表2　　畜産品摂取に伴う残留農薬の暴露量（理論的一日最大摂取量）；ADI比率順

| 順位 | 暫定基準表No. | 農薬名 | ADI mg/kg/day | 評価国/機関，評価年 カッコ内は当該機関によるADI | 暴露量 (mg/day/人) | | | ADI％比 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 国民全体 | 幼小児 | 妊婦 | 国民全体 | 幼小児 | 妊婦 |
| 144 | 525 | FLUDIOXONIL | 0.033 | JPN | 0.0049 | 0.0041 | 0.0054 | 0.3 | 0.8 | 0.3 |
| 145 | 101 | EMAMECTIN BENZOATE | 0.0025 | JPN | 0.0006 | 0.0003 | 0.0006 | 0.5 | 0.8 | 0.4 |
| 146 | 469 | PYRETHRINS | 0.04 | JPN, JMPR1972; confirmed 1999 | 0.004 | 0.0037 | 0.0032 | 0.2 | 0.6 | 0.1 |
| 147 | 544 | FLUROXYPYR | 0.8 | EU 1999 | 0.0857 | 0.0721 | 0.0969 | 0.2 | 0.6 | 0.2 |
| 148 | 483 | FENOBUCARB | 0.012 | JPN | 0.0012 | 0.0009 | 0.0011 | 0.2 | 0.5 | 0.2 |
| 149 | 430 | HALOSULFURON METHYL | 0.01 | JPN | 0.0012 | 0.0007 | 0.0012 | 0.2 | 0.4 | 0.2 |
| 150 | 460 | PYRIDATE | 0.16 | JPN, EU 2001(0.0036) | 0.0173 | 0.0099 | 0.0182 | 0.2 | 0.4 | 0.2 |
| 151 | 185 | CHLORPHENAPYR | 0.026 | JPN | 0.0029 | 0.0016 | 0.003 | 0.2 | 0.4 | 0.2 |
| 152 | 473 | FAMOXADONE | 0.012 | JPN, JMPR 2003 (0.006) | 0.0012 | 0.0006 | 0.0012 | 0.2 | 0.3 | 0.2 |
| 153 | 244 | DIPHENYLAMINE | 0.08 | JMPR 1998 | 0.0057 | 0.0033 | 0.0061 | 0.1 | 0.3 | 0.1 |
| 154 | 645 | MEPIQUAT-CHLORIDE | 0.6 | EPA 1997 | 0.0169 | 0.0164 | 0.0187 | 0.1 | 0.2 | 0.1 |
| 155 | 34 | AZOXYSTROBIN | 0.18 | JPN | 0.0068 | 0.0048 | 0.0075 | 0.1 | 0.2 | 0.1 |
| 156 | 30 | ACEQUINOCYL | 0.027 | JPN | 0.0011 | 0.0006 | 0.0012 | 0.1 | 0.1 | 0.1 |
| 157 | 90 | ETOXAZOLE | 0.04 | JPN | 0.0011 | 0.0007 | 0.0012 | 0.1 | 0.1 | 0.1 |
| 158 | 563 | PROPOXUR | 0.02 | JMPR 1973 and confirmed 1989 | 0.0006 | 0.0003 | 0.0006 | 0.1 | 0.1 | 0.1 |
| 159 | 559 | PROHEXADIONE-CALCIUM | 0.18 | JPN | 0.0046 | 0.0026 | 0.0048 | 0.0 | 0.1 | 0.0 |
| 160 | 246 | DIFENZOQUAT | 0.2 | JPN, EPA 1994 | 0.0039 | 0.0026 | 0.0038 | 0.0 | 0.1 | 0.0 |
| 161 | 149 | CAPTAN | 0.125 | JPN, JMPR 1984; confirmed in 1990, 1995 (0.1) | 0.0029 | 0.0016 | 0.003 | 0.0 | 0.1 | 0.0 |
| 162 | 158 | KRESOXIM-METHYL | 0.36 | JPN | 0.0053 | 0.0045 | 0.0057 | 0.0 | 0.1 | 0.0 |
| 163 | 462 | PYRIPROXYFEN | 0.07 | JPN | 0.0006 | 0.0003 | 0.0006 | 0.0 | 0.0 | 0.0 |

厚生労働科学研究費補助金（食品の安全性高度化推進研究事業）

Ⅱ．分担研究報告書

# 2．食品からのカドミウム曝露と健康影響

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厚生科学研究費補助金(食品安全性高度化推進研究事業)
分担研究報告書

# 食品からのカドミウム曝露と健康影響

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研究要旨

昨年度調査したEおよびF地域，および全国のトータルダイエットスタディーデータをモンテカルロ法で解析し，Cd暴露の確率論的推定を行ったほか，昨年度と同じF地域において，198名の農家女性受診者を得て現地調査を行った。被験者の血中および尿中のCdを測定して，暴露レベルを調査するとともに，尿中α1-およびβ2-ミクログロブリンの測定等により腎機能への影響等，健康影響を検討した。前年度と比較して尿中Cdの分布は余り異ならないが，血中Cd濃度は低いことから，過去の暴露は同程度であるが，近年は暴露が低下している可能性があると考えられた。腎機能への影響はF地域でもほとんど見られないと考えられた。

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## A. 研究目的

平成15年度に行ったカドミウム（Cd）汚染地域で行ったトータルダイエットスタディで得られた結果を基に、Codex Committee on Food Additives and contaminates (CCFAC) がFAO/WHO Joint Expert Committee on Food Additives (JECFA)に対して要請しているCd摂取の確率論的曝露評価を行った。また、これまでの農場従事者の健康影響調査で、最もCd曝露が高かった地域で、198名の追加調査を行った。この集団はProvisional Tolerable Weekly Intake (PTWI)を越えるCd曝露を受けている被験者が半数近く含まれる集団で、腎機能障害、骨粗鬆症などの健康影響を調査し、より正確な摂取許容量算定に有用なデータを得ることを目的とした。

## B. 研究方法

平成１５年度の地域Ｅおよび地域Ｆで行われたトータルダイエットスタディおよび全国のトータルダイエットスタディのCd曝露推定を確率論的な方法（Monte Carlo simulation）を用いて推計した。方法は、新田らがおこなった方法を用いて、統計解析ソフト、クリスタルボールを用いてMonte Carloシュミレーションを行った。

さらに、これまでの全国８カ所の調査地域の中で、最もCd曝露が高い地域Fにて農家女性の腎機能および骨密度などを主体に、健康影響調査を行った。

## C. 研究結果

平成15年度の地域Fでは農家女性224名が健診に参加した（表１）。健診参加者が持参した平成15年産米中のCd濃度の分布は、1.0μg/g以上は０％、0.4μg/g以上1.0 μg/g未満は９％、0.2μg/g以上0.4 μg/g未満は32%、0.2 μg/g未満は59%であった。今年度の地域Fでの受診者数は198名で、受診者各自の検診時に食べていた白米中のCd濃度の平均は、0.114μg/gであり、その分布は1.0μg/g以上は0%、0.4μg/g以上1.0 μg/g未満は1.5%、0.2μg/g以上0.4μg/g未満は13.6%、0.2 μg/g未満は84.8%であった。同じ地域ではあるが16年度産米中のCd濃度は低いことが明らかとなった（表２）。

この集団での健康影響を詳細に調べることは、現在国内で食品からのCd摂取により健康影響が有るかどうか明らかにすることが出来る。さらに、今年度の調査で、昨年度の調査人数と合わせて地域Fで422名となり、隣接する地域Eと合計すると960名の集団となり、コホートとして追跡調査するには適切な母集団となると考えられる。これまで我々の断面的調査により、Cd摂取による健康影響が出ている根拠は見出だせなった。今後長期にわたり、この1,000名弱の比較的高い曝露を受けている集団を追跡調査することにより、日本国内で食品から摂取されるCdにより健康影響が起こっているかどうかを明らかにすることができると推定できる。

