J Musculoskel Neuron Interact 2004; 4(2):301-307

Original Article

Hylonome

Effects of grape seed proanthocyanidins extract on rat mandibular condyle

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Abstract

We investigated the effects of grape seed proanthocyanidins extract (GSPE) on bone formation by examining total and cortical bone mass, density, architecture, and strength non-invasively using mandibular condyles of Ca-restricted rats. Forty Wistar male rats, each 5 weeks old, were divided into control (C), low-Ca diet (LCaD), low-Ca diet·standard diet (LcaD·SD), and low-Ca diet·Estandard diet with supplementary GSPE (LcaD·SD+GSPE) groups. In LCaD·SD group, after the bone debility was induced by low-Ca diet, a standard diet therapy was given. In LCaD·SD+GSPE group, after the bone debility was induced by low-Ca diet, a standard diet therapy with supplementary GSPE was given. Each mandibular condyle was examined using peripheral quantitative computed tomography (pQCT). There were no significant inter-group differences in body weight seen throughout the experimental period. In LcaD·SD+GSPE, cortical bone cross-sectional area and mineral content were not significantly different from C, while bone mineral content was significantly higher in LcaD·SD+GSPE than in LcaD·SD. Cortical bone density of LcaD·SD+GSPE was not significantly different from that of C, however, that value in LCaD and LcaD·SD was significantly lower than that. The cross-sectional (bending) moment of inertia values in LcaD·SD+GSPE were the highest among all groups, though they did not differ significantly from those in C. Further, the cross-sectional (bending) Stress/Strain Index (SSI) values in LcaD·SD+GSPE were statistically similar to those in C, however, not significant higher than in LcaD·SD. These results suggest that GSPE treatment would increase both bone mass and bone strength on the rat mandibular condyles.

Keywords: Calcium, GSPE, Dietary Therapy, Mandibular Condyle, Rats

Introduction

Grape seed proanthocyanidins extract (GSPE) is a type of flavonoid and its active constituents are proanthocyanidins, which represent a variety of flavan-3-ol types, such as catechin[1]. Several experimental and clinical studies have shown that GSPE has a cholesterol-lowering effect[2], cytotoxic effects toward human cancer cells[3], cardioprotective properties[4], and a stimulating action on angiogenesis in dermal wound healing[5]. Further, the compound does not seem to induce any significant toxicological effect[1]. In addition, isoflavone, another flavonoid, was shown microscopically to inhibit bone resorption and enhance bone formation with endochondral ossification[6]. However, the skeletal effects of GSPE, particularly those exerted during the critical period of growth and development that demonstrates a high rate of bone formation, are not known. Age-related bone loss, and the related enhanced fracture incidence are natural phenomena, and the best means to prevent age-related fractures is thought to be achievement of a high bone mass peak at maturity by sufficient calcium intake[7,8]. Low-calcium intake during the growth and development period has been shown to increase bone resorption and decrease bone mass[9], though, several studies have also reported that this condition can only be recovered partially by calcium supplementation[10].

Peripheral quantitative computed tomography (pQCT) is suitable for non-invasive assessment of bone mass, volumetric bone density, architecture, and strength[11,12,13], though few studies have evaluated the bone status of animal mandibular condyles using a pQCT technique[14]. We examined the potential utility of GSPE along with calcium

The authors have no conflict of interest.

