

## 骨粗鬆症による椎体・非椎体骨折リスクのEBM

—骨折リスク評価と危険因子

Risk for spine or non-spine fracture



藤原佐枝子

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◎脊椎骨折の年間発生率は70歳女性で約2%、80歳女性で約6%であり、日本人50歳女性の約14%が生涯に大腿骨頸部骨折を起こすと推定され、高齢者において骨粗鬆症に関連した骨折の頻度は高い。骨粗鬆症診断基準は骨密度値によっているが、骨密度だけでは将来の骨折を十分に予知することはできないことがわかってきた。椎体・非椎体骨折のおもな危険因子は年齢、性、骨密度、既存骨折、ステロイド使用、喫煙、骨折の家族歴であり、これらの危険因子を考慮に入れた絶対リスク評価で骨折高リスク者を判別し、治療介入の指標とする考え方が取り入れられつつある。



発生率、絶対リスク、ライフタイムリスク、危険因子、メタアナリシス

ある事象が発生するであろう確率、すなわち“リスク”という言葉は一般的によく用いられている。“リスク”を表す指標として発生率、死亡率、ライフタイムリスク、相対リスク、絶対リスク、寄与リスクなどがあり、各指標のもつ特性によって使い分けられている(「サイドメモ」参照)。

骨粗鬆症の診断は骨密度値を基準に判定されているが、骨密度だけでは将来の骨折を十分に予知することはできないことがわかってきた。そこで、骨折の危険因子を考慮に入れ、将来、骨折する確率(絶対リスク)を評価して骨粗鬆症治療介入の指標とする考え方が取り入れられつつある。

本稿では、コホート調査から求められた椎体・非椎体骨折の発生率、ライフタイムリスク、および絶対リスク算定のために考慮に入れるべき骨折危険因子を紹介していきたい。

### 椎体・非椎体骨折リスク

#### 1. 発生率

発生率は各年齢、性における一定期間内に疾患が起こる率を示している。脊椎骨折発生率は60代後半から増加し、大腿骨頸部骨折の発生率は70歳

サイド  
メモ

#### 相対リスクと絶対リスク

相対リスクは曝露群と非曝露群との相対的な比であり、曝露によってある疾患の発生が何倍になったかという評価ができる。しかし、たとえば相対リスクが1.3のとき、非曝露群の疾患発生が100であるときの相対リスクが1.3なのか、非曝露群での発生が10であるときの1.3なのか、公衆衛生的にインパクトが大きいのは前者であるが、その区別はできない。絶対リスクは相対リスクに対する用語で、対象集団におけるある疾患発生の確率であり、公衆衛生的なインパクトを表すことができる。集団を対象にした疾患負担や治療介入による費用対効果を検討するには有効な指標である。

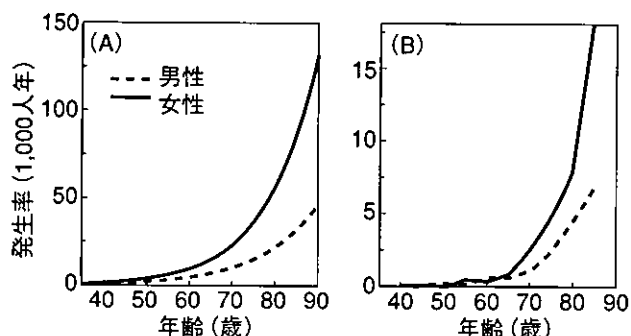


図 1 日本人の脊椎骨折(A), 大腿骨頸部骨折(B)の発生率<sup>2,3)</sup>

代後半から急増する<sup>2,3)</sup>(図 1)。1 年間に新しく骨折を起こす割合は、脊椎骨折については 70 歳女性の約 2%, 80 歳女性の約 6%, 大腿骨頸部骨折では 70 歳女性で約 0.3%, 80 歳女性で約 0.8%である。どちらの骨折も女性は男性の 2 倍の発生率である。

## 2. ライフタイムリスク

ライフタイムリスクはある年齢の人びとが生涯に骨折を起こす確率を示す。ライフタイムリスクは平均余命と発生率から計算されるため、平均余命が長く、発生率が高いとライフタイムリスクは高くなる。日本人女性の 50 歳における脊椎骨折のライフタイムリスクは 37%, 大腿骨頸部骨折では 13.6%と推計される<sup>4)</sup>。大腿骨頸部骨折のライフタイムリスクがもっとも高い国はスウェーデンで 28.5%であり、長寿で発生率の高い北ヨーロッパの国が続く。

## 3. 絶対リスク

現在、WHO ワーキンググループは骨粗鬆症治療介入に“絶対リスク”(10 年間の骨折発生確率; 10-year fracture probability)を取り入れることを検討している。10 年間の骨折発生確率はライフタイムリスクと同様に、発生率、平均余命から算出できる。年齢、性だけを考慮したとき、日本人における大腿骨頸部骨折の 10 年間の発生確率は 70 歳女性で 3.2%, 80 歳で 9.6%, 70 歳男性で 1.3%, 80 歳で 3.3%と推計される<sup>4)</sup>。治療介入のための指標としては、性、年齢だけではなくエビデンスに基づき骨折の危険因子を評価し、危険因子を考慮に入れて計算された“長期の骨折確率”を使うことが検討されている。絶対リスク評価はすでに、高

表 1 骨折の危険因子  
—メタアナリシスから得られた相対リスク<sup>5-14)</sup>

危険因子	相対リスク		
	骨折	骨粗鬆症性骨折	大腿骨頸部骨折
骨密度(1SD 低下)	1.6	1.4~1.9	1.5~2.4
既存骨折	1.8	1.8	1.8
ステロイド使用	1.7~2.0	1.7~2.6	2.5~4.4
喫煙	1.3	1.3	1.8
家族歴	1.17	1.18	1.49

各危険因子の相対リスクに男女差なし。

コレステロール、高血圧治療介入の指標として取り入れられている。

## 骨折の危険因子

疫学調査から、年齢、性以外の骨折危険因子として骨密度、既存骨折、ステロイド使用、やせ、家族歴、喫煙、過剰な飲酒など多くの危険因子が報告されている。ここではエビデンスレベルのもっとも高いメタアナリシスによって骨折との関係が明らかになっている危険因子について述べる。

### 1. 骨密度

低骨密度は骨折リスクを決める重要な因子で、DXA あるいは SXA(dual, single X-ray absorptiometry)で測定した骨密度が 1 標準偏差(SD)低いと、骨折のリスク(相対リスク)は 1.4~2.4 倍になる<sup>5,6)</sup>(表 1)。骨密度の骨折予知力は男女で差がなく、橈骨下端、踵骨、腰椎、大腿骨近位のどの部位の骨密度もほぼ同じ程度に将来の骨折を予測する。著者らは、日本人においても骨密度 1 SD 低下に対する骨折の相対リスクは 1.5~1.8 となり、欧米の結果と差はないことを報告している<sup>2)</sup>。

### 2. 骨折既往

骨折既往があるとそうでない人に比べ将来の骨折リスクは 1.8 倍になる。とくに、脊椎骨折があると将来脊椎骨折のリスクは約 4 倍になる<sup>7,8)</sup>。日本人においても脊椎骨折既往があると将来の脊椎骨折のリスクは女性で約 3 倍、男性で約 4 倍で<sup>2)</sup>、既存骨折の骨折予知力は欧米白人と日本人とに差はなかった。また、既存脊椎骨折の数、変形、程度が大きいほど将来の脊椎骨折リスクは高くなる。

同じ骨密度を示しても骨折既往があると将来骨折リスクは高く、骨折既往は低骨量を示すだけでなく、骨の微細構造の欠陥、転倒のしやすさなどを反映している可能性がある。

骨折既往があった場合の将来の骨折リスクは男女による差はない。年齢別には、骨折既往歴がある人が将来大腿骨頸部骨折を起こすリスクは若年者ほど高いが、その他の骨折については年齢による違いはみられなかった<sup>8)</sup>。

### 3. ステロイド使用

ステロイド使用者の腰椎・大腿骨頸部骨密度は、同じ年齢、性の人に比べ、約10%低下している<sup>9)</sup>。骨折は経口ステロイド治療後3~6カ月以内に増加し、使用中止後は低下する。ステロイド使用があると骨粗鬆症性骨折のリスクを1.7~2.6倍に、大腿骨頸部骨折のリスクを2.5~4.4倍に上げるが<sup>10)</sup>、この相対リスクは骨密度を補正しても変わらない。これらの結果から、ステロイドは骨密度を低下させるだけでなく、骨微細構造、転倒あるいは転倒防御に関連する筋力あるいは代謝に影響を与え、骨折を増加させる可能性を示している。ステロイド使用による骨折リスクは、男女、年齢による差はなかった。

ステロイド量に閾値があるかが議論のあるところである。ステロイド量と非椎体骨折との関係は、プレドニゾン5mg/日以下では骨折の過剰相対リスク(過剰相対リスク=相対リスク-1)は0.2で一定であるが、それ以上になるとステロイド量が多いほど、骨折リスクは高くなる<sup>9)</sup>。また、骨密度測定時にプレドニゾン7.5mg/日以下で、骨密度が低下していたという報告とそうでないという報告があるが、これらの報告は骨密度を測る以前のステロイド量がわかっていないことが多く、解釈は難しい。