一方、被験者の年齢分布は30-39歳11名、40-49歳44名、50-59歳60名、60-69歳91名、70-79歳33名であった（表１）。

血中Cd濃度は、E地域およびF地域の比較では、全年齢でそれぞれ3.61μg/dl、4.13、40歳代で3.45と3.42、50歳代で3.43と3.75、60歳代で3.93と4.49であった。50歳代まではあまり差は見られないが、60歳代になるとF地域が高かった。尿中Cd濃度は、E地域およびF地域の比較では、全年齢でそれぞれ4.08μg/g creと6.37μg/g cre、40歳代で3.59と4.35、50歳代で4.01と6.11、60歳代で4.50と7.65であった。尿中Cdは、過去の曝露を表していることから、F地域は曝露が長年E地域より高かったことが明らかとなった（表２）。

しかし、平均値で比較すると尿中α1-ミクログロブリンおよびβ2-ミクログロブリン濃度はF地域でE地域より高くはなく、特に対照群のA地域と比較しても大きな差は見られなかった（表４）。この結果から、平均的にはF地域はE地域より高い曝露を長年受けていたにもかかわらず、加齢による変化を調整すれば、明らかな腎機能障害はない結果となった。今年度調査したF地域は、尿中Cdの高い被験者が見られるものの尿中β２ミクログロブリンが高値を示す被験者は見られなかった（表５）。

## D. 考察

E地域とF地域は地理的に隣接し交流はあるが、歴史的文化的には異なる地域である。汚染源はほぼ同様な鉱山活動による土壌汚染が主体である。F地域被験者の持参した米中Cd濃度は、E地域のそれと比較すると、Cd濃度の高い米の頻度が

多少高い程度の差のように見えるが、E地域の米の採取は平成13年米の値であり、平成15年は冷夏のため、水管理が行き届いたため米中Cdは例年になくどの地方でも低いことが報告されている。そのため、今回のF地域の米のCd濃度測定値は例年よりかなり低いことが予想され、これまでの曝露量の推定はさらに高いものと考えられる。それは、長期の曝露量を反映する尿中Cd濃度はかなり高く、平均値で見ると明らかな差はないが、個人データを詳しく解析していけば、過去の高いCd曝露のために腎機能障害がある被験者が少数含まれているものと考えられる。今年度の被験者の中には、腎機脳障害がある人は見られなかった。

まとめ

平成15年度に引き続きF地域で調査を行い、198名の農家女性受診者の健康診断を行った。前年度と比較して尿中Cdの分布はあまり異ならないが、血中Cd濃度が低いことを考えると過去の暴露は同程度であるが近年は暴露が低下している可能性があることが考えられた。腎機能への影響はこの地域Fでもほとんど見られないと考えられる。

E. 健康危険情報

なし

F. 研究発表

1. 論文発表

1) Horiguchi H, Oguma E, Sasaki S, Miyamoto K, Ikeda Y, Machida M, Kayama F., Environmental exposure to cadmium at a level insufficient to induce renal tubular dysfunction does not affect bone density among female Japanese farmers., *Environ Res.* 97(1) : 83-92, 2005

G. 知的財産権の出願・登録状況

(予定も含む)

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

表1.調査対象地域の調査対象者人数とその年齢分布

| | F地域H16年 | F地域 | A地域(前回調査) | E地域(前回調査) |
|---|---|---|---|---|
| All ages | | | | |
| N | 198 | 224 | 187 | 538 |
| AM±ASD | 55.2 | 59.1±9.9 | 57.2±9.2 | 57.4±9.5 |
| Max | 71 | 82 | 75 | 78 |
| Min | 36 | 34 | 33 | 30 |
| 30-39 yr | | | | |
| N | 3 | 6 | 3 | 23 |
| AM±ASD | - | 36.7±2.0 | - | 35.3±3.3 |
| 40-49 yr | | | | |
| N | 41 | 41 | 39 | 88 |
| AM±ASD | 46.2 | 45.7±2.7 | 45.8±2.5 | 45.3±3.0 |
| 50-59 yr | | | | |
| N | 95 | 57 | 58 | 175 |
| AM±ASD | 54.3 | 55.1±2.6 | 53.3±2.5 | 54.6±2.9 |
| 60-69 yr | | | | |
| N | 54 | 88 | 71 | 221 |
| AM±ASD | 63.3 | 64.1±2.9 | 64.5±2.8 | 64.5±2.8 |
| 70-79 yr | | | | |
| N | 5 | 31 | 16 | 31 |
| AM±ASD | - | 73.2±2.4 | 71.4±1.5 | 72.9±2.1 |
| 80-89 yr | | | | |
| N | 0 | 1 | 1 | 1 |
| AM±ASD | – | – | – | – |

表2.血液中、尿中、米中Cd濃度

| | F地域H16 | F地域 | A地域（前回調査） | E地域（前回調査） |
|---|---|---|---|---|
| Cd-B (μg/L) | | | | |
| All ages | 3.27 | 4.19 (1.68) | 2.00 (1.58) | 3.61 (1.63) |
| | (range 0.74-18.8) | (1.44-31.2) | (ND-6.81) | (0.55-13.07) |
| 30-39 yr | 2.08 | 3.06 (1.65) | - | 1.86 (1.66) |
| 40-49 yr | 2.88 | 3.41 (1.50) | 1.82 (1.73) | 3.45 (1.61) |
| 50-59 yr | 3.01 | 3.80 (1.62) | 2.07 (1.59) | 3.43 (1.58) |
| 60-69 yr | 3.98 | 4.50 (1.63) | 2.07 (1.50) | 3.93 (1.57) |
| 70-79 yr | 4.48 | 5.73 (1.89) | 2.03 (1.37) | 4.93 (1.49) |
| Cd-U/Cr (μg/g cr.) | | | | |
| All ages | 5.53 | 6.60 (1.67) | 2.63 (1.74) | 4.08 (1.74) |
| | (range 0.14-17.89) | (1.35-29.66) | (ND-7.93) | (0.51-27.26) |
| 30-39 yr | 4.44 | 4.39 (1.77) | - | 2.32 (1.49) |
| 40-49 yr | 4.60 | 4.29 (1.50) | 2.14 (1.59) | 3.59 (1.75) |
| 50-59 yr | 5.29 | 6.11 (1.64) | 2.53 (1.86) | 4.01 (1.68) |
| 60-69 yr | 6.79 | 7.82 (1.53) | 3.10 (1.65) | 4.50 (1.72) |
| 70-79 yr | 4.98 | 9.01 (1.56) | 2.70 (1.73) | 4.83 (1.77) |
| Cd-R (μg/g) | | | | |
| All ages | 0.114 | 0.141 (2.41) | 0.022 (2.29) | 0.156 (2.01) |
| | (ND-0.669) | (range 0.010-0.687) | (0.010-0.178) | (0.005-0.971) |

平成16年度F地域のデータは算術平均、他のデータは GM (GSD).

