Effect of natural menopause on serum levels of IGF-I and IGF-binding proteins: relationship with bone mineral density and lipid metabolism in perimenopausal women

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Abstract
The present study was performed to examine the effect of natural menopause on serum levels of IGF-I, IGF-binding protein (IGFBP)-2 and -3 as well as on bone mass and lipid metabolism in perimenopausal women. One hundred and twenty-one healthy Japanese women, who were 45–55 years old, were studied (71 premenopausal and 50 postmenopausal women 1–9 years after menopause). Bone mineral density (BMD) was measured at the middle third of the radius by using dual energy X-ray absorptiometry. Serum levels of IGF-I, but not those of IGFBP-2 or -3, were significantly reduced in the postmenopausal group compared with the premenopausal group. One year after menopause, serum IGF-I levels were significantly lower, and the biochemical markers of bone turnover, such as serum total alkaline phosphatase level and urinary calcium to creatinine ratio, were significantly higher than the premenopausal levels. Serum levels of IGF-I, but not those of IGFBP-2 or -3, were positively correlated with BMD. Serum levels of IGFBP-2, but not those of IGF-I or IGFBP-3, were negatively correlated with body mass index and body weight. Finally, serum levels of IGFBP-3, but not those of IGF-I, were positively correlated with serum levels of total cholesterol and triglyceride. The present findings suggest that a rapid decrease in serum IGF-I levels after menopause might be partly involved in bone loss following gonadal failure and that IGFBP-2 and -3 might be related to the regulation of body mass and lipid metabolism during perimenopause respectively, although the mechanisms remain unknown.

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Introduction
It has been established (1, 2) that menopause causes marked changes in bone and lipid metabolism, but its exact mechanisms remain unclear. Insulin-like growth factor (IGF)-1 partly mediates the action of growth hormone (GH) (3), and IGF-I is an endocrine factor as well as a local anabolic factor for bone tissue (4, 5). In the circulation, IGF-I is bound to the various kinds of IGF-binding proteins (IGFBPs), mainly to IGFBP-3, with the remaining IGF-I bound to IGFBP-2 or IGFBP-1 (6). In the process of aging, the loss of GH secretion regulates the decrease in serum IGF-I and IGFBP-3 levels (7–12), although data on the perimenopause are not available. Corpas et al. (12) and Johansson et al. (13) have reported that these reductions might be involved in the age-dependent loss of bone mass. On the other hand, there is evidence that the serum IGF-I level is negatively correlated with body mass index (BMI) and low-density lipoprotein cholesterol (14, 15) and that the serum IGFBP-2 level is related to nutritional state (16). It remains unclear, however, whether the changes in circulating IGF-I and/or IGFBPs levels are related to the alterations in bone and lipid metabolism that occur during perimenopause. In the present study, we examined how menopause affects serum levels of IGF-I and IGFBPs, and investigated the possible role of IGF-I and IGFBPs in the regulation of bone and lipid metabolism in the perimenopausal state.

Subjects and methods
Subjects
One hundred and twenty-one healthy Japanese women aged 45–55 years who visited the Kobe West Public Health Center for bone mass examination agreed to participate in this study and gave their informed consent. They all underwent a complete physical
examination, and their health history was documented. None of the subjects had liver or renal dysfunction, nutritional derangement or metabolic diseases which might cause changes in bone mass and/or body composition. None of the subjects was taking drugs known to influence bone and lipid metabolism, including estrogen-replacement therapy.

They were divided into two groups according to their gonadal functional status. One group consisted of 71 premenopausal women with menses. The other group was made up of 50 postmenopausal women who had been without spontaneous menses for 1 to 9 years before the beginning of the study. Serum levels of estradiol in the postmenopausal group were all less than 20 pg/ml.

**Bone mineral density (BMD) and biochemical measurements**

BMD was measured at the middle third of the radius using dual-energy X-ray absorptiometry (DCS-600; Aloca Inc., Tokyo, Japan). The same operator tested all the women during the study to eliminate operator bias. The coefficient of variation (C.V.) of the precision of our measurements was 1-0%.

