Isoflavones and green kiwifruit:
A pilot study assessing the effects on bone turnover and lipid profile in healthy postmenopausal New Zealand women.

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Statement of contribution

Professor MC Kruger – study conception, design and funding.

Mrs CL Booth – Human studies coordinator – ethics application, recruitment, coordination and execution of the study.

Ms C Middlemiss – Support in sample collection and processing, assistance with the DPD and ucOC assays, dietary analyses, and independent data collection, statistical analyses and interpretation.

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Statement of originality

"I hereby declare that this thesis is my own word and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made".

Signed ……..

Date ……..

Abstract

Background: The reduction in estrogen synthesis during menopause heightens the risk of development of osteoporosis and cardiovascular disease in postmenopausal women. Isoflavone (daidzein and genistein) interventions in postmenopausal women have reduced bone loss, and improved the serum lipid profile, which may reduce the risk of fracture and incidence of cardiovascular disease. However, skeletal benefits are inconsistent across interventions. This is partly a result of population heterogeneity in enteric bacterial daidzein metabolism – only ~30% of Caucasian women produce equol, a daidzein metabolite. Equol is more bioactive than its precursor and equol producers have more consistently shown skeletal benefits from isoflavone supplementation. Few interventions have accounted for equol production in postmenopausal study populations. Additionally, there is limited knowledge on how prebiotic foods, such as green kiwifruit, modulate daidzein-metabolising bacteria and equol production in humans. This pilot study aimed to assess bone turnover in response to isoflavone supplementation, and to determine the proportion of equol producers in postmenopausal women.

Objectives: The main objective was to measure the effect of short-term daily isoflavone supplementation alone or with the addition of green kiwifruit on biochemical markers of bone resorption, urinary deoxypyridinoline, plasma cross-linked C-terminal telopeptide of type I collagen, and plasma undercarboxylated osteocalcin in postmenopausal Caucasian women. A secondary objective was to measure the effect of isoflavones and kiwifruit on serum lipid profile. Additionally, equol production was determined in this population and assessed as a main effect.

Methods: This randomised crossover pilot study recruited 33 healthy postmenopausal Caucasian women, 1-10 years postmenopausal, and randomly allocated participants to treatment group A (n=16) or B (n=17) for a 16-week intervention. There were two consecutive 6-week treatment periods with a 2-week lead-in period at intervention commencement and a 2-week washout period between treatments. These treatments prescribed either: (1) daily isoflavone supplementation (50 mg/day aglycone daidzein

and genistein) alone or (2) with two green kiwifruit. Group A and B completed both treatments in opposite order. At treatment baseline and endpoints the following were measured (four time points): bone markers, serum lipid profile and both serum and urinary daidzein and equol. The hormones, serum follicle stimulating hormone, estradiol and thyroid-stimulating hormone, were also measured at baseline and endpoint to monitor potential adverse effects of isoflavones.

Results: Equol producers made up 30% of this study population (equol producers n=10; non-equol producers n=30). Serum equol rose significantly in equol producers. Plasma undercarboxylated osteocalcin decreased by 15.5% after the kiwifruit and isoflavone treatment and increased by 10.8% after the isoflavone only treatment. There were no changes in plasma C-terminal telopeptide of type I collagen or urinary deoxypyridinoline. In non-equol producers high-density lipoprotein cholesterol declined by an average of 4.9% with each treatment; there was no change in serum high-density lipoprotein cholesterol in equol producers following isoflavone treatment alone, and an 8.3% increase in serum high-density lipoprotein cholesterol following the combined kiwifruit and isoflavone combined. There were no other changes to the lipid parameters or hormones.

Conclusions: An aglycone isoflavone dose of 50 mg/day did not reduce bone resorption in the postmenopausal women in this study. Kiwifruit consumption decreased plasma undercarboxylated osteocalcin levels possibly due to the vitamin K content of green kiwifruit; however, alternative bioactive components in kiwifruit may have modulated this effect. The isoflavone treatment inhibited a decline in serum high-density lipoprotein cholesterol in equol producers and had synergistic effect with kiwifruit, which increasing this parameter. Equol and the carotenoid lutein from green kiwifruit may potentially modulate systemic inflammation. Kiwifruit may have a prebiotic effect in equol producers as shown by the increase in log ratio of daidzein to equol, but this requires further study. This equol producer subgroup was too small to detect a change the markers of bone resorption. Larger long-term studies are required to delineate the skeletal and cardiovascular effects of isoflavones and equol production in postmenopausal women.

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“I’d rather have a mind opened by wonder than one closed by belief”

Albert Einstein

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Abbreviations

$1,25(OH)_2D$	Calcitriol
$24,25(OH)_2D$	Dihydroxycholecalciferol
25(OH)D	Calcidiol
AA	Arachidonic acid
AI	Adequate intake
B-ALP	Bone alkaline phosphatase
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMP	Bone morphogenetic protein
BRU	Bone resorptive unit
CaSR	Calcium sensing receptor
COX	Cyclooxygenase
CTx	Cross-linked C telopeptide of type 1 collagen
DBD	Vitamin D binding protein
DEXA	Dual-energy X-ray absorptiometry
DPD	Deoxypyridinoline
E2	Estradiol
ECM	Extracellular matrix
ERα/β	Estrogen receptor alpha/beta
FGF	Fibroblast growth factor
FOS	Fructooligosaccharide
FSD	Functional secretory domain
FSH	Follicle stimulating hormone
GC	Glucocorticoid
HDL-c	High-density lipoprotein cholesterol
HRT	Hormone replacement therapy
ID	Identification
IGF	Insulin-like growth factor
IL	Interleukin

LDL-c	Low-density lipoprotein cholesterol
LRP	Low density lipoprotein-related receptor
M-CSF	Monocyte colony stimulating factor
MCP	Monocyte chemoattractant protein
Mg	Magnesium
NHANES	National Health and Nutrition Examination Survey
NO	Nitric oxide
O-DMA	O-desmethylangolensin
OC	Osteocalcin
OPG	Osteoprotegerin
Ovx	Ovariectomised
PA	Physical activity
PBM	Peak bone mass
PGE	Prostaglandin
PPAR	Peroxisome proliferator-activated receptor
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
RANK	Receptor activator for nuclear factor κβ factor
RANKL	Receptor activator for nuclear factor κβ factor ligand
RB	Ruffled border
RCT	Randomised controlled trial
RDI	Recommended daily intake
SEM	Standard error of the mean
SD	Standard deviation
TAG	Triacylglycerol
TC	Total cholesterol
TC:HDL-c	Ratio of total cholesterol to high-density lipoprotein cholesterol
TGFβ	Transforming growth factor beta
TNF-α	Tumour necrosis factor alpha
TSH	Thyroid stimulating hormone
ucOC	Undercarboxylated osteocalcin
Wnt	Wingless type