258]). Two studies used doses less than 0.5 grams/day; Menon 2009 [257] used 0.4 and Li 2010 [253] used 0.162 grams a day. Those studies with doses of more than 500 mg a day showed a statistical significant evidence favouring calcium supplementation as compared to placebo (MD -0.35 kg, 95% CI -0.54 to -0.16); p = 0.0003 and those studies with doses of 500 mg a day or less showed a no statistically significant effect in the same direction (MD -2.05 kg, 95% CI -4.36 to 0.26); p = 0.08.

Figure 6.10: Oral calcium supplementation versus placebo: Outcome: Body weight by intervention dose



- Type of co-intervention: A subgroup analysis, as specified in the protocol for this review, was conducted to assess the effect that the presence or absence of a co-intervention may have on weight loss. Four trials did not have a co-intervention (Asemi 2015; Li 2010; Palacios 2009; Yanovski 2009)[252–254,258], and eleven studies had a co-intervention for both the calcium and placebo groups. Only one had as co-intervention vitamin D and therefore excluded from this subgroup meta-analysis. Studies without co-intervention or with co-intervention showed a no statistically significant effect favouring calcium. The mean difference for those without co-intervention was MD -0.29, (95% CI -1.42 to 0.84); p= 0.62 with moderate heterogeneity I^2 = 36% and for those with energy restriction or diet counselling was MD -0.17, (95% CI -0.55 to 0.21); p= 0.38; with no heterogeneity I^2 = 0%. There was no difference in the effect of calcium in body weight according to the presence or absence of co-intervention.

Figure 6.11: Oral calcium supplementation versus placebo: Outcome: Body weight by type of co-intervention

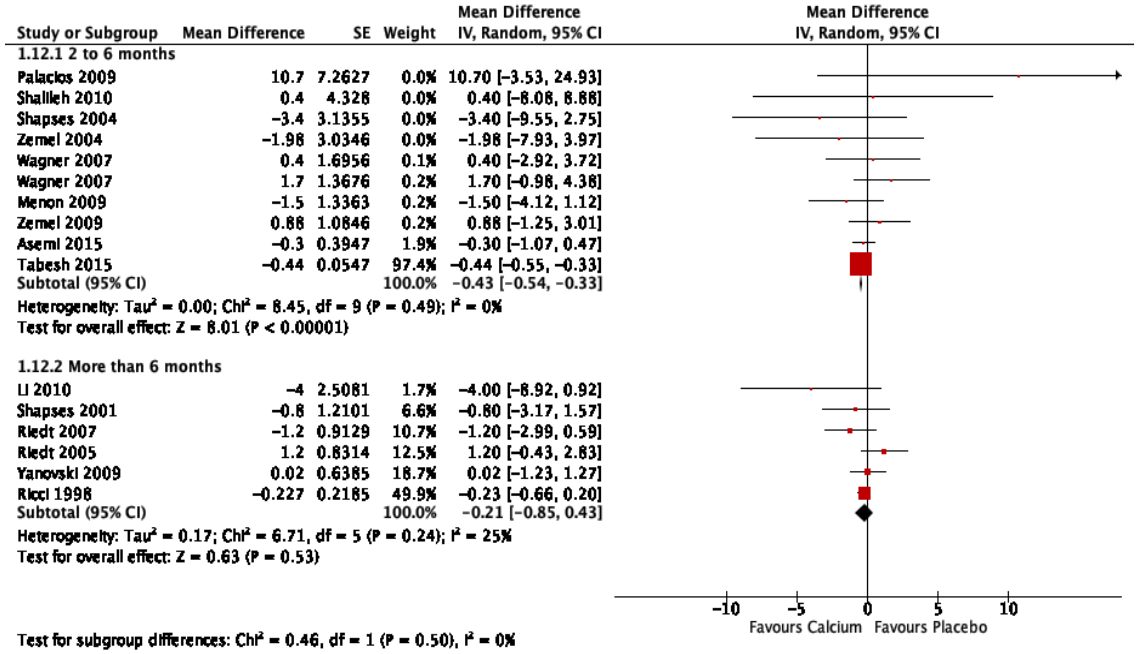


Sensitivity analyses

We performed sensitivity analyses for the following factors:

- Publication status: all trials were published
- Very long or large trials to establish the extent to which they dominate the results. There was a significant effect in the short duration trials of less than 6 months (MD -0.43 kg, 95% CI -0.54 to -0.33; $p < 0.000$) with no considerable heterogeneity detected between the studies ($I^2 = 0\%$; 9 studies) that was not seen in those lasting 6 months or more (MD -0.21 kg, 95% CI -0.85 to 0.43; $p = 0.53$) with higher heterogeneity detected between the studies ($I^2 = 25\%$; 6 studies). However there was only one very long trial lasting 24 month that did not find an effect of calcium supplementation compared to placebo on body weight (difference, 0.02 kg [95% CI, -1.64 to 1.69 kg]; $p = 0.98$), the rest of studies included had a duration of 6 months or less.

Figure 6.12: Oral calcium supplementation versus placebo: Outcome: Body weight by study duration

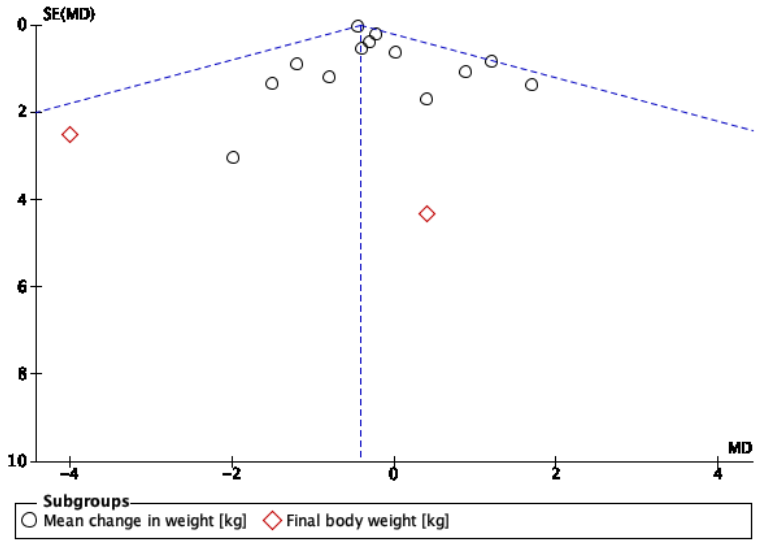


- There were two large trials with a 100 participants or more (Shapses 2001; Yanovski 2009)[254,261] that provided a no statistically significant effect (MD -0.16 kg, 95% CI -1.27; p = 0.78) with no heterogeneity detected between the studies (I^2 = 0%; 2 studies), whereas those with less than a 100 participants provided a marginal effect (MD -0.31 kg, 95% CI -0.61, -0.00; p = 0.05) with no considerable heterogeneity detected between the studies (I^2 = 18%; 13 studies).

Assessment of reporting bias

As there were more than 10 trials included we performed the funnel plot and found no evidence of publication bias.

Figure 6.13: Funnel plot of comparison: Oral calcium supplementation versus placebo, outcome: Body weight (kg)



Ongoing trials

We found eight ongoing RCTs. All had a parallel design. The estimated sample sizes were 10 to 400 participants. Six studies use a supplement dose of 1000 mg of calcium or more [272–277], one study uses enriched foods with calcium without stating the amount of calcium [278] and one a dose of 700 mg a day [279].

The primary outcomes in these studies are body weight or body mass index for five studies [273,276–279]; fat oxidation or lipid metabolism in one study [275], insulin resistance in one study [274] and blood pressure in one study [272]. Three trials did not report recruitment status [272–274] and five [275–279] have completed recruitment.

6.5. Discussion

The systematic review included seventeen studies of which fifteen provided data for the primary outcome body weight. Body weight was reduced with the intervention of calcium supplementation compared to placebo (Mean Difference (MD) -0.33 kg, 95% CI -0.57 to -0.09); (p=0.007); 819 participants; 15 studies). The BMI was also reduced with calcium supplementation compared to placebo (MD -0.17, 95% CI -0.21 to -0.13); p < 0.00001; 695 participants; 10 studies).

The random effect model is recommended to be used in those meta-analysis where the objective, as it was in our study, is to show an effect that is extended to other population groups beyond the ones included in the meta-analysis. The random model is generalizable as it assumes the effect

might vary according to different population groups, it assumes the effect of the included studies in the meta-analysis and some hypothetical variation.[280] The random effect size is a summary of different effect sizes. The random effect size is a summary of different effect sizes. On the other hand, the fixed effect model assumes that there is only one effect size, independent of the population group, and the difference in the included studies is only due to errors in estimating the true effect size.[245]

However if the number of included studies is very small the heterogeneity might not be detected correctly.[281]

All included trials used placebo as comparator, there were no articles measuring food or beverage fortification. None of the included articles reported primary outcomes health-related quality of life or adverse events or secondary outcomes waist circumference, all-cause mortality, morbidity or socioeconomic effects.

