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

A Thesis presented in partial fulfilment of the requirements for the degree of

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in  
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New Zealand.

Catherine Middlemiss  
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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.

Source of funding – This research was funded by New Zealand (Ministry of Business, Innovation and Employment) and Japan (Japanese Science and Technology Agency) for the Strategic Bilateral Agreement Program on Functional Foods.
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