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REGULAR ARTICLE

Association between bone mineral density and lifestyle factors or *vitamin D receptor* gene polymorphism in adult male workers: a cross-sectional study

Yayoi Funakoshi · Hisamitsu Omori · Takahiko Katoh

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Abstract
Objectives The aim of this cross-sectional study was to investigate the association between bone mineral density (BMD) and lifestyle factors, as well as the influence of *vitamin D receptor* (*VDR*) gene polymorphism, in adult male workers.
Methods The subjects were 524 male employees aged 23–49 years (37.3 ± 5.4 years, mean ± standard deviation) working at a large-scale integrated manufacturing facility in Japan. BMD was measured at the nondominant radius by dual-energy X-ray absorptiometry. Lifestyle information was obtained by a questionnaire at the same time, and genomic DNA was isolated from peripheral leukocytes.
Results The genotype frequencies of *VDR* gene polymorphism detected by Taq I digestion were 81.3%, 17.9%, and 0.8% for *TT*, *Tt*, and *tt,* respectively. BMD was 0.56 ± 0.06 g/cm^2. Analysis of covariance with adjustment for age and body mass index (BMI) revealed that subjects who had a past history of exercise, current exercise from 3 to 7 days a week or daily alcohol intake showed significantly higher BMD than subjects without these features (0.56 ± 0.06 versus 0.54 ± 0.06, 0.58 ± 0.06 versus 0.55 ± 0.06, and 0.57 ± 0.06 versus 0.55 ± 0.06, respectively) ($P < 0.05$). Subjects who ate only 2 meals a day or smoked ≥21 cigarettes a day showed significantly lower BMD if they had the *Tt* or *tt* genotype than if they had the *TT* genotype (0.51 ± 0.04 versus 0.56 ± 0.06 and 0.51 ± 0.05 versus 0.57 ± 0.06, respectively) ($P < 0.05$).
Conclusions These findings suggest that the influence of lifestyle on BMD differs according to *VDR* gene polymorphism in adult male workers.

Keywords Bone mineral density · Gene polymorphism · Lifestyle · *Vitamin D receptor*

Y. Funakoshi · H. Omori · T. Katoh (✉)
Department of Public Health, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjou, Kumamoto 860-8556, Japan
e-mail: katoht@gpo.kumamoto-u.ac.jp

Introduction

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of the bone tissue, leading to enhanced bone fragility and consequent increase in the risk of fracture [1]. Osteoporosis is less common in men than in women, but the mortality rate after osteoporotic fracture is higher in men [2–4]. However, male osteoporosis is substantially underdiagnosed, undertreated, and underreported, as well as being inadequately researched [5].

Recently, the American College of Physicians issued new guidelines [5] based on the available evidence about risk factors and screening tests for osteoporosis in men obtained from a systematic review of 269 studies. The guidelines recommend that physicians periodically perform individual assessment of risk factors in older men and test the bone mineral density (BMD) of men who have an increased risk of developing osteoporosis. Thus, the importance of strategies for prevention of osteoporosis in men has been increasingly emphasized.

According to the 2006 Japanese guidelines for the prevention and treatment of osteoporosis [6], BMD can be affected by various environmental factors, such as physical activity, smoking, and alcohol. A diagnosis of osteoporosis is made by measuring the BMD and the most popular method for doing so is dual-energy X-ray absorptiometry

(DXA) [7]. Low BMD is an important risk factor for osteoporotic fracture [8, 9]. Each person's BMD condition is determined by a complex interplay between genetic and environmental factors, including lifestyle habits [10]. Krall et al. estimated that 46–62% of the variation in BMD was attributable to heredity [11]. At least 30 genes are thought to be associated with the development of osteoporosis [12]. Among these, polymorphism of the *vitamin D receptor* (*VDR*) gene has been most widely studied [13], and a meta-analysis [14] has suggested that BMD is influenced by *VDR* gene polymorphism. However, most studies have investigated the effects of *VDR* gene polymorphism and lifestyle factors on BMD separately, so their combined effect is not well understood. Better understanding of this combined effect may allow us to identify a high-risk group for osteoporosis and lead to more effective osteoporosis prevention and to improvement of public health. The purpose of this cross-sectional study was to investigate the association between BMD and lifestyle factors, as well as the influence of *VDR* gene polymorphism, in adult male workers.

