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ORIGINAL ARTICLE

Design of a pragmatic approach to evaluate the effectiveness of concurrent treatment for the prevention of osteoporotic fractures

Rationale, aims and organization of a Japanese Osteoporosis Intervention Trial (JOINT) initiated by the Research Group of Adequate Treatment of Osteoporosis (A-TOP)

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Abstract The aim of osteoporosis treatment is to prevent future fractures. Although concurrent treatment has been used very frequently for osteoporosis in clinical practice, there are no data on accurate and verified effectiveness of concurrent treatment for fracture prevention in patients with osteoporosis. To clarify the clinical usefulness of concurrent treatment, the Japan Osteoporosis Society has authorized the establishment of the A-TOP (Adequate Treatment of Osteoporosis) research group. The objective of this research is to establish a design for a clinical trial to prove whether concurrent treatment using both alfacalcidol (1-alpha-hydroxycholecalciferol) and alendronate is more effective as compared to treatment using alendronate alone

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in terms of fracture prevention. The present study was named JOINT (Japanese Osteoporosis Intervention Trial) and is based on a method using national, prospective, randomized, open-labeled, blinded endpoints focusing on postmenopausal osteoporosis with a high risk for fracture. The patients were mainly selected by practitioners and allocated randomly by a central registration system into two groups, of which one received 5 mg/day of alendronate alone, and the other received 1 μg/day of 1-alpha-hydroxycholecalciferol (alfacalcidol) in addition to the alendronate. The endpoints focused primarily on fracture prevention, and the patients' quality of life (QOL) and change in body height, as well as adherence and the adverse events of the treatments were evaluated secondarily. To obtain sufficient statistical power in the events during a 2-year observation period, the patients who are expected to have higher risk were selected to participate in this study, and it was decided that the final plan would involve 890 patients per group (two-sided alpha = 0.05, power = 0.8). Data collection began in November 2003. Correspondence regarding the registration of the investigator and the progress of the study was conducted through a web system from the Public Health Research Foundation to practitioners.

Keywords Alendronate · Alfacalcidol · Concurrent treatment · Fracture prevention · Osteoporosis

Introduction

Osteoporosis, which is characterized by compromised bone strength and increased susceptibility to fractures, which lead to deterioration in the QOL and increased mortality, is a national burden on an aging society [1, 2]. However, recent studies indicate that treatment with a parathyroid hormone, bisphosphonates or a selective estrogen receptor modulator (SERM) [3–9] may decrease the risk of fractures in patients with osteoporosis.

Although bisphosphonate treatment currently represents the most powerful form of treatment available for fracture prevention in osteoporotic patients, it has not succeeded in completely preventing osteoporotic fractures [3–5, 7–9]. Therefore, concurrent treatment of osteoporosis has been frequently used by Japanese practitioners without any concrete evidence regarding fracture reduction. Since the concept of evidence-based medicine (EBM) has been introduced to clinical practice since the 1990s [10], the Japan Osteoporosis Society and the Japanese Society of Bone Mineral Research have edited the clinical guideline for treatment of osteoporosis (Chief editor: Hajime Orimo [11]). However, the writers recognized that there was a lack of evidence in the effectiveness of concurrent treatment of osteoporosis. Furthermore, it was expected that the patients who visit clinics have varying degrees of risk of fracture, which may differ from the degree of those who participated in development trials for bisphosphonates. This possibility would make it easier to obtain pragmatic evidence in general clinical practice.

Starting in 2000, the Japan Osteoporosis Society had planned to investigate the effectiveness of treatment of osteoporosis in order to provide evidence to general practitioners. Before constructing evidence, some feasibility studies were required to confirm the consensus in the diagnosis of incident fractures among the researchers and to elucidate the risk of future fractures in the patient population. In addition to these efforts in the field of osteoporosis, the Japanese government also established an

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ethical guideline for clinical trials [12], and the International Committee of Medical Journal Editors launched a clinical trial registry [13]. Such kinds of progress in the circumstances of clinical trials have enabled for investigator-initiated clinical trials in general practice.

