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Dietary calcium intake and obesity in adult women: the POWIRS study

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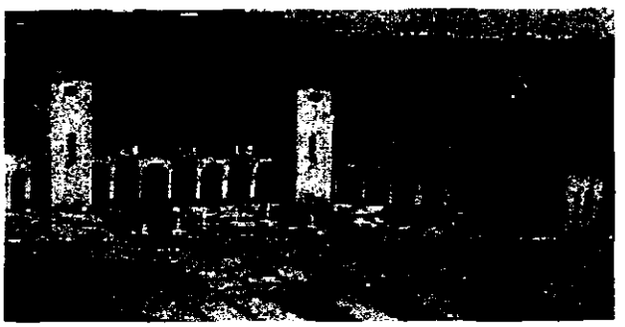

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For my husband, Christo

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Petro Rautenbach

ABSTRACT

Background: The role of dietary calcium in weight management is gaining support in the nutrition research community. It has been hypothesized that high calcium diets protect against fat gain by creating a balance of lipolysis over lipogenesis in adipocytes (Zemel *et al.*, 2000) and that a diet deficient in calcium is associated with higher body weight and that augmenting calcium intake may reduce weight and fat gain or enhance fat loss (Shapses *et al.*, 2004).

Objectives: A lack of baseline data on the physical, physiological and mental effects of obesity on urban African women was the motivation for the POWIRS (Profiles of Obese Women with Insulin Resistance Syndrome) study. The aim of the study was to assess the effects of obesity on health determinants of urban African and white women by comparing the lifestyle and risk factors for non-communicable diseases (NCDs) of lean, overweight and obese subjects. This led to a multi-disciplinary cross-sectional case-control study in which health determinants and health status, as well as the underlying mechanistic relationships between these factors were measured in a sample of African women volunteers. The study was repeated a year later, done in a sample of white women volunteers, POWIRS II. The effect of calcium intake on body composition was assessed during this study.

Methods: One hundred and two apparently healthy urban African women, between the ages of 20 and 50 years participated in the first phase of this case-control cross-sectional survey. For a period of about three weeks, each afternoon ten subjects were to report at a Metabolic Unit Facility (consisting of 10 single bedrooms, 2 bathrooms, a living room and kitchen). Each subject received a "participant sheet" which guided them through the different research 'stations' where the various measurements were done. During the course of the evening demographic questionnaires were filled in and all anthropometric measurements were taken, except weight and height measurements. All participants received an identical light supper which excluded alcohol and caffeine at 20h00, went to sleep before 23h00 and fasted overnight. From 06h00 in the morning weight, height and blood pressure measurements were taken. After a fasting blood sample was taken, a two-hour glucose tolerance test commenced. Subjects received a breakfast and afterwards habitual dietary intake questionnaires were completed.

Results: Mean total dietary calcium intake was significantly higher in white women (POWIRS II), with a mean intake 1053.8 mg per day, as opposed to a mean intake of 494.8 mg calcium per day in the black subjects (POWIRS I). Mean fat intake in the black subjects was 59.3 g per day, and in the white women 103.1 g per day. Thus the calcium:

fat ratio in white women was higher than in black women (11.0 and 8.4 respectively). After adjustment for age and total dietary energy intake, significant negative correlations were found between dietary calcium intake and various variables, only in the white subjects. These were BMI (r=-0.255, p=0.01), percentage body fat (r=-0.252, p= 0.01), fasting insulin (r=-0.205, p=0.05) and fasting glucose (r=-0.199, p=0.046). The calcium: fat ratio correlated negatively with BMI (r=-0.378, p<0.0001), percentage body fat (r=-0.401, p<0.0001), fasting glucose (r=-0.229, p=0.02), fasting insulin (r=-0.212, p=0.04) and plasma leptin (r=-0.284, p=0.004). Adjustment for smoking resulted in slightly different correlation coefficients, but similar significant correlations were still found. The only significant association that was found in the black population, was a negative correlation between dietary calcium intake and systolic blood pressure (p=0.03) as well as diastolic blood pressure (p=0.04). After adjustment for age, smoking and dietary energy intake no significant correlations were found in the black subjects.

Conclusion: The results from the POWIRS study in white women are consistent with the hypothesis that there may be an inverse relationship between adiposity and calcium intake. In our study higher calcium intakes were associated with lower body fat, lower BMI, lower fasting glucose and insulin, as well as plasma leptin in white women. The association seems to be significant in subjects with high intakes of fat and calcium (as seen in the white women).

Key words: Calcium, dietary fat, weight management, lipolysis, lipogenesis, adipocytes, blood pressure, body mass index, cholesterol, body fat, insulin, leptin, glucose.

OPSOMMING

Agtergrond: Die rol van kalsiuminname in die handhawing van gesonde liggaamsmassa word al hoe meer deur voedingsnavorsers ondersteun. Die hipotese is dat 'n dieet hoog in kalsium 'n beskermende effek teen vetstoring kan hê deur die balans ten gunste van lipolise teenoor lipogenese te beïnvloed (Zemel *et al.*, 2000). 'n Dieet laag in kalsium word geassosieer met 'n hoër liggaamsmassa en 'n verhoging van kalsiuminname kan gewig en vetstoring verlaag of verlies daarvan verhoog (Shapses, *et al.*, 2004).

Doelwitte: Onvoldoende inligting oor die fisiese, psigologiese en fisiologiese gevolge van obesiteit in stedelike swart vroue was die motivering agter die POWIRS ("Profiles of Women suffering from the Insulin Resistance Syndrome") studie. Die doel van die studie was om lewenstyl en risikofaktore vir nie-aanmeldbare siektes van maer, oorgewig en obese vroue te vergelyk. Dit het aanleiding gegee tot 'n multi-dissiplinêre dwarsprofiel kruis-kontrole studie waarin die bepalende faktore vir gesondheid, asook die onderligende meganismes van die verwantskap tussen hierdie faktore, gemeet is in 'n steekproef van swart vroue. Dieselfde studie is een jaar later herhaal in 'n steekproef van blanke vroue, die POWIRS II studie. Die effek van kalsiuminname op liggaam samestelling is ondersoek tydens hierdie studie.

Metodes: Een honderd en twee gesonde stedelike swart vroue, tussen die ouderdom van 20 en 50 jaar het aan hierdie gevalkontrole deursnit studie deelgeneem. Vir 'n periode van drie weke moes tien proefpersone elke middag by 'n Metaboliese Eenheid Fasiliteit (bestaande uit 10 enkelkamers, 2 badkamers, 'n leefarea en 'n kombuis) aanmeld. Elke proefpersoon het 'n "deelnamekaart" gekry wat hulle deur die verskillende navorsingspunte gelei het, waar die metings geneem is. Deur die loop van die aand is demografiese en psigologiese vraelyste ingevul en alle antropometriese metings is geneem, behalwe gewig en lengte. Die proefpersone het om 20h00 almal 'n identiese ligte aandete ontvang, sonder kaffeïen en alkohol. Almal het voor 23h00 gaan slaap en het deur die nag gevas. Vanaf 6h00 die volgende oggend is gewig-, lengte- en bloeddrukmetings geneem. Na 'n vastende bloedmonster geneem is, is 'n twee-uur glukosetoleransietoets begin. Die proefpersone het ontbyt ontvang en daarna is dieetinname vraelyste voltooi.

Resultate: Die gemiddelde kalsiuminname van die blanke vroue (POWIRS II) was 1053.8 mg per dag teenoor die gemiddelde 494.8 mg inname by die swart vroue (POWIRS I). Die gemiddelde vetinname was 59.3 g en 103.1 g per dag by die swart vroue en die blanke vroue onderskeidelik. Dus was die kalsium-tot-vet verhouding heelwat hoër in die blanke vroue (11.0) as in die swart vroue (8.4). Na korreksie vir ouderdom en totale dieet

energie-inname is betekenisvolle negatiewe korrelasies tydens hierdie studie gevind tussen dieet kalsiuminname en verskeie veranderlikes, net in die blanke vroue. Dit was met liggaamsmassa-indeks (LMI) (r= 0.255, p=0.01), liggaamsvet persentasie (r= 0.252, p=0.01), vastende insulien (r= 0.205, p= 0.05) en vastende glukose (r=-0.199, p=0.046). Die kalsium tot vet verhouding het betekenisvol negatief gekorreleer met LMI (r= 0.378, p <0.0001), vastende glukose (r=-0.229, p=0.021), liggaamsvet persentasie (r= 0.401, p<0.0001), vastende glukose (r= -0.229, p= 0.02), vastende insulien (r= 0.212, p= 0.04) en plasma leptien (r= 0.03, p=0.004). Korreksie vir rook het min aan hierdie korrelasies verander. Die enigste betekenisvolle verband by die swart vroue was tussen kalsiuminname en bloeddruk. Dieet kalsiuminname het betekenisvol negatief met sistoliese (p=0.031) asook diastoliese (p=0.044) bloeddruk gekorreleer. Na korreksie vir ouderdom, rook en totale energie-inname was hierdie korrelasies nie meer betekenisvol nie.

Gevolgtrekking: Die resultate van die POWIRS studie in blanke vroue bevestig die hipotese dat daar 'n indirekte verwantskap tussen vetsug en kalsiuminname is. In ons studie is hoër kalsiuminname met laer liggaamsvet, laer LMI, laer vastende glukose en insulien, asook laer plasma leptien in wit vroue geassosieer. Die assosiasie blyk sterker te wees met hoër innames van vet en kalsium (soos by die wit vroue). In swart vroue is hoër kalsiuminname met laer bloeddruk geassosieer.

Trefwoorde: Kalsium, dieetvet, liggaamsmassa, vetsug, lipolise, lipogenese, bloeddruk, liggaamsmassa indeks, cholesterol, vetstore, liggaamsvet, insulien, leptien, glukose.

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LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ADP	Air displacement plethysmography
ARIC	Atherosclerosis risk in communities
BMI	Body mass index
CARDIA	Coronary Artery Risk Development in Young Adults
$[Ca^{2+}]i$	Intracellular calcium
Ca	Calcium
CCK	Cholecystokinin
CI	Confidence interval
cm	Centimeters
CSFII	Continuing Survey of Food Intake by Individuals
DEXA	Dual energy x-ray absorptiometry
$1,25(OH)_2D3$	1,25-dihydroxy-vitamin D3
D[1.25(OH)2]	1,25-dihydroxyvitamin D
FAS	Fatty acid synthase.
g	Gram
HDL	High density lipoprotein
IRS	Insulin resistance syndrome
kg	Kilogram
kg/m^2	Kilogram per square meter
kJ	Kilojoules
LDL	Low density lipoprotein
LMI	Liggaamsmassa-indeks
mg	Milligram
mmHg	Millimeter Mercury
mmol/L	Millimol per liter
NCD	Non communicable diseases
ng/ml	Nanogram per milliliter
n	Number of subjects
pmol/L	Picomoles per liter
POWIRS	Profiles of Women suffering from the Insulin Resistance Syndrome
PTH	Parathyroid hormone
P	Level of statistical significance
RAS	Renin-angiotensin system

SD	Standard Deviation
TC	Total cholesterol
µU/ml	Micro units per milliliters

CHAPTER 1: Introduction

1.1 Background

The incidence of obesity is increasing at an alarming rate and with that the chronic diseases associated with obesity such as diabetes mellitus, coronary heart disease and hypertension (Bradshaw *et al.*, 1995). A study that included 547 rural and 468 urban black South African women, concluded that about one quarter (27.5%) to more than half (54%) of the subjects in the different age groups were obese (Mollentze *et al*, 1995). The authors recommended that measures must be taken to prevent a future epidemic of atherosclerotic vascular disease in South Africa's black population and that the adverse effects of obesity in black women should be emphasized. Results from both the coronary artery risk development in young adults (CARDIA) and atherosclerosis risk in communities (ARIC) studies have shown that black and white women should avoid excess adiposity (Folsom *et al.*, 1991).

There is little understanding of the optimal dietary composition necessary to promote weight loss and prevent weight gain. While much attention has been focused on macro nutrient intake and body weight regulation, particularly dietary fat (Astrup *et al.*, 2000), an emerging body of literature suggests that dietary calcium may play a role in the regulation of body weight and body fat (Melanson *et al.*, 2003).

1.2 Problem statement

It has been hypothesized that high calcium diets protect against fat gain by creating a balance of lipolysis over lipogenesis in adipocytes (Zemel *et al.*, 2000). A diet deficient in calcium is apparently associated with higher body weight and augmenting calcium intake may reduce weight and fat gain or enhance fat loss (Shapses *et al.*, 2004). Implicit in the hypothesis that a high calcium intake promotes maintenance of lower body fat mass in humans by enhancing lipolysis is the assumption that high calcium diets promote greater rates of whole-body fat oxidation (Melanson *et al.*, 2003).

The aim of this dissertation was to investigate whether calcium intake is associated with various variables of body composition in South African women. The association between dietary calcium intake and the variables was also assessed, in order to clarify the mechanism underlying this association.