表3-1.尿中Cd濃度分布（μg/g cr.）

| | F地域H16 | | F地域 | | A地域（前回調査） | | E地域（前回調査） | |
|---|---|---|---|---|---|---|---|---|
| | | % | N | % | N | % | N | % |
| ＜10 | 172 | 86.9 | 181 | 80.8 | 187 | 100.0 | 511 | 95.0 |
| 10≦、＜20 | 26 | 13.1 | 38 | 17.0 | 0 | 0.0 | 26 | 4.8 |
| 20≦ | 0 | 0.0 | 5 | 2.2 | 0 | 0.0 | 1 | 0.2 |
| Total | 198 | 100.0 | 224 | 100.0 | 187 | 100.0 | 538 | 100.0 |

表3-2.F地域地域における米中Cd濃度分布（μg/g）

| | H16年 | | H15年 | |
|---|---|---|---|---|
| | N | % | N | % |
| ＜0.2 | 168 | 84.8 | 129 | 57.6 |
| 0.2≦、＜0.4 | 27 | 13.6 | 75 | 33.5 |
| 0.4≦ | 3 | 1.5 | 20 | 8.9 |
| Total | 198 | 100.0 | 224 | 100.0 |

表4.腎機能平均値

| | F地域H16 | F地域 | A地域（前回調査） | E地域（前回調査） |
|---|---|---|---|---|
| Urinary α1MG/Cr (mg/g cr.) | | | | |
| All ages | | 4.35 (2.27) | 4.94 (2.00) | 4.75 (2.08) |
| | | (ND-48.56) | (ND-37.33) | (ND-56.04) |
| 30-39 yr | 1.68 | 1.69 (2.15) | - | 2.43 (1.79) |
| 40-49 yr | 4.83 | 2.93 (1.90) | 3.25 (1.86) | 2.95 (1.79) |
| 50-59 yr | 4.62 | 3.30 (2.09) | 4.88 (1.87) | 4.64 (2.03) |
| 60-69 yr | 5.36 | 5.37 (2.02) | 5.88 (1.95) | 5.79 (1.94) |
| 70-79 yr | 8.85 | 8.93 (2.33) | 7.60 (2.04) | 8.59 (2.13) |
| | | | | |
| Urinary ß2MG/Cr (μg/g cr.) | | | | |
| All ages | 133.5 (2.02) | 173.5 (2.89) | 148 (2.41) | 163 (2.32) |
| | | (ND-15331.8) | (ND-9352) | (ND-5689) |
| 30-39 yr | 84.3 | 76.7 (1.44) | - | 94.5 (1.80) |
| 40-49 yr | 157.6 | 95.3 (2.05) | 94 (1.96) | 111 (1.88) |
| 50-59 yr | 158.5 | 145.1 (2.24) | 147 (2.05) | 164 (2.17) |
| 60-69 yr | 196.6 | 202.3 (2.54) | 169 (2.57) | 178 (2.27) |
| 70-79 yr | 298.4 | 482.0 (4.16) | 266.1 (3.20) | 356.2 (3.25) |

F地域H16年は算術平均、他のデータは GM (GSD).

表5.腎機能分布(Urinary β2MG、μg/g cr.)

| | F地域H16 | | F地域 | | A地域(前回調査) | | E地域(前回調査) | |
|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % |
| <300 | 193 | 97.5 | 172 | 77.1 | 152 | 81.3 | 435 | 80.9 |
| 300≦、<1000 | 5 | 2.5 | 37 | 16.6 | 30 | 16.0 | 83 | 15.4 |
| 1000≦、<10000 | 0 | 0.0 | 14 | 6.3 | 5 | 2.7 | 20 | 3.7 |
| 10000≦ | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Total | 198 | 100.0 | 223 | 100.0 | 187 | 100.0 | 538 | 100.0 |

## III. 研究成果の刊行に関する一覧表

雑誌

| 発表者氏名 | 論文タイトル | 雑誌名 | 巻号 | 頁 | 出版年 |
|---|---|---|---|---|---|
| Horiguchi H, Oguma E, Sasaki S, Miyamoto K, Ikeda Y, Machida M, Kayama F | Environmental exposure to cadmium at a level insufficient to induce renal tubular dysfunction does not affect bone density among female Japanese farmers. | Environ Res. | 97 | 83-92 | 2005 |

# IV. 研究成果の刊行物・別刷

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# Environmental exposure to cadmium at a level insufficient to induce renal tubular dysfunction does not affect bone density among female Japanese farmers ☆

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**Abstract**

Some recent research suggests that environmental exposure to cadmium, even at low levels, may increase the risk of osteoporosis, and that the bone demineralization is not just a secondary effect of renal dysfunction induced by high doses of cadmium as previously reported. To investigate the effect of exposure to cadmium at a level insufficient to induce kidney damage on bone mineral density (BMD) and bone metabolism, we conducted health examinations on 1380 female farmers from five districts in Japan who consumed rice contaminated by low-to-moderate levels of cadmium. We collected peripheral blood and urine samples and medical and nutritional information, and measured forearm BMD. Analysis of the data for subjects grouped by urinary cadmium level and age-related menstrual status suggested that cadmium accelerates both the increase of urinary calcium excretion around the time of menopause and the subsequent decrease in bone density after menopause. However, multivariate analyses showed no significant contribution of cadmium to bone density or urinary calcium excretion, indicating that the results mentioned above were confounded by other factors. These results indicate that environmental exposure to cadmium at levels insufficient to induce renal dysfunction does not increase the risk of osteoporosis, strongly supporting the established explanation for bone injury induced by cadmium as a secondary effect.


*Keywords:* Cadmium; Bone; Kidney; Female; Japanese

## 1. Introduction

Cadmium (Cd) is a toxic heavy metal that induces the deterioration of renal tubular function, which can be detected by the increase in urinary excretion of low molecular weight proteins such as $\alpha_1$-microglobulin ($\alpha$1-MG) or $\beta_2$-microglobulin ($\beta$2-MG), after sustained environmental exposure (Friberg et al., 1986; WHO, 1992). The most severe form of chronic intoxication, called "Itai-itai disease," was endemic among female farmers in the heavily Cd-polluted area of the Jinzu River basin in Japan and was characterized by renal tubular dysfunction, renal anemia, and multiple bone fractures due to osteomalacia, which lead to generalized severe pain (the Japanese word "itai" means "ouch") (Yamagata and Shigematsu, 1970; Kasuya et al., 1992a, b; Horiguchi et al., 1994). The mechanism of the bone injury has been acknowledged as an acquired Fanconi's syndrome: the decrease of the bone calcium (Ca) pool is accelerated by the continuous loss of Ca and

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phosphorus into urine and/or by the disturbance of vitamin D metabolism in the kidneys due to renal tubular dysfunction (Ishizaki and Fukushima, 1968; Akiba et al., 1980; Ruth et al., 1981; Nogawa et al., 1987; Aoshima et al., 1993). In other words, Cd primarily damages the kidneys, followed by osteomalacia as a secondary effect. In fact, Itai-itai disease developed only among the large number of the inhabitants with Cd-induced nephropathy, and vitamin D treatment alleviated the osteomalacia (Kasuya et al., 1992a).

However, some recent epidemiological studies in Europe have challenged this accepted mechanism, suggesting that Cd can induce osteoporosis at much lower exposure levels than previously reported (Järup et al., 1998; Staessen et al., 1999; Alfvén et al., 2000). In addition to these human studies, there are some experimental studies to suggest the direct Cd effect on bone (Bhattacharyya et al., 1988; Ogoshi et al., 1992; Miyahara et al., 1992). This implies that bone demineralization could be induced as a primary Cd effect before the occurrence of renal damage. If this were true, it would constitute a major public health problem among the Japanese, who have a higher Cd body burden than most other people do because they consume Cd-contaminated rice as a staple food. The conflicting views have prompted the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to call for additional studies on the impact of lifetime exposure to Cd on the development of osteoporosis (WHO, 2000). Consequently, we undertook a cross-sectional epidemiological study of 1380 female Japanese farmers with dietary exposure to Cd at levels insufficient to induce renal dysfunction in order to investigate the effects of Cd exposure at these levels on bone mineral density (BMD) and bone metabolism.