The Adequate Treatment of Osteoporosis (A-TOP) study group was established in 2000 [11] in affiliation with the Japan Osteoporosis Society and organized a team for clinical trial management. The team consisted of clinical investigators (planning and analysis), foundation (funding) managers, officers from non-profit organization (data management) and several companies (data collection). This was the first joint team to create the post-making evidence for osteoporosis. In November 2003, A-TOP initiated a randomized clinical trial referred to as the Japanese Osteoporosis Intervention Trial (JOINT). The purpose of JOINT was to confirm the clinical significance of concurrent use of osteoporotic drugs. The first protocol, named JOINT-01, was initiated in 2002, but was suspended the following year due to a change in drug labeling. A second protocol, named JOINT-02, was established to clarify the effect of adding 1-alpha-hydroxycholecalciferol (alfacalcidol) to alendronate (ALN), using the incident fracture rate as the primary endpoint. In this paper, the rationale, organization and study design of JOINT-02 are introduced.

Rationale and aims

In 2002, the Japan Society of Osteoporosis sent a letter to randomly selected practitioners and enclosed a questionnaire regarding whether concurrent treatment using bisphosphonate and another drug was being utilized to treat osteoporosis. Surprisingly, 87.8% (79/90 practitioner) of the doctors who responded did have experience using concurrent treatment [14]. The most frequent drugs used in concurrent treatment with amino-bisphosphonate were alfacalcidol (93.7%), followed by calcitonin (50.6%), as there were expectations for these drugs to exhibit more potent inhibition of fracture occurrence or more significant increase in BMD, even though there was no apparent evidence. In addition to the lack of evidence related to fracture prevention, the safety profile of concurrent treatment had not been evaluated. Thus, evaluations of the effectiveness and safety of concurrent treatment were urgently required. Etidronate [15] and ALN [8, 9] were used as the drugs to confirm anti-fracture effectiveness in comparison to alfacalcidol in Japanese osteoporotic patients. However, these clinical trials were carried out at specific institutions and were initiated by experts in accordance with tight regulations. As a result, there may have been differences in the selected treatment and in the backgrounds of the patients between treatments conducted at these institutions and those conducted in general practice. In addition, the adherence of the treatment is expected to be lower in general practice than in institutions with experts who are committed to developmental trials. Thus, pragmatic study is urgently needed to evaluate whether bisphosphonates are effective to the same extent at the level of general practitioners as compared to the prior study (Phase III study).

Feasibility studies

The Japan Osteoporosis Society started discussions to execute a national clinical trial for obtaining evidence regarding the effectiveness of concurrent treatment in 2000. An executive committee of A-TOP was organized in 2002 and planned on forming a consensus regarding judgment standards for pre-existing fractures and incident vertebral fractures [16]. Morphometric criteria for incident fractures combined with a semi-quantitative assessment were thought to provide useful information on the study of clinical osteoporosis, especially for international comparisons. Next, to assume the number of participants in the clinical trial, the incident fracture rate and the risk of incident fracture were analyzed in the patient population, and the number of participants with sufficient statistical power [17] was calculated. Bone resorption marker was an independent risk factor for incident vertebral fractures in Japanese women. When the newly discovered risk factor was incorporated into the inclusion criteria in addition to conventional selection criteria such as age, prevalent fractures and bone mineral density, a reduction of about 40% in the estimated sample size was achieved. Thus, measurement of bone resorption markers is useful in reducing the sample size and the observation period in fracture-prevention studies carried out for developing drugs used to treat osteoporosis.

Materials and methods

Study design

Objective

JOINT was the first national, prospective, randomized, multicenter, open-labeled, blinded endpoints, controlled trial for osteoporosis made up mainly of practitioners of investigators in Japan. The objective of JOINT-02 was to clarify additive efficacy in terms of fracture prevention and safety, QOL and adherence in simultaneous use of alfacalcidol and ALN.

Subjects, intervention and endpoints

Confirmations regarding the patients were made by practitioners based on the inclusion and exclusion criteria (Table 1) after obtaining written informed consent. The

Table 1 Inclusion and exclusion criteria

Inclusion criteria
Postmenopausal osteoporosis[a]
Over 70 years old
Ambulatory patients who do not require any help
Able to answer QOL questionnaire
Corresponds to more than one of A-TOP's risk factors for fracture[b]
Exclusion criteria
Metabolic bone diseases other than osteoporosis[c]
Contraindication to the drugs (ALN or alfacalcidol)
Dysfunction in communication of intentions
Severe degenerative deformation of vertebra
Abnormal heart function
Abnormal hepatic function
Abnormal kidney function
Treatment of osteoporosis by bisphosphonate within 6 months prior to the present study