1.3 Objectives

The main aim of the study was to investigate whether the hypothesis regarding dietary calcium intake and body composition can be proven. Specific objectives were:

- Assessment of nutritional intake with special attention to total energy, total fat and total dietary calcium intake of adult black and white women, respectively;
- Assessment of dairy product intake (amount of portions per day);
- Assessment of the body composition data regarding body fat percentage and BMI;
- Assessment of the association between body composition and dietary calcium intake;
- Assessment of the association between fasting serum insulin, fasting serum glucose, plasma leptin and serum cholesterol concentrations and dietary calcium intake;
- Assessment of the association between blood pressure and dietary calcium intake.

1.4 Structure of the dissertation

The association between dietary calcium intake and body composition in women is explored and reported in this dissertation.

In Chapter 2, the physiology of obesity; the influence of calcium on body weight; human studies linking calcium to body weight; dairy products, calcium, obesity and insulin resistance syndrome and dairy sources of calcium vs. supplemental calcium are examined in a literature review. Chapter 3 provides a detailed explanation of the methodology that was used in this study as well as the results and discussion. The conclusion and recommendations follow in Chapter 4.

A complete reference list is provided in alphabetical order and all questionnaires used to obtain information during the course of the study are included in the Addendum section.

I am a part-time student at the North-West University and I was responsible for computerizing the demographic data as well as the dietary intakes of the subjects. In order to do so I had to do quality control and I verified weights and other information so that the fieldworkers could establish whether the information is correct. Dietary intake for each subject of every food item had to be converted to gram per day for the program used.

CHAPTER 2

Influence of Calcium status on body composition: A review of the literature

2.1 Obesity: a growing epidemic

Obesity is a growing epidemic with subsequent health consequences leading not only to reduced quality of life, but also to increased medical costs (Teegarden, 2003). The prevalence of increased body weight is rising at an alarming rate.

Although the characterization of several important obesity genes over the past ten years has resulted in an increased insight into the pathophysiology of obesity (Yanovski & Yanovski, 1999), these studies have not led to any significant improvements in the ability to prevent or treat overweight. Genetic factors, it seems, have largely played only a secondary role in the rising prevalence of obesity. Rather, environmental factors affecting diet and activity appear likely to have been the most important determinants of the increasing adiposity (Hill & Melanson, 1999).

There is little understanding of the optimal dietary composition necessary to promote weight loss and prevent weight gain. While much attention has been focused on macro nutrient intake and body weight regulation, particularly dietary fat (Astrup *et al.*, 2000), an emerging body of literature suggests that dietary calcium may play a role in the regulation of body weight and body fat (Melanson *et al.*, 2003).

From recent studies done, the hypothesis was developed that high calcium diets protect against fat gain by creating a balance of lipolysis over lipogenesis in adipocytes (Zemel *et al.*, 2000) and that a diet deficient in calcium is associated with higher body weight and that augmenting calcium intake may reduce weight and fat gain or enhance loss (Shapses *et al.*, 2004). In other words, from the hypothesis that high calcium intakes promotes maintenance of a lower body fat mass in humans by enhancing lipolysis, it could be assumed that high-calcium diets promote greater rates of whole-body fat oxidation (Melanson *et al.*, 2003).

2.2 The physiology of obesity

The amount of fat in the body is precisely regulated as part of the process of energy homeostasis, a process whereby energy intake (food intake) is matched to energy expenditure (metabolism and exercise) and the size of the body's energy stores (the fat mass) (Woods & Seeley, 2002) . The major organ regulating this system is the brain, although multiple organ systems participate in the process. Signals related to the size of the fat mass are integrated with signals from the gastrointestinal system to control energy homeostasis (Woods & Seeley, 2002).

2.2.1 Control of meal size

There is little physiological evidence that appetite and meal initiation are controlled by metabolic or hormonal signals, such as low blood glucose. Rather, the available evidence suggests that, under normal circumstances, meal initiation is based on learned associations, for example, habit and the social environment. Regulation therefore has to involve how much is eaten and there is compelling evidence that meal cessation (that is, meal size) is controlled by pre-absorptive gut signals (Woods & Seeley, 2002).

Gastrointestinal peptides provide signals to tell the brain how much has been eaten, how many energy have accumulated and help to create the feeling of satiety. The best known of these satiety factors is cholecystokinin (CCK). A number of experiments have been carried out in humans in which CCK was given intravenously prior to a test meal and, in every instance, there was a significant reduction of meal size (Muuranhainen *et al.*, 1991). Although the size of individual meals can be manipulated, therapies intended to mimic satiety mechanisms are not in themselves likely to be efficacious for weight loss. There are no studies in which CCK has been given on a chronic basis to humans, but animal studies suggest that this would probably not lead to loss of body weight (Woods & Seeley, 2002).

2.2.2 Control of body fat

There is strong evidence that key hormonal regulatory signals – the adiposity hormones – control both how much is eaten and how much energy is expended. These hormones circulate in the blood in direct proportion to body fat content. They enter the brain and act on receptors in areas of the hypothalamus known to regulate food intake and energy expenditure. If weight is lost, the hormone levels fall, food intake goes up and energy expenditure is reduced. The opposite occurs when an individual has gained excess

weight. Thus body weight tends to be maintained relatively constantly over time (Woods & Seeley, 2002).

Insulin was the first compound described to have this effect. Pancreatic insulin secretion is directly proportional to the size of the fat mass (Woods & Seeley, 2002). Leptin is another adiposity hormone, which is secreted from white fat, again in direct proportion to the size of fat mass, although the amount of fat in the cell is not the exact stimulus for its secretion (Woods & Seeley, 2002).

Administration of either leptin or insulin directly into the brain causes a dose-dependent reduction of food intake, increased energy expenditure and decreased body weight. This suggests that the brain interprets the signal as if more fat has accumulated in the body. Conversely, reducing the amount of insulin or leptin uniquely in the brain causes increased food intake, decreased energy expenditure and increased body weight; in other words, individuals act as if they are underweight (Woods & Seeley, 2002).

From the above mentioned, the fact is realized that the neuro-endocrine control system over energy homeostasis is complex, with multiple possible points of intervention in weight control programmes.

2.3 Influence of calcium on body weight

The role of dietary calcium in weight management is gaining support in the nutrition research community. Among the effects now being attributed to increased consumption of dairy products and dietary calcium are the following:

1. Healthier body weight
2. Greater weight and fat loss on a reduced-energy diet (Zemel, 2001)

2.3.1 The Agouti gene, intracellular calcium and obesity

A compelling mechanism for the antiobesity effect of dietary calcium was provided by studies of the mechanism of action of the *agouti* gene [the first of the obesity genes to be cloned (Bultman *et al.*,1992), which strongly influences whether a fat cell burns energy-containing molecules or converts them to fat (Raloff, 2000) in regulating murine and human adipocyte metabolism. These studies demonstrated a key role for intracellular

calcium ($[Ca^{2+}]i$) in the regulation of adipocyte metabolism, that of modulation of adipocyte triglyceride stores (Jones *et al*, 1996; Shi *et al.*, 1999; Claycombe *et al.*, 2000; Xue *et al.*, 2001). An increase in $[Ca^{2+}]i$ was closely correlated with both the degree of ectopic agouti expression and body weight (Willard *et al.*, 1995), suggesting the possibility of a causal association between $[Ca^{2+}]i$ and obesity. Recombinant *agouti* protein directly increased calcium influx and steady-state ($[Ca^{2+}]i$) in a variety of cell types, including both murine and human adipocytes (Zemel *et al.*, 1995; Kim *et al.*, 1997).

Researchers have conducted animal studies and *in vitro* studies with human fat cells to identify the mechanism by which calcium impacts body weight. Somewhat counter-intuitively, as dietary calcium intake increases, calcium levels within fat cells decrease. In turn, lower calcium levels within cells impact the metabolism of fat, in favour of weight loss. Namely, fatty acid synthase activity, and therefore, fat synthesis (lipogenesis) decreases (decreased triacylglycerol accumulation, Zemel: 2000) and fat breakdown (lipolysis) increases. This shift in fat metabolism may result in less fat storage and a reduction in body weight (Zemel, 2003).

Intracellular calcium concentrations are determined by complex interactions between the flux through voltage-dependent and receptor-stimulated calcium channels, by sequestration with binding proteins, by storage of free calcium in intracellular compartments such as the endoplasmic reticulum, and by active gradient-maintaining ion pumps (Meldolesi & Pozzan, 1998). $[Ca^{2+}]i$ appears to play an important role in the metabolic derangements associated with obesity, hypertension, and insulin resistance (Zemel, 1998). Factors important in obesity, such as insulin (Draznin *et al.*, 1988) and the agouti protein (Xue *et al.*, 1998) – normally expressed in human adipocytes (Kwon *et al.*, 1994) – have been shown to trigger an increase in $[Ca^{2+}]i$ in human adipocytes. (See figure 2.1)

Obese persons have a greater $[Ca^{2+}]i$ than do nonobese age- and sex-matched control persons (Draznin *et al.*, 1988). $[Ca^{2+}]i$ was also found to regulate both lipogenesis and lipolysis in human adipocytes (Zemel, 1998). High $[Ca^{2+}]i$ stimulates the expression and activity of fatty acid synthase, a key enzyme in de novo lipogenesis (Zemel, 1998).

With regard to calcium homeostasis, the calcium-regulating hormones vitamin D and parathyroid hormone (PTH) have both been shown to stimulate a significant and sustained increase in $[Ca^{2+}]i$ concentrations in primary cultures of human adipocytes (Zemel *et al*,

2000).

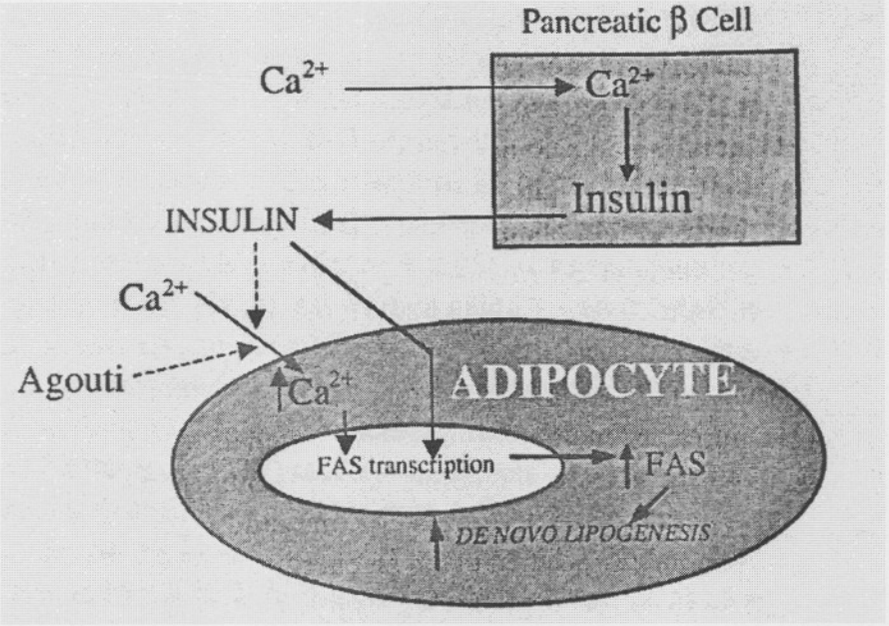

Figure 2.1 Suggested role for the agouti protein, insulin, and intracellular calcium in lipogenesis in human adipocytes. FAS = fatty acid synthase (Zemel, 1998).

2.3.2 Relation between dietary calcium and intracellular calcium

From the afore mentioned, the diametrically opposed effects of increases in $[Ca^{2+}]i$ and increases in dietary calcium, are outlined. Greater $[Ca^{2+}]i$ stimulates lipogenesis and inhibits lipolysis. Greater dietary calcium appears to have opposite effects (Petrov & Lijnen, 1999).

One possible explanation that would link greater dietary calcium to less $[Ca^{2+}]i$ is the effect of dietary calcium on the hormones regulating calcium balance (Parikh & Yanovski, 2003). When serum calcium levels fall below normal (8.5 – 10.5 mg/dl), counter-regulatory increases in PTH promote increased bone resorption, decreased calcium excretion in the kidneys, and increased formation of 1,25-dihydroxy-vitamin D3 ($1,25(OH)_2D3$). Both $1,25(OH)_2D3$ and PTH stimulate increases in $[Ca^{2+}]i$ in human and murine adipocytes (Zemel *et al.*, 2000). Dietary calcium supplementation in humans has been shown to cause significant suppression of intact PTH, $1,25(OH)_2D3$, and the $[Ca^{2+}]i$ in erythrocytes and platelets (Petrov & Lijnen, 1999). Thus, increased calcium intakes lower blood concentrations of calcitropic hormones, such as $1,25(OH)_2D3$ [1,25(OH)2] and PTH, whereas, as well known, diets deficient in calcium stimulate the production and release of $1,25(OH)_2D3$ and PTH (Shi *et al.*, 2001; Parikh & Yanovski, 2003).

Thus, lower dietary calcium intakes can lead to increased concentrations of $1,25(OH)_2D3$ and PTH, which in turn may increase adipocyte calcium. (See figure 2.2) These elevated intra-adipocyte calcium concentrations might then increase the rate of lipogenesis and inhibit lipolysis, consequently leading to increased adiposity. An increased calcium intake would be proposed to prevent this cascade from developing by keeping the calcitropic hormone concentrations low, therefore lowering $[Ca^{2+}]i$ and ultimately the lipid content in adipocytes (Parikh & Yanovski, 2003).