### 4. 喫煙

喫煙者は非喫煙者に比べ、骨折のリスクは約1.3倍、大腿骨頸部骨折は1.4~1.8倍、脊椎骨折は1.8倍になる<sup>11-13)</sup>。骨折リスクは骨密度を考慮すると低下するが、なお有意である。骨折リスクは女性より男性のほうが高く、現在喫煙している人より禁煙した人のほうが低い<sup>11-13)</sup>。

## 5. 家族歴

骨折の家族歴があると骨折、骨粗鬆症性骨折リスクは1.17~1.18倍高くなる<sup>14)</sup>。両親どちらかが大腿骨頸部骨折を起こしていたら、骨粗鬆症性骨折は約1.5倍、大腿骨頸部骨折は約2.3になる。

## おわりに

骨粗鬆症の診断は骨密度がカットオフ値として使われている。しかし、より効果的に骨折の高リスク者を判別するために、エビデンスに基づいて骨密度、既存骨折、ステロイド使用、喫煙などの危険因子を評価し、年齢、性に加えてこれらの危険因子を考慮に入れて将来の骨折発生の確率を絶対リスクで表す方向に進んでいる。骨折の危険因子を包括的に評価することによって、より効果的に治療すべき人を選択し、治療介入を行うことは非常に有効な方法と考えられる。

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# 線維筋痛症とたたかう

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監修／西岡久寿樹 聖マリアンナ医科大学教授・難病治療研究センター長  
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- 線維筋痛症は、欧米では頻度も高く、米国ではACR(米国リウマチ学会)から診断基準も発表されているが、わが国では診断や治療が容易でない数多いリウマチ性疾患の中でも、線維筋痛症の認知度は極めて低く、慢性疼痛を抱え原因不明の痛みで苦しんでいる患者が多い(推定100万人)。
- 日本では2003年、線維筋痛症研究会の発足(3月)と厚生労働省の第1回線維筋痛症調査研究会議(10月)により、現在、線維筋痛症に対するキャンペーンが実施されている。
- 線維筋痛症患者は「手を当てる」ことすら、激痛を誘うため拒否する。現代医療の根本を問う病の多彩な切り口を、はじめて専門医が語り、患者が語った実例から、本症の情報を専門医・臨床医に周知してもらうことと、コメディカルスタッフの教育を緊急の課題として編集した啓蒙書!

## 本書のおもな内容

第一章 わが国の線維筋痛症患者の実態  
第二章 未知の病に出会った医師たち

第三章 線維筋症の診断と治療へのアプローチ  
第四章 これからの患者支援のあり方

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ORIGINAL ARTICLE

# Association of a single-nucleotide polymorphism in the promoter region of leukemia inhibitory factor receptor gene with low bone mineral density in adult women

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**Background:** Osteoporosis is believed to result from the interaction among multiple environmental and genetic determinants that regulate bone-mineral density (BMD).

**Methods:** To investigate a potentially predisposing genetic factor in the onset of osteoporosis, we looked for a possible association between BMD in adult Japanese women and known polymorphisms in the leukemia inhibitory factor receptor gene (LIFR).

**Results:** An association analysis of chromosomes from 384 volunteer subjects revealed significant correlation between the –603T > C variant of LIFR and radial BMD ( $r = 0.11$ ,  $P = 0.032$ ) in this test population. Comparisons of mean values of adjusted radial BMD among separate genotypic groups implied an allelic dosage effect, because homozygous carriers of T alleles of that SNP had the highest adjusted BMDs ( $0.403 \pm 0.054$  g/cm<sup>2</sup>); women homozygous for the C-allele had the lowest ( $0.373 \pm 0.042$  g/cm<sup>2</sup>), and heterozygous individuals had intermediate scores ( $0.394 \pm 0.056$  g/cm<sup>2</sup>).

**Conclusion:** This polymorphism in *LIFR* may be an important determinant of predisposition to postmenopausal osteoporosis.

**Keywords:** bone mineral density, leukemia inhibitory factor receptor (LIFR), osteoporosis, regression analysis, single-nucleotide polymorphism.

## Introduction

Osteoporosis is characterized by low bone-mineral density (BMD) and by deterioration of the microarchitecture of bone tissue, with a consequent increase in fragility and susceptibility to fracture. BMD, an impor-

tant predictor of fracture, is probably determined by genetic as well as environmental factors.<sup>1,2</sup> Any genes that might affect BMD are candidates for involvement in susceptibility to osteoporosis.

Several genes have already been investigated as potential risk factors for osteoporosis.<sup>3,4</sup> For example, cytokine pathways that include interleukin-1 (IL1), interleukin-6 (IL6) and tumor-necrosis factor alpha (TNF $\alpha$ ) are considered to be among the most potent of all bone-resorbing mechanisms.<sup>5–8</sup> However, an extended panel of genes must be examined in detail, in view of the polygenic nature of BMD distribution and the multiplicity of endocrine and local factors that are

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known to influence bone mass and regulate bone turnover.

The leukemia inhibitory factor (LIF) is one such multifunctional cytokine belonging to the IL-6 family.<sup>9,10</sup> It affects the differentiation, survival and proliferation of a wide range of cells, including those in bone tissue. For example, adult mice respond to an excess of circulating LIF with increased numbers of osteoblasts, resulting in overgrowth of mineralized bone.<sup>11</sup> Therefore, we previously searched for common variations in this gene locus (*LIF*) as a candidate gene for osteoporosis susceptibility.<sup>10</sup> However, since only four variations were detected in the 3'-untranslated region of the third exon in our test samples, we excluded *LIF* from the most likely candidates to be examined for association.

Considerable evidence now indicates that LIF activity in bone exerts significant effects through a high-affinity receptor complex composed of a low-affinity LIF-binding subunit (LIFR) and a converter subunit, gp130.<sup>9</sup> Targeted disruption of the LIFR gene in mice causes loss of bone density and increasing numbers of osteoclasts.<sup>1</sup> A linkage study on the rare human diseases Stüve-Wiedemann Syndrome (SWS) and Schwartz-Jampel type 2 syndrome (SJS2) representing long bone bowing and cortical thickening with flared metaphyses identified its mutations in the LIFR gene.<sup>12</sup> Given these observations, we hypothesized that LIFR might be one of the most important elements for determining BMD levels in humans.

To investigate a possible association between genetic variations in LIFR and BMD, we examined five single-nucleotide polymorphisms (SNPs) in the promoter region of this gene, and one missense coding SNP, for association with radial BMD levels among adult women in Japan.

## Materials and methods

### Subjects

DNA samples were obtained from the peripheral blood of 384 Japanese women. All participants were non-related volunteers and gave informed consent prior to

the study. None had medical complications or were undergoing treatment for conditions known to affect bone metabolism, such as pituitary diseases, hyperthyroidism, primary hyperparathyroidism, renal failure or adrenal or rheumatic diseases, and none was receiving estrogen-replacement therapy. Mean ages and body-mass indices (BMI) with standard deviations (SD) were, respectively,  $58.4 \pm 8.6$  years (range, 32–69) and  $23.7 \pm 3.61$  kg/cm<sup>2</sup> (range, 14.7–38.5).

The BMD (expressed in g/cm<sup>2</sup>) of each participant was measured in the distal radius by dual-energy X-ray absorptiometry (DEXA) using a DTX-200 osteometer (Meditech Inc., Hawthorne, CA), according to the Guidelines for Osteoporosis Screening in a health check-up program in Japan. To calculate adjusted BMD, measured BMD values were normalized for differences in age and body-mass index (BMI) by multiple regression analysis using the InStat3 software package (GraphPad Software, San Diego, CA).<sup>13,14</sup> The adjustment equation for the study samples was as follows: (adjusted BMD in g/cm<sup>2</sup>) = (measured BMD in g/cm<sup>2</sup>) - 0.006375 × (58.39 - [age in years]) + 0.008961 × (23.65 - [BMI in kg/cm<sup>2</sup>]).

### Genotyping for molecular variants in the LIFR gene

We examined six SNPs (-1226T > C, -631C > T, -603T > C, -264C > T, -210G > C, and +1899 A > G (Ile633Met), all archived in the NCBI database (<http://www.ncbi.nlm.nih.gov/SNP>). All were confirmed to be polymorphic in our test population (Table 1). A contiguous-sequence, NT\_023195.12 from RefSeq, was referenced for denoting positions of these SNPs.

