骨粗鬆症の疫学
Epidemiology of Osteoporosis

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Key Words 骨粗鬆症（osteoporosis），骨折（fracture），有病率（prevalence），発生率（incidence），危険因子（risk factors）

はじめに

平成13年国民生活基礎調査によると，介護が必要となった原因として，女性では，脳血管障害，高齢による衰弱に続いて，骨折・転倒が第3位であり，男性においても5位の原因となっている。また，最近の報告では，大腿骨頸部骨折後の1年間の死亡率は，男性で約5倍，女性では約3倍であった。高齢者における骨折は，男女に関わらず，生命予後，日常生活動作（ADL），生活の質（QOL）を低下させる一因となり，高齢化社会において重要な医学的・社会的問題となっている。

ここでは，骨粗鬆症・骨折の頻度，骨密度と骨折の関係，危険因子について性差という観点からみていく。

1．骨粗鬆症の有病率

日本骨代謝学会の骨粗鬆症診断基準〔成人骨密度（腰椎）平均値から70%未満〕から日本人の骨粗鬆症の有病率を求めると，40歳代においては，男女とも数パーセン

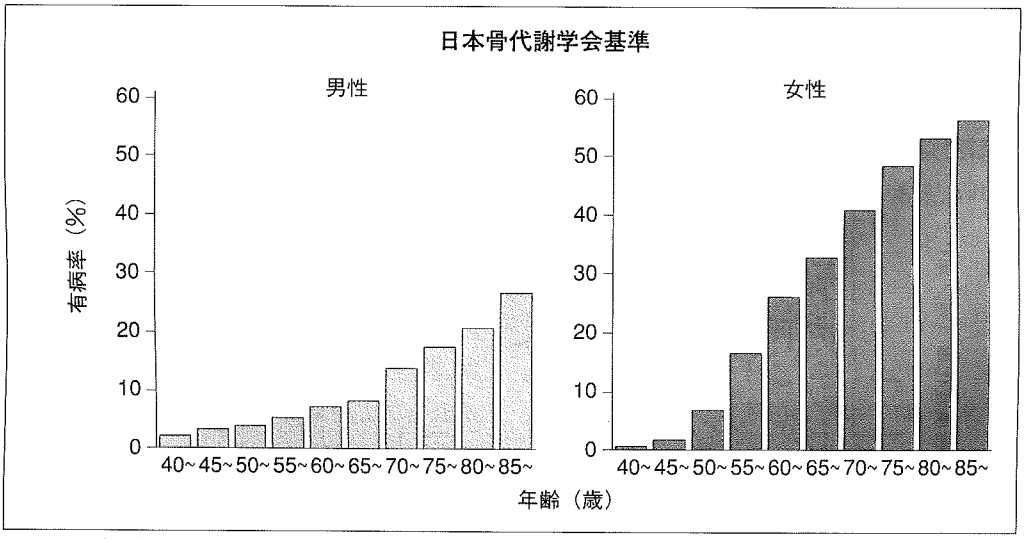

図1 骨粗鬆症の有病率（文献1から引用，改変）

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トであるが，加齢とともに増加し，70歳代後半では男性の約20%，女性の約50%となる（**図1**）[1]。特に，女性は，閉経周辺期である45歳から50歳代にかけて有病率は急に増加し，50歳以降は，女性の有病率は男性の約2～3倍になる。この有病率を用いて，わが国の骨粗鬆症人口を求めると40歳以上の女性で約780万人，男性では約230万人，男女合わせると1,000万人以上が骨粗鬆症と推定される。

2．骨密度の年齢変化

1）年齢別の骨密度平均値

腰椎骨密度を年齢別に比較すると，40歳代までは男女の骨密度平均値は変わらないが，女性は50歳代前半から，男性に比べて低くなり，50歳代後半以降は，男性の各年齢の骨密度平均値から1標準偏差（SD）ほど低い値が女性の骨密度平均値に該当する（**図2**）[2]。男性の腰椎骨密度も，女性ほど急激な低下は見られないものの，年齢とともに徐々に骨密度は低下する。加齢による骨密度低下の男女の違いは，大腿骨頸部骨密度においても同様に見られる。