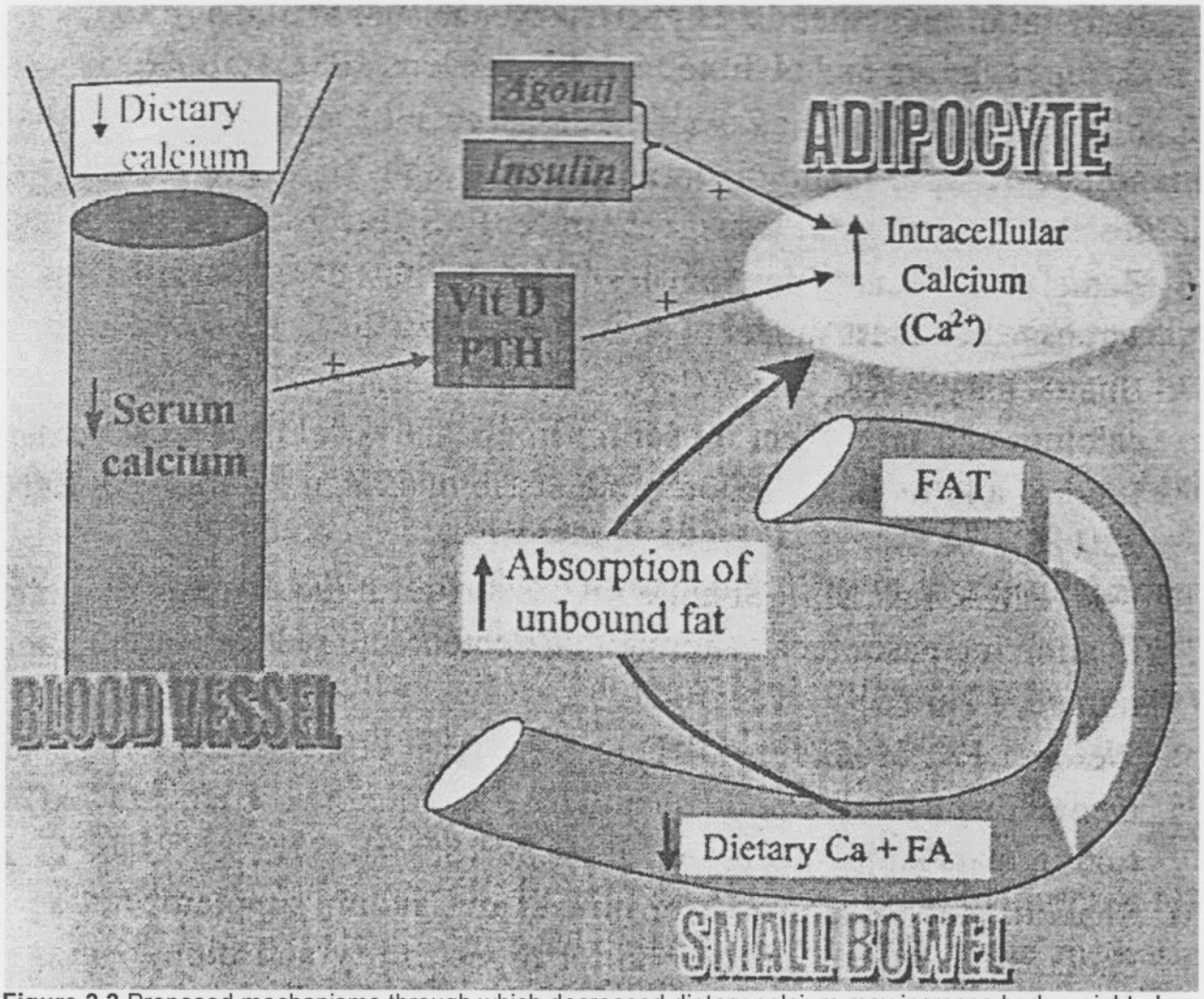

Figure 2.2 Proposed mechanisms through which decreased dietary calcium may increase body weight. Low dietary calcium may increase intra-adipocyte calcium concentrations via stimulation of calcitropic hormones such as vitamin D (Vit D) and parathyroid hormone (PTH). (Parikh & Yanovski, 2003).

2.3.3 Relation between dietary calcium and dietary fat absorption

Dietary calcium has an effect on the absorption of triacylglycerols from the gastrointestinal

tract. This might be a second mechanism by which dietary calcium affects body adiposity. The effect of dietary calcium on fecal fatty acid excretion and serum lipids in a randomized, single-blind, metabolic study of 13 men with moderate hypercholesterolemia, was studied (Denke *et al.*, 1993). In this study, a low calcium diet (410mg elemental Ca/d) was compared with a high-calcium diet (2200mg elemental Ca/d) using calcium citrate maleate as a source for the supplemental calcium for 10 days. Calcium fortification increased the percentage of dietary saturated fat excreted in 72-h fecal collections from 6% to 13% per day.

Welberg *et al.* (1994) studied the effects of calcium supplementation on quantitative and qualitative fecal fat excretion in 24 subjects consuming a controlled diet (1450 – 1880 mg Ca/d) that was supplemented with 0.2 or 4 g elemental Ca/d (given as calcium carbonate). Calcium increased fecal fatty acids in a dose dependent fashion. Total fat excretion increased from 6.8 ± 0.9% of total fat intake with no calcium supplementation to 7.4 ± 1.0% with 2g Ca and 10.2 ± 1.4% with 4g elemental Ca *($P = 0.003$).* Increased fat excretion was due to greater fatty acid excretion; the excretion of neutral fat remained unchanged. High dietary calcium intake increases saponification of fatty acids (FAs) in the gut by calcium and thus may decrease body fat stores by decreasing the absorption of fat (Parikh & Yanovski, 2003).

These studies of calcium's effects on fecal fat excretion predict small effects on total-body lipid flux. The degree of fecal fat loss induced by 2 g elemental Ca in Welberg *et al*'s (1994) study is only ≈ 3% of that induced by lipase inhibitors such as Orlistat (Hollander *et al.*, 1998). In the end, a change in body weight of ≈ -0.4 kg/y can be expected for a person consuming a 2500-kcal diet containing one-third of energy from fat, who took an additional 2g elemental Ca/d. Thus, the effects of calcium on fat excretion are not sufficient to explain the much greater weight differences suggested by some animal and human studies, particularly those supplying calcium in the form of dairy products (Zemel *et al.*, 2000).

2.4 Human studies linking calcium intake to body weight

Many studies identified strong inverse correlations between adiposity and calcium intake. Some of them and those that did not find this association will be looked at.

2.4.1 Studies done on adults

In a cross-sectional study by Melanson *et al.*, (2003) a total of 35 (21 males, 14 females) non-obese, healthy adults participated to determine if total calcium intake and intake of calcium from dairy sources are related to whole-body fat oxidation. Daily energy expenditure, macronutrient oxidation, habitual calcium intake and acute calcium intake were measured. Acute calcium intake (mg/kcal) was positively correlated with fat oxidation over 24 hours (r=0.38, P=0.03), during sleep (r=0.36, P=0.04) and during light physical activity (r=0.32, P=0.07). Acute calcium intake was inversely correlated with 24-h respiratory quotient (RQ) (r= -0.36, P=0.04) and RQ during sleep (r= -0.31, P=0.07). After adjustment for fat mass, fat-free mass, energy balance, acute fat intake, and habitual fat intake, acute calcium intake explained ≈ 10% of the variance in 24-h fat oxidation. Habitual calcium intake was not significantly correlated with fat oxidation. In backwards, stepwise models, total acute calcium intake was a stronger predictor of 24 h fat oxidation than habitual daily calcium intake. According to their findings, higher acute calcium intake is associated with higher rates of whole-body fat oxidation. These effects were not apparent over 24 h, during sleep and, to a lesser extent, during light physical activity. Calcium intake from dairy sources was not a more important predictor of fat oxidation than total calcium intake. Although these results do not show directly that dietary calcium promotes fat oxidation, the findings are consistent with the hypothesis that high intakes of calcium are associated with lower levels of body fat mass.

A relationship was noted in a recent analysis of the Continuing Survey of Food Intake by Individuals (CSFII), which noted a highly significant inverse relationship between body mass index and calcium consumption, and a dose response reduction in obesity prevalence among women as calcium intake increased from the first tertile (<453 mg/day) to the second (453 – 712 mg/day) and third (>712 mg/day) tertiles of calcium intake (Albertson *et al.*, 2003).

Jackmain *et al.* (2003) examined the relationship between calcium intake and body composition in adults participating in Phase II of the Quebec Family study and found that

body weight, body fat, BMI, waist circumference, and total abdominal adipose tissue were significantly greater in adults consuming <600 mg calcium per day than in those consuming higher levels of calcium.

In the HERITAGE Family study, the strongest inverse relationship between dietary calcium and adiposity occurred in black men and white women. Black men in the highest tertile group of calcium intake were significantly leaner than those in the lowest calcium intake group, whereas white women exhibited a significant inverse relationship between calcium intake and BMI, percent body fat, and total abdominal fat area (Loos *et al.*, 2003).

Kamycheva *et al.* (2002) published a study that was designed to investigate whether there is any association between body mass index (BMI) and life-style factors, with a special emphasis on calcium and vitamin D intakes. In the fourth Tromso study 9252 men and 9662 women participated and completed the food-frequency and life-style factors questionnaires. BMI, coffee and alcohol consumption, physical activity, smoking, and calcium and vitamin D intakes were measured. A negative association between physical activity, smoking and BMI, and a positive association between BMI and coffee intake were found in both sexes ($P < 0.001$). BMI and calcium intake were positively related in men ($P < 0.001$), but not in women. BMI and vitamin D intake were negatively associated in both sexes ($P < 0.001$).

Shapses and co-workers (2004) aimed to determine whether calcium supplementation during a weight loss intervention affects body fat or weight loss. Data were combined from three separate 25-wk randomized, double blind, placebo-controlled trials of 1000 mg/d calcium-supplementation in 100 premenopausal and post menopausal women. The primary outcome measures were change in body weight and fat mass adjusted for baseline values. There were no significant differences in body weight of fat mass change between the placebo and the calcium-supplemented groups in the pooled analysis and no significant interactions of calcium supplementation with menopausal/diet status. Calcium supplementation did not significantly affect amount of weight or fat lost by women counseled to follow a moderately energy restricted diet for 25 weeks. Nevertheless, the magnitude and direction of the differences for group means are consistent with a hypothesized small effect (Shapses *et al.*, 2004).

Barr (2003) conducted a MEDLINE search to identify randomized trials of supplementation with calcium or dairy products. Nine studies of dairy product supplementation were

located: In seven, no significant differences in the change of body weight or composition were detected between treatment and control groups. However, two studies conducted in older adults observed significantly greater weight gain in the dairy product groups. Barr (2003) concluded that the data available from randomized trials of dairy product or calcium supplementation provide little support for an effect in reducing body weight or fat mass. However, it is important to realize, these studies reviewed were not specifically designed or powered to address the issue of weight loss.

Teegarden (2003) however, made the comment that if dairy products are added to a diet without compensation for energy intake, one is likely to gain weight. This is shown in a study by Barr *et al.*, 2000, in which 204 men and women, aged 55 – 85 y, were randomized to either a control group or a dairy intervention group. The dairy intervention group was advised to increase skim or 1% milk intake from <1.5 servings to three servings per day. Although their overall nutrient intakes improved substantially, the dairy intervention group also gained 0.6 kg in the 12-wk trial, significantly more than did the control group. However, this gain was less than would be predicted by the increase in dairy products, suggesting that either the subjects altered their diets to compensate for the additional energy in their diet, or potentially that calcium or dairy shifted the energy balance to partially compensate for the additional calories.

In another study done by Lin *et al.* in 2000, healthy normal weight 18–31 year old women were randomized into an exercise or non-exercise group after baseline testing. Three-day diet records were collected at baseline and six-month intervals, and averaged over the two year period of the study. Total body bone mineral content was assessed by dual energy X-ray absorptiometry (DEXA), allowing an analysis of body composition changes as well. The results of 54 women who completed the two year trial were used for analysis. Calcium intakes were low (781 ± 212 mg/d), compared to the dietary reference intakes (1000 mg/d for most of this group), and the primary source of dietary calcium were from dairy sources (67%). When dietary calcium was expressed as nutrient density (calcium/energy, mg/kcal), it negatively predicted changes in body weight and body fat, but not lean mass (Lin *et al.*, 2000).

To further explore why calcium intake predicted the changes only when corrected for energy intake, women were categorized into groups either above or below the mean energy intake of the cohort (1876 kcal/d). Calcium intake did not predict changes in weight or fat mass in the group with calorie intakes above the mean, whereas energy intake

positively predicted these changes; thus the higher the calories, the greater the increase in body fat. On the other hand, calcium, but not calories, negatively predicted changes in weight and fat mass in women with energy intakes below the mean. Between 10 % and 13 % of the variability in weight and fat mass changes were accounted for by calcium or dairy calcium intakes (Lin *et al.*, 2000).