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Accepted 3 November 2003

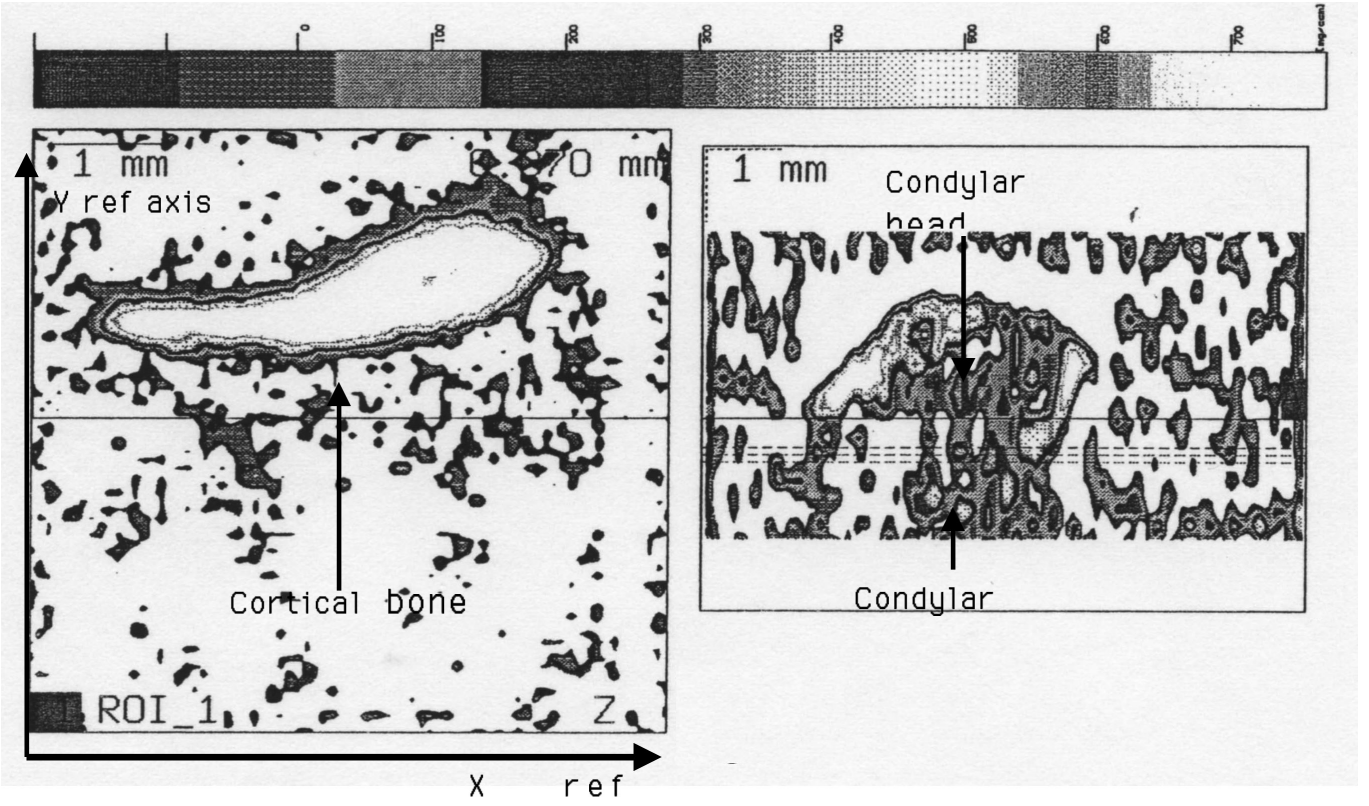

Figure 1. pQCT scans. Left. Tomographic image of bone "slice". Right. Cross-sectional slices scanned 0.4 mm below the condylar head at 3 different positions with 0.1-mm intervals along the neck of the mandibular condyle.

using experimentally-weakened rat mandibular condyles, with a three-dimensional pQCT technique, by which we performed non-invasive measurement of both total and cortical bone separately, as well as assessment of bone architecture and calculation of a bone strength index.

Materials and methods

Materials

Rat food was provided by Oriental Yeast (Tokyo, Japan) and mixed in our laboratory, with the diet components presented in Tables 1-1 ~ 1-3. GSPE was prepared as a powder stock, and had a proanthocyanidins content of more than 90%. The GSPE diet was prepared by additioning 0.3% GSPE w/w to the standard diet in a powder form. The calcium content of the standard diet was 480 mg/100 g and that of the low-calcium diet was 144 mg/100 g (30% of the calcium in the standard diet).