2）年齢別の骨密度変化率

骨密度減少率は，男女および年齢，骨密度測定部位によって違いがある。女性において腰椎骨密度が減少し始

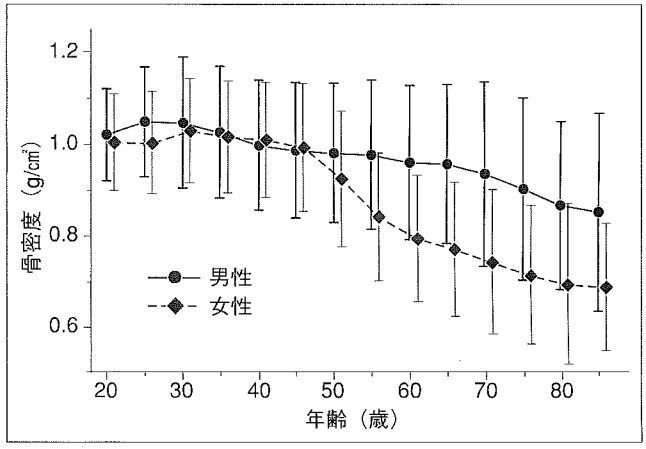

図2　年齢別の腰椎骨密度 平均値と標準偏差（文献2から引用，改変）

めるのは閉経前で，骨密度のピークは20歳代と推定されている。日本人女性を対象として平均追跡期間2年間の腰椎骨密度減少率を求めると，20歳代前半から減少し始め，閉経周辺期で年間約2%の減少率を示した[3, 4]。閉経後は閉経からの年数が経過するに従って骨密度減少率は小さくなった（**図3右図**）[4]。腰椎骨密度変化率は，対象とした集団の違いによって差はあるものの，月経が正常な女性では年間減少率は約0.2%，閉経周辺期で約1.5～3%，閉経後10年以上経つと0.33～1%と報告されている。いずれの調査においても腰椎骨密度減少率が最も大きいのは閉経後早期で，その後減少率は小さくなるという傾向は同じである。