Methods

Subjects and methods

The 1,029 men were recruited from employees working at a large-scale integrated manufacturing facility in Japan. The criteria for entry into this study were no previous diagnosis of osteoporosis, no systemic disease, and no medications known to influence bone or calcium metabolism. Of the 1,029 participants, 841 (81%) agreed to genotyping. Among these 841 participants, we analyzed 524 who were aged 49 years or younger for *VDR* genotyping, lifestyle, body measurements, and laboratory data. Their ages ranged from 23 to 49 years (mean 37.3 ± 5.4 years). They were engaged in office work (49%) or light manual work (51%). Among the participants doing light manual work, 87.6% were on night shift. Night-shift workers accounted for 77.4% of those aged 23–29 years, 59.6% of those aged 30–39 years, and 31.9% of those aged 40–49 years.

Height, weight, BMD, urinary deoxypyridinoline (DPD), and serum bone alkaline phosphatase (BAP) were measured during a comprehensive health check. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Lifestyle assessment was performed at the same time as the above measurements. We followed the ethical guideline for human genome/gene analysis research [15] endorsed by the Japanese government. The study protocol was approved by the ethics committee of Kumamoto University Graduate School of Medical Sciences, and all subjects provided written informed consent.

Bone metabolic markers

Serum BAP and urinary DPD concentrations were analyzed by enzyme immunoassay. Urinary creatinine (Cr) concentration was measured by colorimetric assay. DPD excretion was expressed as a ratio of urinary Cr concentration. DPD has been validated as a useful marker of bone resorption, while BAP is a marker of bone formation [16].

Measurement of bone mineral density (BMD)

BMD was measured at the distal 1/3 site of the radius on the nondominant side using DXA (Osteometer DTX200) according to the manufacturer's protocol (precision error <1.0% CV in vivo). Quality control was carried out in accordance with the manufacturer's guidance. The BMD of the distal 1/3 site of the radius has been validated as being highly predictive of fracture risk [17].

Lifestyle assessment

A self-reporting questionnaire was used to collect the following information: past history of exercise (no, yes), current exercise (no, 1–2 days/week, 3–7 days/week), night-shift work (no, yes), sleeping time (hours), frequency of milk intake (no, sometimes, every day), number of meals per day (3 meals, 2 meals), history of dieting (no, yes), smoking status (no, 1–20 cigarettes/day, ≥21 cigarettes/day), and alcohol intake (no, sometimes, every day). Past history of exercise was determined from exercise habits until the age of 20 years.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by using a DNA Extractor WB Kit (Wako Pure Chemical Industries, Osaka, Japan). Taq I polymorphism of the *VDR* gene was determined by the polymerase chain reaction (PCR) method of Riggs et al. [18]. A 740-base-pair (bp) fragment was generated by PCR with primers located on intron 8 and exon 9. The primer sequences were 5′ cag agc atg gac agg gag caa 3′ (forward) and 5′ gca act cct cat ggc tga ggt ctc 3′ (reverse). PCR was performed for 35 cycles using Taq polymerase (Perkin Elmer Co., Ltd., NJ, USA) under the following conditions: denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min. Then 10 μl of the PCR products were subjected to digestion with Taq I (Takara Shuzo Co., Ltd., Kyoto, Japan) at 65°C for 3 h and were separated on 3% Nusieve agarose gel (FMC Bioproducts, Rockland, ME, USA) [19]. The presence of a C > T substitution at position 3 on codon 352 in exon 9, which codes for isoleucine, is associated with loss of the Taq I restriction site. The

resulting alleles are designated as *T* (Taq I site absent; 2 fragments of 495 bp and 245 bp) or *t* (Taq I site present; 3 fragments of 290 bp, 245 bp, and 205 bp). The subjects were therefore classified as *TT*, *Tt* or *tt* (Fig. 1) [19].

