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## Stimulatory Effect of Wasabi Leafstalk Extract (*Wasabia japonica* MATSUM.) on Bone Calcification: Interaction with Bone Anabolic Factors in Mouse Calvaria Tissue *in Vitro*

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The stimulatory effect of wasabi leafstalk extract (*Wasabia japonica* MATSUM.) on bone calcification was compared with the anabolic effect of insulin, insulin-like growth factor I (IGF-I), 17 $\beta$ -estradiol, and genistein. Mouse calvaria tissues were cultured for 48 h in a serum-free Dulbecco's modified Eagle's medium (high glucose, 4.5%). The presence of wasabi leafstalk extract (25 µg/ml) caused significant increases in bone calcium content. The combination of  $10^{-10}$  M 17 $\beta$ -estradiol and 15 µg/ml wasabi leafstalk extract had an additive effect on bone calcium content, while the increasing effect of phyotoestrogen genistein ( $10^{-9}$  or  $10^{-7}$  M) on this content was not significantly greater in the presence of the same amount of the extract. Insulin ( $10^{-8}$  or  $10^{-7}$  M) had a significant effect on bone calcium content, and the combination of both  $10^{-8}$  or  $10^{-7}$  M insulin with 15 µg/ml wasabi leafstalk extract had an additive effect. Such an effect was not seen in the case of IGF-I ( $10^{-8}$  M), however which increased bone calcium content. The present study demonstrates that wasabi leafstalk extract has an enhancing effect on the anabolic action of  $17\beta$ -estradiol or insulin, which regulates bone formation and calcification *in vitro*.

**Keywords:** <u>wasabi leafstalk</u>, <u>bone calcification</u>, <u>17β-estradiol</u>, <u>insulin</u>, <u>IGF-I</u>, <u>genistein</u>

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