Blood samples were collected after overnight fasting from all subjects. Serum IGF-I levels were measured by RIA after acid-ethanol extraction (17, 18). The intra- and interassay C.V. values were 2-0 to 6-4% and 3-6 to 6-4% respectively. The minimal detection limit was 6-3 ng/ml. Serum IGFBP-3 levels were also measured by RIA (19). The intra- and interassay C.V. values were 5-3 to 6-7% and 4-2 to 8-3% respectively. The minimal detection limit was 0-06 µg/ml. Serum IGFBP-2 levels were also measured by RIA. One hundred microliters standard or sample, and 100 µl anti-bovine IGFBP-2 antibody (1:4000) were mixed and incubated for 48 h at 4°C and 100 µl 125I-human IGFBP-2 were then added. After incubation for 24 h at 4°C, 100 µl goat anti-rabbit serum and 100 µl normal rabbit serum were added. They were incubated for 6 h at 4°C and centrifuged at 3000 r.p.m. for 30 min at 4°C. Precipitates were counted in a γ-counter (ARC-1000; Aloca Inc.). The intra- and interassay C.V. values were 2-7 to 4-7% and 3-4 to 9-3% respectively. The minimal detection limit was 5-0 ng/ml. All assays of serum levels of IGF-I, IGFBP-2 and -3 were run at the same time and at random without distinction between pre- and postmenopausal groups.

Body weight (BW) and body height (BH) were measured, and BMI was calculated by dividing BW (kg) by BH squared (m²).

**Statistical analysis**

The data are expressed as means ± S.E.M. Comparison between the two groups was performed using Mann–Whitney’s U-test for unpaired data. Regression analysis was performed using the statistical computer program Statview (Abacus Concepts Inc., Berkeley, CA, USA). Simple regression analysis was used to assess the linear relationship between study parameters, and Pearson’s correlation coefficients were then calculated. In some cases, multiple regression analyses were also employed. P<0-05 was considered significant.

**Results**

**BMD and biochemical data of perimenopausal women**

Table 1 shows the background data of all the subjects examined. The age of the postmenopausal women was higher than that of the premenopausal women. BMD was significantly lower in the postmenopausal group. On the other hand, serum levels of total alkaline phosphatase (ALP), urinary calcium (Ca) to creatinine (Cr) ratio, and serum levels of Ca and phosphorus (P) were significantly higher in the postmenopausal group. As for lipid metabolism, serum total cholesterol (T-cho) levels were significantly higher in the postmenopausal group.

**Correlation between serum levels of IGF-I, IGFBP-2 and IGFBP-3**

As shown in Fig. 1, serum IGF-I levels were significantly lower in the postmenopausal group compared with the premenopausal group, but there was no significant

| Table 1 BMD and biochemical data in healthy perimenopausal women. Values are means ± S.E.M. |
|---------------------------------|-----------------|-----------------|
|                                | Premenopausal (n = 71) | Postmenopausal (n = 50) |
| Age (years)                    | 47.9 ± 0.3       | 52.3 ± 0.3**    |
| Height (cm)                    | 154.5 ± 0.5      | 153.1 ± 0.7     |
| Weight (kg)                    | 52.9 ± 0.9       | 52.2 ± 0.9      |
| BMI (kg/m²)                    | 22.1 ± 0.3       | 22.3 ± 0.3      |
| BMD (g/cm²)                    | 0.63 ± 0.01      | 0.59 ± 0.01*    |
| Serum                          |                 |                 |
| IGF-I (ng/ml)                  | 197 ± 7          | 168 ± 9*        |
| IGFBP-2 (ng/ml)                | 412 ± 15         | 406 ± 16        |
| IGFBP-3 (µg/ml)                | 2.98 ± 0.06      | 3.07 ± 0.08     |
| Ca (mg/dl)                     | 8.83 ± 0.04      | 9.03 ± 0.04*    |
| P (mg/dl)                      | 3.21 ± 0.04      | 3.43 ± 0.06*    |
| ALP (KA-u)                     | 5.89 ± 0.20      | 7.14 ± 0.16**   |
| GPT (IU/l)                     | 14.7 ± 0.7       | 16.9 ± 1.0      |
| Cr (mg/dl)                     | 0.55 ± 0.01      | 0.57 ± 0.01     |
| T-cho (mg/dl)                  | 210 ± 4          | 226 ± 4*        |
| TG (mg/dl)                     | 95.6 ± 5.4       | 107 ± 7         |
| HDL cho (mg/dl)                | 68.0 ± 1.9       | 69.8 ± 2.4      |
| Urinary Ca/Urinary Cr          | 0.18 ± 0.01      | 0.23 ± 0.01**   |