Most studies were performed in the USA (n= 879), where it is known that the dietary calcium intakes are higher and close to recommendations.[107] There were seven studies with 369 participants performed outside the USA, four of which were in Iran. All trials were performed in adult population and most were performed in women, only one trial was performed in men. There were no studies including children, pregnant women or older adults.

There was an unclear risk of bias, only four studies reported blinding for participants and personnel. Most studies had unclear risk in selection, performance and reporting bias. Ten trials had low attrition rates with possible impact on body weight and BMI outcomes.

All the subgroup analysis performed per protocol showed results in favour of calcium supplementation compared to placebo in the reduction of body weight. However in some subgroups such as those studies including men and women, overweight and obese participants combined or with an intervention of more than 500 mg of elemental calcium the evidence was stronger. On the other hand, there was no statistically significant effect on subgroups such as trials with co-intervention or without co-interventions and those with a duration of 6 months or more. Finally, there were no enough studies to truly show if there was an effect on subgroups such as pre-menopausal or post-menopausal women, doses of 500 mg or less and those with 100 participants or more.

There is a moderate evidence of calcium supplementation compared to placebo in the reduction of body weight shown by the GRADE assessment as the results of the meta-analysis were consistent, the confidence interval were precise, body weight was directly measured and there was no evidence of publication bias.

6.6. Conclusion

There is a moderate evidence of the effect of calcium supplementation as compared to placebo in the reduction of body weight as shown by the random effect meta-analysis (MD -0.33 kg, 95% CI -0.57 to -0.09); (p=0.007); 819 participants; 15 studies) with no considerable heterogeneity detected between the studies; (I^2 = 10%; 819 participants; 15 studies).

Other research articles related to the thesis

Other research articles related to the PhD thesis and developed during the PhD work

1- **Cormick G**, Betrán AP, Romero IB, Lombardo CF, Gülmezoglu AM, Ciapponi A, Belizán JM. Global inequities in dietary calcium intake during pregnancy: a systematic review. BJOG. 2018 Oct 22.
2- Hofmeyr GJ, DSc, Betrán AP, PhD, Singata-Madliki M, PhD, **Cormick G**, Munjanja SP, FRCOG, Fawcus S, Mose S, Hall D, Ciganda A, B Comp Sc, Seuc A, PhD, Lawrie TA, PhD, Bergel E, Roberts J, von Dadelszen P, Belizán JM, PhD, and the Calcium and Pre-eclampsia Study Group. Prepregnancy and early pregnancy calcium supplementation among women at high risk of pre-eclampsia: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2019; 393: 330–39
3- Seijo M, Minckas N, **Cormick G**, Comandé D, Ciapponi A, Belizan JM. Comparison of self-reported and directly measured weight and height among women of reproductive age: a systematic review and meta-analysis. Acta Obstet Gynecol Scand. 2018 Apr;97(4):429-439.
4- Lawrie TA, Betrán AP, Singata-Madliki M, Ciganda A, Hofmeyr GJ, Belizán JM, Purnat TD, Manyame S, Parker C, **Cormick G**; Calcium and Pre-eclampsia Study Group. Participant recruitment and retention in longitudinal preconception randomized trials: lessons learnt from the Calcium And Pre-eclampsia (CAP) trial. Trials. 2017 Oct 26;18(1):500.
5- Hofmeyr GJ, Seuc AH, Betrán AP, Purnat TD, Ciganda A, Munjanja SP, Manyame S, Singata M, Fawcus S, Frank K, Hall DR, **Cormick G**, Roberts JM, Bergel EF, Drebit SK, Von Dadelszen P, Belizan JM; Calcium and Pre-eclampsia Study Group. The effect of calcium supplementation on blood pressure in non-pregnant women with previous pre-eclampsia: An exploratory, randomized placebo controlled study. Pregnancy Hypertens. 2015 Oct;5(4):273-9.

Chapter 7 - Integrated discussion, conclusions and recommendations

7.1. Integrated discussion

The prevalence of overweight and obesity is increasing worldwide in different age groups.[33–35] According to the WHO, the prevalence of obesity doubled between 1980 and 2008 and it is increasing more rapidly in LMICs.[36] In LMICs, obesity prevalence is higher in women than in men for whom the impact of obesity is more deleterious.[51] In South Africa and Zimbabwe the prevalence of overweight and obesity in women doubles that of men.[42,45–47]

Nutritional status and dietary intake before and during pregnancy, and weight gain during pregnancy are the main factors influencing pregnancy outcomes and early child health. Being overweight or obese at the beginning of pregnancy increases the risk of developing complications such as pre-eclampsia, gestational diabetes mellitus, gestational hypertension, depression, foetal macrosomia, stillbirth, preterm birth, birth by caesarean section and infant mortality.[8–13] Excessive weight gain during pregnancy and failure to return to pre-pregnancy body weight have also consequences later in life, as it increases the risk of obesity and cardiovascular disease.[14] Optimal weight management before and during pregnancy is thus of utmost importance for optimal health of the mother and offspring. Although numerous weight loss treatment options exist, the multifactorial causes of obesity can only be addressed with a broad approach and it is likely that strategies at population level are necessary to curb the current trends in obesity prevalence.

A systematic review in 2011 showed that calcium supplementation may play a role in weight management, however the studies included had a short intervention period of around 6 months and the effect during pregnancy was not assessed. [25] Although the effect found was small, at a population level it could help to prevent the observed global trends in obesity. [26] More recent studies have emerged lately and warranted new meta-analyses including different age population group and in different life stages. Furthermore, it has also been reported that calcium intake of women from LMICs is low.[107] However, there are limited number of studies from African countries reporting dietary intake of the population.

This PhD thesis therefore aims to evaluate the effect of calcium intake on body weight and to investigate the pre-pregnancy weight status, weight gain during pregnancy and adequacy of dietary intake of pregnant women participating in the randomised placebo controlled Calcium and Pre-eclampsia (CAP) trial. The thesis involved an intervention study to evaluate the effect of calcium supplementation before and during pregnancy, a systematic review of randomised

control trials evaluating the effect of calcium supplementation on body weight of different age population groups and in different life stages and a descriptive study to assess nutritional status before and during pregnancy and dietary intake of women at mid-pregnancy.

Results of aim 1

The results of the CAP trial showed that supplementation with 500 mg of elemental calcium from before pregnancy until 20 weeks´ gestation as compared to placebo had no effect on weight change of non-pregnant or pregnant women who had history of pre-eclampsia. Although the calcium group had a smaller increase in body weight than the placebo group at week 8 (1.1 kg (SD ± 5.5) vs 1.5 kg (SD ± 6.1)), at week 20 (3.9 kg (SD ±6.0) vs 4.0 kg (SD ±7.0)) and at week 32 (7.7 kg (SD ±6.6) vs 8.3 kg (SD ±7.3)) gestation, none of these differences were statistically significant. In obese women those receiving calcium supplementation had a smaller increase in body weight than those who received placebo, however this smaller increase was not statistically significant. In this trial all women received 1500 mg of elemental calcium from 20 weeks´ gestation until the end of pregnancy as recommended by the WHO.

At 20 weeks´ gestation women randomised to receive calcium supplements or placebo had similar energy and calcium intakes. Women of different age or parity had, at 20 weeks´ gestation, similar energy intake, however calcium intakes were lower in older pregnant women. These results disregard some postulated mechanisms related to a decrease in appetite and thus energy intake in women consuming calcium supplements.

The results of the CAP trial also showed no difference on the infant´s birth weight between those infants born to mothers randomised to calcium 2670 grams (SD ±1021) and those to placebo 2686 grams (SD ±846) (p=0.897). The lack of differences between the birthweight of infants born to mothers that received calcium supplementation and those that received placebo before and in the first half of pregnancy discards any concern about the effect of calcium supplementation on birthweight.

This is the first study assessing the effect of calcium supplementation before and during pregnancy. The results are robust as they are from a randomised controlled trial that followed women from before conception until the end of pregnancy. Even though there was no statistically significant effect, the results were all in the same direction, implying consistency and that there could be an effect if the study had a larger sample size. Unfortunately, the study had a fixed

sample size that was powered to detect a 40% reduction in pre-eclampsia. Before starting the analysis, it was known that the sample size of the CAP trial would only detect a body weight change of 1.2 kg or higher.