Statistical analysis

Results are presented as mean ± standard deviation (SD), and categorical variables are expressed as frequencies. The chi-square test was used to verify Hardy–Weinberg equilibrium of genotype frequencies. Analysis of variance (ANOVA) with a post hoc Tukey's test or Student's *t* test was used to assess the differences between age groups and between *VDR* gene polymorphism (*TT* versus *Tt* plus *tt* genotypes), respectively. Mann–Whitney *U* test was used to compare lifestyle differences between two categorical variables. Analysis of covariance (ANCOVA) was used to adjust BMD for the covariates age and BMI. We then confirmed that these interactions were not significant. The level of statistical significance was set at $p < 0.05$. All analyses were done by using SPSS 15.0 software.

Results

The characteristics of the subjects in each age group are presented in Table 1. Although the age range of the study population was from 23 to 49 years, approximately 50% of the subjects were aged 30–39 years. Compared with the Japan Health and Welfare Statistics (2006) [20], the mean height (169.9 ± 5.8 cm) was approximately the same as the national average (171.9, 171.4, and 170.2 cm for subjects in their 20s, 30s, and 40s, respectively). Although

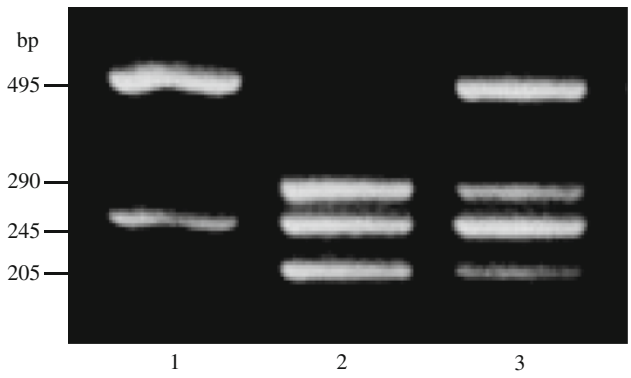

Fig. 1 Restriction fragment length polymorphism (RFLP) analysis of the product of a polymerase chain reaction (PCR) targeting *VDR*. Pattern of fragments for the three possible genotypes after Taq I digestion of the PCR product, a 740-bp amplified region of the *VDR* gene. The 245-bp fragment is constant in all genotypes, being created by cleavage at a nonpolymorphic Taq I site within the range of amplification. *Lane 1 TT* genotype (2 fragments of 495 and 245 bp), *Lane 2 tt* genotype (3 fragments of 290, 245, and 205 bp), *Lane 3 Tt* genotype (4 fragments of 495, 290, 245, and 205 bp)

there was not much difference, the average weight (67.7 ± 9.8 kg) of our subjects was approximately 2% lower than the national average (67.4, 70.5, and 69.7 kg for subjects in their 20s, 30s, and 40s, respectively). Mean BMI was significantly higher in the 40–49 age group than in the 23–29 age group. No significant differences among the age groups were seen with respect to height, weight, BMD, DPD or BAP.

Figure 2 shows the frequencies of *VDR* gene polymorphism detected by Taq I digestion in relation to the BMD (mean ± standard deviation) (*TT* 0.56 ± 0.06, *Tt* 0.55 ± 0.06, and *tt* 0.54 ± 0.06). *VDR* gene polymorphism had a distribution that followed Hardy–Weinberg equilibrium ($P = 0.63$). Similar to a previous Japanese report [21], the genotype frequencies of *VDR* gene polymorphism were 81.3%, 17.9%, and 0.8% for *TT*, *Tt*, and *tt,* respectively. The characteristics of the subjects stratified according to *VDR* gene polymorphism are shown in Table 2. It was necessary to combine the heterozygous (*Tt*) and homozygous (*tt*) genotypes when comparing characteristics due to the low frequency of the *tt* genotype in the Japanese population. When high levels of DPD and BAP are detected, presence of metastatic bone tumor, metabolic bone disease or abnormal calcium metabolism can be suspected, but there were no significant differences in relation to *VDR* gene polymorphism. In addition, no significant differences of *VDR* gene polymorphism were seen in relation to age, height, weight or BMI.