2.4.2 Studies done on children

Carruth & Skinner (2001) conducted a longitudinal study to assess preschool children's food consumption (24 – 60 months) and related their findings to body composition at 70 ± 2 months. Fifty-three white children participated in this study of children's food practices and growth. Using in-home interviews and trained interviewers, 18 days of dietary data and measured height and weight of each child at 6 month intervals were collected. Body composition was determined by DEXA. Dietary fat was 30 – 33% of energy with saturated and mono unsaturated fat intakes >10% and poly unsaturated <10%. Higher mean longitudinal calcium (mg/day) intakes and more servings/day of dairy products were associated with lower body fat percentages. Males had significantly less body fat (p=0.01) than females. They concluded that higher longitudinal intakes of calcium, mono unsaturated fat, and servings of dairy products were associated with lower body fat percentages.

To further investigate the relationship between calcium and childhood weight, Skinner *et al.* (2003) conducted a longitudinal study in 2003. Subjects included 52 8-year old white children (n=25 boys, 27 girls) who participated along with their mothers. Percentages body fat and amount of body fat were assessed using DEXA. In a prospective design, height, weight, and dietary intakes were monitored longitudinally from the ages of 2 months to 8-years.

At 8 years of age, percent body fat was 22.7 ± 6.7 for boys and 26.2 ± 7.9 for girls. Dietary calcium and polyunsaturated fat intake were negatively related to percent body fat (*P = .02 to 0.4)* in three statistical models, which predicted 28% to 34% of the variability in body fat among children. Other variables positively associated with percent body fat were total dietary fat or saturated fat, female gender, sedentary activity, father's BMI, and mothers' body fat percentages. Calcium intakes were significantly correlated over time. Variety of diet was positively related to calcium intake, while intakes of carbonated and other sweetened beverages were negatively related (Skinner *et al.*, 2003).

It was concluded that children should strongly be encouraged to include calcium-rich foods in their diet, as this dietary component may impact upon body weight (Skinner, *et al.*, 2003).

Novotny (2003) at the University of Hawaii studied 321 white, Asian, and mixed-ethnicity girls aged 9 – 14 (average 11.5 years) during 2000 and 2001. For three days, each girl recorded everything she ate and drank and any calcium or multivitamin supplements she took. A researcher recorded the girl's weight and the amount of fat at the iliac site, which is a measure of abdominal fat (Novotny, 2003). Girls who consumed more total energy and exercised less were heavier and had more body fat. However, when the researchers compared groups of girls at comparable age, height, level of maturation, calorie intake and exercise level, they found that girls who consumed more calcium on average weighed less than similar girls who consumed less calcium. It made very little difference if the calcium came solely from dairy products in the diet, or from total calcium including supplementation (Novotny, 2003).

The current and rapidly growing body of evidence is substantial and supports the relationship of dietary calcium intake to reductions in weight and body fat mass. However, it is important to confirm these observations in studies specifically designed to address this issue and in larger trials. It is also important to further understand the underlying mechanism(s) for this effect, and to determine whether the impact is greater in certain subgroups, or while the energy balance is shifting (Teegarden, 2003).

2.4.3 Dairy products, calcium, obesity and insulin resistance syndrome

Insulin resistance is characterized by abnormal intracellular calcium homeostasis in several types of cells, including skeletal myocytes, vascular smooth muscle cells, and adipocytes (Zemel, 1995). Increasing $[Ca^{2+}]i$ inhibits insulin stimulated glucose transport, and calcium channel antagonism improves cellular insulin sensitivity (Draznin, 1993). As previously mentioned, greater dietary calcium can be linked to less $[Ca^{2+}]i$ (Parikh & Yanovski, 2003). In other words, theoretically, less dietary calcium may contribute to insulin resistance.

The dairy intake of 3,157 African-American and white adults (aged 18 – 30 years) who participated in the Coronary Artery Risk Development in Young Adults (CARDIA) study, was assessed by Pereira *et al.* (2002). Insulin resistance syndrome (IRS) was defined as the presence of ≥ two of the following criteria: abnormal glucose homeostasis (fasting plasma insulin level ≥ 20 μU/mL, fasting plasma glucose level ≥ 110 mg/dL, or use of medication to control blood glucose levels), obesity (body mass index [BMI] ≥ 30 or waist-to-hip ratio ≥ 0.85 for women or ≥ 0.90 for men), elevated blood pressure (≥ 130/80 mm Hg or use of antihypertensive medication), and dyslipidemia (high-density lipoprotein cholesterol level </= 35 mg/dL or serum triglyceride level ≥ 200 mg/dL). Dietary intake of dairy products was significantly inversely associated with incidence of IRS components among subjects who were overweight (BMI ≥ 25) at baseline, but not among leaner subjects (BMI < 25). The adjusted risk for development of IRS (≥ 2 components) was 72% lower among overweight subjects with intakes of dairy products in the highest vs the lowest quintile (≥ 35 vs < 10 times/week). Each one serving/day increment in intake of dairy products was associated with a 21% decrease in risk of developing IRS. These associations were similar for African American and whites and for men and women. Other dietary factors, including intake of macronutrients and micronutrients, did not explain the association between dairy intake and IRS (Pereira *et al.*, 2002).

Yu *et al.*, (2003), concluded through their study on Wistar rats, that dietary calcium may improve hyperinsulinemia and also enhance the expression of uncoupling protein 3 (UCP3) mRNA in skeletal muscle, by increasing the serum level of leptin which may play a role in the prevention of obesity.

2.5 Dairy sources of calcium vs. supplemental calcium

In many cases dairy products exert markedly greater effects in attenuating weight and fat gain on obesity-promoting diets and in accelerating fat loss during energy restriction compared with identical levels of inorganic calcium sources (Teegarden & Zemel, 2003). Dairy sources of calcium produced 50% to 70% greater effects on fat loss during energy restriction in both mice and humans (Zemel & Miller, 2004). Although the additional components of dairy products responsible for the differential effects between calcium and dairy products are not yet known, current work is underway to determine their identity. At present, preliminary data suggest that this additional activity resides in the whey fraction of milk. Whey is recognized as a rich source of bioactive compounds (Shah, 2000) that may act independently or synergistically with the calcium to attenuate lipogenesis, accelerate lipolysis, and/or affect nutrient partitioning between adipose tissue and skeletal muscle (Zemel & Miller, 2004). Whey protein has recently been reported to contain significant angiotensin-converting enzyme (ACE) activity (Ha & Zemel, 2003). Although ACE inhibitory activity may appear to be more relevant to an anti-hypertensive effect than to an anti-obesity effect of dairy, recent data demonstrate that adipocytes have an autocrine/paracrine renin-angiotensin system (RAS), and that adipocyte lipogenesis is regulated, in part, by angiotensin II (Ha & Zemel, 2003). Thus, activation or suppression of the adipocyte RAS may exert corresponding effects on adipocyte lipid metabolism independent of the circulating RAS. Indeed, inhibition of the RAS mildly attenuates obesity in rodents, and limited clinical observations support this concept in hypertensive patients treated with ACE inhibitors. These observations suggest that whey derived ACE-inhibitory activity may contribute to the anti obesity effect of dairy products. Consistent with this proposed mechanism, a whey-derived ACE inhibitor was recently found, which augmented the effect of dietary calcium on weight and fat loss in energy-restricted aP2-*agouti* transgenic mice (Causey & Zemel, 2003). However, the combination of calcium and ACE inhibitor was still significantly less potent than milk or whey in reducing body fat, indicating that other whey bioactive compounds may contribute or, alternatively, that a synergistic effect of multiple factors, along with the aforementioned effects of the calcium, are responsible (Zemel & Miller, 2004).

The inverse relationship between dietary calcium and IRS in the CARDIA study was explained solely by dairy intake, whereas the inverse association between dairy consumption and IRS was not altered by adjustment for calcium, indicating an effect of dairy consumption independent of calcium intake (Pereira *et al.*, 2002).

In a previous mentioned study done by Lin *et al.*, (2000), dairy products predicted the changes (lowering of body weight and body fat) as well as did non-dairy calcium. The range of non-dairy calcium intakes however, was low and may not have been sufficient to demonstrate a relationship between lowering of body fat and calcium. Furthermore, in the study by Melanson *et al.*, (2003) calcium intake from dairy sources was not a more important predictor of fat oxidation than total calcium intake. Also, it made very little difference if the calcium came solely from dairy products in the diet or from total calcium including supplementation, when Novotny (2003) studied whether calcium intake had an effect on the weight of adolescent girls.

2.6 Conclusion

Calcium intake or dairy products are not a 'magic bullet' for weight loss or maintenance, and energy balance remains the underlying cause of obesity (Teegarden & Zemel, 2003). However, a substantial body of evidence supporting a beneficial role of dietary calcium and dairy foods in the partitioning of dietary energy has rapidly emerged over the last several years. Increasing dietary calcium intake from the prevailing low levels to levels in the currently recommended range of intakes, results in reductions in body fat in the absence of energy restriction, and acceleration of weight and fat loss during periods of modest energy restriction. Notably, dairy sources of calcium appear to exert markedly greater effects than supplemental or fortified sources. Although there is a strong theoretical framework in place to explain the effects of dietary calcium on adipocyte metabolism and lipid storage, the mechanism(s) whereby dairy products augment these effects are not yet clear. Preliminary evidence suggest that bioactive compounds in whey, including ACE inhibitory activity, may play a role, but cannot fully explain the greater effect of dairy versus calcium (Zemel & Miller, 2004).

CHAPTER 3: The association between calcium intake and body composition of women

3.1 Introduction

A lack of baseline data on the physical, physiological and mental effects of obesity on urban African women was the motivation for the POWIRS (Profiles of Obese Women with Insulin Resistance Syndrome) study. The aim of the study was to assess the effects of obesity on health determinants of urban African and white women by comparing the lifestyle and risk factors for non communicable diseases (NCD) of lean, overweight and obese subjects. A structural outline of the study was developed and is provided in Figure 3.1. This led to a multi-disciplinary cross-sectional case-control study in which health determinants and status as well as the underlying mechanistic relationships between these factors were measured in a sample of African female volunteers. The study was repeated a year later, in a sample of white women, the POWIRS II study. In this paper, the design and methods of the POWIRS study are described, and the association between calcium intakes and body composition is assessed. Only the latter association will be investigated, and the association between some of the health profile variables and calcium intake will also be assessed, in order to understand the possible mechanism whereby calcium intake has an effect on body composition.

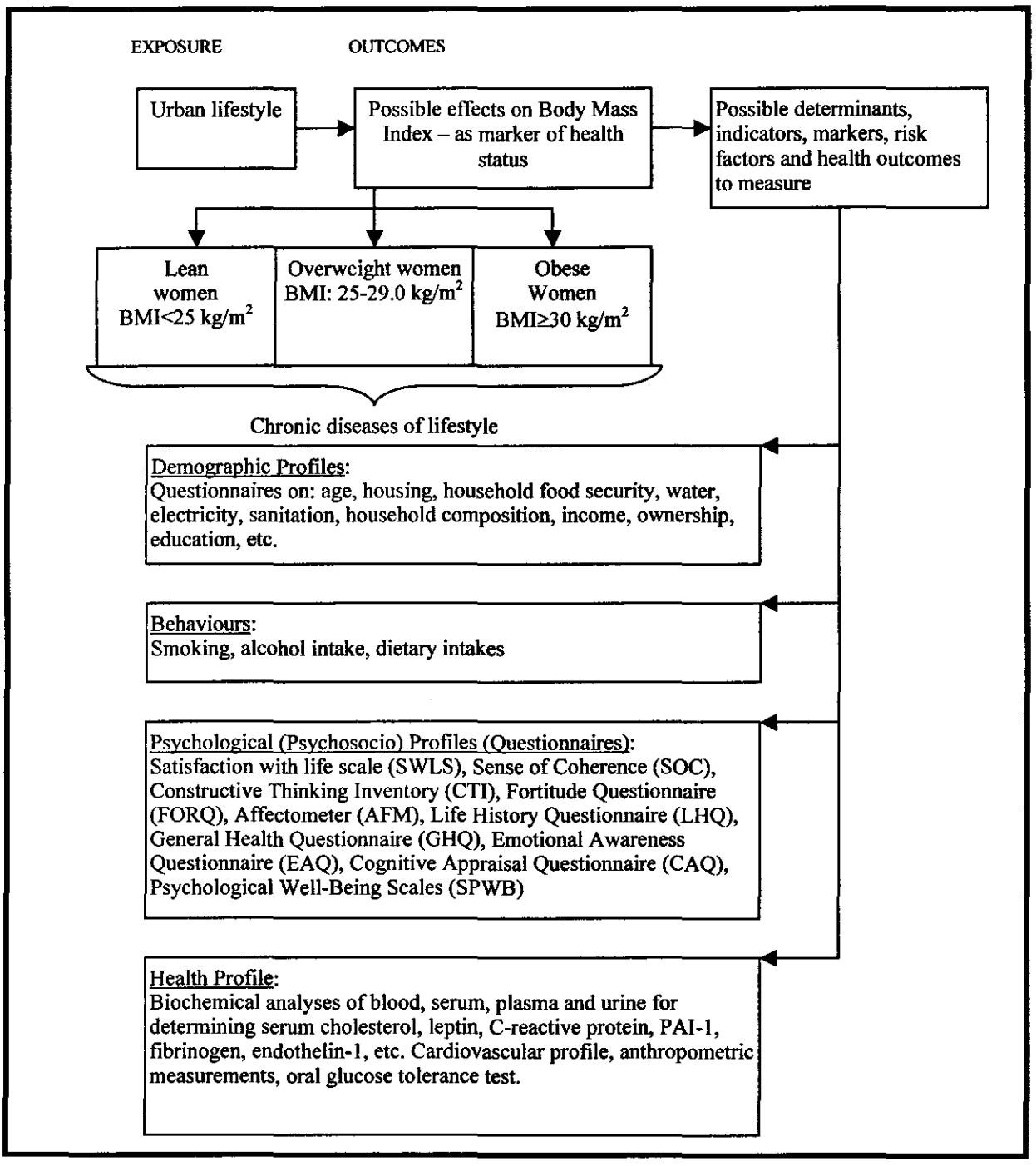

Figure 3.1. Structural outline used to design the POWIRS- study.