Animals and treatment

The experimental protocol was approved by the Committee for the Use of Laboratory Animals of the Kyushu Dental College, Japan. Forty Wistar male rats. each 5 weeks old and weighing approximately 115 g, (maintained by Seiwa Experimental Research Institute), were randomly divided into 4 groups of 10 and individually housed in small cages under similar conditions with a 12-hour light-dark cycle at 22±°C. The control (C) group was fed a standard diet with tap water *ad libitum* for 6 weeks. The low-Ca diet (LCaD) group was given a diet with 30% of the Ca content of the standard diet and distilled water for 6 weeks. The low-Ca diet·standard diet (LCaD·SD) group was given the low-Ca diet and distilled water freely for 3 weeks and then fed the standard diet with tap water for the next 3 weeks. The low-Ca diet·standard diet with supplementary GSPE (LCaD·SD+GSPE) group was given the low-Ca diet and distilled water freely for 3 weeks and then fed the standard diet with supplementary GSPE with tap water for the next 3 weeks. After 6 weeks, the rats were deeply anaesthetized with diethyl ether and killed with thiopental sodium (Ravonal®, Tanabe), after which the mandibular bones were dissected and fixed in 10% neutral buffered formalin.

Body weight

Body weight was recorded once each week.

Ingredients	Standard diet (Ca 480 mg/100g)	Low-calcium diet (Ca 144 mg/100g)
β - corn starch	38.00	37.64
Vitamin-free casein	25.00	25.00
α - potato starch	10.00	10.00
Cellulose powder	8.00	8.00
Soy bean oil	6.00	6.00
Mineral mixture	◇ 6.00	◆ 6.00
Granulated sugar	5.00	5.00
Vitamin mixture	2.00	2.00
$CaCO_3$	0.00	0.36
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	100.00	100.00

◇ = Mineral mixture of standard diet (in Table 1-2)
◆ = Mineral mixture of low-calcium diet (in Table 1-2)

Table 1-1. Composition of experimental diets (%).

◇ Mineral mixture of standard diet (g)	
NaCl	4.66
Kl	0.01
KH_2PO_4	25.72
NaH_2PO_4	9.35
$MgSO_4$	7.17
$CaHPO_4$	14.56
Fe-citrate	3.18
$MnSO_4 \cdot 4 \sim 5H_2O$	0.12
$CuSO_4 \cdot 5H_2O$	0.03
$ZnCO_3$	0.11
Ca-lactate	35.09

Table 1-2. Composition of experimental diets (per 100 g).

◆ Mineral mixture of low calcium diet (g)	
NaCl	4.680
Kl	0.0055
KH_2PO_4	28.333
NaH_2PO_4	9.380
K_2HPO_4	9.550
$MgSO_4$	7.187
Fe-citrate	3.187
$MnSO_4 \cdot E_4 \sim 5H_2O$	0.12817
$CuSO_4 \cdot 5H_2O$	0.03275
$ZnCl_2$	0.10425

Table 1-3. Composition of experimental diets (per 100 g).