男性においては，腰椎骨密度減少率は生涯を通じて小

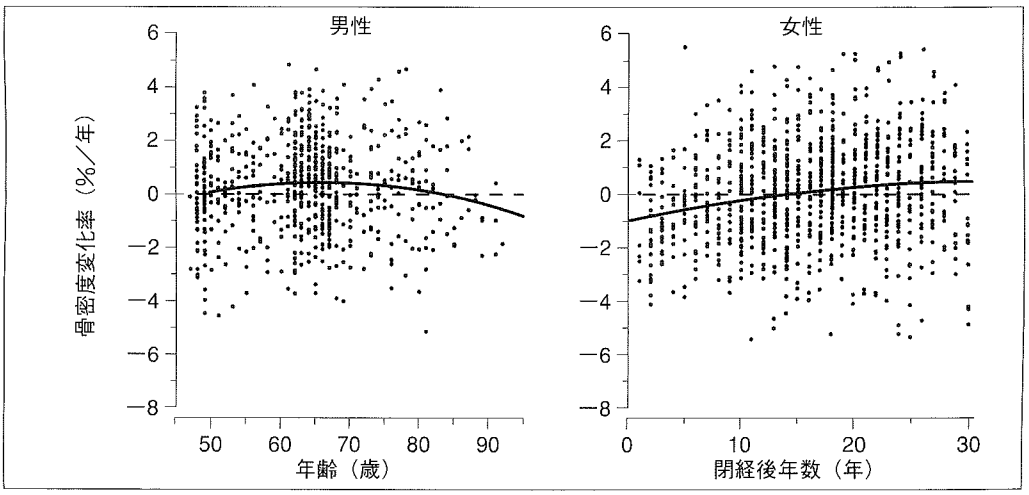

図3　年齢別の腰椎骨密度変化率（文献4から引用，改変）

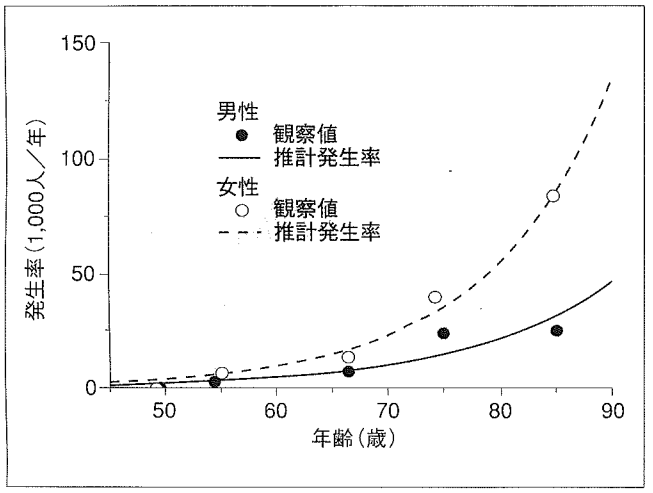

図4　脊椎骨折発生率（文献5から引用，改変）

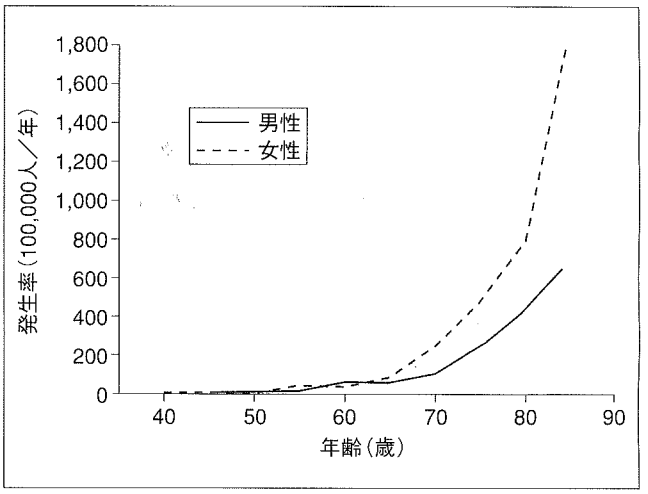

図5　大腿骨頸部骨折の発生率（文献6から引用，改変）

さい，あるいはむしろわずかながら骨密度が増えるという報告もある。図3左図は日本人男性における骨密度変化率を示す。60歳代ではわずかながら骨密度は増え，80歳以降になると減少し始める[4]。中高年男性において腰椎骨密度減少率が小さい，むしろ増加しているのは，加齢に伴う変形性脊椎症の変化によって，骨密度が過大評価されるためと考えられる。変形性脊椎症などの変化が強い場合には，腰椎骨密度は骨密度変化の評価には適していない。

3．骨折発生率の性差

脊椎骨折，橈骨下端骨折，大腿骨頸部骨折の発生率は，男女とも，年齢とともに増加する。脊椎骨折，大腿骨頸部骨折は，50歳までの比較的若い年齢では，男性の発生率が女性より高いが，高齢者では，男性より女性の発生率が約2倍高くなる（図4，5）[5,6]。橈骨下端骨折は，女性では，50歳代に急激に高くなり，それ以降はほぼプラトーになるが，男性では，加齢に伴う発生率の増加はわずかである[6]。いずれの骨折でも，50歳代までの比較的若い年代で男性の発生率が高いのは，事故，転落などに起因した大きな外傷による骨折が含まれている可能性が考えられる。

椎体，橈骨下端は，大腿骨頸部に比べ海綿骨に富み女性ホルモン低下の影響を受けやすく，閉経後比較的早期に骨量減少が始まる。脊椎骨折は，大きな外力が加わらなくて起こり，50歳以降，加齢とともに指数関数的に発生が増える。橈骨下端骨折は，転倒した防護反応として手をついて骨折することが多い。危険因子として活動性が高いことが報告され，活動性が高くかつ骨量減少が始まった女性の50歳代後半に急増する。大腿骨頸部骨折は，転倒して骨折することが多く，バランスや運動能力の低下し，海綿骨量だけでなく皮質骨量の減少も進む高齢者に多く発生すると考えられる。

各国から報告された大腿骨頸部骨折の発生率を比較すると，ほとんどの国で，女性は男性の約2～3倍である[7]。例外的に，中国，韓国，トルコでは，男性の方が女性より発生率が高いが，この性差は，真の差というより調査方法などの違い，あるいは女性は男性に比べて受診しないなどの文化的な背景が考えられる。

4．骨折予知因子

1）骨密度

骨密度の骨折予知については，メタ・アナリシスから，女性では，骨密度が1SD低いと骨折のリスクは約1.5～2倍と報告されている[8]。腰椎，大腿骨頸部，橈骨下端，踵骨のどの部位の骨密度もほぼ同じ程度に，骨折を予測するが，大腿骨頸部骨折を最も予知する部位は，大腿骨頸部骨密度であった。日本人コホートにおいても，男女