Genotypes were determined using the Sd-PCR method, a refined allele-specific PCR, to discriminate polymorphic sequences.<sup>12</sup> In brief, two allele-specific (AS) forward primers and one reverse primer were prepared for each SNP, to transform nucleotide sequences (G, A, T or C) between two alleles at a single site into size-differences. Two different nucleotide mismatches were incorporated at the 3' end of the polymorphic (forward) primers, according to the concept we described elsewhere.<sup>13</sup> AS primers (long and short) have a

**Table 1** Summary of examined polymorphism in the LIFR locus

No	SNP name <sup>†</sup>	JSNP-ID <sup>‡</sup>	dbSNP <sup>§</sup>	Allele frequency	Percent heterozygosity
1	-1226T > C	IMS-JST006591	rs2071233	0.82 : 0.18	30
2	-631C > T	IMS-JST006592	rs2071234	0.81 : 0.19	31
3	-603T > C	IMS-JST006593	rs2071235	0.82 : 0.18	30
4	-264C > T	IMS-JST006594	rs2071236	0.64 : 0.36	41
5	-210G > C	IMS-JST006595	rs2071237	0.67 : 0.33	43
6	Ile633Met	IMS-JST060529	rs2770361	0.96 : 0.04	7

<sup>†</sup>Location of the SNP was defined by NT\_023195.12 from RefSeq; <sup>‡</sup>ID number for JSNP database; <sup>§</sup>ID number for dbSNP (NCBI).

five-base difference between them, and each has a polymorphic nucleotide of the SNP sequence at its 3' end as well as having an additional artificial mismatch introduced near the 3' end. The following primer sets allowed distinct discrimination of alleles:

For SNP -210G > C, long AS-primer; 5'-TTTTTG GTAAAAAGCTTTTGCCTCCCGGC-3', short AS-primer; 5'-CCTAAAAAGCTTTTGCCTCCCGGC-3', reverse primer; 5'-GTTTGCTGCTAAGATTACCTAT TGTGG-3'. For SNP -264C > T, Long AS-primer; 5'-TTTTTGGAGCAGTGTGTTTCAGATGGCAG-3', short AS-primer; 5'-CCAGCAGTGTGTTTCAGATG TTAA-3', reverse primer; 5'-GTTTGCTGCTAAGATT ACCTATTGTGG - 3'. For SNP -603T > C, Long AS-primer; 5'-TTTTTGGATCCACCTGCCTCGGCCTC GCAA-3', short AS-primer; 5'-CCATCCACCTGCCTC GGCCTCCGAG-3', reverse primer; 5'-GTTTGCT GCTAAGATTACCTATTGTGG-3'. For SNP -631C > T, long AS-primer; 5'-TTTTTGGGGGATGACAGG CGTGAGCTACC-3', short AS-primer; 5'-CCGGGAT GACAGGCGTGAGCCGCT-3', reverse primer; 5'-CCAGGAAAGTTTGCATTGCTAATA-3'. For SNP -1226T > C, long AS-primer; 5'-TTTTTGGCAGTG TAAAATCGCCCTTGCCA-3', short AS-primer; 5'-CCCAGTGAAAATCGCCCTTATCG-3', reverse primer; 5'-GTTTGCTGCTAAGATTACCTATTGTGG-3'. For SNP I633M, long AS-primer; 5'-TTTTTGG TCCCATCCCAACAACCTTGCTCT-3', short AS-primer; 5'-CCTCCCATCCCAACAACCTTGCTCC-3', reverse primer; 5'-GCTCTAGGTTTATCTAGTTTGA GCA-3'.

Polymerase chain reactions were performed using 10 ng of each genomic DNA sample and 250 nmol/L of each primer (two polymorphic forward, and a reverse) in a 10-μL reaction mixture containing 10 mmol/L dNTPs, 10 mmol/L Tris-HCl, 1.5 mmol/L MgCl<sub>2</sub>, 50 mmol/L KCl, 1 U Taq DNA polymerase and 0.5 mmol/L fluorescently labeled dCTP (ROX-dCTP; Perkin-Elmer, Norwalk, CT). The reactions and discrimination of alleles on the ABI Prism 377 DNA system (Applied Biosystems, Foster City, CA) were carried out as described previously.<sup>13</sup>

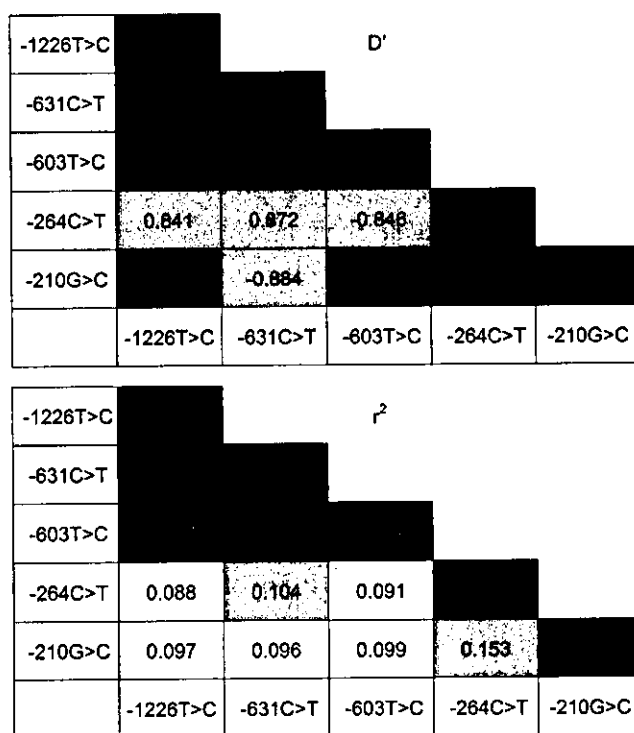
**Statistical analysis**

BMD data for each subject were normalized according to age and BMI.<sup>13</sup> Quantitative associations between genotypes and adjusted BMD values (gm/cm<sup>2</sup>) were evaluated by one-way ANOVA, with regression analysis as a post-hoc test. Three genotypic categories of each SNP were converted into incremental values (0, 1 and 2) corresponding to the number of chromosomes possessing a minor allele. Statistical significance was determined by ANOVA F-tests. We used χ<sup>2</sup> tests to ascertain Hardy-Weinberg equilibrium among genotypes. Haplotypes and indices of linkage disequilibrium (LD) were

calculated using Arlequine software (Genetics and Biometry Laboratory, Geneva, Switzerland).

**Results**

We examined the LIFR gene for possible association with BMD because it was one of the most likely candidates for involvement in susceptibility to osteoporosis. We first confirmed the polymorphic nature of six archived SNPs in that gene among 32 chromosomes from our test population. Since five SNPs in the promoter region (1226T > C, -631C > T -603T > C, -264C > T, -210G > C) and a non-synonymous coding SNP, +1899 A > G (Ile633Met), were moderately polymorphic, we examined them in our entire panel of 384 subjects and clarified allelic and genotypic frequencies (Table 1). For estimating haplotypes we used only the available genotypic data for the five promoter SNPs in 369 subjects. Results for the missense coding SNP were excluded because its minor allele was so rare (4%). LD within the locus was evaluated by two indices, D' and r<sup>2</sup>, calculated for every possible combination of the five SNPs (Fig. 1) LD among these SNPs was evident when D' > 0.5 and r<sup>2</sup> > 0.15.



**Figure 1** Linkage disequilibrium (LD) analysis found significant linkage disequilibrium within the LIFR locus: Indices of LD, D' and r<sup>2</sup> based on 17 estimated haplotypes (covering 100% of the chromosomes) constructed with five promoter SNPs were presented in separate tables. D' greater than 0.4 and r<sup>2</sup> values greater than 0.1 are highlighted with gray half-tone.

**Table 2** Regression analysis of adjusted bone mass density (BMD) among 384 screening subjects

SNP Name	<i>n</i> <sup>†</sup>	Adjusted BMD (g/cm <sup>2</sup> )			Correlation coefficient (r)	<i>P</i> -value <sup>‡</sup>
		Major homozygous ( <i>n</i> )	Heterozygous ( <i>n</i> )	Minor homozygous ( <i>n</i> )		
-1226T > C	382	0.401 ± 0.054 (256)	0.394 ± 0.056 (115)	0.386 ± 0.048 (11)	-0.07	0.16
-631C > T	383	0.402 ± 0.054 (250)	0.395 ± 0.056 (117)	0.377 ± 0.045 (16)	-0.10	0.054
-603T > C	383	0.403 ± 0.054 (255)	0.394 ± 0.056 (115)	0.373 ± 0.042 (13)	-0.11	0.032
-264C > T	382	0.396 ± 0.055 (167)	0.399 ± 0.049 (155)	0.406 ± 0.064 (60)	0.06	0.28
-210G > C	373	0.401 ± 0.055 (169)	0.398 ± 0.053 (160)	0.400 ± 0.060 (44)	-0.02	0.65
Ile633Met	383	0.400 ± 0.054 (354)	0.395 ± 0.056 (28)	(-)	0.02	0.63

<sup>†</sup>Number of genotyped subjects; <sup>‡</sup>*P*-values are calculated for the regression analysis with ANOVA *F*-test.

**Table 3** Summary of characteristics among 383 subjects subgrouped by -603T > C genotypes

	T/T	T/C	C/C	Correlation coefficient (r)
<i>n</i> <sup>†</sup>	255	115	13	NA
Age (year)	58.65 ± 8.68	57.68 ± 8.44	60.23 ± 8.01	0.02
Height (cm)	151.55 ± 6.08	151.55 ± 6.09	151.55 ± 6.10	0.05
Weight (kg)	54.09 ± 9.15	54.57 ± 8.48	54.03 ± 7.80	0.02
Body mass index (kg/cm <sup>2</sup> )	23.54 ± 3.70	23.92 ± 3.43	23.97 ± 3.34	0.05

Values are expressed as means ± SDs. Statistical significance for the correlation was tested by ANOVA *F*-test (*P* < 0.05); none of the correlations were statistically significant.

<sup>†</sup>Number of genotyped subjects.

Regression analysis was performed for each SNP by examining correlations between genotypes and adjusted BMDs. Although no significant correlation was evident for five of the six SNPs examined, one promoter SNP (-603T > C) did reveal a significant correlation (*r* = 0.11, *P* = 0.032) (Table 2). Homozygous carriers of the T allele at this site had the highest adjusted BMDs (0.403 ± 0.054 g/cm<sup>2</sup>); heterozygous individuals were intermediate (0.394 ± 0.056 g/cm<sup>2</sup>); homozygous C-allele carriers had the lowest adjusted BMDs (0.373 ± 0.042 g/cm<sup>2</sup>). The apparent allelic-dosage effect of this variation implied a biological influence on BMD. To examine if -603T > C correlate with the other body status, SNP association was tested for multiple clinical features. As indicated, no significant correlation was detected (age, body weight, height, BMI), indicating a specific effect of this SNP on BMD determination (Table 3).