Table 3 shows the BMD according to *VDR* gene polymorphism stratified by BMI. In the 30–39 age group, mean BMD was significantly lower among subjects with the *Tt* or *tt* genotypes than in those with the *TT* genotype. Subjects were stratified into two groups according to their mean BMI (<23.0 kg/m^2 or ≥23.0 kg/m^2). Among those in the 30–39 age group with BMI ≥23.0 kg/m^2, mean BMD was significantly lower for subjects with the *Tt* or *tt* genotypes than for those with the *TT* genotype.

Table 4 shows the combined effects of lifestyle factors and *VDR* gene polymorphism on BMD. Only variables that presented a statistically significant difference are displayed. According to ANCOVA adjustment for age and BMI, subjects with past history of exercise, current exercise 3–7 days a week or daily alcohol intake had significantly higher BMD than those without these factors. Among subjects who ate 2 meals a day or smoked ≥21 cigarettes a day, BMD was significantly lower if they had the *Tt* or *tt* genotype than if they had the *TT* genotype. On the other hand, there was no significant association between other lifestyle factors (night-shift work, sleeping time, milk intake, and dieting) and BMD (data not shown).

The characteristics of the subjects are presented according to daily number of meals in Table 5. Subjects who ate only 2 meals a day (skippers) had significantly

Table 1 Characteristics of the subjects in each age group

Variable	All ($n = 524$)	23–29 years ($n = 53$)	30–39 years ($n = 280$)	40–49 years ($n = 191$)
Age (years)	37.3 ± 5.4	26.5 ± 2.1	35.8 ± 2.7	42.6 ± 2.3
Height (cm)	169.9 ± 5.8	170.1 ± 6.1	170.2 ± 5.9	169.4 ± 5.7
Weight (kg)	67.7 ± 9.8	65.3 ± 12.7	67.4 ± 10.0	68.6 ± 8.5
BMI (kg/m^2)	23.4 ± 3.0	22.5 ± 3.7	23.2 ± 3.1	23.9 ± 2.6*
BMD (g/m^2)	0.56 ± 0.06	0.55 ± 0.06	0.56 ± 0.06	0.56 ± 0.06
DPD (nmol/mmol CRE)	3.7 ± 1.2	4.0 ± 1.0	3.6 ± 1.2	3.7 ± 1.2
BAP (U/L)	25.5 ± 8.0	26.2 ± 6.4	25.2 ± 7.1	25.8 ± 9.5

Values are mean ± SD

BMI body mass index, *BMD* bone mineral density, *DPD* deoxypyridinoline, *BAP* bone alkaline phosphatase