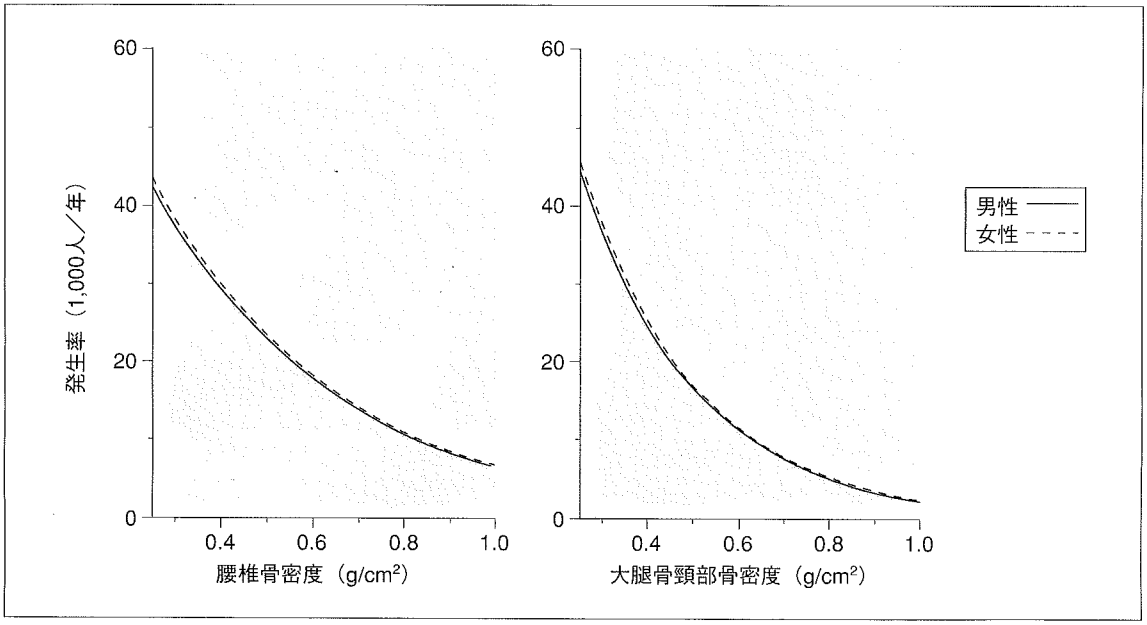

図6　骨密度と脊椎骨折発生率の関係（文献5から引用，改変）

とも骨密度は，脊椎，大腿骨頸部骨折を予知することが報告されている[5]。骨密度の骨折予知力は，欧米の報告と変わりなく，骨密度1SD低下における骨折の相対リスクは男女で差がなかった。

前述したように，女性の脊椎骨折発生率は，男性の約2倍である。しかし，同じ骨密度における男女の脊椎骨折発生率は，ほぼ同じであることがいくつかの報告から認められている（**図6**）[5]。すなわち，脊椎骨折発生率の男女差は，各年齢における骨密度の差を反映しているのにすぎないと考えられる。

2）既存骨折

骨折の既往は，骨密度とともに，将来の骨折を予知する重要な因子である。骨密度が同じであっても，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約2倍，既存脊椎骨折があると将来の脊椎骨折は4倍になる。既存脊椎骨折の将来の脊椎骨折の予知力は最も高いが，その他のどの部位の既存も，ほぼ同じ程度に将来の骨折リスクを予知する。

日本人集団においても，年齢，骨密度を調整しても，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3倍，男性で4倍であった[5]。女性において脊椎骨折既往があると大腿骨頸部骨折リスクは5倍であった[5]。最近のメタ・アナリシスの結果では，既存骨折の将来の骨折の予知力は，男女で差はなかった[9]。

骨折既往が，骨密度と独立して，将来の骨折を予知する理由として，骨折既往は骨の微細構造の欠陥や，転倒しやすさ，転倒した時に骨折を防ごうとする反射的な行動能力の低下などを間接的に示している可能性が考えられている。特に，既存脊椎骨折が，脊椎骨折を強く予知するのは，上記の理由以外に，いったん脊椎骨折を起こすと，姿勢の変化が生じ，脊柱周辺での筋肉の緊張が起こって，新たな脊椎骨折を起こしやすくなる可能性が考えられている。