3.2 Study design and subject selection

The first phase of the study was a case-control cross-sectional survey involving 102 urban African women volunteers working at a governmental institution in Potchefstroom district in the North West Province, South Africa. A dietician, employed at the institution, recruited the subjects according to the initial design of the study. The inclusion criteria were apparently healthy African women aged between 20 and 50 years. The dietician attempted to recruit only HIV negative subjects (according to their status as determined three months before the study), but the negative status of all subjects cannot be guaranteed. Subjects were recruited based on their body mass index (BMI) as measured at the Medical Station at the institution. Three groups of subjects were selected based on guidelines of the Report of a World Health Organization Consultation on Obesity (1997): i) normal range (lean) with BMI: 18.5-24.9 kg/m^2; ii) overweight (pre-obese) with BMI: 25-29.9 kg/m^2; and iii) obese with BMI $\geq$ 30 kg/m^2. Pregnant and lactating women and those with oral temperatures above 37°C, were excluded. The study was repeated a year later, with a sample of 115 white South African women volunteers (POWIRS II).

3.3 Ethical considerations

The study has been approved by the Ethics Committee of the North-West University (project number 03M03). All subjects were fully informed about the objectives and procedures of the study, and assistance was available to provide information in their home language. All subjects signed an informed consent form. Subjects identified with hypertension, diabetes or other abnormalities were referred to local clinics, hospitals or their physicians. All subjects received a short report with their health information. Subjects received supper and breakfast after the glucose tolerance test, as well as a small financial compensation to cover their travel expenses.

3.4 Organisational procedures

Permission to conduct the study with employees from a local governmental institution was obtained from the relevant authorities. A dietician from the institution assisted in the recruitment, selection and screening of subjects. For a period of about three weeks each afternoon ten subjects were to report at a Metabolic Unit Facility (consisting of 10 single bedrooms, 2 bathrooms, a living room and kitchen). They were all introduced to the set-up and after the experimental procedures were explained to them, they signed informed

consent forms. Each subject received a "participant sheet" which guided them through the different research 'stations' where the various measurements were done. This sheet was signed at each station. During the course of the evening demographic questionnaires were filled in and all anthropometric measurements were taken, except weight and height measurements. All participants received an identical light supper which excluded alcohol and caffeine at 20:00, went to sleep before 23:00 and fasted overnight.

From 06h00 in the morning weight, height and blood pressure measurements were done. Fasting blood samples were taken from the *vena cephalica* by a registered nurse using a sterile 21G butterfly infusion set and syringes. Subjects received a breakfast and afterwards habitual dietary intake questionnaires were completed. A personal information sheet was given to each subject regarding their own blood pressure, blood glucose, haemoglobin, etc. to advise each subject and to refer them for further testing and treatment where indicated.

3.5 Questionnaires

The questionnaires were designed or adapted for this study population and were validated with appropriate methods. Questionnaires were issued during individual interviews conducted by the researchers.

Dietary intakes were measured with a *quantitative food frequency questionnaire* developed after a pilot study in which all foods eaten by this population were assessed. This questionnaire was validated in a sub-sample of 100 subjects against a 7-day weighed record and 24-h urine nitrogen excretion (MacIntyre, 1998). Books of photographs of three portion sizes of the most commonly eaten foods, food models, household utensils and food packages were used to assess quantities eaten. Nutrient intakes were analysed with a program based on the South African Food Composition Tables (Langenhoven *et al.*, 1991).

3.6 Anthropometric measurements

Weight was taken to the nearest 0.01 kg on a portable electronic scale (Precision Health scale, A&D Company, Tokyo, Japan). The measurement was taken without shoes and the women wore light nightwear. Height was taken to the nearest 0.1cm with an Invicta IP stadiometer (London, UK), also bare foot and with the head in the Frankfort plane. BMI

was calculated as weight (kg) divided by height (m) squared.
Air displacement plethysmography (ADP) was measured in the BODPOD system (Life Measurement Inc, Concord, CA). The women wore tight-fitting underclothes and swim caps only. The instrument was calibrated each day before measurements. The women were weighed on the BODPOD's electronic scale and body density was calculated. Body density was used to calculate percentage body fat according to the model of Siri (Siri, 1991). BODPOD measurements were done by trained biokineticists.

3.7 Clinical examinations

Two nursing sisters examined the subjects for signs of malnutrition. Oral temperatures were taken and blood pressure recorded in duplicate using a sphygmomanometer (Tycos®, Arden, NC, USA)) with adjustable cuffs of different sizes. The first and fifth Korotkoff sounds were recorded in subjects lying in bed for at least 10 minutes.

3.8 Biochemical analysis

Fasting glucose was measured in capillary blood using the Lifescan Surestep apparatus and fasting plasma glucose was measured using the hexokinase method by the Chemical Pathology laboratory, University of Pretoria. Fasting plasma insulin was measured with the enzyme immunoassay (Biosource Europe SA, Belgium).

3.9 Statistical analysis

All processed data were transferred to Microsoft Excel and further statistically analysed by means of the software computer package Statistica (Statsoft, Inc. 2000) and SPSS for Windows Release 11.0.1 (SPSS Inc., 1989-2001, Chicago, IL, USA). Means, medians, standard deviations, standard errors and 95% confidence intervals were calculated. Data that were not normally distributed were logarithmically transformed. Pearson's partial correlations were performed, while adjusting for age and dietary energy intake, as well as smoking.

3.10 Research results

The results of data from POWIRS I and II and relationships between the variables as determinants of body composition will be discussed in this section.

Table 3.1 indicates how the subjects were divided into the different BMI groups.

Table 3.1 Number of subjects (percentage) in each BMI group

Variables	Lean	Overweight	Obese
POWIRS I (n=102)	39 (38.2%)	25 (24.5%)	38 (37.2%)
POWIRS II (n-106)	38 (35.8%)	29 (27.4%)	39 (36.8%)

Data of only 106 subjects from POWIRS II with complete data were used in the analysis.

Table 3.2 shows how the groups differed regarding sociodemographic characteristics. The majority of the black women had only matric (63) and in the white women, the majority had degrees, or were studying at a university (82). The average monthly income in the black women was lower than in the white group. The majority of black women were single, and approximately half of the white women were married and the other half single. Contraceptive use in both groups was low, with only 43 and 29 in the black and white group respectively, using either the pill or the injection. More white than black women smoked.

Table 3.2 Demographic details of study groups

Variables	POWIRS I (n=102)	POWIRS II (n=115)
Monthly income		
R1000 – R2000	28	31
R2000 – R3000	35	12
R3000 – R4000	13	16
R4000 – R5000	5	16
>R5000	21	40
Educational status		
Standard 6/7	13	1
Standard 8	7	0
Standard 9	7	1
Matric	63	31
Diploma	8	0
Degree	4	82
Marital Status		
Single	81	56
Married	19	51
Divorced	2	5
Widowed	0	3
Contraceptive use		
Injection	27	6
Pill	16	23
Smokers (% subjects)	6.8	13.9

Selected variables that may affect the association between dietary calcium intake and body composition, and related variables are reported here.

Table 3.3 Descriptive statistics of health profile variables from POWIRS I (Black Women)

Variables	Valid N	Mean	95% CI of the Mean	SD
Age (years)	102	31.255	29.557, 32.953	8.643
Weight (kg)	102	70.556	67.444, 73.669	15.848
Height (cm)	102	158.938	157.847, 160.029	5.554
Body Mass Index (kg/m²)	102	27.983	26.738, 29.227	6.334
Systolic Blood Pressure (mmHg)	102	129.815	125.966, 133.664	19.596
Diastolic Blood Pressure (mmHg)	102	77.683	75.583, 79.783	10.692
Fasting plasma glucose (mmol/L)	101	5.184	4.950, 5.418	1.185
Plasma Leptin (ng/ml)	101	57.593	51.631, 63.555	30.200
Fasting Insulin (pmol/L)	101	92.941	84.755. 101.126	41.466

n : study sample
CI : Confidence Interval
SD : Standard Deviation
% : percentage
kg : kilogram
cm : centimeters
kg/m² : kilogram per square meter
mm Hg : millimeter Mercury
mmol/L : milimol per Liter
pmol/L : picomoles per liter
kJ : kilojoule
g : gram
mg : milligram

Table 3.4 Descriptive statistics of health profile variables from POWIRS II (White women)

Variables	Valid N	Mean	95% CI of the Mean	SD
Age (years)	106	31.38	29.59, 33.17	9.293
Weight (kg)	106	80.49	76.35, 84.64	21.528
Height (cm)	106	168.06	166.74, 169.39	6.886
Body Mass Index (kg/m^2)	106	28.45	27.05, 29.85	7.273
Systolic Blood Pressure (mmHg)	106	118.87	116.57, 121.16	11.925
Diastolic Blood Pressure (mmHg)	106	75.29	73.50, 77.08	9.283
Fasting plasma glucose (mmol/L)	106	4.69	4.59, 4.79	0.521
Plasma Leptin (ng/ml)	106	50.84	44.57, 57.11	32.402
Fasting Insulin (pmol/L)	104	92.18	85.71. 98.65	33.28

n	: study sample
CI	: Confidence Interval
SD	: Standard Deviation
%	: percentage
kg	: kilogram
cm	: centimeters
kg/m^2	: kilogram per square meter
mm Hg	: millimeter Mercury
mmol/L	: milimol per Liter
pmol/L	: picomoles per liter
kJ	: kilojoule
g	: gram
ng/ml	: nanogram per milliliter
mg	: milligram

Because the distribution of fasting plasma insulin concentration deviated markedly from the normal distribution, median and 25^{th} and 75^{th} percentiles will also be presented, in addition to descriptive statistics as for the other variables. The median fasting insulin of the black women was 84.76 pmol/L and the 25^{th} and 75^{th} percentiles were 69.0, 107 pmol/L, for white women the median fasting insulin was 86.00 pmol/L and the 25^{th} and 75^{th} percentiles were 70.0, 104 pmol/L.

Table 3.5 Daily dietary intakes of black women (POWIRS I)

Variables	N	Mean	Lower 95%CI	Upper 95%CI	Median	Minimum	Maximum	SD
Total Energy Intake (kJ)	102	7171.843	6793.463	7550.224	6641.500	3968.000	12692.00	1926.398
Fat %	102	31.6	30.8	32.3	31.7	22.0	41.0	3.9
Total Dietary Fat Intake (g)	102	59.345	56.001	62.690	55.700	31.000	109.00	17.028
Ca: Fat Ratio	102	8.434	8.012	8.855	8.098	4.106	14.34	2.145
Total Dietary Calcium Intake (mg)	102	494.784	460.372	529.197	458.500	190.000	1029.00	175.201

kJ : kilojoule
g : gram
mg : milligram
CI : confidence interval
SD : standard deviation

Dairy intake was not calculated during the first POWIRS study. None of the black subjects and only three of the white subjects took calcium supplements, ranging from 300-600mg per day.

Table 3.6 Daily dietary intakes of white women (POWIRS II)

Variables	Valid N	Mean	Lower 95%CI	Upper 95%CI	Median	Minimum	Maximum	SD
Total Energy Intake (kJ)	106	10954.81	10344.89	11564.73	10691.50	4906.000	19880.00	3166.971
Fat %	104	35	34	37	35	18.9	70	8.4
Total Dietary Fat Intake (g)	104	103.09	95.04	111.15	96.45	38.300	240.60	41.427
Ca: Fat Ratio	104	11.00	9.98	12.01	10.21	1.040	30.90	5.221
Total Dietary Calcium Intake (mg)	106	1053.82	951.91	1155.73	961.50	242.000	3446.00	529.155
Total Dairy Intake (portions)	106	2.02	1.80	2.24	1.70	0.160	6.87	1.157

kJ : kilojoule
g : gram
mg : milligram
CI : confidence interval
SD : standard deviation

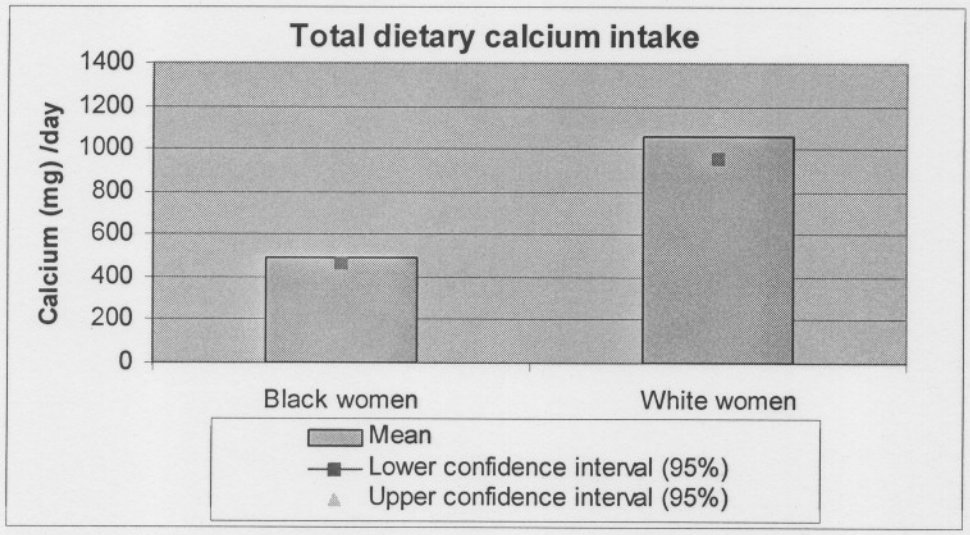

Figure 3.2 Dietary calcium intakes of the women in the POWIRS study.