KA-u, King-Armstrong unit.
* P<0.01, ** P<0.001.
Figure 1 Serum IGF-I and IGFBP levels in healthy perimenopausal women. All values are expressed as means ± s.e.m. PRE, premenopausal group; POST, postmenopausal group; N.S., not significant.

difference in serum IGFBP-2 and -3 levels between the two groups. Figure 2 shows the relationships between serum IGF-I and IGFBPs levels in the perimenopausal women. Serum IGFBP-3 levels were positively correlated with serum IGF-I levels, but negatively correlated with serum IGFBP-2 levels. Serum IGFBP-2 levels were also negatively correlated with serum IGF-I levels. These correlations were also significant, even when analysis was done within the two subpopulations of women (data not shown).

Figure 2 Relationships between serum IGF-I and IGFBP levels in healthy perimenopausal women. Premenopausal women (○); postmenopausal women (●).
**Figure 3** Relationships between BMD and serum IGF-I or IGFBP levels in healthy perimenopausal women. Premenopausal women (○); postmenopausal women (●).

**Figure 4** BMD, serum IGF-I, ALP levels and urinary Ca to Cr ratio versus years since menopause in healthy perimenopausal women. Comparison between premenopausal women (PRE; \( n = 71 \)) and women 1 year after menopause (\( n = 19 \)) was performed. All values are expressed as means ± S.E.M. N.S., not significant; \( u \), urinary.
Correlation of serum IGF-I level to bone mass

Because the serum IGF-I level is reduced after menopause, the possibility has been raised that a decrease in the serum IGF-I level might be partly involved in the bone loss that can occur following gonadal failure. As shown in Fig. 3, serum levels of IGF-I, but not those of IGFBP-2 or -3, were positively correlated with BMD. Multiple regression analysis was employed to explore further whether an age-independent correlation exists between BMD and serum IGF-I levels. Serum IGF-I levels were still positively correlated with BMD even if age was taken into account \((P = 0.03)\). Figure 4 shows the annual changes after menopause in BMD, serum IGF-I levels, and markers of bone turnover such as serum ALP levels and urinary Ca to Cr ratio. One year after menopause, serum ALP levels and urinary Ca to Cr ratio were significantly higher, as compared with those of the premenopausal group. Serum IGF-I levels were also significantly reduced 1 year after menopause and the subsequent IGF-I level was stable during the following years. On the other hand, no significant difference was found in BMD 1 year after menopause, as compared with the premenopausal value. Since there were relatively few subjects \((n = 19)\) who were in their first year of postmenopause, subjects \((n = 30)\) who were in their first and second year of postmenopause were also analyzed. Serum IGF-I levels were also significantly lower, and serum ALP levels as well as urinary Ca to Cr ratio were also significantly higher in these women, as compared with those in the premenopausal women (data not shown).

Correlations of serum IGF-I and IGFBPs levels to body mass and lipid metabolism

Figure 5 shows the relationships between body mass and serum levels of IGF-I or IGFBPs. Serum IGFBP-2 levels were negatively correlated with BMI and BW, while no significant difference was found between these indices of body mass and serum levels of IGF-I or IGFBP-3. These correlations between serum IGFBP-2 levels and indices of body mass were also significant, even when analysis was done within the two subpopulations of women (data not shown). Multiple regression analysis also revealed that serum IGFBP-2 levels were still negatively correlated with...