Furthermore, it is also likely that a higher calcium dose could have had a higher effect as later shown in the systematic review that included non-pregnant adults with higher calcium dosages of more than 1000 mg.

The evidence of this study can be included in a systematic review and meta-analysis to increase the power, and provide novel insights on the effect of calcium in the body weight of pregnant women.

Results of aim 2

The prevalence of overweight and obesity found in the CAP trial population is alarming, with figures reaching 73.7% in South Africa and 60.2% in Zimbabwe. This together with the micronutrient inadequacy at 20 weeks´ pregnancy, show a very poor nutritional status of women that are on high risk of pre-eclampsia and have the possibility of falling pregnant again. For the most basic micronutrients like iron, calcium, folate and zinc, the percentage of women below requirements was above 90%.

Women with normal BMIs at 8 weeks´ gestation, compared to those with overweight or obese BMIs, gained more weight at 32 weeks, which is in accordance to recommendations. However, weight was only assessed up to 32 weeks´ gestation and most gestational weight gain occurs after 20 weeks´ gestation, therefore it was expected that many of these women would exceed the Institute of Medicine recommendations for optimal weight gain during pregnancy. Programmes and interventions to reduce obesity before pregnancy and controlling weight gain during pregnancy would be advisable in view of the findings of this analysis.

Previous studies have shown that obese populations have micronutrient deficiencies and that overweight and obese individuals showed lower blood concentrations of vitamins and minerals compared to non-overweight or obese individuals specially vitamin A, E, D, C, zinc, iron, selenium and calcium.[282] The lower blood concentrations of vitamins and minerals found in overweight or obese populations could be due to higher amounts of micronutrients required to compensate for the extra tissue metabolism or to lower micronutrient intake.[283,284] It has been suggested that overweight and obese individuals could have higher micronutrient requirements.[282,285] Even more, these micronutrient deficiencies may be impairing weight loss, as some of these micronutrients are required for lipid metabolism.[192,286–288] In this way, the

micronutrient deficiency could be contributing to weight gain and development of metabolic problems that, in the case of pregnancy, could also program the foetus to obesity.[289,290] The CAP trial participants reported a rather low energy intake and overall dietary micronutrient inadequacy or the results could possibly be due to underreporting. However we did not measure nutrient status to determine nutrient deficiencies suggested in previous studies. It must be borne in mind that the dietary reference intake values used to evaluate micronutrient inadequacy are set for healthy individuals with a healthy body weight.[99] Individuals with one or more diseases may have different nutrient requirements. As there are no dietary reference intake values available for non-healthy individuals, I used the ones available. The CAP trial participants had history of pre-eclampsia and a high prevalence of overweight and obesity so recommendations for this special group could be different.

Preconceptional care becomes of extreme importance for this population group in order to improve women´s health and that of their children.

Results of aim 3

The systematic review and meta-analysis of calcium supplementation on body weight indicated that there is evidence of the effect of calcium supplementation as compared to placebo in reducing body weight as shown by the random effect meta-analysis (MD -0.33 kg, 95% CI -0.57 to -0.09); (p=0.007); 819 participants; 15 studies) with no considerable heterogeneity detected between the studies; (I^2 = 10%; 819 participants; 15 studies). There was also a significant effect of calcium supplementation compared to placebo in reducing BMI (MD -0.17, 95% CI -0.21 to -0.13); p < 0.00001; 695 participants; 10 studies).

The subgroup analysis showed that there was a statistically significant effect when trials of men and/or women or trials of overweight and obese participants were combined in the meta–analysis. There was a statistically significant effect in those trials with an intervention of more than 500 mg of elemental calcium.

The evidence was moderate according to the GRADE approach that has four levels of evidence: high, moderate, low and very low. The moderate evidence means that although there is evidence from randomised control trials and the true effect is likely to be close to the estimated effect, this evidence has important methodological limitations.[250]

The CAP trial sub-analysis in chapter 4 shows that calcium supplementation compared to placebo does not have an effect in reducing 1.2 kg body weight in women with history of pre-eclampsia

in the last hospital delivery. However the CAP trial sample size did not provide the power to detect smaller effects. On the other hand, the meta-analysis of chapter 6 had the power to detect smaller differences as it included pooled data of fifteen trials with 705 participants. The total mean difference effect size of calcium supplementation versus placebo observed in the meta-analysis was -0.33 kg (95% CI -0.57 to -0.09) and represents an estimate of the true effect.

Despite the number of studies on calcium supplementation during pregnancy, this thesis reports on the first study that investigates the effect calcium supplementation on body weight of pregnant women. There were no studies in children or adolescents. These results warrant further investigation especially on the effect of calcium supplementation on weight management of pregnant women.

The evidence of this systematic review on the effect of calcium supplementation on body weight, together with previous evidence of calcium supplementation in decreasing blood pressure, pre-eclampsia and cholesterol plus the fact that there is a low calcium intake in many regions of the world points to the direction of the need to improve dietary calcium, reach adequate intakes and reduce the risk of these prevalent diseases that are part of the metabolic syndrome.[23,67,77,107]

7.2. Final comments of the Thesis

Strengths

- The data used in this thesis for the first and second aims comes from a well-designed randomised controlled trial as shown by the similarity of women randomised to calcium or placebo. This reflects that any differences found in outcomes between the two groups can be attributed to the intervention.
- The dietary assessment questionnaires were adapted for this study and the fieldworkers were well-trained. I supervised and maintained regular contact with each of them.
- The systematic review was performed using the Cochrane methodology and in communication with the Cochrane Metabolic and Endocrine Disorders Group.

Limitations

The population of the CAP trial involve women with a history of PE. It is thus possible that the nutritional status and dietary intake may be different in women without a history of PE. Women

with history of PE, as the CAP trial women, have a higher risk of recurrent PE and of developing hypertension later in life.[291] Furthermore, it has been shown that, obesity, hypertension and endothelial dysfunction are risk factors of PE. In this way, women in the CAP trial might have had an increase occurrence of these diseases compared to the general population and therefore the physiology of calcium in weight changes may be different. [202,203]

The data of the CAP trial had the limitation of a small sample size for the analysis of the effect of calcium supplementation on body weight; therefore the analysis was not powered to detect an effect smaller than 1.2 kg. Besides the trial was designed to evaluate pre-eclampsia and not to evaluate weight, however weight was included in the original protocol and thus provided the opportunity to conduct this sub-study. Furthermore, all women independently of the group they were allocated to (calcium supplementation or placebo); received calcium supplements with a total of 1500 mg per day after 20 weeks´ gestation. This could have dwindled the effect of the CAP trial intervention -calcium supplementation with a low dose of 500 mg per day- as both groups received calcium supplements at a high dose for almost half of their pregnancy. The same constrain was seen in the CAP trial primary and secondary outcomes, where the effect of calcium supplementation on pre-eclampsia and blood pressure did not reach statistical significance.[170]

Data collection for the CAP trial in Argentina started later than in South Africa and Zimbabwe, consequently, the final sample of Argentina was very small. In this way, although the data collected in Argentina contributed to the main findings in body weight, it was not possible to describe the dietary intake of the participants of Argentina separately.

The combined effect of dietary calcium plus calcium supplements on weight change could not be determined since dietary intake was only evaluated once at 20 weeks´ gestation while supplements were taken from admission to the end of the trial.

7.3. Final conclusions

The results of this thesis show that the majority of women participating in the CAP trial were overweight or obese before starting a new pregnancy and additionally these women had inadequate intakes of micronutrients during pregnancy. The intake of calcium supplements with a dose of 500 mg a day had no significant effect on the body weight of these women before pregnancy nor during pregnancy. Although the calcium group had a smaller increase in body

weight at weeks 8, 20 and 31 of pregnancy, specifically in the group that started pregnancy overweight or obese, these differences were no statistically significant.

The results of the systematic review and meta-analysis are very consistent in showing an effect of calcium supplementation in the reduction of body weight and BMI in adults including non-pregnant women of fertile age and men. However, there were no studies on children and the only study in pregnant women was the one of the CAP trial of this thesis.

7.4. Recommendations

The following recommendations were formulated based on the findings of this research:

- The weight status and dietary intake of women of child bearing age in South Africa and Zimbabwe should receive urgent attention. Preconceptional care needs to be put in place so as to advise body weight management during the inter-pregnancy interval to prevent the adverse effects of overweight and obesity. The health system needs to follow women in the postpartum period, especially those that have finalised a high-risk pregnancy so as to aid them to control their weight and monitor a healthy diet and improve maternal and perinatal outcomes.