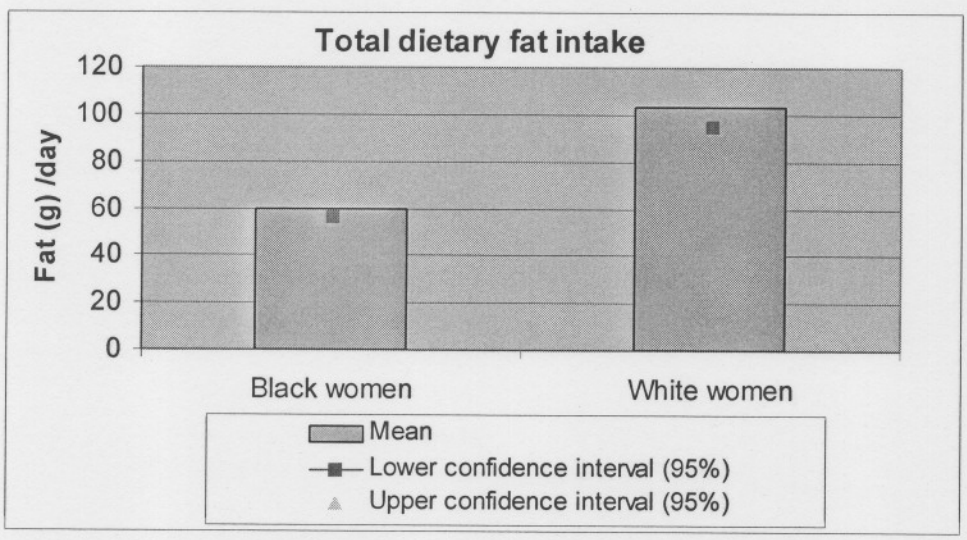

Figure 3.3 Dietary fat intakes of the women in the POWIRS study.

Data that deviated from the normal distribution (blood pressure, age, BMI, leptin, body fat, dietary energy and dietary calcium, fasting plasma glucose and insulin) were logarithmically transformed. Energy intake correlated positively with BMI and body fat percentage in the white women only. Age correlated positively with BMI and body fat percentage and calcium intake correlated positively with dietary energy intake in both black and white women. Thus, there was a positive correlation between calcium intake, BMI and body fat percentage. Therefore a Pearson's partial correlation analysis was done, adjusted for age and dietary energy intake. Table 3.7 and 3.8 show these partial correlations.

No statistically significant correlations were found between number of dairy portions in the diet and BMI ($r=-0.112$, $p=0.28$) or percentage body fat ($r=-0.138$, $p=0.18$) in the white women. Most of the dairy foods consumed by the subjects were high in fat, for example cheese.

Table 3.7 Correlations between dietary, body composition and biochemical variables of POWIRS I subjects (n = 95, correlation coefficient, r, level of significance, p).

Variable	Total Dietary Fat Intake	Body Mass Index	Systolic Blood Pressure	Diastolic Blood Pressure	Plasma Leptin	Fasting Insulin	Fasting Glucose	Total Dietary Calcium Intake	Calcium :Fat Ratio
Total Dietary Fat Intake	1.0000 p=—	.0656 p=0.530	-.2245 p=0.030 #	-.1866 p=0.072	.1638 p=0.115	.0431 p=0.680	.0168 p=0.872	.6633 p≤0.001	-.1605 p=0.122
Body Mass Index	.0656 p=0.530	1.0000 p=—	.1680 p=0.106	.3104 p=0.002	.7178 p≤0.001	.4497 p≤0.001	.3296 p=0.001	-.0451 p=0.666	-.0937 p=0.369
Systolic Blood Pressure	-.2245 p=0.030 #	.1680 p=0.010 6	1.0000 p=—	.7550 p≤0.001	.0788 p=0.450	.0318 p=0.761	.1589 p=0.126	-.2229 p=0.031 #	-.0155 p=0.882
Diastolic Blood Pressure	-.1866 p=0.072	.3104 p=0.002	.7550 p≤0.001	1.0000 p=—	.1707 p=0.100	.1807 p=0.081	.2152 p=0.037	-.2083 p=0.044 #	-.0594 p=0.569
Plasma Leptin	.1638 p=0.115	.7178 p≤0.001	.0788 p=0.450	.1707 p=0.100	1.0000 p=—	.4530 p≤0.001	.2815 p=0.006	.1495 p=0.150	.0497 p=0.635
Fasting Insulin	.0431 p=0.680	.4497 p≤0.001	.0318 p=0.761	.1807 p=0.081	.4530 p≤0.001	1.0000 p=—	.2492 p=0.015	-.0647 p=0.536	-.1406 p=0.176
Fasting Glucose	.0168 P=0.872	.3296 p=0.001	.1589 p=0.126	.2152 p=0.037	.2815 p=006	.2492 p=0.015	1.0000 p=—	-.0521 p=0.618	-.1036 p=0.321
Total Dietary Calcium Intake	.6633 p≤0.001	-.0451 p=0.666	-.2229 p=0.031 #	-.2083 p=0.044 #	.1495 p=0.150	-.0647 p=0.536	-.0521 p=0.618	1.0000 p=—	.6196 p≤0.001
Calcium: Fat Ratio	-.1605 p=.122	-.0937 p=.369	-.0155 p=.882	-.0594 p=.569	.0497 p=.635	-.1406 p=.176	-.1036 p=.321	.6196 p≤0.001	1.0000 p=—

:Significant correlation at $p < 0.05$*

Percentage body fat was measured in only 52 of the black women, correlation between percentage body fat and dietary calcium: r=0.09, p=0.53 (NS).

Table 3.8 Partial Correlations between dietary, body composition and biochemical variables of POWIRS II subjects (n = 95, correlation coefficient, r, level of significance, p).

Variable	Body Mass Index	Systolic Blood Pressure	Diastolic Blood Pressure	Fasting Glucose	Fasting insulin	Plasma Leptin	Total Dietary Calcium Intake	Calcium: Fat Ratio	Body Fat %
Body Mass Index	1.000 p=—	.4431 p<0.001	.2803 p=0.005	.3461 p<0.001	0.473 p<0.001	.8165 p<0.001	-.2552 p=0.010 #	-.3782 p≤0.001 #	.9342 p<0.001
Systolic Blood Pressure	.4431 p<0.001	1.0000 p=—	.7314 p<0..001	.1085 p=0.280	0.349 p=0.001	.3586 p<0.001	-.1131 p=0.260	-.1542 p=0.124	.4716 p<0.001
Diastolic Blood Pressure	.2803 p=0.005	.7314 P<0.001	1.0000 p=—	.0388 p=0.700	0.209 P=0.044	.2930 p=0.003	-.0925 p=0.357	-.1906 p=0.056	.3578 p<0.001
Fasting Glucose	.3461 p<0.001	.1085 p=0.280	.0388 p=0.700	1.0000 p=—	0.483 p<0.001	.3116 p=0.002	-.1992 p=0.046 #	-.2290 p=0.021 #	.3472 p<0.001
Plasma Leptin	.8165 p<0.001	.3586 p<0.001	.2930 p=0.003	.3116 p=0.002	0.459 p<0.001	1.0000 p=—	-.1470 p=0.142	-.2841 p=0.004#	.8804 p<0.001
Total Dietary Calcium Intake	-.2552 p=0.010 #	-.1131 p=0.260	-.0925 p=0.357	-.1992 p=0.05 #	-0.206 p=0.05	-.1470 p=0.142	1.0000 p=---	.8090 p<0.001	-.2520 p=0.011 #
Calcium: Fat Ratio	-.3782 p≤0.001 #	-.1542 p=0.124	-.1906 p=0.056	-.2290 p=0.02 #	-0.212 p=0.04	-.2841 p<0.001#	.8090 p<0.001	1.0000 p=---	-.4007 p≤0.001 #
Body Fat %	.9342 p<0.001	.4716 p<0.001	.3578 p<0.001	.3472 p<0.001	0.440 p<0.001	.8804 p<0.001	-.2520 p=0.011 #	-.4007 p≤0.001 #	p=—

#: Significant correlation at $p < 0.05$

When the additional calcium from supplement intake was taken into account, there was a slightly stronger negative correlation between calcium intake and BMI (r=-0.263, p=0.01), but the correlation between calcium intake and percentage body fat remained the same (r=-0.250, p=0.02).

Significant differences were seen between the two groups regarding their dietary intake, as well as their socio-economic background. The mean energy, fat as well as calcium intakes

of the white women were higher than those of the black women. Economical status may contribute to this as seen from the average income of the groups. Significant negative correlations were seen in the white women, between calcium intake and BMI, body fat percentage, fasting glucose and insulin. In the black women, calcium intake correlated significantly negatively with systolic as well as diastolic blood pressure, but these correlations were no longer significant when adjustments for age and total energy intake were made.

3.11 Discussion

A major finding in this study is that white women with lower intakes of calcium had higher BMI's and body fat percentages than women who had higher intakes of calcium. It has been recently postulated that low calcium diets favour increased adipose tissue energy storage and the converse is true for high calcium intake (Zemel, 2000). Davis and co-workers (2000), reevaluated five clinical studies of calcium intake in women to explore associations between calcium intake and body weight. They found a negative association between calcium intake and weight, and calculated that the odds ratio for being overweight was 2.25 for young women in the lower half of the calcium intake. These authors estimated that a 1000-mg difference in calcium intake was associated with an 8 kg difference in mean body weight and that variations in calcium intake could account for approximately 3% of the variance in body weight. It has been shown that a low calcium intake tends to be a marker for a poor diet and that a poor diet is good predictor of obesity (Miller *et al.*, 2001).

The significant negative correlations between calcium and BMI as well as with percentage body fat were only seen in white women and not in the black women. These differences might be attributed to the differences in dietary composition between the two groups (see tables 3.5 and 3.6). Mean total dietary calcium intake was significantly higher in the POWIRS II study subjects (white women), with a mean intake of 1053.8 mg per day (95%CI 951.9, 1155.7), whereas the mean calcium intake in the black women (POWIRS I) was 494.8 mg per day (95%CI 460.4, 529.2). Mean fat intake in the black population was 59.3 g per day, and in the white women 103.1 g per day. Thus the calcium:fat ratio in white women was higher than in black women (11.0 and 8.4 respectively. See tables 3.5 and 3.6). As described in the literature, dietary calcium has an effect on the absorption of fatty acids (Welberg *et al.*, 1994) and triacylglycerols from the gastrointestinal tract (Parikh & Yanovski, 2003). In the study by Welberg *et al.* (1994) calcium increased fecal fatty

acids in a dose dependent fashion. The relatively low calcium and fat intakes of the black women may be an explanation why calcium intake was not associated with BMI and percentage body fat in black women. The results from our study differ from that of Buchowski *et al.* (2002) in African American women, where a negative correlation was found between calcium intake and BMI.

It has been hypothesized that high calcium diets protect against fat gain by creating a balance of lipolysis over lipogenesis in adipocytes (Zemel *et al.*, 2000). A diet deficient in calcium is associated with higher body weight and augmenting calcium intake may reduce weight and fat gain or enhance fat loss (Shapses *et al.*, 2004). Implicit in the hypothesis that high calcium intake promotes maintenance of lower body fat mass in humans by enhancing lipolysis is the assumption that high-calcium diets promote greater rates of whole-body fat oxidation (Melanson *et al.*, 2003). In our study, consistent with this hypothesis, a negative association was seen between calcium intake and body fat. Again, this effect was not seen in black women.

The ß cells in the body, respond to high blood glucose concentration of the fed and resting states by secreting insulin. These hormones regulate the rate of opposing pathways of lipid metabolism and therefore control whether fatty acids will be oxidized or synthesized. A decrease in glucose concentration in the blood, changes the body's hormone balance. This situation results in long-term increases in the levels of fatty acid oxidation enzymes accompanied by long-term decreases in those of lipid biosynthesis (Voet & Voet, 1995). In the white women in our study, a significant negative correlation was seen between calcium intake and fasting glucose, as well as fasting insulin, which indicates that higher blood glucose concentrations in these women with lower calcium intakes were associated with higher blood insulin concentrations, which could favour fatty acid synthesis and increased percentage body fat.