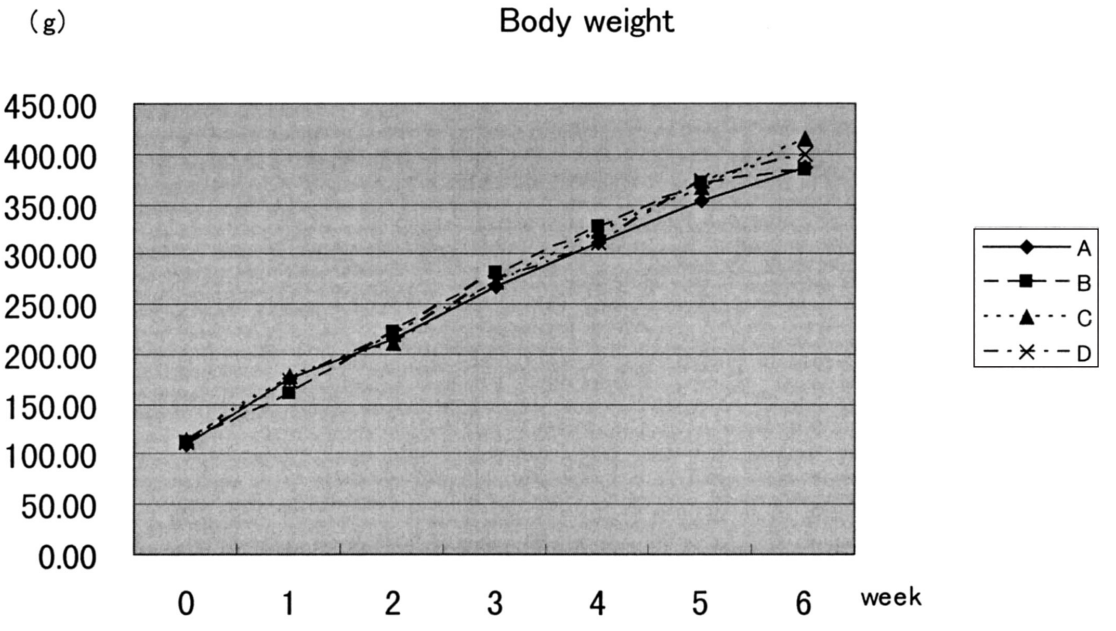

Figure 2. Body weight during the study period. There were no significant differences in body weight between the 4 groups.

Cross-sectional area, bone mineral content, density, and moment of inertia

Using pQCT (XCT Research SA+, Stratec-Medizintechnik GmbH, Pforzheim, Germany), each bone sample was centrally located within the field of the scanner unit with the aid of a support. After producing a scout-view, the tomographic scan was performed. Cross-sectional slices were obtained at about 0.4 mm below the condylar head at 3 different positions separated by 0.1 mm intervals along the neck of the condyle. All scanning was performed with the voxel size 0.07 x 0.07 x 0.26 mm (Figure 1). The total bone region was defined by interactive contour detection, using contour mode 2 to calculate total bone cross-sectional area (ToCSA, mm^2), mineral content (ToBMC, mg/mm), and volumetric mineral density (TovBMD, mg/cm^3). The cortical region was determined using cortical mode 1, setting the density threshold at a 690 mg/cm^3 value, in order to measure the cortical bone CSA (CtCSA, mm^2), BMC (CtBMC, mg/mm), vBMD (CtvBMD, mg/cm^3), and some bone architectural parameters as the second moments of inertia (CSMI) of the CtCSA with respect to the axis Y (yCSMI, mm^4). Most of the customarily mandibular movement is upward-downward. We selected yCSMI to evaluate geometric property in this study because y-axis corresponded to a neutral axis relating to a major loading direction of mandibular open-closed movement.

Non-invasive assessment of bone strength.

The Y Stress/Strain Index (YSSI, a non-invasive indicator of bone mechanical properties of reference axis Y) was assessed by pQCT as SSI = CtvBMD · Z / NCtvBMD, being Z the section modulus (mm^3), and NCBD the maximal possible value of CtvBMD, i.e. 1200 mg/cm^3 with a cortical mode 1 setting a 464 mg/cm^3 threshold value.

Statistical analysis

Data are expressed as means ± SD. All data were analyzed by one-way ANOVA. Statistical differences between any two groups were analyzed using post hoc analyses of paired groups by the same applied ANCOVA.

Results

Body weight

All rats in all groups grew naturally throughout the study period. There were no significant inter-group differences in body weight. Growth curves are shown in Figure 2.

Bone cross-sectional area, mineral content, and density

Bone cross-sectional area, bone mineral content, bone density of the cortical, and the total of mandibular condyle values are summarized in Table 2.

For total bone, the ToBMC of the LCaD·SD+GSPE group was significantly higher than that of the LCaD·SD group ($p < 0.05$).

As for cortical bone, the CtBMC was significantly higher in the LCaD·SD+GSPE group than in LCaD·SD group ($p < 0.05$).

	Control group	Low-calcium diet• group	Low-calcium diet• standard diet group	Low-calcium diet• standard diet+GSPE group
cortical bone cross-sectional area (mm^2)	1.25±0.19	1.01±0.11**	1.19±0.15	1.25±0.16
cortical bone mineral content (mg/mm)	1.21±0.21	0.90±0.11**	1.15±0.11	1.32±0.23
cortical bone density (mg/cm^3)	967.19±52.60	891.03±21.79**	936.67±20.27*	953.44±41.59
total bone cross-sectional area (mm^2)	1.42±0.17	1.34±0.21	1.39±0.10	1.39±0.16
total bone mineral content (mg/mm)	1.31±0.18	1.10±0.09**	1.23±0.15	1.38±0.20
total bone density (mg/cm^3)	920.26±64.51	799.10±39.53**	895.97±20.95	915.82±46.67
y CSMI (mm^4)	0.846±0.179	0.564±0.095**	0.784±0.210	0.903±0.242
ySSI	0.376±0.087	0.309±0.044*	0.396±0.063	0.408±0.125

Data are the mean ± SD (N=40)
*: Compared with control group, $p < 0.05$
**: Compared with control group, $p < 0.01$

Table 2. Bone density, cross-sectional area, bone mineral content, yCSMI and ySSI of mandibular condyle.