## Discussion

In the work reported here we found an association of the -603T > C variation of the LIFR gene with radial BMD in adult Japanese women. Adjusted BMD was highest in homozygous T-allele carriers, intermediate among heterozygotes, and lowest among homozygous C-allele carriers. The data implied that variation(s) in the promoter of LIFR might affect bone metabolism,

eventually introducing variations in BMD among adult women.

Lowered BMD can result from accelerated bone loss and/or lesser acquisition of bone mass.<sup>1,15</sup> Bone-resorbing effects of cytokine signaling pathways involving IL-1, IL-6, and TNF $\alpha$  have been investigated in vivo and in vitro.<sup>5-8</sup> Since members of the IL-6 family, such as LIF, are known to affect the genesis of osteoclasts, promoter variations in the gene encoding the LIF receptor could influence those activities and bring about changes in BMD. Inadequate transcriptional regulation of LIFR in homozygous carriers of the C allele at the -603 position may have led to increased bone loss for those women. We propose that LIF- and LIFR-signaling cascades should be considered in any studies of osteoclastogenesis. Of course, the effects of these molecules on bone formation will need to be clarified as well, because the present study does not address the question of how this particular SNP functionally affects BMD.

In addition to the correlation between one promoter SNP and adjusted BMD in our test population, we demonstrated LD between all five of the promoter SNPs, most significantly between -1226T > C, -631C > T and -603T > C. Although no significant correlation was evident between genotypes of the first two of those three SNPs and adjusted BMD, it remains possible that they can influence regulation of BMD through a combined effect with the -603 site.



The -603T > C variation is important on theoretical grounds, because a predictive analysis of binding motifs for transcription factors using the MatInspector program v2.2<sup>16</sup> revealed that the sequence surrounding this polymorphic site is sufficiently similar to the consensus binding sequence for a transcription factor, Nkx-2.5 (CAAAGTG, where the underlined A is a variant nucleotide). This putative binding site on the promoter of LIFR was intact on chromosomes carrying the -603T allele, but absent in homozygous carriers of C alleles. Although the functions of human Nkx-2.5 in bone tissues are not yet defined, LIFR might be one of its transcriptional targets.<sup>17</sup> Functional studies on this promoter region are ongoing in our laboratory, however, because physiological roles of single *cis*-element or *trans*-factor could not easily be determined by a simple assay, those studies would be presented in a future independent report. There also exists the possibility that the -603 polymorphism could be in linkage disequilibrium with unknown but functional variants nearby.

In summary, we showed a significant association between the -603T > C variation in the promoter region of the LIFR gene and radial BMD among adult Japanese women. Structural inspection proposed a possible contribution of a transcription factor, Nkx-2.5, that might bind to this SNP site. Functional biological studies as well as longitudinal studies may clarify the true mechanism of the association reported here.

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## Alfacalcidol reduces accelerated bone turnover in elderly women with osteoporosis

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**Abstract** To evaluate the effects of alfacalcidol on bone turnover in elderly women with osteoporosis, an open-label, prospective, calcium-controlled study was conducted. A total of 80 patients with osteoporosis were divided into two groups: the control group, group C (mean age, 78.0 years), in which patients were given calcium, and group D (mean age, 77.4 years), in which the patients were given alfacalcidol 1 µg/day together with calcium for 6 months. Calcium regulation, lumbar bone mineral density (LBMD), and markers for bone turnover were assessed. A significant increase in urinary calcium/creatinine ratio (90% increase from baseline at 3 months;  $P = 0.0083$ , and 60% at 6 months;  $P = 0.0091$ ) and a significant decrease in serum parathyroid hormone (30% decrease from baseline at 6 months;  $P < 0.0001$ ) was observed in group D compared with the corresponding changes in group C. Significant decreases of bone resorption markers (deoxypyridinoline and N-telopeptide) at 6 months (about 15% decrease from the baseline values) were observed in group D compared with the corresponding changes in group C. The changes in bone formation markers (bone-derived alkaline phosphatase and osteocalcin) in group D were significantly different at 6 months ( $-21.5\%$ ;  $P = 0.0047$  and  $-13.4\%$ ;  $P = 0.0032$ , respectively) from the values in group C. The magnitudes of the decrease in bone turnover markers were highly correlated with the corresponding baseline values, suggesting that alfacalcidol treatment effectively reduces bone turnover in patients with high bone turnover rates. The LBMD in group D increased by 1.7% and that in group C decreased by 1.6% ( $P = 0.0384$ ). The changes in calcium metabolism and LBMD were in good agreement with those in previous reports. Although the changes in bone turnover markers in group D were slight, significant reduction in bone turnover with alfacalcidol treatment, together with the change in calcium metabolism, may account for the effects of alfacalcidol on BMD and on fracture prevention reported previously. In conclusion, alfacalcidol reduces bone turnover

in elderly women with high-bone-turnover osteoporosis, and it may have beneficial effects on bone.

**Key words** osteoporosis · alfacalcidol · bone turnover · elderly women

### Introduction

Active vitamin D<sub>3</sub> analogues, including the hormonal form of vitamin D<sub>3</sub> (1,25[OH]<sub>2</sub> vitamin D<sub>3</sub>) and 1 α OH vitamin D<sub>3</sub> (alfacalcidol), have been utilized to treat osteoporosis for more than 20 years in Japan. Several studies have shown that alfacalcidol maintains bone mineral density (BMD) [1–3] and prevents bone fractures [2,4,5] in patients with osteoporosis. Alfacalcidol facilitates intestinal calcium absorption, which is known to decrease with advancing age [6]. The negative calcium balance leads to an increase in parathyroid hormone secretion and subsequently accelerates the bone turnover rate. Accelerated bone turnover is a known strong predictor of incident fracture [7], which must be prevented in osteoporosis. These metabolic imbalances are more common in the elderly population than in pre- or recently menopausal women.

Although recent progress in the prevention of incident fracture using bisphosphonates has been reported [8–10], these drugs do not correct the calcium imbalance occurring in the elderly with osteoporosis. When active vitamin D<sub>3</sub> is administered to elderly patients with osteoporosis, the effects mentioned above may have some beneficial effects on bone, such as decreased bone turnover rate. However, few data regarding the effects of alfacalcidol on bone turnover in osteoporosis are available. Recently, several sensitive markers for the evaluation of bone turnover have been established. Thus, we investigated the effects of alfacalcidol on bone turnover in a multicenter, calcium-controlled, randomized study in elderly women with osteoporosis.

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## Patients and methods

### Patient selection

Japanese women with osteoporosis aged 65 years or older, women with osteoporosis who were more than 15 years after menopause were enrolled in this study. Women with metabolic bone diseases other than primary osteoporosis and women with osteoporosis who were being treated with bisphosphonates were excluded. If the patients had been receiving drugs for the treatment of osteoporosis other than bisphosphonates, they underwent an 8-week washout period before study entry. Those receiving hormone replacement therapy underwent at least a 12-week washout period. A diagnosis of primary osteoporosis was made based on the diagnostic criteria proposed by the Japanese Society of Bone and Mineral Metabolism in 1996 [11]. Briefly, women with low lumbar BMD (LBMD;  $\leq 70\%$  of young adult mean) or with LBMD of 80% or less of the young adult mean, together with one prevalent nontraumatic vertebral fracture or more were diagnosed as having osteoporosis.

### Study design

A total of 97 women with osteoporosis met the selection and exclusion criteria for this study. The patients were divided randomly into two groups: the control group (group C) was given calcium aspartate at a dose of 78 mg/day (as elemental calcium), while the alfacalcidol group (group D) was given alfacalcidol 1  $\mu$ g/day together with the same dose of calcium aspartate orally for 6 months. The primary endpoints of the present study were changes in bone turnover markers after therapeutic intervention compared with baseline values. Changes in LBMD and serum levels of calcium-regulating hormones from the baseline value at 1 month (only for calcium regulation) and 3 and 6 months of treatment were also examined. The safety profiles of the intervention in both groups were followed up. Randomization was performed using a random allocation chart prepared by the controller.

The institutional review board of each institution involved approved the study protocol, and written informed consent was obtained from all participants before enrollment.

### Measurements of bone turnover markers and calcium-regulating hormones

Serum and urine samples were collected from the patients in the morning and stored immediately at  $-30^{\circ}\text{C}$  until measurement. For bone resorption markers, urinary excretion of *N*-telopeptide (NTX; as measured by enzyme-linked immunosorbent assay [ELISA];

Osteomark, Ostex International Seattle, WA, USA) and total deoxypyridinoline (DPD; as measured by HPLC after hydrolysis of the urine sample) were measured. For bone formation markers, serum levels of osteocalcin (OC; Cis Radioimmunoassay [RIA], Cis Bio International, Gif/Yvette, France) and bone-derived alkaline phosphatase (BAP; lectin-binding method; Boehringer Mannheim, Mannheim, Germany) were measured.