3）体重

体重は，骨密度に影響する重要な因子であり，男女ともやせは大腿骨頸部骨折の危険因子になることは多くの報告で認められている。しかし，骨密度を調整すると，体重あるいはBMI（body mass index）の脊椎骨折リスクとの関係は消失することが，多くの調査から報告されている。これは，体重が脊椎骨折発生に及ぼす影響は，骨密度を介するもので，骨密度とは独立しては脊椎骨折に与える影響はない，あるいは小さいと考えられる。

しかし，大腿骨頸部骨折については，骨密度とは独立して，体重低下あるいはBMI低下は，骨折リスクを増加させる。その理由として，転倒など外力が加わった時，脂肪組織が厚いことがパットとしての役割をしていて，大腿骨頸部骨折を防ぐ可能性が考えられる。

4）喫煙

メタ・アナリシスの結果から，喫煙者は，非喫煙者に比べて，骨密度が低く，骨折リスクが高いことが認められている[10]。骨密度への影響は，喫煙量に依存し，男性のほうが，女性に比べ喫煙者の骨折リスクが高い。

喫煙が骨密度，あるいは骨折に対する影響する機序として，ニコチンの骨形成に対する抑制，喫煙がカルシウム吸収を低下させる，高齢者の転倒を増加させるなどと考えられている。

おわりに

50歳以降の骨密度は，男性に比べて女性で低く，骨粗鬆症の有病率，脊椎骨折，橈骨下端骨折，大腿骨頸部骨折発生率は男性に比べ女性に高い。しかし，骨密度の骨折予知力は，男女で差はなく，脊椎骨折発生率の違いは，男女の骨密度の違いによって説明できることが分かってきた。さらに，骨折の危険因子は男女とも同じで，喫煙以外は，体重，既存骨折の骨折発生予知力に性差は見られない。

疫学研究によって，骨密度，骨折あるいはその危険因子の性差を明らかにし，その性差が何によって説明することができるのかを解明することは，骨粗鬆症および骨折の病因や危険因子の解明につながる。

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表　題

著者名

醫學のあゆみ　別刷

第　巻・第　号：　　年　月　日号

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

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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．

Key word：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[1]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．

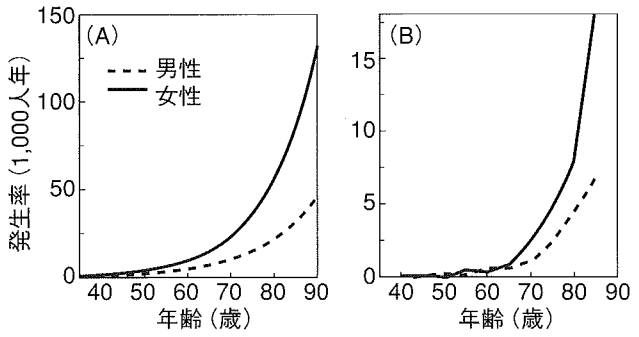

図 1　日本人の脊椎骨折(A)，大腿骨頸部骨折(B)の発生率[2,3]

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[5-14]

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(1 SD 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．

代後半から急増する[2,3](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[4]．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[4]．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[5,6](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[2]．

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[7,8]．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[2]，既存骨折の骨折予測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[8]．

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[9]．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[10]，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，プレドニゾロン5 mg/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[9]．また，骨密度測定時にプレドニゾロン7.5 mg/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[11-13]．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[11-13]．

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[14]．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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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 ± 0.054 g/cm^2); women homozygous for the C-allele had the lowest (0.373 ± 0.042 g/cm^2), and heterozygous individuals had intermediate scores (0.394 ± 0.056 g/cm^2).

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 important predictor of fracture, is probably determined by genetic as well as environmental factors.[1,2] 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.[3,4] For example, cytokine pathways that include interleukin-1 (IL1), interleukin-6 (IL6) and tumor-necrosis factor alpha (TNFα) are considered to be among the most potent of all bone-resorbing mechanisms.[5–8] 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.[9,10] 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.[11] Therefore, we previously searched for common variations in this gene locus (*LIF*) as a candidate gene for osteoporosis susceptibility.[10] 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.[9] Targeted disruption of the LIFR gene in mice causes loss of bone density and increasing numbers of osteoclasts.[1] 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.[12] 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 ± 8.6 years (range, 32–69) and 23.7 ± 3.61 kg/cm^2 (range, 14.7–38.5).