Bone architecture and strength indicators

Values for yCSMI and ySSI are summarized in Table 2. The yCSMI value in the LCaD·SD+GSPE group was higher than that in the LCaD·SD group, however, the difference was not significant. In the LCaD·SD+GSPE group, ySSI was not significantly difference to the LCaD·SD group.

Discussion

Several studies have reported the effects of GSPE in relation to body weight using rats, and those fed a powdered diet[1], GSPE solution[15], and a natural extract from *Vitis* vinifera seeds[1,16] did not show an increase in body weight as compared with their respective control group. However, an excess of GSPE given to rats may reduce body weight, as well as reduce food intake[15]. In the present study, no significant inter-group differences in body weight were observed.

Peak bone mass, generally achieved during childhood and adolescent growth, is one of the major determinants of osteoporosis and fracture risk, and genetic potential, gender, ethnic origin, lifestyle factors (including nutrition), growth patterns, and physical activity each have an influence on the accretion of bone minerals during childhood and contribute to determination of peak bone mass. The relatively small mandibular condyle is a very complex structure[17], in correlation with the loading conditions predominating close to joint surfaces[18]. Remodeling of the condylar head and neck in the case of a condylar fracture takes a long time[19]. Thus, efforts regarding osteoporosis prevention in the mandibular condyle are important.

We analyzed the mandibular condyle by using pQCT. The advantages of pQCT is that it provides volumetric data, and that cortical and cancellous bone can be studied separately. Trabecular bone in the mandibular condyle is generally more robust and denser than that in other locations[18]. However, there are no known studies of the mechanical properties of trabecular tissue using pQCT findings. Cortical bone is thought to respond to mechanical stimulation[20]. The mandibular condyle has a parasagittal plate-like trabecular structure that seems to reflect site-specific additions of bone mass after mechanical loading[21,22]. In the present study, no trabecular parameters were available, though some cortical indicators were determined.

Our results showed that ToBMC and CtBMC in the LCaD·SD+GSPE group were significantly higher than those in LCaD·SD, which suggest that the GSPE compound caused an increase in ToBMC and CtBMC, indicating bone response and facilitation of deposition of mineralized matrix, revealed that the bone mass increased.

However, ToCSA, a better indicator of bone size than the ToBMC[12], did not differ significantly among the groups. Further, increases of bone mass in the present rats were not followed by an increase in bone size.

The yCSMI and ySSI in the LCaD·SD+GSPE group were the highest among the 4 groups, though they were not significantly different from that in the LCaD·SD group. Therefore, we considered that GSPE had a tendency to

increase cortical bone quality and the whole-bone strength.

The present is the first known study of specific condyle tissue mechanical properties as revealed by pQCT. Several previous studies have reported the effects of various flavonoids, such as ipriflavone, an isoflavone synthesized from the soy daidzein, on bone histologically, along with calcium intake and found the combination to be effective for the recovery of debilitated bone in rats[23], inhibiting bone resorption and enhancing bone formation[24], and stimulating osteogenesis of injured perialveolar bones in humans[25]. Those effects are similar to our results. However, since we only performed a non-invasive assessment, further investigation using histomorphometric means should be conducted in the future.

Conclusion

We found that GSPE enhanced total and cortical bone mass in rat mandibular condyles, in which bone fragility had been induced by dietary Ca restriction, and also had a favorable impact on bone architecture and strength. These results suggest a potential usefulness for this compound as a treatment for various types of bone fragility. Further, we recommend that the pQCT technique be employed to assess rat mandibular condyles.

Acknowledgements

We thank Mr Kiichi Nonaka (ELK Corporation Research Laboratory) for his helpful advice and cooperation.

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