- Due to the high proportion of women with inadequate micronutrient intake, there is also a need to approach the problem from a public health perspective rather than individually. Strategies to increase the consumption of healthy foods such as tax reduction and food fortification should be a priority in low and middle-income countries.

- At this stage calcium supplementation for weight management should not be recommended for pregnant women. However, further research is necessary in this regard as this was the first study to investigate this.

- Before making clinical recommendations on the effect of calcium supplementation in reducing body weight further studies are needed to assess diversity of doses within different population groups and in combination with lifestyle treatment.

7.5. Recommendations for future research studies

There is a need to further explore the tendency of a lower body weight gain reduction shown in pregnant women in this thesis. There is also a need to update the evidence with future studies so as to see if the larger effects shown in animal studies can be replicated in humans. The recommendations for future research derived from the findings of this thesis are:

- More research is required to explore barriers and facilitators on the acceptability of nutritional counselling on healthy eating and physical activity interventions in pre-gestational and pregnant women of LMICs. Most of the evidence of interventions such as nutritional counselling on healthy eating and physical activity come from HICs and less is known about the real effects in LMICs. [126]

- There is a need to develop strategies to follow up women to provide nutritional counselling on healthy eating after their pregnancy so as to obtain a better health and nutritional status before facing a new pregnancy. Information from this thesis shows that even after a high risk pregnancy women lose interaction with the health system and start a new pregnancy in poor health and nutritional conditions.

- Evidence of the poor nutritional conditions of women in LMICs from one side the epidemic of obesity and in the other side the poor intake of micronutrient and minerals implies that broad scale interventions should be developed and tested.

- Further RCTs that investigate effect of calcium supplementation on weight management of pregnant women with larger doses than 500 mg of elemental calcium during pregnancy. However, as 1.5 g of elemental calcium is already recommended to pregnant women from mid-pregnancy, a new RCT design should contemplate this. Thus a new RCT design should

be comparing lower to higher doses. Another option would be testing the effect of a calcium-fortified food including some measures of nutrient status.

- Further basic and experimental studies in laboratory animals and humans should be developed to better understand the mechanisms involved in the relationship between calcium intake and weight.

The recommendations for future research derived from the literature of this thesis are:

- Basic and experimental studies to explore if there is a modelling effect of calcium intake during pregnancy on the weight of the progeny in short and long-term basis. These studies would be a major contribution to assess if maternal diet could have a role on the progeny and would derive in strategies to improve maternal diet.

- There is a need to further investigate the role of micronutrients in obesity as inadequate intake of micronutrients may impair weight reduction. There is also a need to establish micronutrient recommended dietary intake values for obese individuals as the currently available recommended dietary intake values are for healthy individuals, and the needs for obese individuals might be different.

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Appendix A Annexure 1: Case Report Forms

World Health Organization

A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia
SCREENING FORM

SCR
page 1/1
V2 (01 Nov 2012)

Center number ☐☐☐☐ Screening number ☐☐☐☐

1. Date of interview: Day ☐☐ Month ☐☐ Year ☐☐☐☐

ELIGIBILITY CRITERIA - Section 1: Unchangeable

	N	Y
2. Did she have pre-eclampsia (high blood pressure and protein in urine during pregnancy) and no term pregnancy without pre-eclamsia afterwards?	1	2
3. DELETED		
4. Is she pregnant? ***Check pregnancy test if unsure.***	1	2
5. Has she been diagnosed with urinary stones?	1	2
6. Has she been diagnosed with persistent renal disease?	1	2
7. Has she been diagnosed with parathyroid disease?	1	2
8. Does she have a serious medical condition (e.g. cancer) which would prevent her from participating in study? Women with HIV may participate.	1	2

If any response (Q1-Q8) falls in a shaded box, she is not eligible for recruitment. Go to Q15 and **end questionnaire.**

ELIGIBILITY CRITERIA - Section 2: Modifiable

	N	Y
9. Is she in a sexual relationship?	1	2
10. Is she using reliable contraception? (pill, injection, IUD or 100% condom)	1	2
11. Is she younger than 18 years of age?	1	2
12. Is she already taking calcium supplementation?	1	2

	N	Y
13. Is she willing to give consent?	1	2

NEW

14.a. Does she have hypertension now AND urine protein 1+ or more?	1	2

If she has hypertension (ie she is on antihypersives or her BP is >140mmHg systolic or >90 mmHg diastolic), but her urine protein is < 1+, the answer is "No".

If any response (Q9-Q14a) falls in a shaded box, she is not eligible at this visit but her status might change in future. Arrange a suitable date for a new assessment, and go to Q19.
If not, continue - she is eligible.

NEW

14.b. Is she on anti-hypertension treatment? ☐
1=No 2=Yes

15. Is she eligible? ☐
1=No 2=Yes
If "No", end questionnaire.
If "Yes", randomize in ALEA.

16. Randomization date: Day ☐☐ Month ☐☐ Year ☐☐☐☐

17. Subject number: ☐☐☐
(generated in ALEA)

18. First supplement pack number given: ☐☐☐☐☐

AFTER RANDOMIZATION, GO TO ADMISSION FORM and end questionnaire.

19. Date of next assessment: Day ☐☐ Month ☐☐ Year ☐☐☐☐

Investigator's name: Investigator's signature:

Date form completed: Day ☐☐ Month ☐☐ Year ☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:
1st data entry:
2nd data entry:

World Health Organization

A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia

ADMISSION FORM

ADM

page 1/1

V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

1. Date of admission: Day ☐☐ Month ☐☐ Year ☐☐☐☐

BASELINE INFORMATION

2. Age: (years) ☐☐

3. Parity (previous pregnancies >24 weeks): ☐☐

4. Date of last birth complicated by pre-eclampsia or eclampsia: Day ☐☐ Month ☐☐ Year ☐☐☐☐ Don't know ☐

PREVIOUS PREGNANCY COMPLICATIONS
(Most recent pregnancy complicated by eclampsia or pre-eclampsia)

5. Is previous hospital record available? ☐
   1= No 2= Yes

6. Onset of pre-eclampsia: (weeks) ☐☐
   (99 if unknown)

7. Highest blood pressure (999 if unknown)
   a) Systolic: (mmHg) ☐☐☐
   b) Diastolic: (mmHg) ☐☐☐

8. Eclampsia: ☐
   1= No 2= Yes 9= Unsure

9. HELLP Syndrome: ☐
   1= No 2= Yes 9= Unsure

10. Labour induction or caesarean section (CS): ☐
    1= No 2= CS 3= Induction
    4= Both 9= Unsure

11. Gestational age at birth: (weeks) ☐☐
    (completed weeks; 99 if unsure)

12. ICU admission (mother): ☐
    1= No 2= Yes

13. Baby born alive: ☐
    1= No 2= Yes

CURRENT VISIT

14. a) Do you have any serious health problems? ☐
    1= No 2= Yes
    If "No", go to Q15.
    b) If "Yes", which of the following does the woman have?
    1= No 2= Yes
    i. Heart disease ☐
    ii. Kidney/bladder disease ☐
    iii. Diabetes ☐
    iv. Severe headaches ☐
    v. Epilepsy/Convulsions ☐
    vi. Other ☐
    If "No" in "vi. Other", go to Q15.
    c) Specify "Other": _____ ICD-10 code ☐☐☐.☐

15. Blood pressure:
    a) Systolic: (mmHg) ☐☐☐
    b) Diastolic: (mmHg) ☐☐☐

16. Weight (999 if not measured): (kg) ☐☐☐

17. Height (999 if not measured): (cm) ☐☐☐

18. First day of last menstrual period: Day ☐☐ Month ☐☐ Year ☐☐☐☐
    Unsure ☐

19. Urine sample collected: ☐
    1= No 2= Yes

20. Date of next visit: Day ☐☐ Month ☐☐ Year ☐☐☐☐

REMARKS:

Investigator's name: Investigator's signature:

Date form completed: Day ☐☐ Month ☐☐ Year ☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:
1st data entry:
2nd data entry:

World Health Organization

A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia

PRE-PREGNANCY VISIT (Every 12 Weeks)

PPV

page 1/1

V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

1. PPV form number: ☐☐

2. Date of visit: Day ☐☐ Month ☐☐ Year ☐☐☐☐

HEALTH VISIT

3. a) Does the woman have any serious health problems? ☐
   1= No 2= Yes
   If "No", go to Q4.
   b) If "Yes", which of the following does she have?
   1= No 2= Yes
   - i. High blood pressure ☐
   - ii. Heart disease ☐
   - iii. Kidney/bladder disease ☐
   - iv. Diabetes ☐
   - v. Severe headaches ☐
   - vi. Epilepsy/Convulsions ☐
   - vii. Other ☐