The BMD (expressed in g/cm^2) 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).[13,14] The adjustment equation for the study samples was as follows: (adjusted BMD in g/cm^2) = (measured BMD in g/cm^2) $- 0.006375 \times (58.39 -$ [age in years]) $+ 0.008961 \times (23.65 -$ [BMI in kg/cm^2]).

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.[12] 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.[13] AS primers (long and short) have a

Table 1 Summary of examined polymorphism in the LIFR locus

No	SNP name†	JSNP-ID‡	dbSNP§	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

†Location of the SNP was defined by NT_023195.12 from RefSeq; ‡ID number for JSNP database; §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′-CCCAGTGTAAAATCGCCCTTATCG-3′, reverse primer; 5′-GTTTGCTGCTAAGATTACCTATTGTGG-3′. For SNP I633M, long AS-primer; 5′-TTTTTGG TCCCATCCCAACAACTTGCTCT-3′, short AS-primer; 5′-CCTCCCATCCCAACAACTTGTCCC-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 $MgCl_2$, 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.[13]

Statistical analysis

BMD data for each subject were normalized according to age and BMI.[13] Quantitative associations between genotypes and adjusted BMD values (gm/cm^2) 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^2 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^2, calculated for every possible combination of the five SNPs (Fig. 1) LD among these SNPs was evident when D′ > 0.5 and r^2 > 0.15.

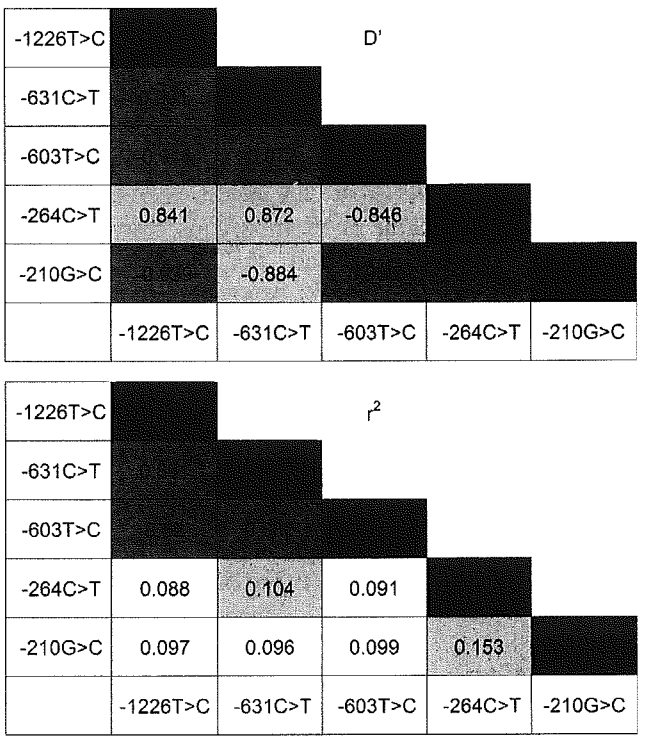

Figure 1 Linkage disequilibrium (LD) analysis found significant linkage disequilibrium within the LIFR locus: Indices of LD, D′ and r^2 based on 17 estimated haplotypes (covering 100% of the chromosomes) constructed with five promoter SNPs were presented in separate tables. D′ greater than 0.4 and r^2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

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

SNP Name	$n^{\dagger}$	Adjusted BMD (g/cm^2) Major homozygous (*n*)	Heterozygous (*n*)	Minor homozygous (*n*)	Correlation coefficient (r)	*P*-value‡
−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

†Number of genotyped subjects; ‡*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)
$n^{\dagger}$	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^2)	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.
†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^2); heterozygous individuals were intermediate (0.394 ± 0.056 g/cm^2); homozygous C-allele carriers had the lowest adjusted BMDs (0.373 ± 0.042 g/cm^2). 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.[1,15] Bone-resorbing effects of cytokine signaling pathways involving IL-1, IL-6, and TNFα have been investigated in vivo and in vitro.[5–8] 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[16] 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.[17] 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

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Introduction

Active vitamin D_3 analogues, including the hormonal form of vitamin D_3 (1,25[OH]$_2$ vitamin D_3) and 1 α OH vitamin D_3 (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_3 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.

