Interaction of the effects between vitamin D receptor polymorphism and exercise training on bone metabolism

ORIE TAJIMA, NORIKO ASHIZAWA, TOMOO ISHII, HITOSHI AMAGAI, TOMOKO MASHIMO, LI JING LIU, SHINICHI SAITO, KUMPEI TOKUYAMA, AND MASASHIGE SUZUKI.

Interaction of the effects between vitamin D receptor polymorphism and exercise training on bone metabolism. J Appl Physiol 88: 1271–1276, 2000.—Bone metabolism is strongly influenced by heredity and environmental factors. To investigate interaction of the effects between vitamin D receptor polymorphism by Fok I and resistance exercise training on bone metabolism, young male subjects with FF genotype (F, n = 10) and Ff or ff genotypes (f, n = 10) followed 1 mo of weight training, and changes in bone metabolism were compared. An additional 14 subjects served as a sedentary control. Biomarkers of bone formation, bone-specific alkaline phosphatase, and osteocalcin were significantly increased by training in both F and f groups. 1,25-Dihydroxyvitamin D3, known to upregulate bone formation, was also increased by the training in f but not in the F group. Bone resorption assessed by cross-linked NH2-terminal teropeptide of type I collagen was significantly suppressed by the training, and the decrease in F was greater and longer lasting than that in f group. In conclusion, stimulation of bone formation and suppression of bone resorption occurred within 1 mo in young men. Despite a significant increase in 1,25-dihydroxyvitamin D3 in the F group but not in the f group, the response of bone metabolism to the training in the F was similar to or greater than that in f group, suggesting a functional difference between vitamin D receptor genotypes f and F.

Fok I; bone formation; bone resorption

BONE MINERAL DENSITY (BMD) is influenced by environment (35) and heredity (28, 31). Mechanical load is one of the major environmental factors to influence BMD and bone metabolism (12). We have shown that resistance exercise training for 4 mo increased bone formation, whereas it transiently suppressed bone resorption, and such adaptive changes of bone metabolism to the training occurred during the early period of the training in young men (13).

BMD and bone turnover in adults are under genetic influence; in particular, serum osteocalcin concentration is strongly correlated in monozygotic twin pairs (19). The vitamin D receptor (VDR) is a good genetic candidate for a prime regulator of BMD and bone metabolism (8) because of its acting to mediate the vitamin D endocrine system through binding of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] and subsequently controlling the transcription of target genes relating to calcium homeostasis (32). Common allelic variants in the VDR gene have been associated with BMD (24). The VDR gene has two potential translation initiation sites (ATG), and a thymine/cytosine polymorphism has been noted in the first ATG. This polymorphism was first found in the patients with vitamin D-dependent rickets type II (30). Recent studies have reported that the polymorphism at the translation initiation site of the VDR gene correlates significantly with BMD or with a rate of bone loss in the healthy population, whereas some have not observed these associations (1, 2, 7, 10, 11, 14, 15, 18, 23).

Although the effects of mechanical load and genetic factors on bone metabolism have been intensively studied, there is a paucity of literature relating these two factors (27). As the first attempt in human study, we recruited young men with different VDR genotypes and compared the response of biomarkers of bone metabolism between the two training groups during the first month of resistance exercise training.

MATERIALS AND METHODS

Subjects. Allele frequencies for the VDR polymorphism at the translation initiation site were determined among 81 unrelated Japanese young adult male volunteers (18–31 yr) who had not participated in a regular exercise program for at least 1 year. The response of bone metabolism to resistance exercise training between groups with different VDR genotypes was investigated in 34 normal, healthy randomly selected male volunteers (22–26 yr) who were taking no medications that would alter calcium or bone homeostasis. Ten men previously identified to have the FF genotype were selected as the F-group training subjects, and another 10 men with f allele, including 7 ff and 3 Ff, were selected as the f-group training subjects. An additional fourteen subjects (8 FF and 6 Ff) were selected as the sedentary control. There

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Table 1. Physical characteristics of the subjects of training (F, f) and sedentary groups