These thresholds were decided by risk analysis by the A-TOP research group

[a] Over 1 year after menopause

[b] Pre-existing vertebral fracture number ≥1; BMD ≤ Young Adult Mean -3 SD; Urinary DPD ≥7.6 nmol/mmol Cr; or NTX ≥ 54.3 nmol BCE/mmol Cr

[c] Hyperparathyroidism and hyperthyroidism were excluded

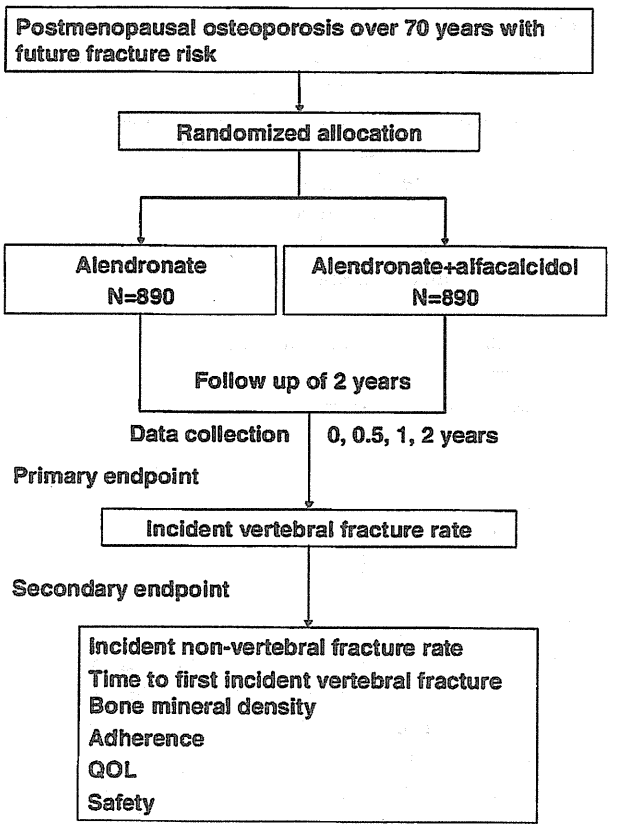

Fig. 1 Study design and outcomes

participants were selected by the practitioners and registered by Japan Clinical Research Support Unit (JCRSU), and then randomly allocated with a modified minimization method using age, number of pre-existing vertebral fracture number, bone mineral density (BMD) and value of bone metabolic marker into the group to be administered only ALN or the group that was to be administered both ALN and alfacalcidol. Registration and allocation of the participants were carried out on the Internet. After initiation of the assigned treatment, clinical data were collected at intervals of half a year for 2 years by I'cros Co., Ltd., through their visiting data collection service. Data were input to the database using a web system developed by ING Corporation. The primary endpoint was to compare the incident vertebral fracture rate between the intervention arms. The secondary endpoints were to compare the differences in the time to first incident vertebral fracture, non-vertebral fracture rate, bone mineral density, adherence, QOL and safety (Fig. 1). In addition, sub-group analyses categorized by baseline characteristics such as age, body mass index (BMI), serum 25-hydroxyl vitamin D levels, the number of pre-existing vertebral fractures and fracture grade were candidate factors. If participants wanted to change the designated treatment because of side effects or occurrence of fractures, they were permitted to do so, and follow-up observations were continued. Please see Table 1 and Fig. 1.

Sample size

Assumptions regarding the fracture rate in the ALN group were made based on a paper by Kushida et al. (Phase III trial for alendronate), in which it is reported that there was a 12.2% fracture rate during observations conducted over 2 years [8]. Since there are not much data on concurrent use of ALN and alfacalcidol [18], the authors' expectations were such that the effects of ALN would be added to those of alfacalcidol and that the hazard ratio of the alfacalcidol combined arm to ALN alone would be 0.64 [19]. The sample size was then estimated to be 890 cases per arm (two-sided alpha = 0.05, power = 0.8), taking account of a dropout rate of 10% referring to the value of the prior clinical trial of fracture intervention [4].