For the evaluation of calcium metabolism before and after the administration of calcium or alfacalcidol plus calcium, serum levels of calcium (Ca), phosphorus (P), albumin, intact parathyroid hormone (I-PTH; immunoradiometric assay [IRMA], Nichols Institute, San Juan Capistrano, CA, USA), and the urinary Ca/Creatinine ratio (Ca/Cr) were measured. In addition, the serum level of 25-hydroxy vitamin D (25-D) was measured before intervention, using a competitive protein-binding assay after extraction and purification of the sample had been done using HPLC to determine the presence of vitamin D deficiency. All samples were measured at one laboratory (SRL Teijin Bio, Tokyo, Japan) without any information on the intervention.

### Measurement of LBMD

LBMD was measured by dual-energy X-ray absorptiometry (DXA), using two apparatuses (QDR; Hologic, Bedford, MA, USA and DPX; GE-Lunar, Madison, WI, USA) at the L2-4 region according to the routine technique of anterior-posterior (A-P) view. The coefficients of variation at each institution and each DXA apparatus ranged from 1% to 2%. The LBMD values in patients with severe vertebral deformity caused by degenerative change or fracture at the L2-4 region were excluded from the analysis.

### Safety profile

The safety profiles in groups C and D were recorded.

### Statistical analysis

For comparisons between the two groups, the data were analyzed by analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD). The differences in the serum and urine parameters before and after the interventions were analyzed using paired Student's *t*-test. The level of significance was set at less than 0.05. Data values are expressed as means  $\pm$  SD.

## Results

### Patient details and background characteristics

A total of 97 patients with osteoporosis were enrolled and divided randomly into two groups. Groups C and D

**Table 1.** Patient background characteristics

Characteristic	Group C	Group D	P value
n	41	39	
Age (years)	78.0 ± 8.1	77.4 ± 6.8	NS
YSM (years)	28.6 ± 9.6	28.6 ± 8.1	NS
Body mass index (kg/m <sup>2</sup> )	21.7 ± 2.9	22.0 ± 3.0	NS
Prevalent fracture (%)	51.2	56.4	NS
S-Ca (mg/dl)	8.9 ± 0.4	8.9 ± 0.4	NS
S-P (mg/dl)	3.5 ± 0.4	3.4 ± 0.4	NS
U-Ca/Cr	0.18 ± 0.11	0.18 ± 0.14	NS
Albumin (g/dl)	4.1 ± 0.3	4.2 ± 0.3	NS
Total Al-P (IU)	202 ± 67	208 ± 67	NS
OC (ng/ml)	13.4 ± 5.0	14.1 ± 5.0	NS
BAP (IU)	75.5 ± 37.2	82.5 ± 29.4	NS
DPD (nM/mMCR)	9.2 ± 3.7	10.3 ± 3.4	NS
NTX (nM/mMCR)	62.4 ± 34.3	70.4 ± 34.8	NS
CTX (nM/mMCR)	286 ± 149	338 ± 158	NS
25-D (ng/ml)	17.4 ± 7.1	18.1 ± 5.3	NS
I-PTH (pg/ml)	36.5 ± 11.3	38.8 ± 16.9	NS
LBMD (% YAM)	59.8 ± 11.7	63.7 ± 12.3	NS

The background data were obtained in the baseline period.

YSM, years since menopause; YAM, young adult mean of lumbar bone mineral density (LBMD); NS, not significant. Because of the differences in the dual-energy X-ray absorptiometry (DXA) equipment, the baseline LBMD is expressed as a percentage of the YAM. Group C, calcium control; group D, alfacalcidol plus calcium-treated group

**Table 2.** Changes in bone resorption markers (percent change from baseline value)

Months of treatment	Group C		Group D	
	DPD	NTX	DPD	NTX
1	1.6 ± 26.0	6.3 ± 36.8	-6.8 ± 16.2	-0.1 ± 36.9
3	1.3 ± 28.2	-1.9 ± 43.7	-10.9 ± 23.7*	-6.8 ± 33.2
6	12.0 ± 37.1	13.6 ± 48.6	-14.3 ± 22.3**	-14.3 ± 32.8***

\*  $P = 0.0400$ ; \*\*  $P = 0.0018$ ; \*\*\*  $P = 0.0129$  vs group C

Values are expressed as means ± SD

Bone resorption marker levels were measured before and after treatment. The percent changes in the markers from the baseline values are listed. Group C, calcium control; group D, alfacalcidol plus calcium-treated group

consisted of 49 and 48 patients, respectively. Three patients were excluded from all analyses because they did not take the designated medication. Fourteen patients were excluded from the efficacy evaluation because of protocol violations ( $n = 3$ ) or were lost to follow-up ( $n = 11$ ), and the findings in the remaining 80 patients (group C;  $n = 41$ ; group D;  $n = 39$ ) were analyzed for efficacy. Ninety-four patients were included in the safety evaluation.

Table 1 shows the background characteristics of the two groups included in the efficacy analysis. There were no significant differences between the two groups in any characteristic.

#### Changes in bone resorption markers

The mean levels of bone resorption markers in group C were maintained at the baseline values during the study period. While, these values in group D had decreased by

approximately 15% at 6 months after the treatment. Thus, the significant differences in these indices between groups C and D were observed at 6 months ( $P = 0.0018$  for DPD and  $P = 0.0129$  for NTX) (Table 2).

#### Changes in bone formation markers

The mean serum level of OC in the group C did not change during the study period, although, in group D, it had decreased by  $13.4 \pm 16.2\%$  at 6 months. The changes in serum BAP in groups D and C at 6 months were significantly different ( $-21.5 \pm 25.6\%$  versus  $9.3 \pm 47.2\%$  of the baseline value, respectively; Table 3).

#### Changes in calcium regulation

The urinary Ca/Cr ratios at 0, 1, 3, and 6 months in group C were  $0.186 \pm 0.105$ ,  $0.167 \pm 0.139$ ,  $0.158 \pm 0.122$ , and  $0.162 \pm 0.127$  mg/mg, respectively. The corre-

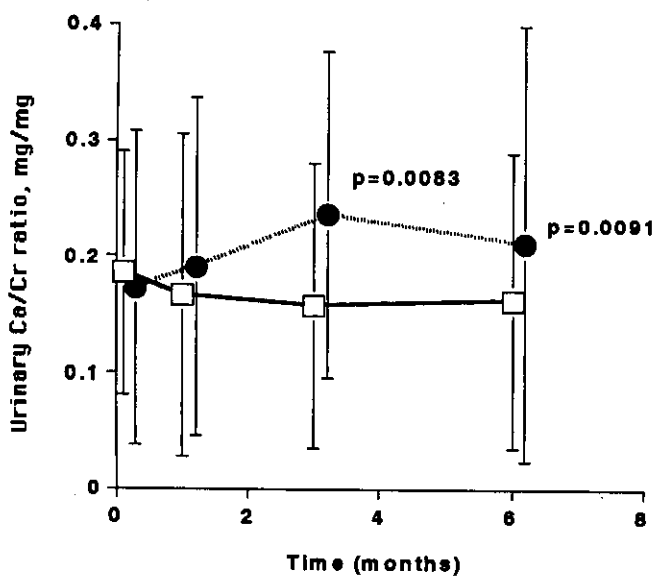
**Table 3.** Changes in bone formation markers (percent change from baseline value)

Months of treatment	Group C		Group D	
	BAP	OC	BAP	OC
1	10.4 ± 47.6	8.5 ± 39.8	-6.5 ± 31.6	11.7 ± 27.6
3	1.0 ± 30.7	6.8 ± 62.3	-6.2 ± 43.1	9.6 ± 33.1
6	9.3 ± 47.2	7.9 ± 35.3	-21.5 ± 25.6*	-13.4 ± 16.2**

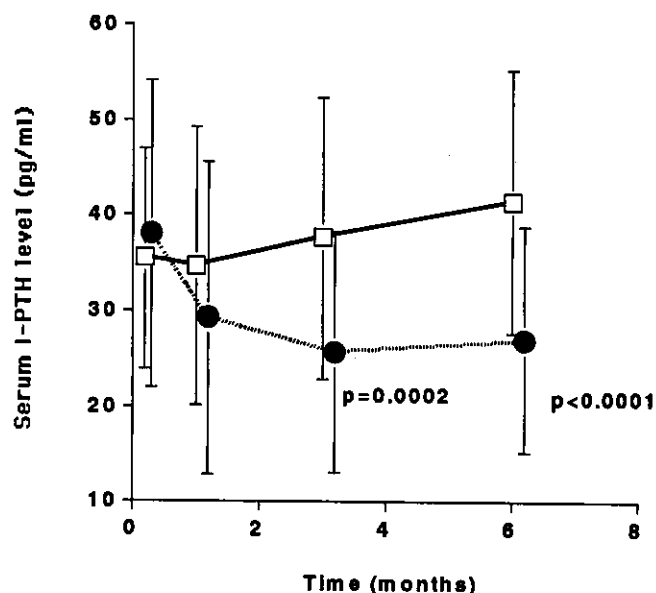
\* $P = 0.0032$ ; \*\* $P = 0.0047$  versus group C

Values are expressed as means ± SD

Serum levels of bone formation markers were measured before and after treatment. Group C, calcium control; group D, alfacalcidol plus calcium-treated group



**Fig. 1.** Effects of alfacalcidol on urinary excretion of calcium. Urinary excretion of calcium in group D was increased by about 60% to 100% from the baseline value, and that in group C was maintained at the baseline value during the study period. Data values are expressed as means ± SD. Open squares, group C; closed circles, group D. Significant differences between groups C and D were observed at 3 and 6 months of treatment. Ca/Cr, urinary calcium/creatinine