<table>
<thead>
<tr>
<th>VDR Group</th>
<th>Sedentary Group (n = 14)</th>
<th>F (n = 10)</th>
<th>f (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.8 ± 0.4</td>
<td>23.5 ± 0.4</td>
<td>23.7 ± 0.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173.8 ± 0.7</td>
<td>174.2 ± 2.0</td>
<td>175.1 ± 1.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.8 ± 0.9</td>
<td>66.0 ± 2.9</td>
<td>65.8 ± 1.4</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>17.3 ± 0.5</td>
<td>17.1 ± 1.1</td>
<td>16.3 ± 0.9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.8 ± 0.3</td>
<td>21.7 ± 0.6</td>
<td>21.5 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. F, training group of subjects with FF genotype (vitamin D receptor alleles lacking FokI site); f, training group of subjects with Ff or ff genotype (vitamin D receptor alleles containing FokI site).

There were no significant differences in physical characteristics among these groups (Table 1).

VDR genotype. VDR gene polymorphism at the translation initiation site was determined by a restriction fragment length polymorphism by using the restriction endonuclease FokI.

DNA was extracted from whole blood by using the sodium iodide method (DNA extractor WB kit, Wako Pure Chemical Industries, Osaka, Japan) and was amplified by the PCR method (2). PCR products were digested with FokI and then electrophoresed through a 2% agarose gel containing ethidium bromide. VDR alleles containing the FokI site were denoted by f and alleles lacking the site were denoted by F, and individuals were scored as FF homozygotes, Ff heterozygotes, or ff homozygotes according to the digestion pattern (15).

Training program. The two training groups followed a weight training program consisting of eight exercises (3, 13). The training was performed 12 times at 3 times per week for 1 mo. During the first half of the training period, training subjects performed two sets of 10 repetitions of each exercise (60% of one repetition maximum (1RM) for the first set and 75% of 1RM for the second set, respectively). During the latter half of the training period, training subjects performed three sets of exercise to further load the skeleton (60% of 1RM for the first set, 75% of 1RM for the second and third sets, respectively). All exercises were performed on a weight machine (Uesaka). Weights were increased on an individual basis as strength improved. Improved strength was measured by a 1RM test and adjusted to accommodate strength gains for each individual after six training periods.

Diet. Each subject was given a daily supplement of 600 mg of calcium (Ozaka) to ensure an adequate calcium intake of more than 800 mg/day during the 1-mo experimental period. Control diets containing 2,421 ± 86 kcal (58.3 ± 1.9% carbohydrate, 13.2 ± 0.6% protein, and 28.5 ± 2.0% fat) and 902 ± 35 mg of calcium were given for 3 days, after which blood and urine samples were collected.

Biochemical analysis. To evaluate the effects of the resistance exercise training, biomarkers of bone metabolism were measured before and after the training period on resting days in all groups. Additionally, the biomarkers in the training groups were assessed at three points after exercise days during the training period. Fasting blood and 24-h urine samples were stored at −60°C to be run simultaneously at the conclusion of the study to eliminate interassay variations.

As bone formation markers, serum bone-specific alkaline phosphatase (B-ALP) activity was measured by an ELISA (Alkphase-B, Metra Biosystems, Palo Alto, CA), and serum osteocalcin was determined by specific two-site RIA for human osteocalcin (ELSA-OSTEO, CIS Biointernational, Bagnoles, France). As bone resorption markers, urinary deoxypyridinoline (DPYR) was measured by an ELISA (Pyrilinks-D, Metra Biosystems), and urinary cross-linked NH2-terminal teropeptide of type I collagen (NTx) was measured by an ELISA (Osteomark, Ostex International, Seattle, WA). In addition, 1,25(OH)2D3, the active hormonal form, was measured by radio receptor assay [1,25(OH)2D3 kit (SRL), Yamasa, Chiba, Japan]. Duplicate measurements were made of all samples.

Data analysis. Values are means ± SE, and Mann-Whitney’s U tests were used to compare differences in BMD and biochemical measurements between F- and f-group subjects before the training intervention.

The response of each biochemical measurement to resistance exercise was expressed as the percentage change from the baseline value, and the data are means ± SE. To determine the effect of training for 1 mo, paired Wilcoxon’s tests were used. Multifactor analysis of variance (MANOVA) with Fisher’s post hoc test was used to evaluate the interaction of effect to bone metabolism between exercise training and VDR genotype. Statistical significance was taken at the P < 0.05 level.