## 2. Materials and methods

### 2.1. Study populations

We conducted health examinations on female Japanese farmers from five districts of Japan in 2001–2002 as previously described, named "Japanese Multicentered Environmental Toxicants Study" (JMETS) (Horiguchi et al., 2004). Briefly, we investigated 202, 202, 203, 204, and 569 participants—1380 in total—from one non-contaminated district (A) as a control, where highly Cd-contaminated rice has never been detected, and four Cd-contaminated districts (B, C, D, and E), where such rice is often detected, respectively. The participants were thought to have been exposed to the sustained levels of Cd through the consumption of the rice grown in their own fields ever since birth. The geometric means (GMs) of urinary Cd levels (Cd-U) were 2.6, 3.5, 3.2, 3.2, and 4.1 μg/g cr. in the five districts, respectively, with only 1% over the threshold at which Cd induces renal dysfunction (10 μg/g cr.) (Roels et al., 1979; Ikeda et al., 2003). In addition, the GM of urinary β2-MG in the total population was less than 150 μg/g cr., with less than 3% showing $\beta_2$-microglobulinuria (over 1000 μg/g cr. of urinary β2-MG, a cutoff value considered as the threshold of irreversible tubular dysfunction; Teranishi et al., 1992). Thus, the Cd exposure levels of the investigated subjects can be described as "moderate" and insufficient to induce renal dysfunction.

### 2.2. Health examinations

At the examinations, we collected peripheral blood and second-morning urine samples before breakfast and measured the participants' weight, height, grasping power, and BMD. Body mass index (BMI) was calculated by dividing weight (kg) by height (m) squared. The grasping power of a participant's nondominant hand was measured three times with a hand dynamometer, and the highest value was used as an indicator of physical activity. We measured BMD by dual energy X-ray absorption (DXA) in the participant's nondominant forearm with a DTX-200 (Osteometer), which scanned DXA at distal sites of the radius and ulna between the 8 and 24 mm points. Subjects with BMD less than 80% of the young adult mean (YAM) (20–44 years old) were classified as having "decreased BMD" according to the criteria of the Japanese Society for Bone and Mineral Research (The Committee on Standard Criteria of Primary Osteoporosis, 1996).

We obtained medical information about each participant's health status, including present and past medical history, using a self-administered questionnaire. We investigated the intakes of Ca and vitamin D by another questionnaire, named diet history questionnaire (DHQ), which was designed to determine food and nutrient intake levels in the previous month with regard to the quantity and semi-quantitative frequency of consumption of 110 food items commonly consumed in Japan (Sasaki et al., 1998).

### 2.3. Analysis of blood and urine samples

Two urinary proteins, α1-MG and β2-MG, Ca, and creatinine (Cr) in urine were determined by a latex agglutination method, the *o*-cresolphthalein complexone method, and the Jaffé reaction method, respectively, with one drop of sodium hydrogen carbonate solution added to the β2-MG sample just after collection to prevent destruction by low pH (Donaldson et al., 1989). Luteinizing hormone (LH), bone-specific alkaline phosphatase (BAP), and bone Gla protein (BGP) in serum

were measured by immunoradiometric assay (IRMA), ELISA, and IRMA, respectively. *N*-telopeptide cross-linked collagen type 1 (NTx) and deoxypyridinoline (D-Pyr) in urine were determined by ELISA. The heparinized whole blood samples were decomposed with nitric acid by a microwave device, after which Cd concentrations (Cd-B) were measured using HP 4500 series ICP-MS (Yokokawa Analytical Systems). We measured Cd-U by flameless atomic absorption spectrometry, SIMAA 6000 (Perkin–Elmer), after holding urine samples with nitric acid for 24 h. All items, such as plastic bottles, tubes, or syringes, which would be in contact with these samples, were shown before use to be free of any detectable Cd contamination. Metocean Environment Inc. (Shizuoka, Japan) conducted all Cd determinations.

### 2.4. Statistical analysis

We used data of 1243 subjects for the analysis, excluding 137 from the original participants for the following reasons: past or current smoking, 52; chronic renal failure treated with hemodialysis, 1; collagen diseases, 13; spinal caries, 1; oophorectomy, 5; insufficient ovary growth, 1; early menopause, 7; hyperthyroidism, 7; use of oral contraceptives, 4; hormone replacement therapy, 40; steroid hormone therapy, 2; extremely high serum β2-MG, 1; insufficient urine sample volume, 3. When values were less than the limits of detection (blood Cd, 0.4 /μg/L; urinary Cd, 0.3 μg/L; urinary α1-MG, 0.9 mg/L; urinary β2-MG, 70 μg/L), we used half values for statistical calculation. Data that appeared to follow a normal distribution are presented as arithmetic means (AM) and arithmetic standard deviations (ASD). GM and geometric standard deviations (GSD) were used for data with a log-normal distribution, which were also converted into base-10 logarithms before the following statistical analysis. Single regression analysis as well as Bonferroni's multiple comparison procedure following one-way ANOVA were used to test the trend in Cd-U-divided subgroups. The prevalence of subjects with decreased BMD among the subgroups was examined by the $\chi^2$-test, followed by Bonferroni's multiple comparison procedure. In multiple regression models, we selected age, BMI, grip power, Cd-B, Cd-U, α1-MG, β2-MG, Ca-U, and Ca and vitamin D intakes as independent variables. We further added dummy variables for the five districts with district A as a reference into the models. We judged a factor as significant when the standard partial regression coefficient (SPRC) showed a relatively high value with a partial correlation coefficient (PCC) greater than 0.2, since the statistical $P$ value is inclined to produce false positives at higher degrees of freedom (Armitage and Berry, 1994).

## 3. Results

### 3.1. Grouping subjects based on Cd-U and age-related menstrual status

We first divided the population using three cutoff values of Cd-U (2.5, 3.5, and 5.0 μg/g cr.) into four subgroups with similar numbers of participants, although the mean ages increased Cd-U-dependently, reflecting the close relation between age and Cd accumulation (Table 1). Since age and menstrual status could affect bone metabolism, we further divided each Cd-U-based subgroup into four classes by age-related menstrual status: premenopause (41–48 years old), perimenopause (49–55), younger postmenopause (56–65), and older postmenopause (66–75). Subjects less than 41 years of age or more than 75 years of age were excluded because of small numbers. We verified menstrual status using serum LH, the secretion of which increases in response to the decrease of estrogen levels due to menopause. All subjects in the premenopausal class had menses and low LH, but the perimenopausal class included both subjects with and without menstruation, showing higher LH levels with wider ASD. All subjects in both postmenopausal classes had sustained high LH levels and no menstrual periods. As a result, each age class lost difference in mean age between Cd-U-divided subgroups. On the other hand, the increasing trends of Cd exposure, indicated by Cd-B and Cd-U, did not disappear even after age-classification (Table 2). Thus, this grouping method allowed us to observe the effects of Cd exposure not confounded by age and menstrual status.

### 3.2. Cd effect on BMD

We first observed the effects of Cd and age-related menstrual status on BMD (Table 3). The BMD levels declined age-dependently in every Cd-U subgroup, with a notable sudden drop from peri- to postmenopause. Although BMD levels showed a clear, negative correlation with Cd-U when subjects of all ages were considered together, this correlation disappeared when the pre- and perimenopausal groups were considered individually, but remained significant in both postmenopausal groups. These results indicate that Cd exposure might accelerate bone demineralization after menopause, although the effect of aging on the decline of BMD was much stronger than the effect of Cd exposure. We observed a similar, but weaker and statistically nonsignificant, trend in the prevalence of subjects with decreased BMD (Table 4). BMI, however, which is well known to affect BMD, showed significant decreasing trends along with Cd-U after menopause in a very similar pattern to BMD (Table 3), suggesting that the observed Cd effect might be confounded by it.