**Fig. 2.** Effects of alfacalcidol on serum intact parathyroid hormone (I-PTH) level. The serum level of I-PTH in group D rapidly decreased, by about 30% from the baseline value and the inhibition of I-PTH secretion was maintained during the observation period, while the level in group C was maintained at the baseline value. Data values are expressed as means ± SD. Open squares, group C; closed circles, group D. Significant differences between groups C and D were observed at 3 and 6 months of treatment

sponding values in the group D were  $0.173 \pm 0.135$ ,  $0.191 \pm 0.145$ ,  $0.236 \pm 0.140$  ( $P = 0.0083$  versus group C), and  $0.271 \pm 0.187$  mg/mg ( $P = 0.0091$  versus group C), respectively (Fig. 1). There were four patients whose urinary Ca/Cr ratio exceeded 0.4 mg/mg in group D. However, none exhibited hypercalcemia. The mean serum level of I-PTH decreased by about 30% from the baseline value in group D ( $38.0 \pm 16.1$ ,  $29.2 \pm 16.3$ ,  $25.7 \pm 12.6$ , and  $26.9 \pm 11.8$  pg/ml at 0, 1, 3, and 6 months, respectively). No changes in serum I-PTH levels were observed in group C ( $35.5 \pm 11.5$ ,  $34.7 \pm 14.6$ ,  $37.6 \pm 14.8$ , and  $41.4 \pm 13.8$  pg/ml at 0, 1, 3, and 6 months, respectively; Fig. 2). The differences in serum I-PTH level between groups C and D were significant at 3 and 6 months.

#### Baseline calcium regulation and changes in bone turnover markers in group D

To evaluate the baseline metabolic states associated with changes in bone turnover markers, the correlation coefficients between the baseline serum levels of I-PTH and 25-D, and the changes in bone turnover markers at 6 months in group D were calculated (Table 4). The baseline serum I-PTH level was significantly correlated with changes in serum BAP and in NTX levels, while there was no association between the baseline 25-D level and changes in bone markers. Furthermore, the correlation coefficients between the changes in serum I-PTH and bone turnover marker levels were calculated, and no correlation was found between them (Table 5).

### Baseline data and changes in bone turnover markers in group D

The correlation coefficients between the baseline level of each bone turnover marker and changes after 6 months of alfacalcidol treatment were calculated. The baseline values of NTX ( $r^2 = 0.253$ ;  $P = 0.0054$ ), and BAP ( $r^2 = 0.253$ ;  $P = 0.0054$ ) were highly correlated with changes in these markers at 6 months (Fig. 3), as were those of DPD ( $r^2 = 0.224$ ;  $P = 0.0095$ ). The baseline serum OC level did not correlate with the change in OC level at 6 months ( $r^2 = 0.015$ ;  $P = 0.5273$ ).

**Table 4.** Correlation coefficients between baseline I-PTH and 25-D levels and changes in bone turnover markers after alfacalcidol treatment

	DPD	NTX	BAP	OC
25-D	0.185	0.301	-0.057	-0.206
	NS	NS	NS	NS
I-PTH	0.012	-0.427	-0.373	-0.297
	NS	0.0209	0.0461	NS

To evaluate whether changes in bone turnover markers at 6 months of treatment with alfacalcidol were associated with the baseline levels of 25-D or I-PTH, their correlation coefficients were calculated. The baseline 25-D level did not correlate with changes in bone markers after the treatment, while the baseline level of I-PTH significantly predicted changes in NTX and BAP after 6 months of treatment. NS, not significant

### Changes in LBMD

The change in LBMD in the group C was  $-1.6 \pm 4.3\%$  of the baseline value and that in group D  $+1.7 \pm 5.5\%$  after 6 months of treatment ( $P < 0.05$ ).

### Safety profiles

There was no serious adverse event during the study period in either group. Only one patient, in group D, showed a slight increase in serum levels of aspartate transaminase and alanine transaminase.

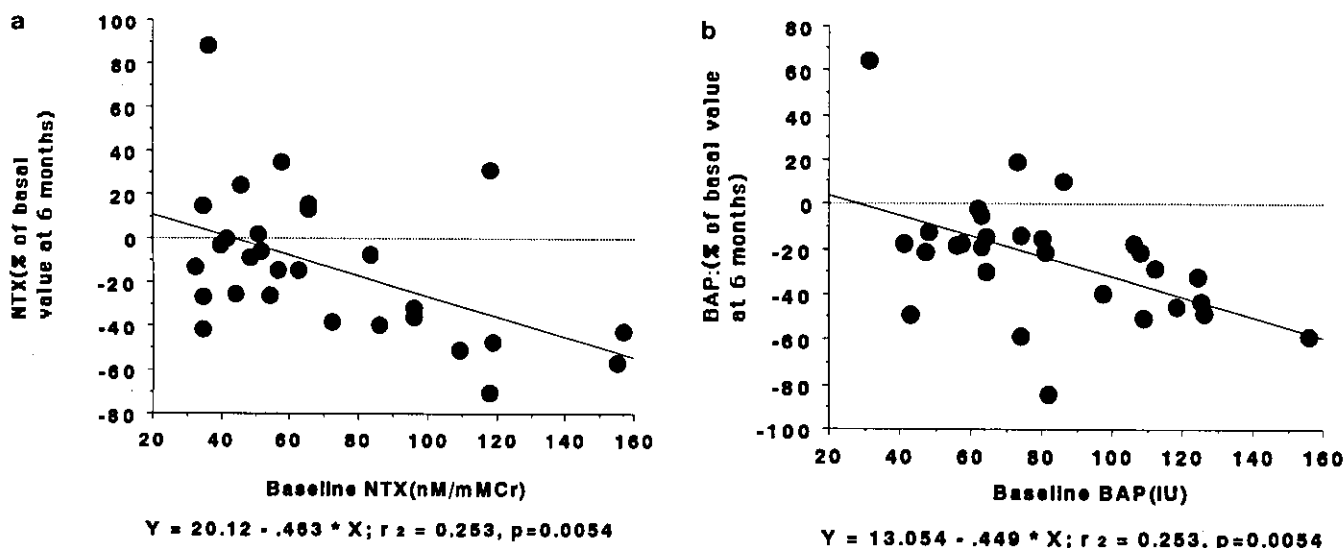
### Discussion

Treatment of osteoporosis with alfacalcidol has been reported to be effective in maintaining BMD and reducing incident fracture [1-5]. The mechanisms of action of alfacalcidol in osteoporosis are considered to involve improvement of the calcium balance through enhancement of intestinal calcium absorption and subsequent reduction of PTH secretion. Secondary hyperparathyroidism is frequently seen in the elderly population, and this may be related to the high bone turnover rates that are a risk for future fracture [7]. Therefore, the reduction of bone turnover and inhibition of secondary hyperparathyroidism are important in the prevention of

**Table 5.** Changes in serum I-PTH and bone marker levels after alfacalcidol treatment

Urinary DPD (percent change from baseline)				
Correlation coefficients between changes in serum I-PTH and urinary excretion of DPD				
	Month	1	3	6
Serum I-PTH (percent change from baseline)	1	0.056	0.213	0.212
		NS	NS	NS
	3	NA	0.141	0.094
	6	NA	NA	0.108
				NS
Serum BAP (percent change from baseline)				
Correlation coefficients between the changes in serum I-PTH and BAP				
	Month	1	3	6
Serum I-PTH (percent change from baseline)	1	0.126	0.247	0.170
		NS	NS	NS
	3	NA	0.289	0.243
	6	NA	NA	0.069
				NS

To investigate the relationship between changes in serum I-PTH and bone turnover marker levels, their correlation coefficients were calculated, in group D. Considering the time-course difference among the changes in I-PTH and in bone markers, we assessed the correlation at the same time, as well as the remote effect of the decrease in serum I-PTH level after alfacalcidol treatment on changes in bone markers (1 month versus 1, 3, and 6 months). The correlations between the change in I-PTH and the changes in NTX and OC were also not significant (data not shown). NS, not significant; NA, not applicable



**Fig. 3.** Reduction of bone turnover by alfacalcidol treatment is dependent on the baseline bone turnover rate. **a** Correlation between baseline *N*-telopeptide (*NTX*) level and change in *NTX* after 6 months of treatment. **b** Correlation between baseline bone-derived alkaline phosphatase (*BAP*) level and change in *BAP* after 6 months treatment. Correlations between baseline levels of bone turnover markers and changes in these markers after 6 months of treatment with alfacalcidol

were calculated. A highly significant negative correlation between the baseline level of *NTX* and the change in *NTX* after intervention was observed. ( $Y = 20.12 - 0.463 * X; r_2 = 0.253; P = 0.0054$ ). The same trend was also found for *BAP* ( $Y = 13.054 - 0.449 * X; r_2 = 0.253; P = 0.0054$ ). The results indicate that alfacalcidol reduces bone turnover in patients with osteoporosis with high bone turnover

incident fracture. Bisphosphonates are thought to be the most powerful agents for reducing bone resorption, and many recent reports have shown their beneficial effects on bone fracture prevention in osteoporosis [8–10]. However, bisphosphonate treatment induces at least transient secondary hyperparathyroidism, especially in elderly patients, because it inhibits bone resorption [3]. In this regard, alfacalcidol may still play an important role in the treatment of osteoporosis.