Yu *et al.*, (2003), concluded through their study on Wistar rats, that dietary calcium may improve hyperinsulinemia and also enhance the expression of uncoupling protein 3 (UCP3) mRNA in skeletal muscle, by increasing the serum level of leptin which may play a role in the prevention of obesity. In our study we found an inverse correlation between the calcium: fat ratio and leptin in the white women.

A recently published multi center, population-based, prospective observational study found that increased dairy consumption had a strong inverse association with the 10-years

cumulative incidence of obesity (i.e., BMI = 30) and with the insulin-resistance syndrome in overweight adults (BMI 25 at baseline; n = 923). The odds of obesity, abnormal glucose homeostasis, and elevated blood pressure were 20% lower at each additional daily increment of dairy consumption (Zemel, 2000).

This association was not found in the white population of our study; however a negative correlation was seen in the black population between dietary calcium intake and systolic blood pressure, and dietary calcium intake and diastolic blood pressure (table 3.7). A possible reason for the lack of association between number of dairy portions per day and BMI or percentage body fat could be that most of the dairy portions were also high in fat, for example cheese.

We do not have a clear explanation for the previous mentioned differences seen between black and white women. However, a higher calcium and fat intake than those of the black women in this study seems to be necessary for an effect on body composition and fasting glucose and insulin. Black women may have reported their dietary intakes less accurately that white women.

Given the increasing prevalence of obesity along with its significant medical consequences, the importance of environmental factors in the rapid rise in the prevalence of obesity, and the relative cost-effectiveness and safety profile of calcium and dairy supplementation, we believe that more research is necessary to determine whether the body weight of overweight adults can be altered by either dietary calcium or dairy supplementation.

3.12 Summary

The incidence of obesity is increasing at an alarming rate and with that the chronic diseases associated with obesity such as diabetes mellitus, coronary heart disease and hypertension (Bradshaw *et al.,* 1995). In the past decade, the percentage of adults in the United States, aged 20–74 years who are overweight or obese has increased to 61% (Sterchi *et al.,* 1990). The same tendency is seen in South Africa. A study done by Mollentze *et al.* (1995) concluded that about one quarter to more than half of urban black South African women (in different age groups) were obese.

It has been hypothesized that dietary calcium intake may influence various variables of

body composition and therefore may play an important role in the treatment and prevention of obesity. Literature has shown a relationship between calcium and body fat percentage (Shahkhalili *et al.,* 2001), BMI (Carruth & Skinner, 2001) and blood pressure (Zemel, 2000). Epidemiologic and limited experimental data from some studies suggest that differences in calcium intake may be associated with changes in body weight of 0.35 kg/y (Davies *et al,* 2000).

The results from the POWIRS studies are consistent with the hypothesis that there may be an inverse relationship between adiposity and calcium intake. However, most of the associations were only seen in white women. We speculate the reason for this might be the fact that the black women had significant lower intakes of calcium and fat than the white women, and that higher calcium and fat intake is necessary for this effect.

CHAPTER 4: Conclusion and Recommendations

4.1 Introduction

The prevalence of obesity and its health consequences is increasing globally at epidemic rates. Reduced quality of life and increased medical costs because of this, are reasons for major concern (Teegarden, 2003). Macro nutrient intake have received much attention in body weight regulation (Astrup *et al.*, 2000), but an emerging body of literature suggests that dietary calcium may play a role in the regulation of body weight and body fat (Melanson *et al.*, 2003).

A multi-disciplinary cross-sectional case-control study (POWIRS study) was conducted in which health determinants and status as well as the underlying mechanistic relationships between these factors was measured in a sample of African women volunteers.

One hundred and two apparently healthy urban African women, between the ages of 20 and 50 years participated in this case-control cross-sectional survey. Demographic, lifestyle and psychological questionnaires were filled in, anthropometric measurements, urine samples, fasting blood samples and dietary histories were taken. The study was repeated a year later, done in a sample of white women volunteers, POWIRS II

Our aim was to investigate the hypothesis that calcium intake may play a role in the treatment of obesity and to determine whether there is any link between dietary calcium intake and variables associated with body composition.

The two groups differed with regards to their educational status and also their income. The majority of the white women had degrees whereas the majority of the black women had only matric. Mean income in the white group was higher than that of the black women.

Dietary intake differed significantly between the women in POWIRS I (black women) and POWIRS II (white women). This could be due to the difference in income seen in the two groups. Total dietary calcium as well as total fat intake was significantly higher in white women than in the black women. Thus the calcium:fat ratio in white women was higher than in black women.

Significant negative correlations were seen between dietary calcium intake and various variables of health status. These were BMI, body fat, fasting insulin and fasting glucose. The calcium:fat ratio correlated negatively with BMI, fasting glucose, fasting insulin, percentage body fat and plasma leptin. These results however, were only seen in the white population. After adjustment for age, smoking and dietary energy intake, no significant correlation were found in the black subjects.

It has been recently postulated that low calcium diets favour increased adipose tissue energy storage and the converse is true for high calcium intake (Zemel, 2000).

4.2 Conclusion

Higher intakes of calcium predicted lower BMI and body fat percentage in white women. A higher calcium:fat ratio was associated with lower plasma leptin, fasting glucose, fasting insulin, body fat percentage and BMI. Our findings were in contrast with that of Buchowski *et al.,* (2002) where calcium intake was negatively associated with BMI in African American women. In the black population from our study, higher calcium intakes were significantly associatd with systolic as well as diastolic blood pressure, but had no influence on any variables of body composition.

Dietary calcium has an effect on the absorption of triacylglycerols (Parikh & Yanovski, 2003), and fatty acids (Welberg *et al.* (1994) from the gastrointestinal tract which may be another mechanism by which dietary calcium affect body adiposity. We however did not measure fecal fatty acid excretion from the gastrointestinal tract.

From the initial results, we could see that calcium intake correlated positively with energy intake, and that energy intake correlated positively with body fat and BMI. This finding underlines the comment made by Teegarden (2003), that if dairy products are added to a diet without compensation for energy intake, one is likely to gain weight. This may suggest that women with moderate energy intakes may benefit more from high calcium intake and control weight better than women with higher energy intakes. Indeed, it has been postulated that higher energy intakes could overwhelm the impact of calcium on changes in body composition in adult women (Lin *et al.*,2000).

However to conclude, through our study it became apparent that higher levels of calcium

intake may have various positive effects on body composition and blood glucose and insulin levels and may play a role in body weight regulation.

4.3 Recommendations

- In general, higher dietary intake of calcium may be beneficial to several variables of health status such as BMI, body fat percentage, plasma leptin, fasting plasma insulin and fasting glucose.
- In order to increase dietary calcium consumption, without adding extra energy, it is suggested that low fat or fat free dairy products are used, as part of the usual diet and not in addition to what is normally eaten.
- More research is necessary for the effect of calcium supplementation in addition to the diet as well as the effect of calcium on fecal fat excretion.

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ADDENDUM 1

Recruitment and informed consent form

Potchefstroomse Universiteit
vir Christelike Hoër Onderwys

POWIRS PROJECT

RECRUITMENT AND INFORMED CONSENT FORM

Title of the project: The profiles of obese women suffering from the insulin resistance syndrome

Ethics Committee no: 03M03

Name: _____ **Subject number:** ____ _____

Adress: _____

Tel no: _____

Age: _____

Are you pregnant ?_____

Are you breastfeeding ? _____

Do you suffer from diabetes ? hypertension ? other disease ?

_____

INFORMED CONSENT

I, the undersigned _____
(full names in print), have read the details of the project or have listened to the oral explanation thereof, and declare that I understand it. I have had the opportunity to discuss relevant aspects with the researcher and declare that I voluntarily participate in the project. I hereby give consent to participate in the project.

_____
Signature of participant

Witnesses:

1. _____ 2._____

Signed at _____ on _____

ADDENDUM 2

Demographic and lifestyle questionnaire

Demographic and lifestyle questionnaire

Information given in this questionnaire is confidential

1. Subject number			
2. Date			
3. Age			
4. Date of birth	D | M | Y		
5. First language (mark correct block with an X)	Afrikaans		(1)
	English		(2)
	Other		(3)
6. Second language	Afrikaans		(1)
	English		(2)
	Other		(3)
7. What is your marital status?	Never married		(1)
	Married		(2)
	Divorced		(3)
	Widowed		(4)
8. What is your highest qualification?	Matric		(1)
	Matric + university / technikon./ college		(2)
9. What is your occupation?			
10. Do you work: shifts, e.g. night shifts		Yes (1)	No (2)
office hours (± 8 hours per day)		Yes (1)	No (2)
mornings / part time (± 5 hours per day)		Yes (1)	No (2)
11. Are you pregnant?		Yes (1)	No (2)
12. Are you breastfeeding?		Yes (1)	No (2)
13. When did you have your last menstrual period (please give the date of the first day of your last period):		D D M M Y Y	
Do you suffer from any of the following?			
14. Hypertension		Yes (1)	No (2)
15. Diabetes		Yes (1)	No (2)
16. Stroke		Yes (1)	No (2)
17. Heart disease		Yes (1)	No (2)

18. Gout	Yes (1)	No (2)
19. Arthritis	Yes (1)	No (2)
20. Malaria	Yes (1)	No (2)
21. TB	Yes (1)	No (2)
22. Sexually transmitted disease	Yes (1)	No (2)
23. Head injury (previously)	Yes (1)	No (2)

Does anyone in your family suffer from:

24. Hypertension	Yes (1)	No (2)	Uncertain (3)
25. Diabetes	Yes (1)	No (2)	Uncertain (3)
26. Stroke	Yes (1)	No (2)	Uncertain (3)
27. Heart disease	Yes (1)	No (2)	Uncertain (3)
28. Gout	Yes (1)	No (2)	Uncertain (3)
29. Arthritis	Yes (1)	No (2)	Uncertain (3)
30. Malaria	Yes (1)	No (2)	Uncertain (3)
31. TB	Yes (1)	No (2)	Uncertain (3)
32. Sexually transmitted disease	Yes (1)	No (2)	Uncertain (3)

33. Do you take any medication?	Yes (1)	No (2)
34. If yes, please list medication: _____ _____ _____ _____		
35. Do you take birth control tablets?	Yes (1)	No (2)

36. Do you get a birth control injection?	Yes (1)	No (2)
37. Have you ever been on anti-depressants ?	Yes (1)	No (2)
38. Have you ever been hospitalized for any psychiatric illness (eg depression, anxiety, panic attacks)?	Yes (1)	No (2)
39. How many pregnancies did you have? _____		
40. Are your parents still alive?		
Mother	Yes (1)	No (2)
Father	Yes (1)	No (2)
41. If your mother has died, what was the cause of death?		
42. If your father has died, what was the cause of death?		
43. Do you have other sources of income other than your job?	Yes (1)	No (2)
44. If yes, please name the source of income: _____		
45. Give an indication of your income per month (mark the correct block with a X):		
R 1 000 – R 2 000		(1)
R 2 000 – R 3 000		(2)
R 3 000 – R 4 000		(3)
R 4 000 – R 5 000		(4)
> R 5 000		(5)
46. Does your work offer any benefits?	Yes (1)	No (2)
47. If yes, please mark the benefits:		(1)
Pension		(2)
Medical aid		(3)
Housing		(4)
Car		(5)
Allowance for car / housing / medical aid		(6)
Food (please list the foods)	Yes (1)	No (2)

48. Do you own property?	Yes (1)	No (2)

49. If yes, what type of property, e.g. house, flat?

50. How many people eat in your house (give the number of people):	Number
Children under 11 years	(1)
Children under 18 years	(2)
Adults (children older than 18 years, grand parents, brothers, sisters, wives, husbands, etc)	(3)

51. Do they contribute to your household costs?	Yes (1)	No (2)
52. In what way: food / money	Food (1)	Money (2)

53. If money, how much money? _____

54. Please name the members of your household:

Member	Age	Education	Present job
(1)	(2)	(3)	(4)
(5)	(6)	(7)	(8)
(9)	(10)	(11)	(12)
(13)	(14)	(15)	(16)
(17)	(18)	(19)	(20)
(21)	(22)	(23)	(24)
(25)	(26)	(27)	(28)
(29)	(30)	(31)	(32)
(33)	(34)	(35)	(36)

55. Does any member of your household have the right to use any property as his / her own ?	Yes (1)	No (2)

56. In what type of area do your live (please tick the appropriate block with a X):	
Rural area	(1)
Farm	(2)
Town/City	(3)

57. How long have you been living here (years)? _____		
58. Where have you been living before? Rural area		(1)
Farm		(2)
Town/City		(3)
59. Do you smoke?	Yes (1)	No (2)
60. If yes, mark what you smoke and indicate the Amount per day / week:		Amount per day / week
Cigarettes		
61. For how long are you smoking (years)? _____		
62. If you don't smoke at the moment, have you been smoking regularly before?	Yes (1)	No (2)
63. Do you use alcohol?	Yes (1)	No (2)
64. If yes, mark the type of alcohol you use:		
Beer (commercial)		(1)
Spirits		(2)
Wine		(3)
Liqueur		(4)

Try to tell the amount of alcohol you use per day / per week:		Per day	Per week
65. Beer (commercial)	quart / tin /dumpy	(1)	(2)
66. Spirits	tot / bottle	(1)	(2)
67. Wine	glass / bottle	(1)	(2)
68. Liqueur	glass / bottle	(1)	(2)

Please answer yes or no to the following questions:

69. Was there a period in your *childhood* (o – 18 y) when you didn't have enough food in the house?	Yes (1)	No (2)
70. Was there a period in your *adulthood* (> 18 yr) when there was not enough food in the house?	Yes (1)	No (2)

ADDENDUM 3

Food frequency questionnaire

FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

SUBJECT NO: .....