Data were analyzed by analysis of variance (ANOVA) and Tukey's test

* $P < 0.05$ compared with 23–29 years

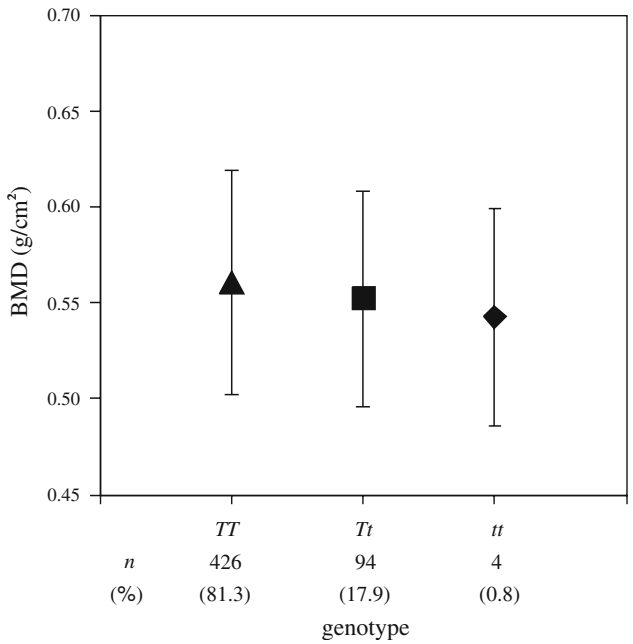

Fig. 2 Genotype frequencies of *vitamin D receptor* gene polymorphism detected by Taq I digestion and mean bone mineral density ± standard deviation

lower age, weight, and BMI than subjects who ate 3 meals a day. In addition, skippers tended to perform less physical activity, had a low intake of milk, and smoked more.

There was no significant association between lifestyle factors and DPD or BAP.

Discussion

In the present study, we found that subjects who only ate 2 meals a day or smoked ≥21 cigarettes a day had a significantly lower BMD if they possessed the *Tt* or *tt* genotype than if they possessed the *TT* genotype. *VDR* gene polymorphism has been reported to be associated with BMD in Japanese females [21], but no previous studies have exclusively targeted Japanese men. To our knowledge, this is the first report to reveal an association between BMD and lifestyle factors or *VDR* gene polymorphism in Japanese adult men. Vitamin D plays a central role in calcium homeostasis by regulating calcium absorption, bone resorption, bone cell differentiation, and parathyroid hormone secretion [13], and the *VDR* gene regulates bone turnover as the receptor for vitamin D [22].

Table 2 Characteristics of the subjects stratified according to *vitamin D receptor* gene polymorphism

Variable	All ($n = 524$)	*TT* ($n = 426$)	*Tt* + *tt* ($n = 98$)
Age (years)	37.3 ± 5.4	37.5 ± 5.5	36.6 ± 5.0
Height (cm)	169.9 ± 5.8	169.8 ± 5.9	170.4 ± 5.7
Weight (kg)	67.7 ± 9.8	67.9 ± 10.0	66.6 ± 9.0
BMI (kg/m^2)	23.4 ± 3.0	23.5 ± 3.1	22.9 ± 2.6
DPD (nmol/mmol CRE)	3.7 ± 1.2	3.7 ± 1.2	3.6 ± 1.1
BAP (U/L)	25.5 ± 8.0	25.6 ± 8.3	25.1 ± 6.9

Values are mean ± SD

BMI body mass index, *DPD* deoxypyridinoline, *BAP* bone alkaline phosphatase

Subjects who had past history of exercise showed significantly higher BMD than those without such a history, and this relationship was unrelated to *VDR* genotype. Subjects with current exercise 3–7 days/week had significantly higher BMD than those without exercise among all subjects and in the case of the *TT* genotype. The American College of Sport Medicine has developed guidelines for exercise to improve bone health; their recommendation is to exercise 3–5 times per week [23]. A meta-analysis of 8 studies has shown that exercise has a beneficial effect on

Table 3 Mean bone mineral density according to *vitamin D receptor* gene polymorphism by quintile of BMI

	Age group (years)	All		*TT*		*Tt* + *tt*	
		n	BMD (mean ± SD)	*n*	BMD (mean ± SD)	*n*	BMD (mean ± SD)
All subjects	All ages	524	0.56 ± 0.06	426	0.56 ± 0.06	98	0.55 ± 0.06
	23–29	53	0.55 ± 0.05	39	0.55 ± 0.06	14	0.55 ± 0.04
	30–39	280	0.56 ± 0.06	226	0.56 ± 0.06	54	0.55 ± 0.06*
	40–49	191	0.56 ± 0.06	161	0.56 ± 0.06	30	0.56 ± 0.05
BMI by quintile (kg/m^2)							
<23.0	All ages	237	0.54 ± 0.06	188	0.54 ± 0.06	49	0.54 ± 0.06
	23–29	29	0.53 ± 0.04	20	0.52 ± 0.04	9	0.54 ± 0.03
	30–39	139	0.54 ± 0.06	109	0.54 ± 0.06	30	0.54 ± 0.06
	40–49	69	0.55 ± 0.06	59	0.55 ± 0.06	10	0.55 ± 0.05
≥23.0	All ages	287	0.57 ± 0.06	238	0.58 ± 0.06	49	0.56 ± 0.05
	23–29	24	0.57 ± 0.06	19	0.57 ± 0.07	5	0.57 ± 0.05
	30–39	141	0.58 ± 0.05	117	0.58 ± 0.05	24	0.56 ± 0.06*
	40–49	122	0.57 ± 0.06	102	0.57 ± 0.06	20	0.57 ± 0.05

