Beneficial effects of vitamin K on morphometric and material bone properties during growth

Vitamin K (VK) facilitates collagen accumulation, osteoblast differentiation, and bone structural integrity, and furthermore, exerts an antiresorptive action on bone. Thus, the VK status is deeply associated with healthy bone growth [1]. However, during childhood and adolescence, bone metabolic activity is high for bone radical extension, and dietary VK intake may be insufficient to satisfy the metabolic VK requirement. Indeed, dietary VK intake and the metabolic VK requirement are reported to be imbalanced in children compared with those in adults [2]. This suboptimal vitamin K status will be responsible, at least in part, for vulnerability to frsacture during growth, which is not only detrimental to healthy bone development but also poses a potential risk of osteoporosis later in life [3]. Thus, supplemental VK during childhood and adolescence may be beneficial for healthy bone growth; in fact, better VK status contributes to the enhancement of bone growth [2]. However, the VK effect on bone strength or quality has not been entirely explored, and there is a lack of experimental insight in this regard. We thus tested the hypothesis that VK is beneficial to the mechanical properties of bones in growing rats [4].

Experiments were conducted with the approval of the Animal Research Committee of Osaka University Graduate School of Engineering Science. Sixteen female Wistar rats at 5 weeks of age were divided into two groups, VK2 diet and control diet (n=8 each), fed with normal diet with and without VK2 treatment, respectively. VK2 (menatetrenone, kindly donated by Eizai, Japan) was given as a dietary supplement; the actual VK2 intake was 22 mg/kg of body weight per day. VK2 was used rather than VK1 because of its advantage over the latter in half-life time. The animals were kept for 9 weeks under standard housing conditions and then euthanized. Serum analysis by sandwich enzyme-immunoassay (EIA) at the end of the feeding period showed a lower ratio of undercarboxylated to γ-carboxylated serum osteocalcin (Glu/Gla-OC) in VK2 diet than in control diet (0.68 *vs* 0.93%, $p<0.05$), indicating improved VK status in VK2 diet. Urinary deoxypyridinoline, a bone resorption marker, did not differ between the two groups. That is, in the present setting, VK2 seemed beneficial to bone formation rather than antiresorption.

Tibial bone was transected, and bone material properties were analyzed by Fourier transform infrared microspectroscopy (FTIR-MS) and nanoindentation testing and bone morphometry by micro-computed tomography (μCT). Figure 1 shows three bone segments measured for each analysis. FTIR spectra were collected in the reflection mode from three regions each at the anterior and posterior cortical midlayers for determining the mineral-to-matrix ratio, mineral crystallinity, and collagen maturity.

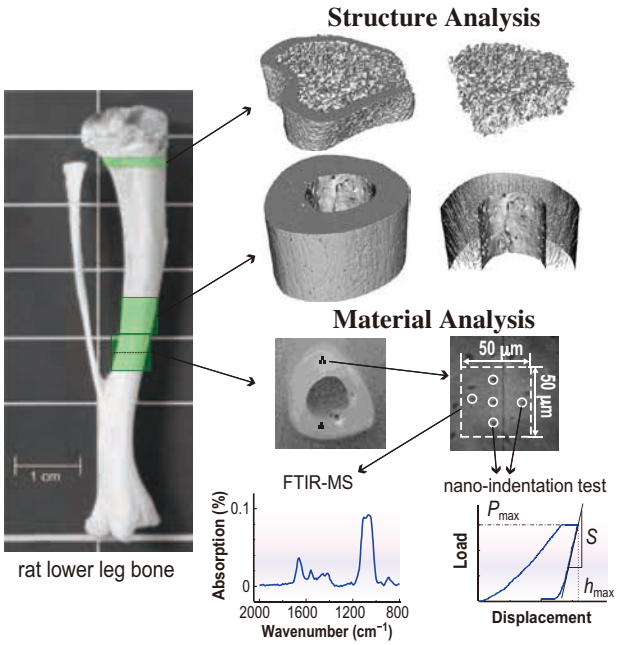

Fig. 1. Bone segments measured for morphometric and material analyses. The indentation test included 5 indents in each of 3 anterior and 3 posterior regions where FTIR spectra were obtained.

Subsequently, the specimens were subjected to the nanoindentation test. Five points were tested with a trapezoidal loading waveform in each region where the FTIR spectra were collected, and the indentation modulus and hardness were obtained from a resulting force-displacement curve according to the method described by Oliver and Pharr [5].

