# Increased intake of vegetables, herbs and fruit: effects on bone in postmenopausal women

---

**A thesis**

**Presented in partial fulfilment of the requirements**

**for the degree**

**of**

**Doctor of Philosophy**

**in Nutritional Science**

**Massey University,**

**Manawatu,**

**New Zealand.**

**Caroline Ann Gunn**

**2013**

# ABSTRACT

Dietary approaches to address bone loss at midlife usually involve supplementation or fortification. We aimed to investigate a food based approach to reduce bone turnover in post-menopausal (PM) women in two studies. In the first study, we investigated whether daily inclusion of specific vegetables attributed with bone resorbing inhibiting properties was feasible. We hypothesised increased intake of fruit/vegetables to ≥9 servings/day would lower potential renal acid load (PRAL) significantly (~20mEq/day) and increase urine pH (0.5 pH units) sufficiently to affect bone markers. The results of the first study confirmed the feasibility of daily inclusion of specific vegetables, reduction in renal acid load and increased urine pH. The subsequent Scarborough Fair Study (SF) used a randomised, active comparator design to increase specific vegetable/herb/fruit intake in two groups (A and B) of 50 PM women, from ≤ 5 servings/day to ≥ 9 servings/day for 3 months while a control group consumed their usual diet (n=43). Primary outcome variables were plasma bone markers which were assessed at baseline, six weeks and twelve weeks. Secondary outcome variables were plasma inflammation markers including adiponectin, urinary electrolytes (calcium, magnesium, potassium and sodium) and dietary intake assessed at baseline and 12 weeks and urinary pH assessed twice weekly. Increased intake of vegetables/herbs/fruit reduced P1NP and CTX (osteopenia) in Group B (SF) and urinary calcium loss in both intervention groups A and B (SF) with reduced PRAL. Adiponectin, tumour necrosis factor, interleukin 6 and 10 reduced in all groups. This study showed the SF vegetables/herbs/fruit may influence bone turnover and inflammatory markers. Few human intervention studies demonstrate reduction in plasma bone resorption markers with diet. Even fewer studies demonstrate reduction without supplementation with calcium, vitamin D, alkaline substrates, concentrated extracts or consumption of large quantities of a single functional food. The SF vegetables/herbs/fruit may protect against high bone turnover and subsequent bone loss in women with osteopenia and may have possibilities as an adjuvant to pharmaceutical therapies or a holistic dietary approach to reduce bone turnover and bone loss. **Trial registration** ACTRN 12611000763943

# ACKNOWLEDGEMENTS

I gratefully acknowledge the support of Professor Marlena Kruger. I am indebted to you for being instrumental in obtaining significant research funding for this project but most of all I would like to thank you for your generosity of time, guidance and outstanding academic expertise even when under considerable work pressure. I would also like to thank my co-supervisor Dr Janet Weber for her innovative suggestions, discussions and critique of papers, Dr Anne-Thea McGill for prompt and expert medical guidance on clinical study aspects and discussion on adiponectin, Associate Professor David Woodward for reviewing the manuscript from a biochemist's perspective, Dr Carolyn Lister for expert advice on phytochemicals and Associate Professor Lisa Emerson for providing invaluable writing assistance.

I am thankful for the expertise and friendship of key laboratory staff: Anne B, Chris B, Michelle and Simon. Additional laboratory assistance from Alison, Caroline D, Ellie, Jasmine, Jenny, Maria, Mark and Ying was instrumental in the success of this project. Thanks to Louise and Karen for technical advice and helpful tips, Catherine E and Laurel for data entry, Neil for IT support and Catherine LW and Associate Professor Welma Stonehouse for statistical help. To all the women participants in both studies I am indebted for your enthusiasm for the study idea and participation.

To my family and friends, your love and support was much appreciated.

# Statement of originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

# Table of Contents

# TABLE OF FIGURES

# LIST OF TABLES

# ABBREVIATIONS

3DDD; 3 Day Diet Diary

AdipoR1; Adiponectin Receptor1

AdipoR2; Adiponectin Receptor2

AI; Adequate Intake

ALP; Alkaline Phosphatase

ANOVA; Analysis of Variance

AP-1; Activator Protein-1

APOSS; Aberdeen Prospective Osteoporosis Screening Study

ARE; Antioxidant Response Elements

ATF-4; Activating Transcription Factor 4

BAP; Bone Alkaline Phosphate

BMD; Bone Mineral Density

BMI; Body Mass Index

BMP; Bone Morphogenetic Protein

BMU; Bone Multicellular Unit

BRIFs; Bone Resorption Inhibiting Foods

BSA; Body Surface Area

BSP; Bone Sialoprotein

CAT; Catalase

CATK; Cathepsin K

CI; Confidence Interval (95%)