RESULTS

VDR polymorphism. The frequency of VDR genotypes by FokI in this study was 50.6% (n = 41) FF, 40.7% (n = 33) Ff, and 8.7% (n = 7) ff. There were no statistically significant differences between the two groups of VDR genotype by FokI, FF and one or more F allele (Ff or ff), in physical characteristics of the subjects (Table 2).

Effects of resistance exercise training. In evaluating the effects of the resistance exercise training, all of the bone metabolism markers except urinary DPYR excretion in trained subjects were significantly changed after the training period compared with baseline values (Table 3). Additionally, serum concentration of 1,25(OH)2D3 seemed to be increased after the training period in the trained subjects, but the difference was

Table 2. Characteristics of the subjects by VDR groups

<table>
<thead>
<tr>
<th>VDR Group</th>
<th>B-ALP, IU/l</th>
<th>Osteocalcin, ng/ml</th>
<th>1,25(OH)2D3, pg/ml</th>
<th>NTx, nmol/day</th>
<th>DPYR, nmol/day</th>
<th>Total BMD, g/cm²</th>
<th>BMC, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (n = 18)</td>
<td>18.3 ± 1.1</td>
<td>20.3 ± 3.0</td>
<td>34.2 ± 3.5</td>
<td>691 ± 84</td>
<td>37.7 ± 4.6</td>
<td>1.32 ± 0.03</td>
<td>3080 ± 120</td>
</tr>
<tr>
<td>range</td>
<td>12.0–30.9</td>
<td>5.0–45.4</td>
<td>11.4–58.2</td>
<td>209–1268</td>
<td>12.1–79.2</td>
<td>1.15–1.48</td>
<td>2393–4323</td>
</tr>
<tr>
<td>f (n = 16)</td>
<td>20.0 ± 1.6</td>
<td>18.3 ± 2.6</td>
<td>31.2 ± 2.5</td>
<td>644 ± 196</td>
<td>41.3 ± 6.3</td>
<td>1.30 ± 0.02</td>
<td>3027 ± 62</td>
</tr>
<tr>
<td>range</td>
<td>11.3–32.4</td>
<td>6.8–36.7</td>
<td>17.8–53.3</td>
<td>386–1004</td>
<td>11.6–93.9</td>
<td>1.16–1.38</td>
<td>2649–3473</td>
</tr>
<tr>
<td>P value</td>
<td>0.51</td>
<td>0.6</td>
<td>0.95</td>
<td>0.08</td>
<td>0.64</td>
<td>0.93</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. VDR, vitamin D receptor; B-ALP, bone-specific alkaline phosphatase; 1,25(OH)2D3, 1,25-dihydroxyvitamin D3; NTx, cross-linked NH2-terminal teropeptide of type I collagen; DPYR, deoxypyridinoline; BMD, bone mineral density; BMC, bone mineral content.
not statistically significant (P = 0.10). There were no significant changes in any biochemical measurements in sedentary group subjects after the experimental period.

Gene-environment interaction. When the two training groups were separately analyzed, similar changes in the bone metabolism markers in each group were observed, but the increase in serum B-ALP activity in the f group was not statistically significant (P = 0.14). Interestingly, the response of circulating 1,25(OH)2D3 to exercise training depends on VDR genotypes, and an increase in 1,25(OH)2D3 after 1 mo training was observed in the f but not in the F group (Fig. 1). Furthermore, gene-environment interaction in 1,25(OH)2D3 as evaluated by MANOVA was statistically significant (P < 0.05).

During the exercise training period, the time course of changes in biomarkers of bone metabolism and circulating 1,25(OH)2D3 was compared between the F and f groups (Figs. 2–5). Serum B-ALP activity and serum osteocalcin concentration were similarly increased in both training groups during the training period. 1,25(OH)2D3 was significantly increased during the latter half of the training period in type f but not in type F. Urinary NTx excretion was significantly decreased by the training in both type F and type f during the early period of the training but that in type f seemed to gradually return to its baseline value.