Table 1
Grouping of the study population by urinary Cd, age, and menstrual status

| Age classes | Urinary Cd (μg/g cr.) | | | | |
|---|---|---|---|---|---|
| | <2.5 | ⩾2.5, <3.5 | ⩾3.5, <5.0 | ⩾5.0 | Total |
| All subjects | | | | | |
| Number | 323 | 272 | 321 | 327 | 1243 |
| Age | 51.9±10.1 | 55.1±8.8* | 58.6±8.4* | 60.1±8.1* | 56.5±9.4 |
| Maximum age | 76 | 76 | 78 | 75 | 78 |
| Minimum age | 30 | 32 | 36 | 36 | 30 |
| Serum LH | 18.2±14.8 | 20.5±13.1 | 23.0±13.1 | 22.8±11.6 | 21.2±13.3 |
| Premenopause (41–48 y.o.) | | | | | |
| Number | 97 | 56 | 35 | 30 | 218 |
| Age | 45.0±2.2 | 45.0±2.3 | 45.5±2.4 | 45.1±2.4 | 45.1±2.3 |
| Serum LH | 9.7±11.7 | 8.7±11.8 | 9.5±15.8 | 8.7±10.1 | 9.3±12.2 |
| Perimenopause (49–55 y.o.) | | | | | |
| Number | 80 | 88 | 82 | 69 | 319 |
| Age | 51.8±1.9 | 52.0±1.8 | 51.9±1.7 | 52.7±2.0 | 52.1±1.9 |
| Serum LH | 27.4±17.1 | 24.2±13.7 | 26.6±15.7 | 26.2±12.1 | 26.0±14.8 |
| Younger postmenopause (56–65 y.o.) | | | | | |
| Number | 68 | 77 | 123 | 129 | 397 |
| Age | 60.8±2.7 | 60.9±2.9 | 61.2±2.8 | 61.1±2.9 | 61.1±2.8 |
| Serum LH | 23.5±8.9 | 25.6±10.0 | 26.1±9.9 | 25.5±11.0 | 25.4±10.1 |
| Older postmenopause (66–75 y.o.) | | | | | |
| Number | 38 | 40 | 73 | 97 | 248 |
| Age | 68.9±2.2 | 68.5±2.6 | 68.7±2.2 | 68.9±2.5 | 68.8±2.4 |
| Serum LH | 24.0±10.3 | 20.9±7.6 | 21.7±7.1 | 21.7±8.4 | 21.9±8.3 |

*Note.* The values of age and serum luteinizing hormone (LH) (mIU/mL) are presented as arithmetic mean±arithmetic standard deviation.
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).

Table 2
Cd concentrations in peripheral blood and urine in subgroups divided by urinary Cd levels and age classes

| Age classes | Urinary Cd (μg/g cr.) | | | | | Regression coefficients |
|---|---|---|---|---|---|---|
| | <2.5 | ⩾2.5, <3.5 | ⩾3.5, <5.0 | ⩾5.0 | Total | |
| Peripheral blood Cd (μg/L) | | | | | | |
| All subjects | 1.72 (1.94) (range 0.62–7.47) | 2.33 (1.69)* (ND-7.89) | 2.71 (1.78)* (ND-8.69) | 3.75 (1.62)* (0.92–13.07) | 2.54 (1.89) (ND-13.07) | 0.394** |
| Premenopause (41–48 y.o.) | 1.57 (1.94) | 2.21 (1.67)* | 3.58 (1.58)* | 4.45 (1.61)* | 2.26 (2.00) | 0.590** |
| Perimenopause (49–55 y.o.) | 1.72 (1.97) | 2.22 (1.80)* | 2.54 (1.70)* | 3.42 (1.54)* | 2.37 (1.85) | 0.348** |
| Younger postmenopause (56–65 y.o.) | 1.85 (2.00) | 2.42 (1.59)* | 2.67 (1.71)* | 3.62 (1.66)* | 2.72 (1.81) | 0.337** |
| Older postmenopause (66–75 y.o.) | 2.12 (1.72) | 2.50 (1.69) | 2.56 (2.04) | 4.02 (1.58)* | 2.96 (1.85) | 0.369** |
| Urinary Cd (μg/g cr.) | | | | | | |
| All subjects | 1.66 (1.46) (range ND-2.50) | 2.98 (1.10)* (2.50–3.50) | 4.17 (1.10)* (3.50–4.98) | 6.75 (1.29)* (5.01–27.26) | 3.46 (1.78) (ND-27.26) | — |
| Premenopause (41–48 y.o.) | 1.57 (1.45) | 3.02 (1.09)* | 4.21 (1.11)* | 6.67 (1.32)* | 2.66 (1.81) | — |
| Perimenopause (49–55 y.o.) | 1.67 (1.37) | 2.97 (1.11)* | 4.11 (1.11)* | 6.49 (1.24)* | 3.31 (1.68) | — |
| Younger postmenopause (56–65 y.o.) | 1.66 (1.60) | 2.94 (1.10)* | 4.18 (1.10)* | 6.78 (1.31)* | 3.90 (1.74) | — |
| Older postmenopause (66–75 y.o.) | 1.86 (1.34) | 3.03 (1.10)* | 4.17 (1.11)* | 6.97 (1.30)* | 4.28 (1.67) | — |

*Note*: The values are presented as geometric mean (geometric standard deviation), and converted into base-10 logarithms for analysis.
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).
**Significant single regression coefficient ($P<0.05$).
ND, not detected (blood Cd, less than 0.4 μg/L; urinary Cd, less than 0.3 μg/L).

Table 3
BMD and BMI in subgroups divided by urinary Cd levels and age classes

| Age classes | Urinary Cd (μg/g cr.) | | | | | Regression coefficients |
|---|---|---|---|---|---|---|
| | <2.5 | ⩾2.5, <3.5 | ⩾3.5, <5.0 | ⩾5.0 | Total | |
| BMD (g/cm$^2$) | | | | | | |
| All subjects | 0.449 (0.078) (range 0.185–0.623) | 0.430 (0.077)* (0.213–0.624) | 0.412 (0.083)* (0.210–0.644) | 0.392 (0.086)* (0.219–0.680) | 0.421 (0.084) (0.185–0.680) | −0.011** |
| Premenopause (41–48 y.o.) | 0.489 (0.051) | 0.484 (0.051) | 0.495 (0.050) | 0.490 (0.052) | 0.489 (0.051) | 0.001 |
| Perimenopause (49–55 y.o.) | 0.466 (0.064) | 0.455 (0.063) | 0.470 (0.065) | 0.460 (0.075) | 0.463 (0.066) | 0.000 |
| Younger postmenopause (56–65 y.o.) | 0.407 (0.078) | 0.399 (0.072) | 0.388 (0.063) | 0.378 (0.070)* | 0.390 (0.070) | −0.006** |
| Older postmenopause (66–75 y.o.) | 0.362 (0.073) | 0.359 (0.069) | 0.349 (0.068) | 0.332 (0.062)* | 0.346 (0.067) | −0.006** |
| BMI | | | | | | |
| All subjects | 23.9 (3.5) (range 16.8–34.9) | 23.7 (3.0) (17.3–32.8) | 23.7 (3.3) (15.7–41.5) | 23.6 (3.2) (15.7–36.7) | 23.7 (3.3) (15.7–41.5) | −0.042 |
| Premenopause (41–48 y.o.) | 23.7 (3.3) | 23.1 (3.1) | 22.6 (3.0) | 23.5 (3.1) | 23.3 (3.2) | −0.033 |
| Perimenopause (49–55 y.o.) | 23.6 (3.3) | 23.1 (3.0) | 23.8 (4.1) | 24.0 (3.1) | 23.6 (3.4) | 0.114 |
| Younger postmenopause (56–65 y.o.) | 24.5 (3.5) | 24.3 (2.8) | 23.8 (3.1) | 23.5 (3.2) | 23.9 (3.2) | −0.205** |
| Older postmenopause (66–75 y.o.) | 25.1 (3.1) | 24.7 (3.1) | 24.1 (2.9) | 23.6 (3.3) | 24.2 (3.2) | −0.289** |

*Note*: The values are presented as arithmetic mean (arithmetic standard deviation).
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).
**Significant single regression coefficient ($P<0.05$).