Values are mean ± SD

BMI body mass index, *BMD* bone mineral density, *DPD* deoxypyridinoline, *BAP* bone alkaline phosphatase

Data were analyzed by Student's *t* test

* $P < 0.05$ compared with *TT*

BMD [24]. In particular, past history of exercise was reported to have a strong influence on BMD [6]. In a recent study of 263 Japanese subjects (127 boys and 136 girls) aged 12–15 years, exercise performed two or more times weekly during adolescence was associated with higher BMD [25]. A review of studies performed on European and American men and women to assess the effect of exercise on BMD over the long term suggested that the most beneficial effect of exercise on BMD is obtained during growth [26]. Bone formation is known to be stimulated by mechanical stress [27] and this effect is especially important during periods of growth [23]. However, much remains uncertain about the mechanism of bone formation by mechanical stress.

There have been no previous studies investigating the influence of *VDR* gene polymorphism and past or current exercise on BMD in men. Japanese women performing continuous exercise from the age of 12 years display a positive effect on bone metabolism irrespective of *VDR* gene polymorphism [21]. In addition, a study that assessed the effect of high-impact exercise on BMD in premenopausal Finnish women aged 35–45 years who participated in an 18-month exercise program suggested that such exercise was beneficial for bones irrespective of *VDR* genotype [28]. These data show that BMD is not only influenced by calcium metabolism but also by other factors such as mechanical stress. However, these studies were all performed on women, so further epidemiological studies are needed to determine the effect of exercise on BMD in men. In this study, there was no significant association between current exercise and BMD in the *Tt* plus *tt* group. It should be noted that we did not assess the duration or intensity of exercise. Therefore, further research is needed to explore the interaction between physical activity (current exercise) and *VDR* genotypes.

In the *Tt* plus *tt* group, subjects who ate 2 meals a day had significantly lower BMD than those who ate 3 meals. Furthermore, among subjects who ate 2 meals daily, BMD was significantly lower if they had the *Tt* or *tt* genotype than if they had the *TT* genotype. While approximately 80–90% of bone mineral is comprised of calcium and phosphorus, other dietary components, such as protein, magnesium, zinc, copper, iron, fluoride, and vitamins D, A, C, and K are required for normal bone metabolism [29]. According to the 2002 National Nutrition Survey in Japan, intake of these nutrients required for normal bone metabolism was lower in skippers than in nonskippers [30]. In addition, higher incidence of hip fracture was associated with low intake of vitamins D and K in both men and women [31]. Based on these studies, we speculate that skipping meals may cause nutritional deficiencies that affect bone metabolism. We also found that skippers had lower weight and BMI, tended to perform less physical activity, had a low intake of milk, and smoked more. These unhealthy behaviors may have had an adverse effect on bone metabolism and resulted in poor physique.