However, very little information has been available on changes in bone turnover rates after treatment with alfacalcidol [12,13]. The hormonal form of vitamin  $D_3$  ( $1,25[OH]_2$  vitamin  $D_3$ ) is known to stimulate bone resorption in *in vitro* and animal studies [14], although previous clinical reports have shown no effect on bone resorption after active vitamin  $D_3$  treatment at a clinical dosage [2,3]. We have reported that alfacalcidol  $0.75 \mu\text{g}/\text{day}$  increased the urinary excretion of calcium without a concomitant increase in the urinary excretion of hydroxyproline [2]. These results indicate that alfacalcidol may not stimulate bone resorption as seen in *in vitro* studies, and thus the increase in urinary excretion of calcium probably resulted from enhanced intestinal calcium absorption [6].

In the present study, we attempted to investigate the effects of alfacalcidol on bone turnover in elderly patients with osteoporosis who were expected to have a calcium imbalance due to low intestinal calcium absorption or low intake of calcium [15]. The baseline charac-

teristics in the present study revealed that the elderly patients with osteoporosis had markedly high levels of bone turnover markers and, thus, accelerated bone resorption rates.

Alfacalcidol effectively inhibited PTH secretion by about 30% compared with the baseline value, and it increased urinary calcium excretion by approximately 60%–100%. These results are in good agreement with those from previous studies [2,3]. Therefore, we can conclude that alfacalcidol is effective in improving the calcium imbalance in elderly women with osteoporosis.

Bone turnover marker levels decreased after alfacalcidol administration. However, the exact mechanism(s) by which alfacalcidol inhibits bone turnover in patients with osteoporosis remains unclear. The baseline level of I-PTH and the decrease in PTH after alfacalcidol treatment may account for the decrease in resorption, because PTH is known to facilitate resorption [16]. The baseline level of serum I-PTH was significantly correlated with the decrease in *NTX* and *BAP* levels (Table 4), suggesting that patients with secondary hyperparathyroidism respond well to alfacalcidol treatment in terms of reduction of bone turnover.

However, direct correlations between changes in bone resorption markers and serum I-PTH levels after treatment were not identified at any time point. The remote effects of the decrease in PTH on bone turnover were also not significant (Table 5). We have to consider the possibility that the present assay system for PTH

cross-reacts with both 1–84 PTH and N-truncated PTH fragments and is antagonistic to the whole PTH molecule [17]. Thus, whole PTH measurements are required before concluding whether the decreases in bone marker were a result of the reduced PTH secretion.

As shown in Table 1, the patients in the present study had very high levels of bone turnover markers. The same trend in the effect of alfacalcidol treatment on bone turnover in women immediately after menopause who had high bone turnover rates was reported [18], suggesting that alfacalcidol may depress bone resorption only in patients with accelerated bone turnover. No inhibitory effect of alfacalcidol on the urinary excretion of DPD was found in a phase III study of alendronate [3]. The mean baseline DPD excretion in group D in the present study was 10.3 nM/mM Cr, while it was 6.8 nM/mM Cr in the alendronate phase III study, even though osteoporotic patients were selected. To confirm the hypothesis that alfacalcidol reduces bone turnover only in osteoporotic patients with high bone turnover, we calculated the correlation coefficients between the baseline level of bone markers and their changes after 6 months of treatment. The results clearly demonstrate that the declines in bone resorption markers after the intervention are highly dependent on baseline values. Therefore, we may conclude that alfacalcidol inhibits bone resorption in patients with osteoporosis with high bone resorption rates.

BAP and OC levels were significantly decreased after 6 months of alfacalcidol treatment. The magnitude of the decrease in the serum level of BAP after alfacalcidol treatment also depended on its baseline level. However, there was no correlation between the baseline level of OC and the decrease in serum OC after 6 months of treatment. A possible explanation for this discrepancy between BAP and OC levels may be the time-course difference between them. That is, the time-course change in serum OC level after alfacalcidol administration tended to be delayed comparing with that in serum BAP. A longer observation period would be required to confirm this possibility. The effects of alfacalcidol on bone turnover were statistically significant but slight, and all changes were within the minimal significant change for each marker (22.5% for DPD, 24.7% for NTX, and 17.0% for BAP; from the study of the Committee of Guidelines for Bone Markers of the Japanese Society of Osteoporosis [16]). Therefore, changes in bone turnover after alfacalcidol treatment may be difficult to recognize in individual patients.

The effects of alfacalcidol on BMD in the present study are in good agreement with those found in previous studies. Alfacalcidol treatment increased LBMD by approximately 2%. This increase in LBMD was consistent in previous studies, regardless of bone turnover rates [1–3]. Because baseline BMD and bone turnover

have been reported to be independent risk factors for future fracture [7,19,20], the slight inhibition of bone resorption together with the improvement of the calcium balance with alfacalcidol may explain the effects of alfacalcidol on BMD maintenance and fracture prevention.

In conclusion, alfacalcidol increased the urinary excretion of calcium and decreased the serum level of PTH, possibly through enhancement of intestinal calcium absorption. Alfacalcidol slightly decreased the accelerated bone turnover rate without a direct correlation to the effects on PTH metabolism. Thus, these metabolic effects of alfacalcidol may yield beneficial effects on BMD and on the prevention of osteoporotic fracture.

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## Absolute height reduction and percent height ratio of the vertebral body in incident fracture in Japanese women

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**Abstract** Vertebral fractures are the most common complication associated with osteoporosis. The definition of prevalent or incident vertebral fractures is important in epidemiological evaluation and in clinical trials of osteoporosis treatment. There have been few reports regarding the morphometric cutoff criteria for incident fractures in Asians, and only a few reports of their occurrence in Caucasians. The aim of this study was to establish a definition for incident fractures in Japanese women. A total of 279 women, aged  $64.8 \pm 9.9$  (mean  $\pm$  SD) years, were recruited, and repeated thoracolumbar spinal radiographs were performed at a mean of  $1.45 \pm 0.44$  years after the baseline film. Vertebral fractures were graded from 0 to 3 in both baseline and follow-up films using a semiquantitative assessment by the consensus of three readers. Incident fractures were defined as an increase of grade, except grade 3, and a vertebral body height of less than 12mm on the baseline film. As morphometric criteria, both absolute height reduction (AHR) and the percent height ratio (PHr) were calculated. Prevalent fractures were observed in 207 vertebrae (6.2%) and 85 cases (30.5%), and incident fractures in 42 vertebrae (1.3%) and 29 cases (10.4%). As morphometric criteria for incident fracture, both a PHr  $\geq 15\%$  and an AHR  $\geq 3$ mm or  $\geq 4$ mm indicated sensitivity of 49.17% and 50.83% and specificity of 99.92% and 99.90%, respectively. In conclusion, the morphometric criteria of incident fracture combined with a semiquantitative assessment will provide useful information in the study of clinical osteoporosis, especially for international comparisons.

**Key words** osteoporosis · incident fracture · cutoff value · vertebral height

### Introduction

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural

deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures [1]. Fractures in the spine, hip, and forearm are known as fragility fractures. Although vertebral fractures occur at a higher rate than other osteoporotic fractures, one-third of them are asymptomatic [2]. Therefore, accurate diagnosis of vertebral fractures depends on radiologic visual assessment or quantitative vertebral morphometry [3,4]. The definition of prevalent or incident vertebral fractures on radiograms is important not only in clinical trials of osteoporosis for drug therapy [5] but also in epidemiological studies of osteoporosis [6]. There are ethnic differences between Japanese and Caucasians in the number of vertebral fractures [7], incidence of hip fractures [8,9], and dosages of bisphosphonates, alendronate [10,11], and risedronate [12,13] for increased effects on lumbar bone mineral density (BMD) in osteoporotic patients. These ethnic differences are important in clarifying the pathoetiology of osteoporosis. In particular, it is necessary to employ the same criteria for vertebral fractures in comparative studies between different races. Many criteria, both visual semiquantitative and morphometric quantitative, for vertebral fractures have been reported [4,14–16].

In the present study, we determined the morphometric criteria for absolute height reduction (AHR) and the percent height ratio (PHr) of the vertebral body for incident fractures in Japanese women.

### Materials and methods

#### Subjects

A total of 279 Japanese women, aged  $64.8 \pm 9.9$  (mean  $\pm$  SD) years, who visited the Research Institution and Practice for Involutional Diseases, Nagano, Japan, were enrolled in the study. Written informed consent was obtained from all subjects. Their mean lumbar BMD

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was  $-1.80$  of the T score. The exclusion criteria were severe scoliosis, vertebral deformities other than nontraumatic fractures, and a history of disease known to cause secondary osteoporosis. All participants received no treatment or operation for osteoporosis.

*Radiographs*

Conventional lateral radiographs of the lumbar and thoracic vertebrae were taken at both baseline and follow-up time points, with a mean interval of  $1.45 \pm 0.44$  years between them. The tube-to-film distance was 105cm, and the X-ray beam was centered over T7 for the thoracic film and L3 for the lumbar film.

*Vertebral fracture*

*Semiquantitative assessment*

Both prevalent and incident vertebral fractures were initially evaluated using semiquantitative assessment (Fig. 1).

A visual semiquantitative grading of 0–3 of the vertebral body of T4 through L5 was done by three independent observers: grade 0 (normal); grade 1 (mildly deformed, approximately 20%–25% reduction in anterior, middle, and/or posterior height and a reduction of area of 10%–20%); grade 2 (moderately deformed, approximately 25%–40% reduction in any height and a reduction in area of 20%–40%); and grade 3 (severely deformed, approximately 40% reduction in any height and area) (Fig. 2) [3].