Table 4
Prevalence of subjects with decreased bone mineral density (<80% of Japanese young adult mean)

| Age classes | Urinary Cd (μg/g cr.) | | | | | | | | | | $P$ value ($\chi^2$ test) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | < 2.5 | | ⩾2.5, <3.5 | | ⩾3.5, <5.0 | | ⩾5.0 | | Total | | |
| | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | |
| All subjects | | | | | | | | | | | |
| Total | 323 | 100.0 | 272 | 100.0 | 321 | 100.0 | 327 | 100.0 | 1243 | 100.0 | 0.000 |
| ⩾80% | 260 | 80.5 | 198 | 72.8 | 211 | 65.7 | 169 | 51.7 | 838 | 67.4 | |
| <80% | 63 | 19.5 | 74 | 27.2 | 110 | 34.3* | 158 | 48.3* | 405 | 32.6 | |
| Premenopause (41–48 y.o.) | | | | | | | | | | | |
| Total | 97 | 100.0 | 56 | 100.0 | 35 | 100.0 | 30 | 100.0 | 218 | 100.0 | 0.782 |
| ⩾80% | 96 | 99.0 | 55 | 98.2 | 35 | 100.0 | 30 | 100.0 | 216 | 99.1 | |
| <80% | 1 | 1.0 | 1 | 1.8 | 0 | 0.0 | 0 | 0.0 | 2 | 0.9 | |
| Perimenopause (49–55 y.o.) | | | | | | | | | | | |
| Total | 80 | 100.0 | 88 | 100.0 | 82 | 100.0 | 69 | 100.0 | 319 | 100.0 | 0.083 |
| ⩾80% | 74 | 92.5 | 76 | 86.4 | 76 | 92.7 | 56 | 81.2 | 282 | 88.4 | |
| <80% | 6 | 7.5 | 12 | 13.6 | 6 | 7.3 | 13 | 18.8 | 37 | 11.6 | |
| Younger postmenopause (56–65 y.o.) | | | | | | | | | | | |
| Total | 68 | 100.0 | 77 | 100.0 | 123 | 100.0 | 129 | 100.0 | 397 | 100.0 | 0.147 |
| ⩾80% | 38 | 55.9 | 42 | 54.5 | 71 | 57.7 | 57 | 44.2 | 208 | 52.4 | |
| <80% | 30 | 44.1 | 35 | 45.5 | 52 | 42.3 | 72 | 55.8 | 189 | 47.6 | |
| Older postmenopause (66–75 y.o.) | | | | | | | | | | | |
| Total | 38 | 100.0 | 40 | 100.0 | 73 | 100.0 | 97 | 100.0 | 248 | 100.0 | 0.272 |
| ⩾80% | 15 | 39.5 | 15 | 37.5 | 24 | 32.9 | 24 | 24.7 | 78 | 31.5 | |
| <80% | 23 | 60.5 | 25 | 62.5 | 49 | 67.1 | 73 | 75.3 | 170 | 68.5 | |

*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).

### 3.3. *Cd effect on bone metabolism*

We next looked into the effect of Cd on bone metabolism (Table 5). Urinary Ca level (Ca-U), which reflects Ca loss from bones, increases significantly along with Cd-U in every age class, with especially notable rises at higher Cd-U levels in perimenopause. The pattern of BAP, an osteogenic marker, was similar to

Table 5
Urinary Ca excretion and markers of bone metabolism in subgroups divided by urinary Cd levels and age classes

| Age classes | Urinary Cd (μg/g cr.) | | | | | Regression coefficients |
|---|---|---|---|---|---|---|
| | <2.5 | ⩾2.5, <3.5 | ⩾3.5, <5.0 | ⩾5.0 | Total | |
| Urinary Ca/Cr (mg/g cr.) | | | | | | |
| All subjects | 102.2 (1.80) (range 9.7–619.4) | 118.1 (1.76)* (12.3–405.9) | 128.9 (1.83)* (12.2–451.7) | 149.7 (1.77)* (10.7–1022.2) | 123.8 (1.82) (9.7–1022.2) | 9.167** |
| Premenopause (41–48 y.o.) | 87.2 (1.67) | 94.7 (1.90) | 98.4 (1.94) | 109.4 (1.65) | 93.7 (1.77) | 4.279** |
| Perimenopause (49–55 y.o.) | 117.2 (1.82) | 122.8 (1.64) | 140.5 (1.66)* | 161.9 (1.66)* | 133.4 (1.71) | 9.725** |
| Younger postmenopause (56–65 y.o.) | 118.5 (1.73) | 126.1 (1.74) | 130.4 (1.89) | 143.9 (1.64) | 131.6 (1.76) | 4.866** |
| Older postmenopause (66–75 y.o.) | 125.3 (1.76) | 144.2 (1.68) | 131.9 (1.82) | 169.8 (1.90)* | 146.5 (1.84) | 7.953** |
| BAP | | | | | | |
| All subjects | 25.0 (1.5) (range 9.0–145.0) | 26.6 (1.4)* (10.1–73.4) | 29.3 (1.4)* (9.1–74.3) | 30.8 (1.4)* (9.8–81.1) | 27.9 (1.45) (9.0–145) | 1.165** |
| Premenopause (41–48 y.o.) | 20.0 (1.4) | 19.7 (1.4) | 19.5 (1.4) | 19.2 (1.3) | 19.7 (1.4) | −0.157** |
| Perimenopause (49–55 y.o.) | 27.2 (1.4) | 26.8 (1.4) | 28.8 (1.4) | 28.7 (1.4) | 27.8 (1.4) | 0.380 |
| Younger postmenopause (56–65 y.o.) | 31.9 (1.4) | 30.6 (1.4) | 32.0 (1.4) | 32.9 (1.3) | 32.0 (1.4) | 0.291 |
| Older postmenopause (66–75 y.o.) | 31.5 (1.3) | 32.4 (1.4) | 32.6 (1.3) | 34.6 (1.4) | 33.2 (1.4) | 0.593** |
| Urinary NTx (nmol/mmol cr.) | | | | | | |
| All subjects | 42.3 (1.7) (range 12.2–552.0) | 48.7 (1.6)* (13.6–175.0) | 56.0 (1.6)* (8.8–220.0) | 61.7 (1.6)* (14.4–200.0) | 51.8 (1.7) (8.8–552.0) | 3.803** |
| Premenopause (41–48 y.o.) | 31.0 (1.5) | 33.2 (1.4) | 31.0 (1.6) | 31.8 (1.6) | 31.7 (1.5) | 0.016 |
| Perimenopause (49–55 y.o.) | 48.6 (1.5) | 50.0 (1.6) | 56.6 (1.6)* | 65.8 (1.6)* | 54.4 (1.6) | 3.782** |
| Younger postmenopause (56–65 y.o.) | 57.5 (1.6) | 57.2 (1.5) | 62.0 (1.5) | 66.1 (1.5)* | 61.5 (1.5) | 1.859** |
| Older postmenopause (66–75 y.o.) | 55.1 (1.5) | 63.3 (1.4) | 63.8 (1.5) | 67.3 (1.5) | 63.6 (1.5) | 2.048 |

*Note*: Cr, creatinine; BAP, bone-specific alkaline phosphatase; NTx, *N*-telopeptide cross-linked collagen type 1. Data are presented as geometric mean (geometric standard deviation), and converted into base-10 logarithms for analysis.
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).
**Significant single regression coefficient ($P<0.05$).

that of BMD, whereas NTx, an osteoclastic marker, corresponded to that of Ca-U. Other markers, BGP and D-Pyr, did not show any significant increasing trends with Cd-U (not shown). Thus, Cd might accelerate urinary Ca excretion and the subsequent decrease of BMD due to high bone turnover after menopause. However, urinary α1-MG and β2-MG showed the increasing trends along with Cd-U in the parallel patterns with Ca-U, although the levels were much less than the threshold of irreversible renal tubular dysfunction (Table 6). These results suggest that the increase of Ca-U by Cd exposure observed in the grouping analyses might in fact be due to the decreased renal tubular reabsorptional ability.