On the other hand, there were no such adverse effects on the bone in subjects with the *TT* genotype. This difference might be mediated through *VDR* gene polymorphism, because vitamin D and calcium deficiency alter the

Table 4 Mean bone mineral density according to lifestyle factors and *vitamin D receptor* gene polymorphism

Characteristic	All (n = 524)		*TT* (n = 426)		*Tt* + *tt* (n = 98)	
	n (%)	BMD (mean ± SD)	n (%)	BMD (mean ± SD)	n (%)	BMD (mean ± SD)
Past history of exercise						
No	104 (19.9)	0.54 ± 0.06	82 (19.3)	0.54 ± 0.06	22 (22.7)	0.53 ± 0.06
Yes	418 (80.1)	0.56 ± 0.06[a]	343 (80.7)	0.57 ± 0.06[b]	75 (77.3)	0.56 ± 0.05[c]
Current exercise						
No	278 (54.2)	0.55 ± 0.06	220 (52.8)	0.55 ± 0.06	58 (60.4)	0.55 ± 0.05
1–2 days/week	180 (35.1)	0.56 ± 0.06	149 (35.7)	0.56 ± 0.06	31 (32.3)	0.56 ± 0.06
3–7 days/week	55 (10.7)	0.58 ± 0.06[a]	48 (11.5)	0.58 ± 0.05[b]	7 (7.3)	0.55 ± 0.07
Number of meals daily						
3 meals/day	427 (81.5)	0.56 ± 0.06	347 (81.5)	0.56 ± 0.06	80 (81.6)	0.56 ± 0.06
2 meals/day	97 (18.5)	0.55 ± 0.06	79 (18.5)	0.56 ± 0.06	18 (18.4)	0.51 ± 0.04[d,e]
Smoking status						
No	200 (38.7)	0.56 ± 0.06	160 (38.1)	0.56 ± 0.06	40 (41.2)	0.55 ± 0.05
1–20 cigarettes/day	254 (49.1)	0.56 ± 0.06	204 (48.6)	0.56 ± 0.06	50 (51.6)	0.55 ± 0.06
≥21 cigarettes/day	63 (12.2)	0.56 ± 0.06	56 (13.3)	0.57 ± 0.06	7 (7.2)	0.51 ± 0.05[f]
Alcohol intake						
No	155 (30.4)	0.55 ± 0.06	129 (31.3)	0.55 ± 0.06	26 (26.5)	0.55 ± 0.06
Sometimes	141 (27.6)	0.55 ± 0.06	116 (28.2)	0.56 ± 0.06	25 (25.5)	0.54 ± 0.06
Every day	214 (42.0)	0.57 ± 0.06[a]	167 (40.5)	0.57 ± 0.06	47 (48.0)	0.56 ± 0.05

Data are mean ± SD

BMD bone mineral density

BMD was adjusted for age and BMI by analysis of covariance (ANCOVA)

[a] $P < 0.05$ compared with "No" in all subjects

[b] $P < 0.05$ compared with "No" in *TT* subjects

[c] $P < 0.05$ compared with "No" in *Tt* plus *tt* subjects

[d] $P < 0.05$ compared with 2 meals/day in *TT* subjects

[e] $P < 0.05$ compared with 3 meals/day in *Tt* plus *tt* subjects

[f] $P < 0.05$ compared with ≥21 cigarettes/day in *TT* subjects

efficiency of calcium absorption [32, 33]. Further studies will be required to determine how nutritional status affects BMD, including the influence of *VDR* gene polymorphism.

In this study, there were no significant differences of BMD in relation to smoking status among all subjects. Among heavy smokers (≥21 cigarettes daily), however, BMD was significantly lower in subjects with the *Tt* or *tt* genotype than in those with the *TT* genotype. A previous population-based study of 1,068 young men showed that smokers had significantly lower BMD than nonsmokers [34]. Hagiwara et al. suggested that smoking is a risk factor for osteoporosis after they investigated 1,736 male Japanese office workers [35]. Though the mechanism by which smoking affects bone metabolism remains unclear, several studies have detected a decrease of intestinal calcium absorption in smokers [36] and the serum level of 25-hydroxyvitamin D has been shown to be lower in smokers than in nonsmokers [37, 38]. Furthermore, a study of 402 elderly men and women revealed that calcium absorption was lower in smokers, and those smoking at least 20 cigarettes daily had the lowest mean absorption rate [36]. Although the exact mechanism underlying the relation between a lower BMD and the *Tt* or *tt VDR* genotypes remains unclear, these genotypes seem to have a more adverse impact on BMD.

