

Summer Meeting, 4–6 July 2011, 70th Anniversary: From plough through practice to policy

The citrus flavanone hesperetin decreases osteoclast formation and function *in vitro*

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A diet rich in fruit and vegetables may help prevent bone diseases such as osteoporosis⁽¹⁾. There is growing evidence to suggest that phytochemical compounds such as flavanoids may play a role. The citrus fruit glycoside hesperidin has been shown to decrease osteoclast cell number and prevent bone loss in mice and rats *in vivo*^(2,3) and similar results have been seen with rats fed citrus pulp⁽⁴⁾. However, the mechanism is uncertain and little is known about the effects of hesperetin (aglycone) on bone cells *in vitro*. It has been postulated that the bone sparing effects of hesperetin may be due to inhibition of HMG-CoA reductase, a rate limiting enzyme of the mevalonate pathway⁽²⁾. The aim of this study was therefore to study the effects of hesperetin on osteoblasts and osteoclasts *in vitro*.

To assess effects of hesperetin on human subjects osteoclast formation, human subjects osteoclasts derived from peripheral blood mononuclear cells (PBMC) were quantified by vitronectin receptor (VNR) expression. Osteoclast function was determined by F-actin ring number and resorption pit area of osteoclasts generated from human subjects PBMC, or mature rabbit osteoclasts, seeded on dentine. Calvarial mouse osteoblast differentiation and mineralisation were determined by alkaline phosphatase activity and Ca nodule formation, respectively. Effects on the mevalonate pathway were determined by detection of unprenylated Rap1A by Western blotting. In all experiments, a range of hesperetin (0.1, 1, 10, 50 and 100 µM) was tested and compared with control using one-way ANOVA with Bonferroni correction.

Hesperetin dose-dependently decreased human subjects osteoclast formation. Reductions of 50% and 70% in mononucleated and multinucleated VNR positive cells, respectively were observed when treated with 100 µM hesperetin compared with control ($P = 0.007$ and $P = 0.039$, respectively). No effect of hesperetin on Rap1A prenylation was observed. Above 50 µM, hesperetin significantly inhibited the activity of mature rabbit and human subjects osteoclasts *in vitro*. In human subjects osteoclast culture, 100 µM hesperetin decreased F-actin ring number by 90% ($P < 0.001$) and resorption pit area was reduced by 80% and 90% with 50 µM ($P = 0.016$) and 100 µM ($P = 0.003$), respectively. In mature rabbit osteoclast culture, F-actin ring number was decreased by 70% with 100 µM hesperetin ($P < 0.001$) and resorption pit area was reduced by 40 and 70% with 50 and 100 µM, respectively, relative to control. Osteoclast number was also decreased by 20% ($P = 0.001$) and 50% ($P < 0.001$) with 50 and 100 µM hesperetin, respectively, compared with control, suggesting a toxic effect at these concentrations. Hesperetin had no effect on mouse osteoblast differentiation or mineralisation.

In summary, the citrus flavanone hesperetin may help prevent osteoporosis by decreasing osteoclast formation and function. However, this effect was seen at doses that may not be relevant as part of a normal diet and may only be achieved by supplementation. This effect appears to be unrelated to inhibition of HMG-CoA reductase. Further work is required to investigate the bioavailability and pharmacokinetics of hesperetin, to determine if flavonoids may work in synergy with other compounds in a balanced diet and to elucidate the cellular mechanism of action of these compounds on bone cells.

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