The proximal metaphysis and distal diaphysis were scanned for imaging of the cancellous trabecular architecture and cortical porous network, respectively, by laboratory μCT (12-μm cubic voxel) and by synchrotron radiation μCT (SR μCT, 2.74-μm cubic voxel) at beamline **BL20B2**. By calibration using K_2HPO_4 phantom solutions, bone mineral density was quantitated from SR μCT images. In the cancellous bone, trabecular volume fraction, trabecular thickness, trabecular number density, and trabecular connectivity density were calculated. In cortical porous structure, cortical porosity, mean porous diameter, porous density in the inner and outer cortical surfaces, and density of porous connections were calculated.

There was no effect of VK2 on bone mineral density or mineral-to-matrix ratio, but VK2 increased both the mineral crystallinity (1.7 *vs* 1.5, $p<0.05$) and collagen maturity (3.2 *vs* 2.5, $p<0.05$). Thus, VK2 can contribute to the maturation of cortical bone tissue. An increasing effect of VK2 on bone hardness (1.6 *vs* 1.3, $p<0.05$) was also found. Figure 2 shows the plots of mineral crystallinity, collagen maturity, and hardness for each specimen. Increased mineral crystallinity, rather than increased collagen maturity, seems to be implicated in the increased hardness because collagen maturity affects mainly on the ultimate strength and toughness of bone rather than on the strength. No effect of VK2 was found on cortical porosity or the other indices of porous structure; however, VK2 increased the trabecular volume fraction with increasing trabecular thickness (Fig. 3). Trabecular number also tended to be increased by VK2. A different activity of bone modeling/remodeling between cancellous and cortical bone or a strong linkage between cortical microstructure and intracortical vascularization may be involved in the site-dependent VK2 effect.

In summary, supplemental VK2 improved the VK status, reinforced the trabecular bone architecture, and promoted the maturation of cortical bone tissue in growing rat tibia. Thus, VK supplementation could be beneficial for enhancing bone quality and reducing the risk of fracture during growth spurts.

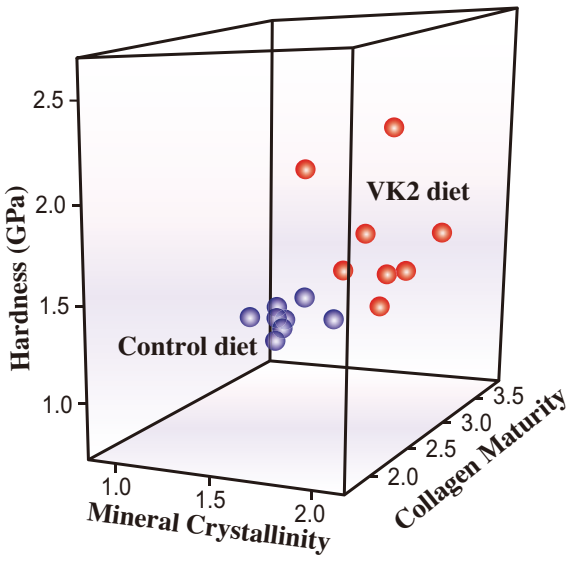

Fig. 2. Scatter diagram of mean values of hardness, mineral crystallinity, and collagen maturity. Blue circles: control diet; red circles: VK2 diet.

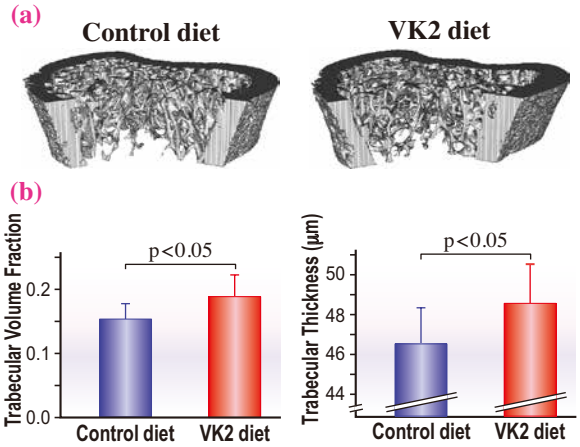

Fig. 3 **(a)** Three-dimensional displays of tibial metaphyses harvested from control- and VK2-diet rats. **(b)** Increases in trabecular volume fraction and thickness with VK2 intake. Bars represent standard deviation. Statistical analysis was performed using the unpaired t-test.

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