Fracture evaluation

X-ray films of conventional lateral radiographs of lumbar and thoracic vertebrae were taken and collected by I'cros Co., Ltd. After masking the patient's information, two

independent readers (orthopedist, TN and radiologist, MF) simultaneously reviewed films from T4 to L4 in chronological sequence and graded vertebral fracture based on a semi-quantitative method [20]. Before the start of the study, these two observers held meetings to make adjustments between their own criteria for grading vertebral fractures. When the diagnosis of pre-existing fractures made by the reviewers differed from those made by the practitioners, the reviewers' diagnosis was adopted preferentially. If inconsistencies arose between the readers in diagnosing pre-existing and incident vertebral fractures, the two readers negotiated between themselves to reach a consensus. Incident bone fractures other than those of the vertebrae were comprehended from the chart, and the occurrence of fractures was confirmed based on X-ray films or a record of the operation.

Clinical data

BMD at the lumbar vertebrae, hip (proximal femur), distal radius (dual energy X-ray absorptiometry) or left-sided second metacalpal bone (microdensitometry) was measured at baseline and at 6-month intervals for 2 years at each institute. The data of BMD obtained from the different machines and from different bone sites were calculated as the percentage change in each time point from the baseline value. The statistical difference in change of BMD between the group that received combined treatment and the group that was administered only ALN was compared based on each set of data for BMD for the different bone sites. Body height was measured at baseline, 12 and 24 months. Bone turnover markers (urinary type I collagen cross-linked N-telopeptide or urinary excretion of deoxypyridinoline) were measured at baseline and again 6 months after initiating treatment. QOL was assessed by using self-administered questionnaires (JOQOL and EQ-5D) at baseline and at 6, 12 and 24 months after initiating treatment [21]. Serum samples were sent to the central laboratory (SRL Co., Japan), and 25-hydroxyl vitamin D concentrations were measured. Other routine biochemical examinations were carried out at baseline and at 2 years after initiating treatment in order to estimate biochemical adverse events. All adverse events were reported to JCRSU, coded by MedDRA, and categorized as either "known" or "unknown." If an unknown adverse effect occurred, it was reported to the investigator and ethical committee.

Ethics and registration

Ethical issues regarding protocol were reviewed by the ethical committee for JOINT under the Declaration of Helsinki (Dr. Rikushi Morita, Chairman). If it turned out that a patient was at a disadvantage under observation, the ethical committee was given permission to stop the protocol. This study was registered at UMIN-CTR (University Hospital Medical Information Network—Clinical Trial Registry) with the number C000000001.

Statistical analysis

Analysis of the intent to treat principle was applied to the statistical analysis. Efficacy analysis uses a full analysis set (FAS), and all of the enrolled patients are applied to the analysis except for patients without efficacy data, patients who do not correspond to inclusion criteria and patients who do not receive treatment. The PPS (protocol per set) group is defined as consisting of patients without any serious protocol violation.

Recruitment of the practitioner

The explanatory meeting of the protocol and registration was held in all of Japan. The executive members of the A-TOP research group were responsible for the presentation of the protocol and for the recruitment of study institutions and practitioners. The A-TOP committee created the WEB site on the Internet so that the registration of the study could be executed directly. I'cros Co., Ltd., was also involved as a collaborator in calling practitioners.

Results and discussion

Several principles had to be considered for acceptance of concurrent treatment: firstly, the concurrent treatment should be clinically and statistically significantly more effective than the basic treatment; secondly, the concurrent treatment should be of the same level of safety as single treatment; thirdly, the concurrent treatment should be cost-effective. Although these principles should be evaluated before adopting concurrent treatment, the authors have applied concurrent treatment to osteoporosis widely, without any background evidence. Among these principles, the authors have decided to evaluate the first two issues, effectiveness and safety, in the present study. Since this type of evaluation is absolutely required by a clinician, a researcher-initiative study was considered as being the most suitable type of evaluation. This was the reason why the authors decided to use a researcher-initiative clinical trial in determining the effectiveness of concurrent treatment for osteoporosis. The JOINT-02 protocol was the first randomized, controlled trial conducted nationwide for osteoporosis in Japan initiated by researchers, and its scale was also the largest ever. It was therefore necessary for various organizations to collaborate together, and there

have been no previous reports on how to manage the PROBE trial in Japan. This is why we wanted to report the design of JOINT-02. In this paper, we have presented the organization of the A-TOP research group and execution of the JOINT 02 protocol. This is because we believe that this report should help a researcher who is willing to build a new nationwide investigation that is constructed by an organization of clinical research work.