### 3.4. Multivariate analyses

Since these results suggest that the grouping analyses could not exclude confounding factors sufficiently, we performed multivariate analyses for BMD and Ca-U using possible confounding factors as well as Cd exposure as independent variables. Because of collinearity, we considered it inappropriate to include both Cd-B and Cd-U as markers for Cd exposure or both urinary α1-MG and β2-MG as indicators of renal tubular function in the same model. We therefore made four multiple regression models, each including one of the markers for Cd exposure and one of the indicators of renal tubular function, as shown in Table 7. In every model on BMD, age and BMI were the first and second significant factors, respectively, but neither Cd-B nor Cd-U was significant. Multiple logistic regression models also did not indicate any significant contribution of Cd exposure to decreased BMD (Table 8). These results indicate that Cd exposure would have no actual contribution to BMD, suggesting that the Cd effect on BMD observed in the grouping analyses is confounded by other factors. On the other hand, the multiple regression models on Ca-U revealed significance only for α1-MG and β2-MG (Table 7), indicating that the observed Cd-U-dependent Ca-U increase was confounded strongly by renal tubular function.

## 4. Discussion

The grouping analyses allowed us to see the effect of Cd on bones independent of age and menstrual status, but other possible confounding factors could not be excluded sufficiently. This indicates the necessity of multivariate analyses to explore the real causes of osteoporosis in our population. We deliberately made

Table 6
Urinary proteins in subgroups divided by urinary Cd levels and age classes

| Age classes | Urinary Cd (μg/g cr.) | | | | | Regression coefficients |
|---|---|---|---|---|---|---|
| | <2.5 | ⩾2.5, <3.5 | ⩾3.5, <5.0 | ⩾5.0 | Total | |
| Urinary α1-MG/Cr (mg/g cr.) | | | | | | |
| All subjects | 3.68 (1.95) (range ND-30.72) | 4.39 (1.91)* (ND-19.35) | 5.17 (1.93)* (ND-37.33) | 5.74 (1.94)* (0.86–56.04) | 4.70 (1.97) (ND-56.04) | 0.403** |
| Premenopause (41–48 y.o.) | 3.07 (1.78) | 3.52 (1.88) | 3.04 (1.84) | 3.44 (1.59) | 3.23 (1.79) | 0.047 |
| Perimenopause (49–55 y.o.) | 4.05 (1.95) | 4.15 (1.85) | 4.32 (1.73) | 4.82 (1.96) | 4.30 (1.87) | 0.166** |
| Younger postmenopause (56–65 y.o.) | 4.47 (1.85) | 4.89 (1.85) | 5.63 (1.86)* | 5.87 (1.86)* | 5.34 (1.87) | 0.279 |
| Older postmenopause (66–75 y.o.) | 5.30 (1.85) | 6.28 (1.91) | 7.01 (1.89)* | 7.48 (1.89)* | 6.76 (1.90) | 0.403 |
| Urinary β2-MG/Cr (μg/g cr.) | | | | | | |
| All subjects | 118.5 (1.93) (range ND-1235.77) | 133.5 (2.11) (ND-1555.56) | 153.3 (2.39)* (ND-9352.03) | 187.9 (2.43)* (ND-5911.11) | 146.7 (2.26) (ND-9352.03) | 13.840** |
| Premenopause (41–48 y.o.) | 101.9 (1.84) | 112.2 (2.06) | 99.7 (1.85) | 114.0 (1.84) | 105.7 (1.90) | 1.749 |
| Perimenopause (49–55 y.o.) | 126.6 (1.81) | 131.6 (2.07) | 123.0 (1.93) | 159.8 (2.09) | 133.6 (1.98) | 6.654 |
| Younger postmenopause (56–65 y.o.) | 132.9 (2.02) | 128.7 (2.09) | 164.6 (2.20) | 174.6 (2.14)* | 154.2 (2.15) | 9.348 |
| Older postmenopause (66–75 y.o.) | 173.5 (2.16) | 195.2 (2.25) | 202.3 (3.11) | 279.1 (2.85)* | 222.8 (2.76) | 20.622** |

*Note*: α1-MG, $\alpha_1$-microglobulin; β2-MG, $\beta_2$-microglobulin; Cr, creatinine. Data are presented as geometric mean (geometric standard deviation), and converted into base-10 logarithms for analysis.
*Significantly different from the value in the lowest urinary Cd group (<2.5 μg/g cr.) judged by multiple comparison ($P<0.05$).
**Significant single regression coefficient ($P<0.05$).
ND, not detected (α1-MG, less than 0.9 mg/L; β2-MG, less than 70 μg/L).

Table 7
Multiple regression analyses on BMD and urinary Ca excretion ($n = 1243$)

| Dependent variable | Independent variables | Model 1 | | | Model 2 | | | Model 3 | | | Model 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SPRC | PCC | *P* value | SPRC | PCC | *P* value | SPRC | PCC | *P* value | SPRC | PCC | *P* value |
| BMD | Age | −0.573 | −0.541 | 0.000 | −0.582 | −0.556 | 0.000 | −0.577 | −0.540 | 0.000 | −0.586 | −0.553 | 0.000 |
| | BMI | 0.286 | 0.367 | 0.000 | 0.287 | 0.368 | 0.000 | 0.286 | 0.367 | 0.000 | 0.288 | 0.368 | 0.000 |
| | Grip | 0.106 | 0.129 | 0.000 | 0.108 | 0.131 | 0.000 | 0.106 | 0.129 | 0.000 | 0.108 | 0.131 | 0.000 |
| | Cd-B* | −0.004 | −0.005 | 0.869 | −0.004 | −0.005 | 0.867 | | | | | | |
| | Cd-U/Cr* | | | | | | | 0.015 | 0.019 | 0.509 | 0.012 | 0.015 | 0.601 |
| | α1-MG/Cr* | −0.045 | −0.057 | 0.044 | | | | −0.047 | −0.059 | 0.038 | | | |
| | β2-MG/Cr* | | | | −0.030 | −0.038 | 0.180 | | | | −0.031 | −0.039 | 0.167 |
| | Ca-U/Cr* | −0.087 | −0.115 | 0.000 | −0.088 | −0.114 | 0.000 | −0.089 | −0.117 | 0.000 | −0.090 | −0.116 | 0.000 |
| | Ca intake/E | −0.001 | −0.001 | 0.976 | −0.001 | −0.001 | 0.964 | −0.001 | −0.001 | 0.960 | −0.001 | −0.002 | 0.951 |
| | VD intake/E | 0.020 | 0.027 | 0.337 | 0.019 | 0.026 | 0.359 | 0.021 | 0.028 | 0.325 | 0.020 | 0.027 | 0.349 |
| | District B | −0.069 | −0.073 | 0.011 | −0.070 | −0.073 | 0.010 | −0.071 | −0.074 | 0.009 | −0.071 | −0.074 | 0.009 |
| | District C | −0.056 | −0.059 | 0.039 | −0.055 | −0.058 | 0.041 | −0.058 | −0.061 | 0.031 | −0.057 | −0.060 | 0.035 |
| | District D | −0.063 | −0.065 | 0.022 | −0.064 | −0.065 | 0.022 | −0.065 | −0.067 | 0.019 | −0.065 | −0.067 | 0.019 |
| | District E | −0.133 | −0.117 | 0.000 | −0.130 | −0.114 | 0.000 | −0.140 | −0.125 | 0.000 | −0.136 | −0.121 | 0.000 |
| | | $R' = 0.717$ | | | $R' = 0.717$ | | | $R' = 0.717$ | | | $R' = 0.717$ | | |
| Ca-U/Cr* | Age | 0.116 | 0.098 | 0.001 | 0.114 | 0.101 | 0.000 | 0.108 | 0.090 | 0.002 | 0.104 | 0.090 | 0.002 |
| | BMI | 0.051 | 0.053 | 0.063 | 0.047 | 0.050 | 0.081 | 0.051 | 0.053 | 0.063 | 0.047 | 0.050 | 0.079 |
| | Grip | −0.085 | −0.078 | 0.006 | −0.089 | −0.083 | 0.003 | −0.077 | −0.071 | 0.013 | −0.080 | −0.075 | 0.008 |
| | Cd-B* | 0.100 | 0.092 | 0.001 | 0.094 | 0.088 | 0.002 | | | | | | |
| | Cd-U/Cr* | | | | | | | 0.095 | 0.093 | 0.001 | 0.094 | 0.093 | 0.001 |
| | α1-MG/Cr* | 0.216 | 0.207 | 0.000 | | | | 0.206 | 0.196 | 0.000 | | | |
| | β2-MG/Cr* | | | | 0.276 | 0.274 | 0.000 | | | | 0.270 | 0.267 | 0.000 |
| | Ca intake/E | 0.031 | 0.031 | 0.271 | 0.042 | 0.043 | 0.130 | 0.029 | 0.030 | 0.300 | 0.040 | 0.041 | 0.148 |
| | VD intake/E | 0.009 | 0.009 | 0.742 | 0.021 | 0.022 | 0.440 | 0.008 | 0.008 | 0.787 | 0.020 | 0.020 | 0.476 |
| | District B | 0.139 | 0.110 | 0.000 | 0.145 | 0.117 | 0.000 | 0.118 | 0.093 | 0.001 | 0.125 | 0.100 | 0.000 |
| | District C | 0.074 | 0.058 | 0.040 | 0.080 | 0.065 | 0.023 | 0.073 | 0.058 | 0.041 | 0.080 | 0.064 | 0.024 |
| | District D | 0.126 | 0.098 | 0.001 | 0.130 | 0.103 | 0.000 | 0.098 | 0.076 | 0.008 | 0.103 | 0.081 | 0.005 |
| | District E | 0.169 | 0.112 | 0.000 | 0.153 | 0.103 | 0.000 | 0.178 | 0.120 | 0.000 | 0.160 | 0.110 | 0.000 |
| | | $R' = 0.376$ | | | $R' = 0.413$ | | | $R' = 0.376$ | | | $R' = 0.414$ | | |

