Effects of Soy Phytoestrogens and New Zealand Functional Foods on Bone Health

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Summary New Zealand is a rich source of food components that may have bioactivity on bone. Docosahexaenoic acid (DHA) from fish oil has been shown to maintain bone in ovariectomised (OVX) rats. Kiwifruit, a source of fibre and carotenoids, may also affect bone via a prebiotic as well as direct cell-based mechanisms. We aimed to 1) ascertain the effects of DHA on two cell models, including interactions with soy isoflavones; 2) and investigate the specific effects of carotenoids from kiwifruit as well as whole kiwifruit in cell-based and rodent models as well as in a human study. RAW 264.7 mouse monocytes or mouse bone marrow was used to generate osteoclasts (OC). Cells were exposed to the agents between 5 and 21 d and formation and activity of OC measured, including molecular markers. DHA inhibited OC formation in both cell models, including expression of cathepsin K, NFATc1 as well as actin ring formation. Combination with isoflavones enhanced these effects. In OVX rats and mice fed with kiwifruit for 8 wk, green kiwifruit reduced the rate of bone loss after OVX, and in mice it reduced C-telopeptide of Type 1 collagen (CTX) levels and RANKL expression while in menopausal women, green kiwifruit affected blood lipids and bone markers positively.

Key Words kiwifruit, omega 3 fats, osteoclasts, bone, soy phytoestrogens

Osteoporosis is a critical disorder involving bone loss due to estrogen deficiency in postmenopausal women. With the loss of estrogen, bone turnover increases while being uncoupled from the process of formation and the result is a net loss of bone. Hormone replacement therapy is most effective for the prevention of postmenopausal osteoporosis; however, hormone replacement therapy can cause adverse effects (1). Soybean isoflavones have similar structures to those of estrogen and have a weak affinity for the estrogen receptors, specifically estrogen receptor β, which is predominant in bone, brain, thymus, bladder and prostate (2). Genistein, one of the isoflavones, has been reported to have inhibitory effects on bone resorption in vitro, similar to estrogen, which is known for suppressing bone resorption activity (3). We also demonstrated that soy isoflavones, such as daidzein, genistein, and equol, suppressed osteoclast formation in vitro (4). A recent meta-analysis indicates that isoflavone interventions significantly attenuate bone loss in postmenopausal women (5).

Daidzein is metabolized to equol in the intestine by gut microflora and equol possesses a stronger estrogenic activity than daidzein (6). For example, equol administration inhibited femoral bone loss in ovariectomized (OVX) mice without estrogenic activity in the reproductive organs (7). Furthermore, intake of equol-containing soy fermented food inhibited bone resorption in postmenopausal women not producing equol (8). Therefore, the clinical effectiveness of isoflavones, especially daidzein, is due to the further metabolism in the gut producing equol. The latter depends on various factors including the microbiota diversity in the large intestine as well as diet and ethnicity (9). In humans, only 30–50% of the population can produce equol (10). Recent animal studies indicate that gut bacteria can be manipulated to modify the metabolism of isoflavones in the intestine. Fructooligosaccharides (FOS) increase the bioavailability of isoflavones, leading to cooperative effects in the prevention of bone loss in OVX mice (11). Polydextrose and raffinose stimulate equol production, and enhance the bone-protective effects of daidzein in OVX mice (12). A small cross-over study in Japanese women supplemented with 37 mg isoflavones plus/minus 5 g FOS per day for 2 wk observed no effect of the prebiotic on metabolism of daidzein (13).

The purpose of our research programme was to 1) investigate whether kiwifruit or omega 3 fatty acids have specific modulatory effects on bone cells in vitro, and to 2) investigate whether the metabolism of the isoflavones could be affected by selected foods including
Kiwi fruit and omega 3 fatty acids.

**Kiwi fruit**

Kiwi fruit contain several components that may have bioactivity in bone including calcium (20–34 mg/100 g) and magnesium, as well as vitamin C (93–105 mg/100 g) and vitamin K (0.006–0.04 mg/100 g) as well as dietary fibre (14).

In an animal feeding study using pigs aged 28 d, the animals were fed fresh kiwi fruit at 15 g of fruit/kg of body weight/day for 4 wk. Green as well as gold kiwi fruit improved calcium retention significantly in comparison to an ascorbic acid-fed control group (14). This study indicated that carotenoids and vitamin C could affect mineral uptake and a few studies report a relationship between vitamin C and bone density (BMD) (15). Epidemiological reports have shown an inverse relationship between carotenoid intake and low BMD, risk of fracture and risk for developing osteoporosis (16). In a recent study beta-carotene, lutein and zeaxanthin suppressed osteoclast formation (4).