Since the primary aim of JOINT-02 was to determine whether concurrent treatment using ALN and alfacalcidol is superior to treatment using ALN alone in terms of fracture prevention, true ("hard") endpoints such as vertebral fractures or long bone fractures were selected as the primary endpoint. The diagnosis of whether vertebral fractures were present or not on the X-ray films was made by two independent reviewers who did not have any information about the patient; when the judgment of fractures was split between the two reviewers, the reviewers negotiated with each other. Identifying vertebral fractures is more difficult than identifying long bone fractures due to some cases of new vertebral fractures not showing clinical symptoms and the shape of the vertebral body making it difficult at times to recognize whether there is a fracture. Therefore, to avoid misdiagnosis, diagnosis of pre-existing and incident vertebral fractures was made by two different observers. In cases where there was a discrepancy in the diagnosis of vertebral fractures between the reviewers and the practitioner, which occurred with regard to pre-existing fractures, the reviewers' judgment was given priority over that of the practitioner.

Surrogate ("soft") endpoints such as change in BMD or bone turnover markers were considered to be inadequate as primary endpoints in making conclusive statements regarding the efficacy of concurrent treatment. In this type of study, the soft endpoint (BMD or biomarkers) will connect dropout bias less effectively than the case-selected hard endpoint, because such markers are not able to be made blind to the clinicians. Furthermore, previous studies indicate that changes in BMD or bone markers do not predict future fractures [22–24].

In recent literature, it has been reported that poor adherence of bisphosphonates leads to a decline in the beneficial effects of this drug on bone [25–28]. It is expected that in contrast to prior developmental trials, this current study may have a higher dropout rate, since in a researcher-initiative study, the registered practitioner is not forced to maintain adherence very strictly. As a result, adherence in the present study may resemble the actual circumstances of adherence to bisphosphonate treatment by a general practitioner. It will be interesting to determine whether adherence in this study modifies fracture prevention by alendronate.

Although a careful plan for this study has been set up, the results will be applied to osteoporosis patients with the same background as the present study population, but not adapted to the entire osteoporosis population. Despite this limitation, we believe that the results will give us very important information regarding the concurrent treatment of osteoporosis.

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Effects of long-term vitamin K_1 (phylloquinone) or vitamin K_2 (menaquinone-4) supplementation on body composition and serum parameters in rats

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ABSTRACT

Vitamin K is a cofactor for γ-glutamyl carboxylase, which is an essential enzyme for the γ-carboxylation of vitamin K-dependent proteins such as osteocalcin and matrix Gla protein. Although it has been suggested that vitamin K plays an important role in the improvement of bone metabolism, the relationship between dietary vitamin K intake and bone metabolism has not been thoroughly investigated. Moreover, vitamin K is thought to have other actions beyond influencing the γ-carboxylation status. In the present study, we examined the effects of the long-term addition of phylloquinone (PK) or menaquinone-4 (MK-4) to a control diet on bone mineral density, bone strength, body composition, and serum parameters in rats. A total of 23 female Sprague–Dawley strain rats (6 weeks old) were divided into three groups: basic control diet group, PK diet (PK: 600 mg/kg diet) group, and MK diet (MK-4: 600 mg/kg diet) group. Three months after starting the experimental diet, the addition of PK to the basic control diet significantly increased the bone mineral density (BMD) of the femur ($p<0.05$). In the MK group, there was no significant difference in the BMD of the femur. However, two types of bone strength parameter: the minimum cross-sectional moment of inertia and the polar moment of inertia, were significantly higher in the MK group than in the control ($p<0.05$, respectively). Furthermore, the femoral bone parameters (the width, dry weight and ash weight, and cortical, cancellous, trabecular, and total bone mineral contents) in the MK group were increased significantly compared with the control. Interestingly, the addition of PK or MK-4 significantly decreased the total fat accumulation ($p<0.01$ and $p<0.05$, respectively), and serum triglycerides were reduced by 48% in the PK group and 29% in the MK group compared with the control. There were no significant differences in the levels of serum calcium, phosphorus, alkaline phosphatase, growth hormone, insulin-like growth hormone-1, insulin-like growth hormone binding protein-3, and cross-linked N-teleopeptide of type I collagen among the three groups. This is the first study to demonstrate the effect of the long-term addition of PK or MK-4 to the control diet on body composition and serum parameters in an *in vivo* system using rats. Further studies on the mechanism of vitamin K supplementation in the regulation of bone metabolism would provide valuable data on the prevention of lifestyle-related disorders, including osteoporosis.