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; ≤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 μg/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°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; Boerhinger 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 recorted.

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 ± 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/m²)	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 ± 16.2% at 6 months. The changes in serum BAP in groups D and C at 6 months were significantly different (−21.5 ± 25.6% versus 9.3 ± 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 ± 0.105, 0.167 ± 0.139, 0.158 ± 0.122, and 0.162 ± 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

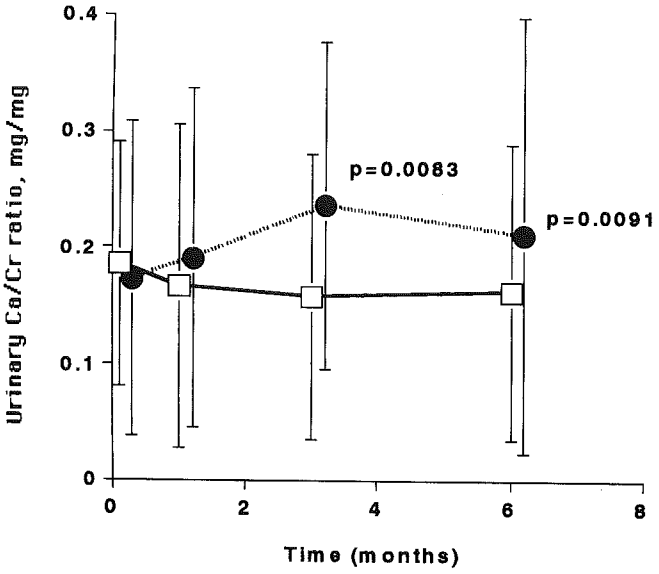

Fig. 1. Effects of alfacalcidol on urinary excretion of calcium. Urinary excretion of calcium in group D was increased by about 60% to 100% from the baseline value, and that in group C was maintained at the baseline value during the study period. Data values are expressed as means ± SD. *Open squares*, 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

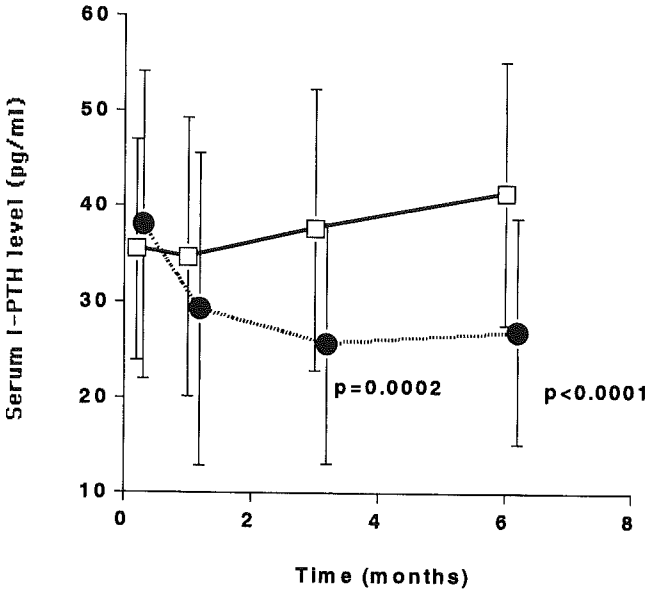

Fig. 2. Effects of alfacalcidol on serum intact parathyroid hormone (*I-PTH*) level. The serum level of I-PTH in group D rapidly decreased, by about 30% from the baseline value and the inhibition of I-PTH secretion was maintained during the observation period, while the level in group C was maintained 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 ± 0.135, 0.191 ± 0.145, 0.236 ± 0.140 (P = 0.0083 versus group C), and 0.271 ± 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 ± 16.1, 29.2 ± 16.3, 25.7 ± 12.6, and 26.9 ± 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 ± 11.5, 34.7 ± 14.6, 37.6 ± 14.8, and 41.4 ± 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 ($r^2 = 0.015$; $P = 0.5273$).

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.

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

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
			NS	NS
	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
			NS	NS
	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