*Note*: SPRC, standard partial regression coefficient; PCC, partial correlation coefficient; $R'$, multiple correlation coefficients adjusted for the degrees of freedom; BMD, bone mineral density; BMI, body mass index; Cd-B, blood Cd level; Cd-U, urinary Cd level; α1-MG, urinary α1-microglobulin; β2-MG, urinary β2-microglobulin; Ca-U, urinary Ca level; Cr, creatinine; Ca intake/E, energy adjusted calcium intake; VD intake/E, energy adjusted vitamin D intake. The SPRCs of the four districts (B, C, D, and E) represent contrasts between each district and a reference area, district A.
*Converted into base-10 logarithms for analysis.

Table 8
Multiple logistic regression analysis on the subjects with reduced BMD (the subject number is 1243)

| Dependent variable | Independent variables | Model 1 | | | Model 2 | | | Model 3 | | | Model 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SPRC | *P* value | OR (95% CI) | SPRC | *P* value | OR (95% CI) | SPRC | *P* value | OR (95% CI) | SPRC | *P* value | OR (95% CI) |
| The subjects with reduced BMD | Age | 1.856 | 0.000 | 1.218 (1.185–1.251) | 1.879 | 0.000 | 1.220 (1.188–1.254) | 1.867 | 0.000 | 1.219 (1.186–1.252) | 1.888 | 0.000 | 1.222 (1.189–1.255) |
| | BMI | −0.672 | 0.000 | 0.814 (0.769–0.862) | −0.675 | 0.000 | 0.813 (0.768–0.861) | −0.669 | 0.000 | 0.815 (0.770–0.862) | −0.672 | 0.000 | 0.814 (0.769–0.862) |
| | Grip | −0.259 | 0.008 | 0.949 (0.913–0.986) | −0.264 | 0.007 | 0.948 (0.912–0.985) | −0.256 | 0.008 | 0.949 (0.913–0.987) | −0.261 | 0.007 | 0.949 (0.913–0.986) |
| | Cd-B | 0.097 | 0.305 | 1.058 (0.950–1.178) | 0.099 | 0.297 | 1.059 (0.951–1.179) | | | | | | |
| | Cd-U/Cr | | | | | | | 0.021 | 0.795 | 1.009 (0.942–1.080) | 0.028 | 0.725 | 1.012 (0.946–1.083) |
| | α 1-MG/Cr | 0.022 | 0.782 | 1.005 (0.971–1.040) | | | | 0.020 | 0.808 | 1.004 (0.970–1.040) | | | |
| | β 2-MG/Cr | | | | −0.080 | 0.313 | 1.000 (0.999–1.000) | | | | −0.080 | 0.311 | 1.000 (0.999–1.000) |
| | Ca-U/Cr | 0.256 | 0.003 | 1.003 (1.001–1.005) | 0.282 | 0.001 | 1.003 (1.001–1.005) | 0.259 | 0.002 | 1.003 (1.001–1.005) | 0.284 | 0.001 | 1.003 (1.001–1.005) |
| | Ca intake/E | −0.001 | 0.990 | 0.990 (0.212–4.614) | −0.008 | 0.926 | 0.930 (0.200–4.329) | −0.005 | 0.951 | 0.953 (0.205–4.425) | −0.012 | 0.887 | 0.895 (0.193–4.153) |
| | VD intake/E | −0.025 | 0.758 | 0.740 (0.109–5.036) | −0.030 | 0.709 | 0.693 (0.101–4.755) | −0.027 | 0.737 | 0.720 (0.105–4.909) | −0.032 | 0.694 | 0.679 (0.099–4.663) |
| | District B | 0.185 | 0.091 | 1.668 (0.922–3.018) | 0.175 | 0.108 | 1.626 (0.899–2.941) | 0.178 | 0.104 | 1.639 (0.903–2.975) | 0.168 | 0.125 | 1.595 (0.879–2.895) |
| | District C | 0.182 | 0.105 | 1.661 (0.899–3.066) | 0.169 | 0.132 | 1.603 (0.868–2.963) | 0.192 | 0.087 | 1.709 (0.926–3.155) | 0.179 | 0.110 | 1.648 (0.892–3.044) |
| | District D | 0.187 | 0.114 | 1.688 (0.882–3.229) | 0.176 | 0.138 | 1.636 (0.854–3.131) | 0.183 | 0.124 | 1.670 (0.869–3.211) | 0.171 | 0.151 | 1.615 (0.839–3.106) |
| | District E | 0.417 | 0.002 | 2.336 (1.363–4.002) | 0.405 | 0.003 | 2.280 (1.331–3.908) | 0.460 | 0.000 | 2.553 (1.525–4.274) | 0.447 | 0.001 | 2.485 (1.486–4.157) |
| Correlation ratio ($R^2$) | | | 0.413 | | | 0.413 | | | 0.411 | | | 0.412 | |

*Note*: SPRC, standard partial regression coefficient; OR, Odd's ratio; YAM, Japanese youg adult mean; BMD, bone mineral density; BMI, body mass index; Cd-B, blood Cd level; Cd-U, urinary Cd level; α-MG, urinary α1-microglobulin; β2-MG, urinary β2-microglobulin; Ca-U, urinary Ca level; Cr, creatinine; Ca intake/E, energy adjusted calcium intake; VD intake/E, energy adjusted vitamin D intake. The dependent variable is divided into two groups, reduced BMD (<80%) and normal BMD (≥80%). The SPRCs and ORs of the four districts (B, C, D and E) represent contrasts between each district and a reference area, district A.