   If "No" in "vii. Other", go to Q4.
   c) Specify "Other": _____ ICD-10 code ☐☐☐.☐

4. Is she pregnant? ☐
   1= No 2= Yes
   If "Yes", go to Q7.
   If "Unsure", please check pregnancy test.

5. Is she having regular sexual intercourse? ☐
   1= No 2= Yes

6. a) Is she using condoms? ☐
   1= Never 2= Sometimes 3= Always

   b) Is she using other contraception? ☐
   1= No 4= IUD
   2= Depot (DMPA or nur-isterate) 5= Other
   3= Oral contraceptives
   If "No", go to Q7.

   c) Is she planning to stop contraception in the future? ☐
   1= No 2= Yes 9= Unsure
   If "No" or "Unsure", go to Q7.

   d) When, approximately, does she want to stop contraception? (months) ☐☐

7. Number of antihypertensive medicines being taken: (9 if unsure) ☐

8. Blood pressure (999 if not measured)
   a) Systolic: (mmHg) ☐☐☐
   b) Diastolic: (mmHg) ☐☐☐

9. First day of last menstrual period: Day ☐☐ Month ☐☐ Year ☐☐☐☐
   Unsure ☐

SUPPLEMENTATION

10. a) Old supplement bottle returned? ☐
    1= No 2= Yes
    If "No", go to Q10d.
    b) Old supplement bottle number returned by the woman: ☐☐☐☐☐
    c) Number of tablets returned: ☐☐
    Go to Q11.
    d) If not returned, estimate number of tablets taken since last visit: (99 if unable to estimate) ☐☐

11. Does she continue in the study? ☐
    1=No 2=Yes
    If "No", fill in EOS form and end questionnaire.

12. New supplement bottle number: ☐☐☐☐☐

13. Date of next visit (in 12 weeks): Day ☐☐ Month ☐☐ Year ☐☐☐☐

Investigator's name: Investigator's signature:

Date form completed: Day ☐☐ Month ☐☐ Year ☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:

1st data entry:

2nd data entry:

World Health Organization

A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia
DURING PREGNANCY VISIT (<8, 8, 20 and 32 weeks)

DPV
page 1/2
V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

Hospital record number (9 if not known): ☐☐☐☐☐☐☐☐☐☐

1. Type of DPV form: ☐
   1= less than 8 weeks
   2= 8 weeks
   3= 20 weeks
   4= 32 weeks

2. Date of visit: Day ☐☐ Month ☐☐ Year ☐☐☐☐

INFORMATION FROM ANTENATAL CARD SINCE LAST TRIAL VISIT

3. Highest blood pressure (999 if unknown):
   a) Systolic (mmHg) ☐☐☐
   b) Diastolic (mmHg) ☐☐☐

4. Highest proteinuria on dipstick: ☐
   0= 0 2= ++ 9= Unknown
   1= + 3= +++

CURRENT VISIT

5. a) Do you have any serious health problems? ☐
   1= No 2= Yes
   If "No", go to Q6.

   b) If "Yes", which of the following does the woman have?
   1= No 2= Yes
   i. High blood pressure ☐
   ii. Heart disease ☐
   iii. Kidney/bladder disease ☐
   vi. Diabetes ☐
   v. Severe headaches ☐
   vi. Epilepsy/Convulsions ☐
   vii. Other ☐
   If "No" in "vii. Other", go to Q6.

   c) Specify "Other":
   _____ ICD-10 code ☐☐☐.☐

6. Number of antihypertensive medicines being taken: ☐
   (9 if unknown)

7. Are you taking routine calcium supplementation (apart from trial tablets)? ☐
   1= No 2= Yes

8. Blood pressure (999 if not measured):
   a) Systolic: (mmHg) ☐☐☐
   b) Diastolic: (mmHg) ☐☐☐

9. Proteinuria on dipstick: ☐
   0= 0 2= ++ 9= Not done
   1= + 3= +++

10. Weight (999 if not measured): (kg) ☐☐☐

11. If visit at 8 weeks, date of last normal menstrual period: Day ☐☐ Month ☐☐ Year ☐☐☐☐
    Unsure ☐

12. First ultrasound report (record only once):
    a) Date: Day ☐☐ Month ☐☐ Year ☐☐☐☐
    Not done ☐
    If "Not done", go to Q13.

    b) Gestational age at time of ultrasound:
    weeks ☐☐ days ☐

13. If visit at 8 weeks, best clinical estimate of gestational age:
    weeks ☐☐ days ☐

14. If visit at 20 weeks, estimated dietary calcium intake:
    (9999 if unknown) (mg/day) ☐☐☐☐

15. If visit at 20 or 32 weeks, urine sample collected: ☐
    1= No 2= Yes

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

Hospital record number (9 if not known): ☐☐☐☐☐☐☐☐☐☐

INFORMATION FROM ANTENATAL CARD SINCE LAST TRIAL VISIT PLUS HOSPITAL RECORD

1. Highest blood pressure (999 if unknown):
   a) Systolic (mmHg) ☐☐☐
   b) Diastolic (mmHg) ☐☐☐

2. Highest proteinuria on dipstick: ☐
   0= 0 2= ++ 9= Unknown
   1= + 3= +++

3. Number of antihypertensive medicines taken: (9 if unsure) ☐

4. First ultrasound report (record only once):
   a) Date: Day ☐☐ Month ☐☐ Year ☐☐☐☐
   Not done ☐
   If "Not done", go to Q5.
   b) Gestational age at time of ultrasound:
   weeks ☐☐ days ☐

5. Best clinical estimate of gestational age at delivery/end of pregnancy:
   weeks ☐☐ days ☐

BIRTH OUTCOME

6. Date of birth, miscarriage or abortion: Day ☐☐ Month ☐☐ Year ☐☐☐☐
   Unknown ☐

7. a) Onset of Labour: ☐
   1= Spontaneous
   2= Induced
   3= No Labour
   If not "Induced", go to Q8a.

   b) Reason for induced labour: ☐
   1= Hypertensive disorder
   2= Other medical condition
   3= APH
   4= PROM
   5= Fetal distress
   6= Other
   If not "Other", go to Q8a.
   c) Specify other reason for induced labor:
   _____ ICD-10 code ☐☐☐.☐

8. a) Final mode of delivery: ☐
   1= Vaginal
   2= Assisted (vacuum or forceps)
   3= Caesarean section before labour
   4= Caesarean section in labour
   5= Laparotomy for ectopic pregnancy
   6= Medical or surgical procedures for miscarriage or abortion
   7= Other
   If "Vaginal", go to Q9.
   If "Laparotomy", go to Q16.

   b) Reason for intervention: ☐
   1= Hypertensive disorder
   2= Other medical condition
   3= APH
   4= PROM
   5= Fetal distress
   6= Poor progress of labour
   7= Woman's request
   8= Other
   If not "Other", go to Q9.
   c) Specify other reason for intervention:
   _____ ICD-10 code ☐☐☐.☐

9. Total number of births: ☐
   If more than one baby, complete ABO form(s).

10. Pregnancy outcome: ☐
    1= Live birth
    2= Miscarriage
    3= Fresh stillbirth
    4= Macerated stillbirth
    5= Termination of pregnancy
    If "Miscarriage", "Fresh/Macerated stillbirth" or "Termination", go to Q16.