**Figure 5** Relationships between the indices of body mass and serum IGF-I or IGFBP levels in healthy perimenopausal women. Premenopausal women (○); postmenopausal women (●).
BMI ($P < 0.001$) or BW ($P < 0.001$), even if age was taken into account. We also analyzed these relationships in the subjects ($n = 90$) whose BMI was within normal limits (19 to 24 kg/m$^2$). Significant negative correlations were also found in these subjects (serum IGFBP-2 level vs BMI: $r = -0.325$, $P = 0.0018$; serum IGFBP-2 level vs BW: $r = -0.242$, $P = 0.0215$).

As shown in Fig. 6, serum IGFBP-3 levels were positively correlated with serum T-cho levels, while no significant difference was found between serum levels of T-cho and IGF-I or IGFBP-2. Multiple regression analysis revealed that the serum IGFBP-3 level was correlated with the serum T-cho level, even if age was taken into account ($P = 0.02$). Serum IGFBP-3 levels were also positively correlated with serum triglyceride (TG) level. Multiple regression analysis also revealed that serum IGFBP-3 levels were still positively correlated with TG, even if age was taken into account ($P < 0.001$). The negative correlation between serum IGFBP-2 and TG levels was also significant, but this correlation was weaker, as compared with that between serum IGFBP-3 and TG levels. This correlation between serum IGFBP-3 and TG levels, but not that between serum IGFBP-3 and T-cho levels, was also significant, even when analysis was done within the two subpopulations of women (data not shown).

**Discussion**

There have been several indications (7–12) that serum IGF-I and IGFBP-3 levels decline with aging. The subjects were therefore perimenopausal women who were 45 to 55 years old to control for age-related variations. Serum ALP levels and urinary Ca to Cr ratio significantly increased immediately after menopause, as was evident 1 year after menopause, but BMD gradually decreased after menopause. The present findings suggest that bone turnover rate was accelerated immediately after menopause, and this was followed by the loss of bone mass.

In the present study, serum IGF-I levels were significantly lower in the postmenopausal women, agreeing with a previous report (20). Schwander et al. (21) reported that circulating IGF-I is derived, at least in
part, from the liver, and that its level is related to the amount of GH secreted. Serum levels of glutamate pyruvate transaminase and γ-glutamyl transpeptidase were all within normal limits, although levels of proteins, such as albumin and choline esterase, which reflect hepatic biosynthesis, were not obtained. It is likely that liver dysfunction is not responsible for this decrease in serum IGF-I levels after menopause. There has been previous evidence (22–25) that the GH secretion rate declines after menopause and estrogen exerts stimulatory actions on multiple sites of the GH/IGF-I axis. Taken together, the present findings suggest that a decrease in GH secretion rate would be partly involved in the decrease in the serum IGF-I level, although we do not have data about the GH secretion rate.

The present study revealed a positive correlation between serum IGF-I levels and BMD, although serum IGF-I levels accounted for a small amount of the variability in BMD during the perimenopause. Our finding is compatible with previous reports on adults with acquired GH deficiency (26), men with osteoporosis (27), and postmenopausal women (20). A significant decrease in serum IGF-I levels was evident 1 year after menopause, and this was followed by a decrease in BMD. It is possible, therefore, that IGF-I plays some role in maintaining BMD during perimenopause, although we cannot rule out the possibility that our finding merely reflects the effects of GH on liver and bone. Recently, local factors such as interleukins 1 and 6 have been reported to play an important role in the pathogenesis of postmenopausal osteoporosis (28–31). Our data suggest that a decrease in serum IGF-I levels might also be partly involved in bone loss secondary to gonadal failure, although the evidence for a role of IGF-I in the pathogenesis of postmenopausal osteoporosis still remains scanty. The present study was a cross-sectional one. Longitudinal studies of natural menopause or studies including a period of treatment with sex steroids are necessary to clarify the functional role of IGF-I in the pathogenesis of postmenopausal osteoporosis.