There is a significant amount of vitamin K in green kiwi fruit specifically, up to 60% of the RDI. Osteocalcin is a vitamin K-dependent protein produced by osteoblasts during bone formation and is the primary non-collagenous protein in bone. Osteocalcin functions as a regulator of bone mineral maturation (17). The γ-carboxylation of osteocalcin is the primary mechanism underlying the protective influence of vitamin K on bone. Vitamin K can also modulate certain cytokines involved in bone turnover. Kiwifruit is also a rich source of dietary fibre and has been shown to have a prebiotic effect of promoting the content of faecal lactobacilli and bifidobacteria in healthy female adults (18). In a rat study we reported synergistic effects of daidzein and kiwifruit on bone in ovariectomised (OVX) rats (19). The results showed that the combination of daidzein with green kiwifruit reduced ovariectomy-induced decline in bone mineral density (BMD) compared to the OVX control rats, but kiwifruit did not affect equal production in the rats. In a further study we investigated the effects of green and gold kiwifruit in the absence of daidzein on bone markers and molecular markers for bone formation/resorption in OVX mice fed freeze-dried kiwifruit as 3% of the diet for 8 wk. Green kiwifruit significantly reduced levels of C-Telopeptide of Type I collagen (CTX-1) in comparison to the OVX control mice while RANK-L expression was significantly reduced in the mouse bones by green kiwifruit (Katsumata et al., 201; unpublished results).

The preliminary data obtained from a rat study (19) and the mouse study described above prompted further investigation of an effect by green kiwifruit in menopausal women. In a cross-over design, we supplemented women aged between 50 and 65 with 50 mg isoflavones and analysed for microflora diversity, equol production, blood lipid levels and bone turnover markers. Preliminary data indicate that the green kiwifruit in combination with the isoflavones was bone protective (Kruger et al., 2015; unpublished data).

**Omega 3 Fatty Acids**

There is a substantial body of evidence that has accumulated over the past year that dietary long-chain polyunsaturated fatty acids (LCPUFAs) with a chain length longer than 18C are beneficial for bone health. Research over the past 20 y has suggested that PUFA's of the n-3 series, may prove beneficial to bone health when consumed in appropriate amounts (20). In addition, it has been shown that a reduction of the n-6/n-3 PUFA ratio could result in increased bone strength in animals and reduced bone loss in humans (21, 22).

Rahman et al. (23) reported that in RAW 264.7 cells, n-3 PUFA's reduce NF-kappa-β expression and modulate RANKL. The observed inhibition correlated with inhibition of several osteoclast-specific genes such as TRAP, cathepsin K, and c-FOS as well as TNF-α.

We recently assessed the effects of EPA, DHA, GLA and AA on osteoclastogenesis using the RAW 264.7 cell model. The cells were treated with 5–20 μg/mL of each PUFA in the presence of RANKL to assess osteoclast differentiation using tartrate-resistant staining (TRAP). All the PUFA's inhibited RANKL-induced osteoclast differentiation with the strongest effect observed for DHA and AA (24). Actin ring formation was significantly reduced by AA and in the presence of DHA actin rings were completely absent. Investigation of gene expression using RT-PCR and Western blot analyses indicated that both AA and DHA suppress mRNA expression of both cathepsin K and TRAP but not matrix metalloproteinase, with the effect of DHA stronger that AA (24). More recent work also indicated that DHA at the concentration of 30 μM, is able to significantly suppress expression of NEATc-1 and TRAP in mouse bone marrow cells (Katsumata et al., 2015; unpublished data). We also investigated the effects of the soy isoflavones genistein and daidzein on osteoclast formation and the possible interactions with DHA using mouse bone marrow cultures. DHA suppressed the number of TRAP-positive mononucleated cells (TRAP(+)MNCs) as well as TRAP activity in a stepwise manner. Individually, daidzein as well as genistein between 0.1 and 10 μM significantly inhibited TRAP activity but both only had a significant inhibitory effect on the number of mononucleated cells at 10 μM. When 20 μM DHA was combined with either 10 μM genistein or daidzein, the inhibitory effects of genistein and daidzein were enhanced by DHA (Katsumata et al., unpublished data).

**Conclusion**

Long-chain fatty acids have significant effects on reducing osteoclastogenesis and disrupting function of.
the osteoclast, and daidzein and genistein enhance the effects. Green kiwifruit modulates molecular markers of bone resorption and in older women may be bone protective.

REFERENCES