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

11. Birthweight: (g) ☐☐☐☐
(9999 if not measured)

12. Apgar score at 5 min: ☐☐

13. NICU admission > 24h: ☐
1= No2= Yes

14. a) Any baby complications: ☐
1= No 2= Yes 3= Died
If "No" or "Died", go to Q15.

b) If "Yes", specify baby complications:
1= No 2= Yes
- i. Birth asphyxia ☐
- ii. Encephalopathy ☐
- iii. Respiratory complications ☐
- iv. IVH ☐
- v. Sepsis ☐
- vi. NEC ☐
- vii. Anomalies ☐
- viii. Other ☐

If "No" in "viii. Other", go to Q15.

c) Specify "Other":

_____ ICD-10 code ☐☐☐.☐

15. Baby discharge or death date: Day ☐☐ Month ☐☐ Year ☐☐☐☐

MOTHER OUTCOME

16. Placental abruption: ☐
1= No 2= Yes

17. ICU admission >24h: ☐
1= No 2= Yes

18. Cerebrovascular accident: ☐
1= No 2= Yes

19. Pulmonary oedema: ☐
1= No 2= Yes

20. Ventilation > 24 hours: ☐
1= No 2= Yes

21. Cardiac arrest: ☐
1= No 2= Yes

22. Pre-eclampsia? ☐
1= No 2= Yes

23. Eclampsia? ☐
1= No 2= Yes
If "No" in both Q22 and Q23, go to Q37.

FOR WOMEN WITH PRE-ECLAMPSIA/ECLAMPSIA

24. Date of first diagnosis of hypertension: Day ☐☐ Month ☐☐ Year ☐☐☐☐
Not diagnosed or unknown ☐

25. Date of first diagnosis of pre-eclampsia/ eclampsia: Day ☐☐ Month ☐☐ Year ☐☐☐☐
Not diagnosed or unknown ☐

26. Laboratory urine protein:
(9999 if not done) a) (mg/l) ☐☐☐☐
b) (mg/24h) ☐☐☐☐

27. Laboratory urine protein/creatinine ratio: (9.99 if not done) ☐.☐☐

28. Highest Creatinine level: (µmol/l) ☐☐☐☐
(9999 if not done)

29. Lowest platelet count: (1000/µl) ☐☐☐
(999 if not done)

30. a) Highest Urate: (mmol/l) ☐☐.☐☐
(99.99 if not done)
If "not done", go to Q31.

b) Date of urate test: Day ☐☐ Month ☐☐ Year ☐☐☐☐
Unknown ☐

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

31. Highest ALT: (U/l) ☐☐☐
(999 if not done)

32. Highest AST: (U/l) ☐☐☐
(999 if not done)

33. Highest LDH: (U/l) ☐☐☐☐
(9999 if not done)

34. If eclampsia, number of convulsions: ☐
(9 if unknown)

35. HELLP Syndrome: ☐
1= No2= Yes

36. Use of MgSO4: ☐
1= No 3= Treatment
2= Prophylaxis 9= Unknown

37. a) Any other maternal complications? ☐
1= No 2= Yes 3= Died
If "No", go to Q38.
If "Died", fill in Serious Adverse Event form and go to Q38.

b) If "Yes", please specify other maternal complications:

_____ ICD-10 codes ☐☐☐.☐

_____ ☐☐☐.☐

38. Mother's discharge or death date:

Day	Month	Year
☐☐	☐☐	☐☐☐☐

Unknown ☐

REMARKS:

_____

_____

_____

_____

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_____

_____

_____

_____

Investigator's name: Investigator's signature:

Date form completed:

Day	Month	Year
☐☐	☐☐	☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:
1st data entry:
2nd data entry:

World Health Organization

A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia

ADVERSE EVENT REPORT

AE R

page 1/1

V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

Hospital record number (9 if not known): ☐☐☐☐☐☐☐☐☐

1. AER report number: ☐

2. Date event first notified to investigator: Day ☐☐ Month ☐☐ Year ☐☐☐☐

3. Date of report: Day ☐☐ Month ☐☐ Year ☐☐☐☐

4. Description of event:

ICD-10 codes

a) _____ ☐☐☐.☐

b) _____ ☐☐☐.☐

5. Date of onset: Day ☐☐ Month ☐☐ Year ☐☐☐☐

6. Date ended: Day ☐☐ Month ☐☐ Year ☐☐☐☐

Not ended ☐

7. Duration: hours ☐☐ mins ☐☐ (if less than 24 hours)

8. Frequency: ☐
1= Intermittent 2= Continuous

9. Severity: ☐
1= Mild 2= Moderate 3= Severe

10. a) Treatment to manage event: ☐
1= No 2= Yes
If "No", go to Q11.

b) Treatment provided: ICPM codes

_____ ☐-☐☐☐

_____ ☐-☐☐☐

11. Current status or outcome: ☐
1= Resolved 4= Sequelae
2= Partially resolved 5= Death
3= Not yet resolved

12. Measures taken:

a) Study drug: ☐
1= No change
2= Study drug stopped
3= Not applicable

b) Participation in the study: ☐
1= Continuing
2= Discontinued or released from the study

13. a) Was the adverse event related to the study treatment? ☐
1= Not related 4= Probably
2= Unlikely 5= Highly probably
3= Possibly 9= Not assessable
If "Not related" or "Unlikely", go to Q14.

b) If "Not assessable", explain:

_____

c) If "Possibly", "Probably" or "Highly Probably", is the adverse event unexpected? ☐
1= No 2= Yes

Report all Unexpected Adverse Events to WHO immediately (Dr Ana Pilar Betran, Tel +41.22.791.4205, Fax +41.22.791.4171, email: betrana@who.int)

14. Is the adverse event a Serious Adverse Event? ☐
1= No 2= Yes
If "Yes", complete SAE form.

REMARKS:

Please sign, scan and upload form to Open Clinica.

Investigator's name: Investigator's signature:

Date form completed: Day ☐☐ Month ☐☐ Year ☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:

1st data entry:

2nd data entry:

World Health Organization

A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia
SERIOUS ADVERSE EVENT REPORT

SAE
page 1/2
V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

Hospital record number (9 if not known): ☐☐☐☐☐☐☐☐☐

1. SAE report number: ☐

2. Date event first notified to investigator: Day ☐☐ Month ☐☐ Year ☐☐☐☐

3. Date of report: Day ☐☐ Month ☐☐ Year ☐☐☐☐

SUBJECT

4. Weight: (kg) ☐☐☐

5. a) Previous intolerance to medication: ☐
1= No 2= Yes 9 = Unknown
If "No" or "Unknown", go to Q6.

b) Specify medication:
ICPM codes
_____ ☐ - ☐☐☐
_____ ☐ - ☐☐☐

ADVERSE EVENT

6. Description of event:
ICD-10 codes
a) _____ ☐☐☐.☐
b) _____ ☐☐☐.☐

7. Date of onset: Day ☐☐ Month ☐☐ Year ☐☐☐☐

8. Date ended: Day ☐☐ Month ☐☐ Year ☐☐☐☐
Not ended ☐

9. Duration: hours ☐☐ mins ☐☐
(if less than 24 hours)

10. Hospitalization (or prolongation of hospitalization) ☐
1= No 2= Yes

11. Treatment given to manage the event:
ICPM codes
_____ ☐ - ☐☐☐
_____ ☐ - ☐☐☐

12. a) Current status or outcome: ☐
1= Resolved 4= Sequelae
2= Partially resolved 5= Death
3= Not yet resolved
If answer is not "Death", go to Q13a.

b) If "Death", date of death: Day ☐☐ Month ☐☐ Year ☐☐☐☐

c) Cause of death:
ICD-10 codes
_____ ☐☐☐.☐
_____ ☐☐☐.☐

d) Autopsy performed: ☐
1= No 2= Yes

CASE SUMMARY

13. a) Clinical description:
_____
_____
_____

b) Relevant medical history:
_____
_____
_____

World Health Organization

A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia

TELEPHONE FOLLOW UP (6 weeks after delivery)

TEL

page 1/1

V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

Hospital record number (9 if not known): ☐☐☐☐☐☐☐☐☐

1. Date of telephone contact: Day ☐☐ Month ☐☐ Year ☐☐☐☐

2. a) Does the mother have any health problems? ☐
   1=No 2=Yes 3=Died
   If "No", go to Q3.
   If "Died", go to Q2d.

   b) If "Yes", which of the following does the woman have?
   1=No 2=Yes
   - i. High blood pressure ☐
   - ii. Heart disease ☐
   - iii. Kidney/bladder disease ☐
   - iv. Depression ☐
   - v. CNS disease (e.g. stroke) ☐
   - vi. Other ☐

   If "No" in "vi. Other", go to Q3a.

   c) Specify "Other":
   _____ ICD-10 code ☐☐☐.☐

   Go to Q3a.

   d) If mother has died:
   i) Date of mother's death: Day ☐☐ Month ☐☐ Year ☐☐☐☐

   ii) Specify cause of mother's death:
   ICD-10 codes
   _____ ☐☐☐.☐
   _____ ☐☐☐.☐

3. a) Does the baby have any serious health problems? ☐
   1=No 2=Yes 3=Died
   If "No", go to Q4.
   If "Died", go to Q3d.

   b) If "Yes", which of the following does the baby have?
   1=No 2=Yes
   - i. Chest problems ☐
   - ii. Feeding problems ☐
   - iii. Floppy baby ☐
   - iv. Convulsions ☐
   - v. Other ☐

   If "No" in "v. Other", go to Q4.

   c) Specify "Other":
   _____ ICD-10 code ☐☐☐.☐

   Go to Q4.

   d) If baby has died:
   i) Date of baby's death: Day ☐☐ Month ☐☐ Year ☐☐☐☐

   ii) Specify cause of baby's death:
   ICD-10 codes
   _____ ☐☐☐.☐
   _____ ☐☐☐.☐

   If baby has died, end questionnaire.

