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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.

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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
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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.
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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-Telopeptide 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

TLR; 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