Subjects who drank alcohol every day had significantly higher BMD than nondrinkers. A previous study of 632 elderly Japanese women suggested that regular alcohol intake was associated with higher BMD [39], but the influence of alcohol in men has not been investigated until now. Alcoholism is a risk factor for osteoporotic fracture and low BMD, but the effects of moderate alcohol consumption on bone are unknown. A recent meta-analysis revealed that persons of both sexes with moderate alcohol intake (0.5–1.0 drinks per day) have lower risk of hip fracture compared with abstainers or heavier drinkers [40]. However, the precise mechanism by which moderate alcohol intake alters bone metabolism is still unknown.

Table 5 Characteristics of the subjects according to daily number of meals

	3 meals/day ($n = 427$)	2 meals/day ($n = 97$)	P
Age (years)	37.6 ± 5.3	36.1 ± 5.8	<0.001
Height (cm)	170.1 ± 5.8	169.1 ± 5.9	NS
Weight (kg)	68.3 ± 9.8	65.2 ± 9.6	<0.001
BMI (kg/m^2)	23.6 ± 3.0	22.8 ± 2.9	<0.001
BMD (g/m^2)	0.56 ± 0.06	0.55 ± 0.06	NS
DPD (nmol/mmol CRE)	3.7 ± 1.2	3.8 ± 1.2	NS
BAP (U/L)	25.6 ± 8.2	25.0 ± 7.4	NS
Lifestyle			
Past history of exercise (%)			
No	19.3	22.7	NS
Yes	80.7	77.3	
Current exercise (%)			
No	51.1	67.7	<0.05
Yes	48.9	32.3	
Smoking status (%)			
No	42.3	22.9	<0.001
Yes	57.7	77.1	
Daily alcohol intake (%)			
No	58.9	54.3	NS
Yes	41.1	45.7	
Daily milk intake (%)			
No	78.9	88.7	<0.05
Yes	21.1	11.3	

Values are the mean ± SD

BMI body mass index, *BMD* bone mineral density, *DPD* deoxypyridinoline, *BAP* bone alkaline phosphatase

Data were analyzed by Student's *t* test or Mann–Whitney *U* test

Thus, further detailed studies about the association between alcohol consumption and BMD are required.

Vitamin D is produced endogenously when ultraviolet rays in sunlight strike the skin and trigger its synthesis [41]. Night work is associated with lack of sunlight, but there was no influence of the shift work pattern. In a study of 220 young Finnish men, vitamin D status (serum level of 25-hydroxyvitamin D) varied according to season and was lower in winter, with lower serum levels of 25-hydroxyvitamin D being associated with low BMD [42]. However, the subjects were from North Europe, which receives fewer hours of sunlight, and there is no evidence of this effect in the Japanese population.

Our study had several limitations. First, there was no questionnaire to assess environmental factors in detail or for complete assessment of factors related to osteoporosis. However, the questionnaire was designed to cover the well-known risk factors for fracture [6]. We should develop a reliable and valid questionnaire to assess environmental factors contributing to genetic susceptibility to osteoporosis. Also, our study subjects were relatively young. We could not find a relationship between *VDR* gene polymorphism and bone loss. Clearly, further studies will be needed to define the relation between bone loss and *VDR* gene polymorphism.

In conclusion, our results suggest that the influence of lifestyle factors on BMD depends on *VDR* gene polymorphism in adult male workers. Genetic diagnosis of *VDR* gene polymorphism may be helpful for researching the risk of, and identifying individuals susceptible to, osteoporosis. Such studies could lead to more effective prevention of osteoporosis and improvement of public health. A large-scale prospective study will be needed to define the relation between BMD and lifestyle factors, as well as the influence of *VDR* gene polymorphism.

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