4. What feeding method is baby using? ☐
   1= Breastfeeding only
   2= Formula feeding only
   3= Mixed breast and formula feeding

REMARKS:

_____

Investigator's name: Investigator's signature:

Date form completed: Day ☐☐ Month ☐☐ Year ☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:

1st data entry:

2nd data entry:

World Health Organization

A65750 - Long Term Calcium Supplementation in Women
ADDITIONAL BIRTH OUTCOME
(repeat for each baby in addition to first)

ABO
page 1/1
V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

1. Birth order: ☐

2. Pregnancy outcome: ☐
   1= Infant alive
   2= Miscarriage
   3= Fresh stillbirth
   4= Macerated stillbirth
   5= Termination of pregnancy
   If "Miscarriage", "Fresh/Macerated stillbirth", or "Termination", end questionnaire.

3. Birthweight: (g) ☐☐☐☐
   (9999 if not measured)

4. Apgar score at 5 min: ☐☐

5. NICU admission >24h: ☐
   1= No 2= Yes

6. a) Any baby complications: ☐
   1= No 2= Yes 3= Died
   If "No" or "Died", go to Q7.

   b) If "Yes", describe baby complications:
   1= No 2= Yes
   - i. Birth asphyxia ☐
   - ii. Encephalopathy ☐
   - iii. Respiratory complications ☐
   - iv. IVH ☐
   - v. Sepsis ☐
   - vi. NEC ☐
   - vii. Anomalies ☐
   - viii. Other ☐

   If "No" in "viii. Other", go to Q7.

   c) Specify "Other":

   _____ ICD-10 code ☐☐☐.☐

7. Baby discharge or death date:

Day	Month	Year
☐☐	☐☐	☐☐☐☐

REMARKS:

_____

_____

Investigator's name: Investigator's signature:

Date form completed:

Day	Month	Year
☐☐	☐☐	☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:
1st data entry:
2nd data entry:

World Health Organization	A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia END OF STUDY FORM	EOS page 1/1 V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

1. Date of end of study:

Day	Month	Year
☐☐	☐☐	☐☐☐☐

2. a) Status of subject: ☐

1= Completed study with pregnancy
2= Completed study without pregnancy
3= Discontinued while pregnant
4= Discontinued while not pregnant
5= Lost to follow-up

If "Completed study with pregnancy", "Completed study without pregnancy" or "Lost to follow-up", end questionnaire.

b) If "Discontinued", what is main reason: ☐

1= Medical reasons
2= Personal reasons

REMARKS:

Investigator's name: Investigator's signature:

Date form completed:

Day	Month	Year
☐☐	☐☐	☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:

1st data entry:

2nd data entry:

World Health Organization

A65750 - Long Term Calcium Supplementation in Women at High Risk of Pre-Eclampsia

UNSCHEDULED VISIT FORM

(not related to end of pregnancy)

UNV

page 1/1

V1 (10 Oct 2011)

Center number ☐☐☐☐ Subject number ☐☐☐ Screening number ☐☐☐☐

Hospital record number (9 if not known): ☐☐☐☐☐☐☐☐☐

1. UNV form number: ☐

2. Date of visit: Day ☐☐ Month ☐☐ Year ☐☐☐☐

3. Was this visit scheduled by doctor: ☐
   1= No 2= Yes

4. a) Main reasons for this visit: ☐
   1= Needs tablets
   2= Other
   If "Needs tablets", go to Q12.

   b) Specify "Other" reason: ICD-10 codes
   _____ ☐☐☐.☐
   _____ ☐☐☐.☐

5. Blood pressure (999 if not measured):
   a) Systolic (mmHg) ☐☐☐
   b) Diastolic (mmHg) ☐☐☐

6. Proteinuria on dipstick: ☐
   0= 0 1= + 2= ++ 3= +++ 9= Not done

7. Date of last menstrual period: Day ☐☐ Month ☐☐ Year ☐☐☐☐
   Unsure ☐

8. a) Is she pregnant? ☐
   1= No 2= Yes
   If "No", go to Q10.
   b) Has ultrasound been done? ☐
   1= No 2= Yes
   If "No", go to Q9.
   c) Date of ultrasound: Day ☐☐ Month ☐☐ Year ☐☐☐☐
   d) Gestational age at time of ultrasound: weeks ☐☐ days ☐

9. Best clinical estimate of gestational age at this visit: weeks ☐☐ days ☐

10. a) Treatment given at this visit? ☐
    1= No 2= Yes
    If "No", go to Q11.
    b) Specify treatment given: ICPM codes
    _____ ☐ - ☐☐☐
    _____ ☐ - ☐☐☐

11. a) Is subject hospitalized? ☐
    1= No 2= Yes
    If "No", go to Q12.
    b) If "Yes", specify main reasons: ICD-10 codes
    _____ ☐☐☐.☐
    _____ ☐☐☐.☐

SUPPLEMENTATION

12. a) Old supplement bottle returned? ☐
    1= No 2= Yes
    If "No", go to Q12d.
    b) Old supplement bottle number returned by the woman: ☐☐☐☐☐
    (88888 if routine calcium)
    c) Number of tablets returned: ☐☐
    Go to Q13.
    d) If not returned, estimate number of tablets taken since last visit: ☐☐
    (99 if unable to estimate)

13. Does she continue in the study? ☐
    1=No 2=Yes
    If "No", fill in EOS form and end questionnaire.

14. New supplement bottle number: ☐☐☐☐☐
    (99999 if not issued; 88888 if routine calcium)

15. Date of next trial visit: Day ☐☐ Month ☐☐ Year ☐☐☐☐

Investigator's name: Investigator's signature:

Date form completed: Day ☐☐ Month ☐☐ Year ☐☐☐☐

OPEN CLINICA DATA ENTRY SIGN & DATE:

1st data entry:

2nd data entry:

Annexure 2: Consent Form

INFORMATION LEAFLET AND INFORMED CONSENT

Each ***participant*** must receive, read and understand this document **before** any study-related procedure

STUDY NUMBER: Calcium trial

STUDY TITLE: Long-term calcium supplementation in women at high risk of pre-eclampsia: a randomized, placebo-controlled trial

SPONSOR: Bill & Melinda Gates Foundation

INVESTIGATOR: Professor GJ Hofmeyr (National Principal Investigator)

Dr A. Oyebajo (Wits Principal Investigator)

INSTITUTION: Effective Care Research Unit, East London Hospital Complex/ Department of Obstetrics and Gynaecology, Chris Hani Baragwanath Hospital

DAYTIME AND AFTER HOURS

TELEPHONE NUMBER(S): 011-933-8155/6 or 073-248-8211

To the potential Participant: *This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.*

INTRODUCTION:

Good day. My name is Dr A. Oyebajo, I am a consultant obstetrician at Chris Hani Baragwanath hospital. I would like to ***invite*** you to consider participating in a research study, entitled '**Long-term calcium supplementation in women at high risk of pre-eclampsia: a andomized, placebo-controlled trial', or the 'Calcium trial' (shortened name) which is being conducted by researchers in South Africa, Zimbabwe, Canada, Argentina and The World Health Organisation.**

1. Before agreeing to participate, it is important that you read and understand the following explanation of the purpose of the study, the study procedures, benefits, risks, discomforts, and precautions as well as the alternative procedures that are available to you, and your right to withdraw from the study at any time.

   This information leaflet is to help you to decide if you would like to participate. You should fully understand what is involved before you agree to take part in this study.
2. If you have any questions, do not hesitate to ask me.
3. You should not agree to take part unless you are satisfied about all the procedures involved.
4. You may not participate in another investigational medicine research study, nor take any other investigational medicine during your participation in this study.
5. You should not have participated in an investigational medicine research study within the past 14 days.
6. Please be completely truthful with me regarding your health history, since you may otherwise harm yourself by participating in this study.
7. If you decide to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will be given a copy to keep.
8. If you have a personal doctor, please discuss with or inform him/her of your possible participation in this study. If you wish, I can also notify your personal doctor in this regard.

PURPOSE OF THE STUDY:

We understand that you have indicated you might wish to have another baby. In your previous pregnancy, we know you suffered from high blood pressure, which can affect the health of the mother and baby. We are interested in finding out whether increased calcium in the mother's diet before and during pregnancy can improve the outcomes of pregnancy. We are asking you to take part in a study in which you will be given one tablet to chew every day until you become pregnant, and for the first 5 months of your pregnancy. Half of the women in the study will be given tablets which contain calcium, a natural ingredient in foods such as milk that you may normally eat. The other half of the women in the study will be given tablets which are very similar but which will provide no extra calcium. Neither you nor I will know which of the two types of tablet you are on. The tablets will be kept in the clinic, and we will ask you to visit our clinic every 3 months to collect them. 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 the birth. We will ensure that you receive the food

supplements which are known to improve the outcome of pregnancy, such as iron, folate, and calcium in the second half of your pregnancy.