Introduction

Vitamin K was originally recognized as a factor involved in blood clotting, and is known to be important in bone metabolism. In nature, vitamin K exists in two forms: vitamin K_1 (phylloquinone: PK), and vitamin K_2 (menaquinone: MK-n), which comprises a family of naphthoquinones with differing numbers of isoprenoid residues (1 to 14) at the 3-position of naphthoquinone. PK is found in leafy, green vegetables, and menaquinone-4 (MK-4) is present in meat, eggs, and dairy products. Japanese fermented beans (referred to as natto) *Bacillus natto*, contain large amounts of menaquinone-7 (MK-7) synthesized by the bacteria.

Several epidemiologic studies have shown the association between biological makers of bone metabolism and vitamin K intake. A low dietary phylloquinone intake was associated with an increased risk of hip fracture in the elderly [1]. Kaneki et al. reported a significant inverse correlation between the incidence of hip fracture in women and the consumption of natto, one of the major sources of vitamin K_2 [2].

Vitamin K is a cofactor for vitamin K-dependent carboxylase, known as γ-glutamyl carboxylase (GGCX), which facilitates the post-translational modification of glutamic acid (Glu) to γ-carboxyglutamic acid (Gla) residues in selected proteins [3–7]. Three vitamin K-dependent proteins, osteocalcin (OC), matrix Gla protein (MGP), and protein S, are found in

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bone; OC is the most abundant [8–10]. OC is produced in osteoblasts, and fully carboxylated OC binds the calcium ions of hydroxyapatite [11].

Recently, Hosoi et al. clarified a significantly higher association between the single nucleotide polymorphism (SNP) of GGCX (R325Q, 974G>A) (rs699664) and bone mineral density (BMD) among postmenopausal women [12]. Moreover, we suggested that the requirement of vitamin K for γ-carboxylation may be different depending on the GGCX genotypes in healthy young males [13].

Vitamin K is thought to have other actions beyond the function of a coagulation factor associated with the γ-carboxylation status.

In this study, we investigated the effect of long-term PK or MK-4 supplementation on the BMD, bone strength, fat accumulation, serum parameters and bone metabolism markers in an *in vivo* system using rats.

Materials and methods

Experimental animals

The care and use of rats in the present study followed the guidelines of governmental legislation in Japan on the proper use of laboratory animals. Six-week-old female Sprague Dawley rats were used, and all rats were initially fed the control diet (AIN-93 diet) [14] for eight days. Then, they were divided into three groups, and were each fed experimental diets for eighty-five days: control (Cont.) diet, vitamin K_1 (phylloquinone: PK) diet, or vitamin K_2 (menaquinone-4: MK-4) diet. The vitamin K diets were modified from AIN-93 and contained PK or MK-4 at 600 mg/kg, respectively [15]. PK and MK-4 were kindly supplied by Eisai Co., Ltd. (Tokyo, Japan). Ca, P, protein, and lipid contents were identical in these diets. The animals were housed individually in wire cages with free access to ion-exchanged distilled water. Twelve-hour light/dark cycles, a constant temperature (23 ± 1 °C), and constant humidity (50 ± 5%) were maintained. All rats were observed each day. Their food intake was monitored, and body weight measurements were obtained every second day.

X-ray computed tomography (CT) scanning

Eighty-two days after starting the experimental diet, the body composition (fat and muscle amounts) and bone mineral contents, bone volume, and bone mineral density (BMD) were measured using an X-ray CT system for small experimental animals with a rat mode (LaTheta LCT-100, Aloka, INC., Tokyo, Japan) [16]. The visceral and subcutaneous fat volumes computed automatically were compared with those after the radiologist's adjustments. Ratios of volumetric visceral fat-to-total fat and visceral fat-to-subcutaneous fat were compared on average and with single-slice measurements obtained at L4 and L5 vertebral body levels. The visceral and subcutaneous fat volumes were computed on the tomographic scanning images at 1.5-mm intervals in the measurement area presented with 2 yellow lines in Fig. 1A.