CL; Confidence Limit

Colla 1; Collagen Type 1 alpha

Colla 2; Collagen Type 2 alpha

CBFA-1; Core Binding Factor-1

COX-1; Cyclooxygenase (constitutive)

COX-2; Cyclooxygenase (inducible)

CRP; C-Reactive Protein

CSF1; Cytokine Stimulating Factor 1

μCT; Micro computed Tomography

CTX; C-Terminal Telopeptide of Type I Collagen

DASH; Dietary Approaches to Stopping Hypertension

DHA; Docasahexaenoic Acid

DKK-1; Dickkopf-1

DPD; Deoxypyridinoline

DXA; Duel-Energy X Ray Absorptiometry

EAR; Estimated Average Requirement

ECF; Extracellular Cellular Fluid

EPA; Eicosapentaenoic Acid

EpRE; Electrophile Response Elements

FFQ; Food Frequency Questionnaire

FOX01; Forkhead Box Protein 01

GLA; Gamma Linolenic Acid

GPCS; -L-Glutamyl–trans-S-1-Propenyl-L-Cysteine Sulfoxide

GPx: Glutathione Peroxidase

GR; Glutathione Reductase

GSH; Reduced Glutathione

GSSH; Glutathione Disulphide

GST; Glutathione S-Transferase

HFI; Hip Fracture Incidence

Hoxa-2; HomeoboxA-2

IGF-1; Insulin Like Growth Factor-1

IHH; Indian Hedgehog Signalling

IL-6; Interleukin 6

IL-10; Interleukin 10

IFCC; International Federation of Clinical Chemistry and Laboratory Medicine

IOF; International Osteoporosis Foundation

JAK-STAT; Janus Kinase-Signal Transducer and Activator of Transcription Pathway

KEAP1; Kelch like ECH-Associated Protein

LPR5; Lipoprotein Receptor 5

MAPK; Mitogen-Activated Protein Kinase

MCP-1; Monocyte Chemoattractant Protein-1

M-CSF; Macrophage-Colony Stimulating Factor

MMP; Matrix Metalloproteinase

MOH; Ministry of Health (NZ)

MSC; Mesenchymal Stem Cell

MC3T3-E; Mus Musculus Calvaria

NAE; Net Acid Excretion

NEAP; Net Endogenous Acid Load

NFATc-1; Nuclear Factor of Activated T-cells

NF-κB; Nuclear Factor kappa B

NO; Nitric Oxide

NQO1; NAD(P) H-Quinone Oxidoreductase-1

Nrf2; Nuclear Factor-Erythroid 2 Related Factor-2

NSAID ; Non-Steroidal Anti-Inflammatory Drug

NTX; Cross-linked N-Ttelopeptide of Type I Collagen

NZ; New Zealand

OA; Organic Acid

OHP; Hydroxyproline

OPG; Osteoprotegerin

OSCAR; Osteoclast Associated Receptor

P; Test statistic probability considered significant at the level 0.05

PI3K; Phosphatidylinositide 3-Kinases

PERK; Protein kinase RNA-like Endoplasmic Reticulum Kinase

PGE2; Prostaglandin-2

PKC; Protein Kinase C

P1CP; Procollagen Type I C Propeptide

P1NP; Procollagen Type I N Propeptide

PAI-1; Plasminogen Activator Inhibitor

pQCT; peripheral Quantitative Computed Tomography

PRAL; Potential Renal Acid Load

PYD; Pyridinoline

PTH; Parathyroid Hormone.

r ; Pearson's Correlation (2 tailed)

RANKL; Receptor Activator of Nuclear Factor kappa B Ligand

RCF; Relative Centrifugal Force

RDI; Recommended Daily Intake

RNA; RiboNucleic Acid

ROS; Reactive Oxidative Species.

RPM; Revolutions per Minute

Runx2; Runt Related Transcription Factor

SA-BMC; Size Adjusted Bone Mineral Content

SATB-2; Special AT-rich Sequence Binding Protein

SD; Standard Deviation

SDT; Suggested Dietary Target

SEM; Standard Error of Mean

SF; Scarborough Fair

SIRT-1; Sirtuin-1

SOD; Superoxide Dismutase

SPSS; Statistical Package for Social Sciences

SPARC; Sport and Recreation New Zealand

SWAN; Study of Women's Health Across the Nation

TA; Titratable Acidity

TAC; Total Antioxidant Capacity

TLRs; Toll-Like Receptors

TRAP; Tartrate Resistant Phosphatase

TREM-2; Triggering Receptor Expressed by Myeloid Cells-2

TNF; Tumour Necrosis Factor

WHI; Women's Health Initiative

Wnt/ catenin; Wingless/ Catenin Pathway