DISCUSSION

The coupling of bone resorption and formation plays an important role for the maintenance of the adult skeleton (29). Many investigators have suggested that exercise affects BMD (4, 6, 20, 21) and changes bone metabolism (3, 5, 9, 22, 26, 33). In our present study, resistance exercise training enhanced bone formation and suppressed bone resorption markers, concordant with our previous study in young men (13). When data from all the trained subjects were combined, the changes in bone formation assessed by serum osteocalcin concentration and B-ALP activity were significantly increased. Concerning response of bone resorption to the exercise, urinary NTx excretion was significantly decreased by the training but urinary excretion of DPYR did not decrease. This difference in changes in the two biomarkers of bone resorption was similar to the report that the significant decrease of urinary excretion of

| Table 3. Effects of resistance exercise training on bone metabolism |
| --- | --- | --- | --- | --- |
|   | B-ALP | Osteocalcin | 1,25(OH)2D3 | NTx | DPYR |
| Sedentary (n = 14) | −0.2 ± 6.1 | 0.3 ± 3.2 | 14.8 ± 8.7 | 5.5 ± 15.8 | 19.7 ± 18.1 |
| Training (n = 20) | 10.9 ± 3.3* | 13.1 ± 3.6* | 15.5 ± 7.9 | −32.6 ± 6.8* | 9.0 ± 13.9 |

Values are means ± SE. *Significant changes from baseline values (P < 0.01).
NTx was observed by 5-wk endurance training in adolescent male subjects but that of DPYR did not significantly change (9). Moreover, it was reported that changes in urinary NTx excretion were greater than those of DPYR in bisphosphonate treatment of hypercalcemia (34), suggesting that NTx may be a more sensitive indicator of bone resorption than is DPYR. It is suggested that stimulation of bone formation and suppression of bone resorption, taken together, began within the first month of resistance training in young men.

To investigate the interaction between the effect of genetic factor and exercise on bone metabolism, response of bone metabolism to resistance training was compared in groups F and f. Screening 81 young men revealed a distribution of VDR genotypes similar to that reported for middle-aged Japanese women (2, 23), and the subjects with VDR genotype ff were rare. Low statistical power of the present study because of a limited number of subjects prevented reaching a clear conclusion, but our results obtained during the first month of the training suggest that markers of bone metabolism except urinary NTx excretion in both groups responded similarly to the training. The decrease of urinary NTx excretion seems to be greater and longer lasting in type F than that in type f. Thus resistance exercise training seems to be more effective in the group of VDR type FF for suppression of bone resorption than in the group of f, but the molecular mechanism by which the difference in VDR genotypes affects NTx excretion remains to be determined.

1,25(OH)\(_2\)D\(_3\) is known to upregulate B-ALP and osteocalcin in these mRNA levels (17), and especially the gene expression of the latter is stimulated by 1,25(OH)\(_2\)D\(_3\) through a vitamin D-responsive element in the promoter region of its gene (25). A previous study (5) has reported that serum concentration of 1,25(OH)\(_2\)D\(_3\) was significantly higher in muscle-building exercisers than in the sedentary controls. Our present data suggest an increase in serum 1,25(OH)\(_2\)D\(_3\) concentration by training, but the change was not
statistically significant (P = 0.10). When the two training groups were analyzed separately, however, serum 1,25(OH)2D3 concentration was increased by training in the f but not in the F group. Thus response of circulating 1,25(OH)2D3 to resistance training is smaller in the F than in the f group. A similar response of bone formation to training was observed, suggesting higher sensitivity of F than f VDR. The polymorphism, detected with Fok I, at the translation initiation site of the VDR gene results in a structural change that could potentially alter the function of the VDR protein. It was reported that the longer allele of the VDR, designated f, may be less efficient in vitamin D-mediated transactivation in a transfected cell system (2, 23), although the ligand affinity of each genotype receptor have not been detected (16, 23). The physiological mechanism by which exercise training reveals functional difference in the two VDR isoforms remains to be clarified.

In conclusion, suppression of bone resorption begins during the early period of resistance training and is followed by stimulation of bone formation in young Japanese men. Despite a significant increase in 1,25(OH)2D3 in the f group but not in the F group, the response of bone metabolism to the training in the F group was similar to or greater than that in the f group, suggesting a functional difference between vitamin D-receptor genotypes f and F.

Address for reprint requests and other correspondence: K. Tokuyama, Lab. of Biochemistry of Exercise and Nutrition, Inst. of Health and Sport Sciences, Univ. of Tsukuba, Tsukuba 305-8574, Japan (E-mail: tokuyama@taiiku.tsukuba.ac.jp).

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