PLACEBO

- **A placebo is an inactive substance** and it does not contain any medicine.
- You will be randomly allocated to one or other treatment (i.e. like spinning a coin). Neither you nor I will know which treatment you are receiving during your participation in the study. This procedure helps to ensure that the information gathered during the study is accurate. In case of an emergency, it will however be possible to determine which treatment you have been receiving.

LENGTH OF THE STUDY AND NUMBER OF PARTICIPANTS:

- The study will be performed in three other hospitals
- Approximately 660 participants will participate in this study around the world.
- In South Africa about 165 Participants will be enrolled at each study site
  The participants will be 18 years old or older.
- The total amount of time required for your participation in this study will be a maximum of 18 hours over a 2-year period.
- You will be asked to visit me every three months, and if you become pregnant during the trial, you will be followed up once a month during pregnancy until baby is delivered. Depending on whether you fall pregnant or not, you will visit me between 3 and 12 times during the study.

PROCEDURES:

- If you agree to take part in this study, you will first be asked questions and examined to see if you qualify for this study.
- Before receiving your first dose of study medicine, you will have your vital signs (blood pressure, pulse rate) checked. You will have a general check up by a study doctor.

At each following visit you will undergo a general check up, including blood pressure measurement. When your blood pressure is measured, an inflatable cuff is wrapped around your upper arm, inflated and then slowly deflated. It causes some mild discomfort for a few minutes when cuff is inflated.

Blood will not be drawn as part of the study, but results of blood tests performed as part of your routine pre-pregnancy and antenatal care at the hospital will be recorded.

RISKS AND BENEFITS OF THE STUDY MEDICINE:

There is no known risk of taking the calcium tablets and the amount of calcium is equal to that which is in 500ml (one small bottle) of milk. Participation in this research study may not benefit you directly, but might benefit pregnant women in the future, as it may help us to understand what foods women need to eat while they are pregnant. There are no alternative treatments.

- If you decide not to take part in this study you will still receive the best current care, from your usual doctor.

INTERACTIONS:

- It is important that you let me know of any medicines (both prescriptions and over-the-counter medicines), alcohol or other substances that you are currently taking.
- Please ensure you notify me if you have any history of kidney stones, kidney disease or parathyroid disease.

RIGHTS AS A PARTICIPANT IN THIS STUDY:

Your participation in this study is entirely voluntary and you can decline to participate, or stop at any time, without stating any reason. Your withdrawal will not affect your access to other medical care.

- ***Discontinuation of study treatment.***

You must inform me if you wish to stop taking your study medicine. I will supervise any discontinuation with your health as first priority.

New findings:

- I will provide you with any additional information that becomes available during the study, which may affect your willingness to continue on the study .

Withdrawal:

- Your withdrawal will not affect your access to other medical care.
- I retain the right to withdraw you from the study if it is considered to be in your best interest. If your participation is ended early, you may be asked to return for study-ending tests and procedures for your safety
- If you did not give an accurate history or did not follow the guidelines of the study and the regulations of the study facility, you may be withdrawn from the study at any time.

EMERGENCY CARE AND HOSPITALISATION:

- If you seek emergency care or if hospitalisation is required at any time during the study or up to 2 month/s after taking the last dose of study medicine, please tell the treating doctor **that you are/were enrolled in this research study** and that **I must be informed.**

FINANCIAL ARRANGEMENTS:

- Neither you nor your medical scheme will be expected to pay for any study medication, study related visit or study procedures.

REIMBURSEMENT FOR STUDY PARTICIPATION:

The trial treatment will be provided to you free of costs. You will be given a small payment (R50) to cover your transport costs to visit the hospital on your routine visits. You will not receive any payment for taking part.

INSURANCE:

The University of British Columbia in Canada has obtained insurance for you and me in the event of study related injury or illness. A study-related injury or illness is one that occurs as a direct result of the

administration of the study medicine or of study-specific procedures.

ABPI STATEMENT ON COMPENSATION:

The University of British Columbia will provide compensation for reasonable medical expenses incurred as a result of study-related injury or illness, determined according to the guidelines laid down by the **Association of the British Pharmaceutical Industry (ABPI Guidelines)**, and **Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa**.

- You must notify me immediately of any complications, side effects and/or injuries during the study and the nature of the expenses to be covered.
- If a research related injury occurs, you have not waived any of the legal rights which you otherwise would have as a participant in this study by signing this form.

Further detailed information on the payment of medical treatment and compensation due to injury can be obtained from me. I have a copy of the ABPI Guidelines and the Insurance Certificate, should you wish to review them.

ETHICAL APPROVAL:

- This clinical study protocol has been submitted to the University of the Witwatersrand, **Human Research Ethics Committee (HREC)** and written approval has been granted by that committee.
- The study has been structured in accordance with the **Declaration of Helsinki** (last updated: October 2008), which deals with the recommendations guiding doctors in biomedical research involving human participants. A copy may be obtained from me should you wish to review it.
- This study is sponsored by the Bill and Melinda Gates Foundation.
  I do not have any financial or personal interests with this organisation that may bias my actions.

SOURCE OF ADDITIONAL INFORMATION:

For the duration of the study, you will be under the care of Dr Oyebajo. If at any time between your visits, you feel that any of your symptoms are causing you any problems, or you have any questions during the study, please do not hesitate to contact me.

The 24-hour telephone number through which you can reach me or another authorised person, is or 073-248-8211.

- If you want any information regarding your **rights as a research participant, or complaints regarding this research study**, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee established to help protect the rights of research participants at (011) 717 2301.

- For **research information** you can contact Professor Hofmeyr, national principal investigator on 043- 709-1111 or 0832809402.

MEDICINES CONTROL COUNCIL

SOUTH AFRICA - MCC

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the Medicines Control Council (MCC) South Africa at:

The Registrar
Medicines Control Council SA
Department of Health
Private Bag X828
PRETORIA
0001

CONFIDENTIALITY:

- All information obtained during the course of this study, including hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study.
- This information will be reviewed by authorised representatives of Bill and Melinda Gates Foundation.
- The information might also be inspected by the University of the Witwatersrand, Human Research Ethics Committee (HREC), the South African Medicines Control Council (MCC) and/or the United States Food and Medicine Administration (FDA), as well as your personal doctor. Therefore, you hereby authorise me to release your medical records to the Bill and Melinda Gates foundation, its employees or agents, domestic and foreign regulatory health authorities, the South African Medicines Control Council and the University of the Witwatersrand, Human Research Ethics Committee (HREC).
- These records will be utilised by them only in connection with carrying out their obligations relating to this clinical study.
- Any information uncovered regarding your test results or state of health as a result of your participation in this study will be held in strict confidence. You will be informed of any finding of importance to your health or continued participation in this study but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission. The only exception to this rule will be cases of communicable diseases where a legal duty of notification of the Department of Health exists. In this case, you will be informed of my intent to disclose such information to the authorised state agency.

PERSONAL DOCTOR / SPECIALIST NOTIFICATION OPTION:

Please indicate below, whether you want me to notify your personal doctor or your specialist of your participation in this study:

- **YES**, I want you to inform my personal doctor / specialist of my participation in this study.
- **NO**, I do not want you to inform my personal doctor / specialist of my participation in this study.
- **I do not have** a personal doctor / specialist

PARTICIPANT QUESTIONS ?:

Did the participant raise any questions? **YES / NO**

If YES – What where they:

_____

_____

_____

INFORMED CONSENT:

- I hereby confirm that I have been informed by the study doctor, Dr Oyebajo, about the nature, conduct, benefits and risks of clinical study- the Calcium trial
- I have also received, read and understood the above written information (Participant Information Leaflet and Informed Consent) regarding the clinical study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system by the Bill & Melinda Gates Foundation or on their behalf.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

PARTICIPANT:

Printed Name	Signature / Mark or Thumbprint	Date and Time

I, _____*(NAME OF STUDY DOCTOR)*, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY DOCTOR:

Printed Name	**Signature**	**Date and Time**

TRANSLATOR / OTHER PERSON EXPLAINING INFORMED CONSENT.....(DESIGNATION):

Printed Name	**Signature**	**Date and Time**

WITNESS (If applicable):

Printed Name	**Signature**	**Date and Time**