I shall now ask you about the type and the amount of food you have been eating in the last few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize porridge.

Do you eat maize porridge? YES 1 NO 2
If YES, what type do you have at home now?

Brand name: .....
Don't know: 2
Grind self: 3

If brand name given, do you usually use this brand?
YES 1 NO 2 DON'T KNOW 3
Where do you get your maize from? (May answer more than one)

Shop	1
Employer	2
Harvest and grind self	3
Other – specify	4
Don't know	5

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				FOR OFFICIAL USE CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
Maize meal porridge	Stiff ('pap')						e4225 4250	
Maize meal porridge	Soft ('pap')						e4225 4250	
Do you pour milk on your soft porridge? YES 1 NO 2 If YES, what type of milk (whole fresh, sour, 2 %, fat free, milk blend)? ..... INSTRUCTIONS: Show subject examples.								
If YES, how much milk?								
Do you pour sugar on your soft porridge? YES 1 NO 2								
If YES, how much sugar?							9012	
Maize meal porridge	Crumbly (phutu)						e4225 4250	
Ting								
Mabella Coarse Fine Rice	Stiff						4082	
Mabella Coarse Fine Rice	Soft						4082	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
Do you pour milk on your mabella porridge? YES 1 NO 2 If YES, what type of milk (whole fresh, sour, 2 %, fat free, milk blend)? ..... INSTRUCTIONS: Show subject examples.								
If YES, how much milk?								
Do you pour sugar on your mabella? YES 1 NO 2								
If YES, how much sugar?							9012	
Oats							4032	
Do you pour milk on your oats YES 1 NO 2 If YES, what type of milk (whole fresh, sour, 2 %, fat free, milk blend)? ..... INSTRUCTIONS: Show subject examples.								
If YES, how much milk?								
Do you pour sugar on your oats? YES 1 NO 2								
If YES, how much sugar?							9012	
Breakfast Cereals	Brand names of cereals at home now: Don't know ...						4036	
Do you pour milk on your cereal? YES 1 NO 2 If YES, what type of milk (whole fresh, sour, 2 %, fat free, milk blend)? ..... INSTRUCTIONS: Show subject examples.								
If YES, how much milk?								
Do you pour sugar on your cereal? YES 1 NO 2								
If YES, how much sugar?						9012		
Samp	Bought Self ground with fat Without fat					4043		
Samp and Beans						A014		
	Are the amounts of samp and beans the same as in the pictures? YES NO If NO, do you use more beans than in the picture or less? MORE LESS							
Samp and peanuts						A013		
	Are the amounts of samp and peanuts the same as in the pictures? YES NO If NO, do you use more peanuts than in the picture or less? MORE LESS							
Rice	White Brown Maize rice						4040 4134 4043	
Pastas	Macaroni Spaghetti Other						4062	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
You are being very helpful. Can I now ask you about meat? CHICKEN, MEAT, FISH How many times per day /week do you eat meat, fish or chicken? .....x/day .....x/week								
Chicken:	Boiled, nothing added						1521	
	Fried: in butter/ crumbs Not coated						1634 1520	
	Roasted, grilled						1520	
	Stewed						1520	
	What vegetables are in the stew?							
	Don't know							
Do you eat chicken skin? ALWAYS 1 SOMETIMES 2 NEVER 3								
Chicken feet	How do you cook it?						A004 1609	
Chicken offal	How do you cook it?						1610	
Where do you get your MEAT from? (May answer more than 1) Shop, supermarket, spaza Employer Slaughter own Gift Other specify: Do you eat red meat								1 2 3 4 5 6
Red meat:	How do you like meat? With fat Fat trimmed							
Beef	Fried – with bone							
	Fried – without bone							
	Stewed – with bone						A001	
	Stewed – without bone						A001	
	Grilled – with bone							
	Grilled – without bone							
	Minced						1585	
Mutton	Fried – with bone						1522	
	Fried – without bone						1571	
	Stewed – with bone						1511	
	Stewed – without bone						1511	
	Grilled – with bone							
	Grilled – without bone							
	Minced						1662	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
Pork	Fried – with bone							
	Fried – without bone							
	Stewed – with bone							
	Stewed – without bone							
	Grilled – with bone							
	Grilled – without bone							
Beef offal	Intestines: boiled, nothing added						161	
	Stewed with vegetables							
	Tripe						1546	
	Heart						1565	
	Lungs							
	Liver						1515	
	Kidneys						1518	
	Other specify:							
What vegetables are usually put into meat stews?								
Wors sausage	Fried Grilled						1526	
Bacon							1501	
Cold meats	Polony						1514	
	Ham						1564	
	Viennas						1531	
	Other specify:							
Canned meat	Bully beef						1535	
	Other specify:							
Meat pie	Home made Bought						1548	
Hamburger	Home made Bought						A015	
Dried beans, peas, lentils	How do you prepare them?							
Soya products e.g. Toppers	Brands at home now Don't know Show examples						3527	
Pilchards in tomato chili brine	Whole						2557	
	Mashed with fried onion						A005	
Fried fish	With batter/crumbs Without batter/crumbs						2523	
	Without batter/crumbs						2509	
Other canned fish	Tuna							
	Pickled fish Other:						2562	
Fish cakes	Home made (describe) Frozen Bought						2531	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
Eggs	Boiled poached Scrambled Fried						1001 1025 1003	
WE NOW COME TO VEGETABLES AND FRUIT How many times per day/week do you eat vegetables? .....x/day .....x/week								
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						8066	
	Boiled with potato and onion and fat						A006	
	Fried, nothing added						A007	
	Boiled, then fried with potato, onion						A006	
	Other							
	Don't know							
	How do you cook spinach?							
	Boiled, noting added						8071	
	Boiled fat added						8209	
	Boiled with – onion, tomato & fat						A011	
	- onion, tomato & potato						8212	
	- with peanuts							
	Other:							
	Don't know							
Tomato and onion 'gravy'	Home made - with fat - without fat						A012 A016	
	Canned 9Is this the amount of pap you eat? How much more or less?)						8221	
Pumpkin	How do you cook pumpkin?							
	Cooked in fat & sugar						A010	
	Boiled, little sugar and fat						A009	
	Other:							
	Don't know							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
Carrots	How do you cook carrots?							
	Boiled, sugar & fat							
	With potato/ onion							
	Raw, salad Chakalaka							
	Other:							
	Don't know							
Mealies/ Sweet corn	How do you eat mealies? On cob – with fat - without fat							
	Off cob – with fat - without fat							
Beetroot salad	Home made Bought							
Potatoes	How do you cook potatoes?							
	Boiled/ baked - with skin - without skin							
	Mashed							
	Roasted							
	French Fries							
	Salad							
	Other:							
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/ baked - with skin - without skin							
	Mashed							
	Other:							
	Don't know							
Salad vegetables	Raw tomato						8059	
	Lettuce						8031	
	Cucumber						8025	
Other vegetables specify:								
FRUIT: Do you like fruit? YES NO How many times per day/week do you eat fruit? .....x/day .....x/week								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
Apples/Pears	Fresh						7001	
Pears	Fresh Canned						7053 7054	
Bananas							7009	
Oranges/ naartjies							7031	
Grapes							7020	
Peaches	Fresh Cnned						7036 7038	
Apricots	Fresh Canned						7003 7004	
Mangoes	Mangoes						7026	
Guavas	Fresh Canned						7021 7023	
If subjects eats canned fruit: Do you have custard with canned fruit? YES 1 NO 2								
Custard	Home made Ultramel						0004	
Wild fruit/ berries	Stamvrugte						7070	
	Noen-noen							
	Klappers							
	Maroelas							
	Nastergals							
	Other - specify							
Dried fruit:	Types							
Other fruit:								
BREAD AND BREAD SPREADS								
Bread Bread rolls	White						4001	
	Brown						4002	
	Whole wheat						4003	
Do you spread anything on the bread? ALWAYS 1 SOMETIMES 2 NEVER 3								
Margarine	What brand do you have at home now? ..... Don't know Show examples						6508 6521	
Butter	What brand do you have at home now? ..... Don't know Show examples						6502	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
Peanut butter								
Jam/syrup/honey								
Marmite/Fray Bentos etc								
Fish/ meat paste								
Cheese								
Atchar								
Polony								
Other spreads: specify								
Dumpling								
Vetkoek								
Provita, crackers etc								
FATS: What fats do you use and where do you use them?								
Margarine	Where used: on bread							
	With vegetables** Number of spoons ..... /number in family							
Holsum/ vegetable fat	Number of spoons ..... /number in family						6508	
Oil	Number of spoons ..... /number in family						6510	
Dripping	Number of spoons ..... /number in family							
Mixed fat (makhuru)	Number of spoons ..... /number in family							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
Lard	Where used: Number of spoons ..... /number in family						6520	
Mayonnaise/ Salad dressing	Number of spoons ..... /number in family						6573	
Cream	Fresh/ Long life/ canned Orley whip						6503	
DRINKS:								
Tea							9514	
Sugar/ cup tea							9012	
Milk/ cup tea	What type of milk do you use in tea?							
	Fresh/ long life whole						0006	
	Fresh/ long life 2%							
	Fresh/ long life fat free						0072	
	Whole milk powder Brand						0009	
	Skimmed milk powder Brand						0008	
	Milk blend Brand						0068	
	Whitener Brand						0039	
	Condensed milk						0002	
	Evaporated milk						0003	
	None							
Coffee								
Sugar / cup coffee							9012	
Milk/ cup coffee	What type of milk do you use in coffee?							
	Fresh/ long life whole						0006	
	Fresh/ long life 2%							
	Fresh/ long life fat free						0072	
	Whole milk powder Brand						0009	
	Skimmed milk powder Brand						0008	
	Milk blend Brand						0068	
	Whitener Brand						0039	
	Condensed milk						0002	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
	Evaporated milk							
	None							
Milk as such	What type of milk do you drink as such?							
	Fresh/ long life whole						0006	
	Fresh/ long life 2%							
	Fresh/ long life fat free						0072	
	Whole milk powder Brand						0009	
	Skimmed milk powder Brand						0008	
	Milk blend Brand						0068	
Milk drinks Brand	Nestle Milo Other						0023	
Yoghurt	Drinking yoghurt Thick yoghurt						0044 0020	
Squash	Sweeto SixO Oros/Lecol - with sugar - artificial sweetener Kool Aid Other						9013 9013 9002 9013 9002	
Fruit juice	Fresh/Liquifruit/Ceres Tropica Concentrates e.g. Halls Nectars Flavour							
Fizzy drinks Coke, Fanta	Sweetened Diet						9001 9013	
Magau/Motogo							9562	
Home Brew							9516	
Tlokwe							9516	
Beer							9506	
Spirits							9510	
Wine red							9508	
Wine white							9518	
Liquer							9517	
Other: specify								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
SNACKS AND SWEETS								
Potato crisps							4275	
Cheese curls Niknaks etc.							4067	
Peanuts	Raw Roasted						9001 6007	
Raisins							7022	
Peanuts and raisins								
Chocolates	Name						9024	
Candies	Sugars, gums, hard sweets						9009	
Sweets	Toffees, fudge, caramels						9014	
Biscuits	Type							
Cakes & tarts	Type							
Scones							4029	
Rusks							4160	
Savouries	Sausage rolls Samoosas Biscuits e.g. Bacon Kips Other						1534 4196 4162	
PUDDINGS:								
Canned fruit	Type							
Jelly							9004	
Custard	Homemade Ultramel						0004	
Baked pudding							4181	
Instant pudding							4066	
Ice cream Sorbet							6507 6516	
Other: specify								
SAUCES/ GRAVIES/ CONDIMENTS:								
Atchar							3004	
Tomato sauce							3027	
Worcester sauce								
Chutney							9524	
Pickles							8176	
Packet soups							3046	
Others:								
INSECTS:								
Locusts								
Mopani worms								
Others:								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT
			Per day	Per week	Per Month	Seldom Never		
WILD BIRDS OR ANIMALS (hunted in rural areas or on farms)								
MISCELLANEOUS: Please mention any other foods used more than once/ two weeks which we have not talked about:								

1. What type of salt do you use? Fine=1, coarse=2		1	2
2. Do you add salt to food during cooking?	Always=1	Sometimes=2	Never=3
3. Do you add salt to food at the table?	Always=1	Sometimes=2	Never=3
4. Do you eat salty foods (chips/salted peanuts)?	Often=1	Sometimes=2	Never=3
5. Do you take any vitamin tablets or syrup other than those supplied by the clinic?			
If yes, specify:			