Although there has been evidence that estrogen stimulates the production of IGF-I and IGFBP-3 in osteoblasts (32, 33), the decrease in serum IGF-I levels after menopause is unlikely to reflect a reduced production of IGF-I in bone. The liver is the major source of circulating IGF-I (21) and previous studies have shown that peroral and transdermal administration of estrogen had distinct effects on serum IGF-I levels as well as hepatic protein synthesis (24, 34–36). It does not appear, therefore, that the effects of estrogen on bone cells are responsible for the detectable changes in serum IGF-I levels.

IGFBP-3 has been reported to either potentiate or inhibit the action of IGF-I in bone, depending on the experimental conditions (37, 38). IGFBP-2 has been shown to inhibit IGF-I action (39). In the present study, there were no significant differences in serum levels of IGFBP-2 and -3 between the premenopausal and postmenopausal groups. It seems unlikely, therefore, that the decrease in IGF-I levels can be attributed to variations in IGFBPs, although we have no data concerning other IGFBPs.

Serum IGFBP-3 levels did not significantly change after menopause; this is compatible with the recent evidence (40) that estrogen treatment did not change serum IGFBP-3 levels in healthy postmenopausal women. Blum & Ranke (41) reported that IGFBP-3 is primarily regulated by GH, and its measurement is useful as an index of integrated GH secretion. However, Corpas et al. (42) reported that the serum IGFBP-3 level is related to IGF-I, but not to spontaneous GH release in elderly people. Supporting these findings, a significant positive correlation existed between serum levels of IGF-I and IGFBP-3 in our perimenopausal women. Therefore, the serum level of IGF-I might be a more sensitive indicator of the changes in GH secretion than the serum level of IGFBP-3, which might contribute to an explanation of the differences in the changes in serum levels of IGF-I and IGFBP-3 after menopause. Although there has been previous evidence about the correlation between IGFBP-3 and BMD in healthy men (13) and in postmenopausal women with osteoporosis (8), no correlation was found between them in the present study. The differences in sex and age might contribute to an explanation of these conflicting results.

In the present study, serum IGFBP-2 levels were negatively correlated with serum levels of IGF-I and IGFBP-3. There has been evidence (6) that serum IGFBP-2 levels increase under conditions where IGFBP-3 is insufficient to bind to the available IGFs. Serum IGFBP-2 levels appear to be related to nutritional state. For example, the IGFBP-2 level is significantly increased in emaciated patients with anorexia nervosa or malnutrition and, after weight recovery, it decreases to the level in normal young women (43). The present study revealed that serum levels of IGFBP-2, but not those of IGF-I or IGFBP-3, were negatively correlated with BW and BMI in perimenopausal women. This correlation was also found in subjects whose BMI was within the normal limit. Insulin is the major regulator of serum IGFBP-2 levels (44). It is possible, therefore, that correlations between serum IGFBP-2 levels and BMI might simply reflect differences in insulin secretion, although we do not have any data on insulin secretion.

In our study, the serum TG level was significantly higher in the postmenopausal group, as compared with that of the premenopausal group; this was compatible with a previous report (2). Moreover, serum TG levels also tended to be higher in the former group, but there was no significant difference in serum high-density lipoprotein (HDL) cholesterol levels between the two groups. To our knowledge, there have been no reports on the role of IGFBP-3 in the regulation of lipid metabolism during perimenopause. The present study revealed that the serum level of IGFBP-3, but not that of
IGF-I, was positively correlated to serum T-cho and TG levels. Because Friedman et al. (45) reported that GH is negatively correlated with serum cholesterol level, our finding was the opposite of what we expected. It is possible, therefore, that IGFBP-3 might be related to lipid metabolism during perimenopause through a mechanism that is independent of the GH/IGF-I axis. However, this cross-sectional study did not clarify whether or not IGFBP-3 would really regulate lipid metabolism. Moreover, more detailed analyses of other lipids and lipoproteins as well as fat mass are necessary to clarify the exact role of IGFBP-3 in lipid metabolism.

In conclusion, estrogen plays an important role in the regulation of serum IGF-I levels and a rapid decrease in serum IGF-I levels after menopause might be partly involved in a decrease in bone loss secondary to gonadal failure. IGFBP-2 and -3 might be related to body mass and lipid metabolism during perimenopause respectively, although the mechanisms remain unknown.

References