Three readers simultaneously viewed both the baseline and the follow-up films from T4 to L5 in chro-

nological sequences, and graded vertebral fractures. After these independent readings, both films were reviewed by three observers. When inconsistency between the readings was recognized, the three readers negotiated among themselves to reach an agreement regarding the degree of vertebral fracture and made a consensus reading decision.

*Morphometric assessment*

The radiographs were digitized using a laser scanner (Hitachi Digitizer HDG-1717BL), and the digitized images were then displayed on a CRT monitor. Six points, superior and inferior points of anterior, middle, and posterior sites, were marked for each vertebral body by an independent trained assistant in a standard fashion (Fig. 3) [14]. With six pointization, the four

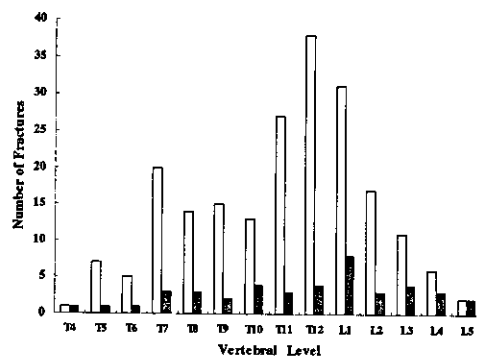


Fig. 1. Distribution of prevalent and incident fractures after consensus reading on semiquantitative assessment. □, Prevalent fracture; ■, Incident fracture

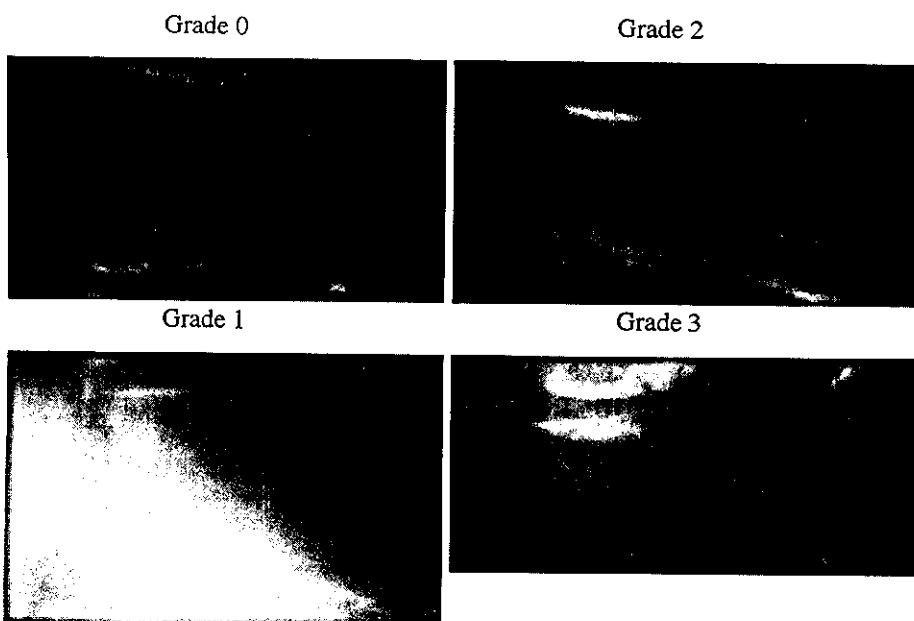
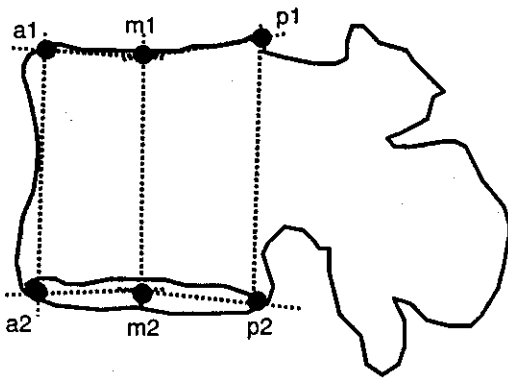


Fig. 2. Semiquantitative grading of vertebral deformity: examples of grades 0 to 3



**Fig. 3.** Pointing method in morphometric assessment. *a1*, anterosuperior; *a2*, anteroinferior; *m1*, middle superior; *m2*, middle inferior; *p1*, posterosuperior; *p2*, posteroinferior; anterior vertebral height (Ha), *a1* to *a2*; middle vertebral height (Hm), *m1* to *m2*; posterior vertebral height (Hp), *p1* to *p2*

corner points, anterosuperior (*a1*), anteroinferior (*a2*), posterosuperior (*p1*), and posteroinferior (*p2*), of the vertebral body were marked.

An additional point, middle-superior (*m1*) or middle-inferior (*m2*), was placed on the middle on a line bisecting the two projections of the upper and lower endplate: two lines, *a1* to *m1*, and *m1* to *p1*, were equal in length, and *a2* to *m2*, and *m2* to *p2* were also equal. The anterior (Ha), middle (Hm), and posterior (Hp) heights and anteroposterior ratio (Ha/Hp), middle-posterior ratio (Hm/Hp), and middle-anterior ratio (Hm/Ha) were calculated.

The reproducibilities of morphometry were evaluated by measuring 20 randomly selected radiographs, 10 of grade 0 and 10 of grade  $\geq 1$ , on semiquantitative assessment on 10 occasions in 2 weeks; the coefficients of variation (RMS) were 1.26%, 1.10%, and 1.31% for Ha, Hm, and Hp for grade 0, and 1.70%, 1.66%, and 1.65% for Ha, Hm, and Hp for grade  $\geq 1$ , respectively.

Morphometric assessments, in addition to semiquantitative assessments, were done of all baseline and follow-up radiographs.

#### Definition of incident fractures

Incident fractures were defined as those in which vertebral bodies showed a distinct alteration in morphology resulting in a higher deformity grade on the follow-up radiograph, except for a vertebral deformity of grade 3 and a vertebral body height of less than 12mm on the baseline radiograph.

The results for which a consensus was obtained in the reading grade by semiquantitative assessment were considered as the "gold standard" for incident fractures. Chronological changes in absolute height reduction

(AHR) and the percentage height ratio (PHr) were calculated in all cases with and without vertebral fracture by morphometry. In incident fractures as diagnosed by a consensus reading, the cutoff values for AHR and PHr were evaluated from sensitivity and specificity.

## Results

### Morphometry of vertebral heights and vertebral height ratios

The values of Ha, Hm, and Hp from T4 to L5 in grade 0 are shown in Table 1. The mean values of the vertebral heights (3348 vertebrae, 279 cases) were  $25.3 \pm 4.7$  mm,  $25.2 \pm 4.2$  mm, and  $27.2 \pm 4.2$  mm for Ha, Hm, and Hp, respectively.

The values of Ha/Hp, Hm/Hp, and Hm/Ha from T4 to L5 in grade 0 are shown in Table 2. The mean values of the vertebral height ratios (3348 vertebrae, 279 cases) were  $0.93 \pm 0.08$ ,  $0.93 \pm 0.06$ , and  $1.00 \pm 0.05$ , respectively.

### Prevalent fractures

Prevalent fractures as determined in 3338 vertebrae in 279 cases by consensus reading on semiquantitative assessment were observed in 207 vertebrae (6.2%) in 85 cases (30.5%). See Fig. 1 for the distribution of prevalent fractures. Prevalent fractures occurred more commonly in the thoracolumbar regions: 13.0%, 18.4%, and 15.0% in T11, T12, and L1, respectively.

**Table 1.** Values of Ha, Hm, and Hp from T4 to L5 in grade 0 at the baseline

Level	<i>n</i>	Ha (mm)	Hm (mm)	Hp (mm)
T4	215	$19.9 \pm 1.3$	$19.9 \pm 1.2$	$21.7 \pm 1.3$
T5	240	$20.5 \pm 1.3$	$20.6 \pm 1.2$	$22.5 \pm 1.3$
T6	255	$20.5 \pm 1.4$	$21.1 \pm 1.2$	$23.2 \pm 1.3$
T7	246	$21.0 \pm 1.4$	$21.5 \pm 1.2$	$23.8 \pm 1.3$
T8	255	$21.8 \pm 1.6$	$22.0 \pm 1.4$	$24.1 \pm 1.3$
T9	253	$23.0 \pm 1.5$	$22.8 \pm 1.3$	$24.6 \pm 1.3$
T10	250	$24.0 \pm 1.8$	$23.8 \pm 1.4$	$25.9 \pm 1.4$
T11	231	$24.7 \pm 1.7$	$25.2 \pm 1.5$	$28.2 \pm 1.7$
T12	219	$26.8 \pm 1.8$	$27.4 \pm 1.6$	$30.8 \pm 1.9$
L1	242	$28.7 \pm 1.9$	$29.0 \pm 1.7$	$32.5 \pm 1.8$
L2	256	$30.4 \pm 2.2$	$29.7 \pm 1.9$	$32.6 \pm 2.0$
L3	260	$31.3 \pm 2.5$	$30.4 \pm 2.0$	$32.1 \pm 2.0$
L4	259	$30.8 \pm 2.8$	$30.5 \pm 2.3$	$30.3 \pm 2.2$
L5	167	$31.2 \pm 2.9$	$29.8 \pm 2.3$	$27.9 \pm 2.0$
Totals	3348	$25.3 \pm 4.7$	$25.2 \pm 4.2$	$27.2 \pm 4.2$

Ha, anterior vertebral height; Hm, middle vertebral height; Hp, posterior vertebral height  
Values are mean  $\pm$  SD