Bone parameters (bone mineral contents, bone volume, and BMD) were computed on the tomographic scanning images at 1.0-mm intervals in the measurement area between the proximal and distal epiphyses of the right femur [17]. The minimum cross-sectional moment of inertia and polar moment of inertia that represent the flexural rigidity and torsional rigidity, respectively, were also calculated automatically employing the software provided with the device. According to the manufacturer, the precision error (as % CV) was within 2% range for all measurements.

The length of the femur and the width of the femur were measured using a dial caliper, and the fresh weight of femur was measured. Then, the femurs were dried at 95 °C for 24 h to measure the dry weight of the femur. All the femurs were burnt to ash at 550–600 °C for 24 h, and the ash weight was measured. The ashed bone was dissolved in 1 M nitric acid to determine the calcium and phosphorus content in the femur.

Biochemical analysis of serum

Eighty-five days after starting the experimental diet, the animals were fasted overnight and sacrificed by bleeding from the abdominal aorta under anesthesia. Blood was collected and centrifuged at 2500 rpm for 15 min to extract the serum. Sera were collected and stored at −80 °C until being thawed for analyses.

Calcium was measured employing the o-cresol-phthalein complexion color development method [18], and inorganic phosphorus was determined using the method of p-methylaminophenol reduction [19]. Total amounts of protein were measured applying the Biuret method [20]. Alkaline phosphatase was determined employing the method of Bessey et al. [21]. Cross-linked N-teleopeptide of type I collagen (NTx) was measured using an enzyme-linked immunosorbent assay (Mochida Seiyaku Co., Ltd., Tokyo, Japan). Serum glucose, triglycerides, and total cholesterol were assayed using the glucose-enzyme [22], GK-GPO (glycerokinase-glycerol-3-phosphate oxidase) [23], and enzymatic determination [24] methods, respectively.

Serum growth hormone (GH) was measured employing the immuno-radiometric assay (TFB Inc., Tokyo, Japan). Insulin-Like Growth Factor-1 (IGF-1) was measured using the immuno-radiometric assay (Mitsubishi Kagaku Yatoron Inc., Tokyo, Japan). Insulin-Like Growth Factor Binding Protein 3 (IGFBP-3) was measured by radioimmunoassay (Bioclone Australia Pty Ltd., Sydney, Australia).

Statistical analysis

Values are shown as the mean ± standard error (S.E.). Comparisons between treatments (Cont. vs. PK, Cont. vs. MK) were conducted using the unpaired Student's t-test. Differences were considered significant at $p<0.05$. Analysis was conducted using SPSS17.0J (SPSS Inc., USA).

Results

Animals and diets

There were no significant differences in the final body weight at 85 days among the Cont. (mean ± S.E., 287.3 ± 7.0 g), PK (272.0 ± 3.7 g), and MK (281.5 ± 3.7 g) groups. In addition, there were no significant differences in the food intake (g/day) among the Cont. (mean ± S.E., 15.6 ± 0.3 g/day), PK (15.2 ± 0.4 g/day), and MK (16.2 ± 0.2 g/day) groups. No significant differences among the three groups (Cont., PK and MK) in the body weight gain (g/day) or food efficiency (body weight gain/food intake) were noted (data not shown). The vitamin K diets contained 0.06% PK or MK-4, respectively. Therefore, the amount of vitamin K intake from the experiment diets was calculated approximately 9–10 mg/day (30–35 mg/kg body weight).

Bone mass and bone mineral density of femur

As shown in Table 1, the width of the femur in the MK group was significantly higher than in the Cont. group ($p<0.05$). The dry and ash weights of the femur in the MK group were also significantly higher than those of the Cont. group ($p<0.05$, respectively). There was no significant difference in the width, dry weight, and ash weight of the femur between the Cont. and PK groups.

Moreover, the addition of MK-4 led to a significant increase in the total, cortical, cancellous, and trabecular volumes of the femur in the MK group compared with the control group ($p<0.01$, $p<0.05$, $p<0.01$, and $p<0.05$, respectively, Table 2). There was no significant difference in the total, cortical, cancellous, and trabecular volumes of the femur between the Cont. and PK groups.

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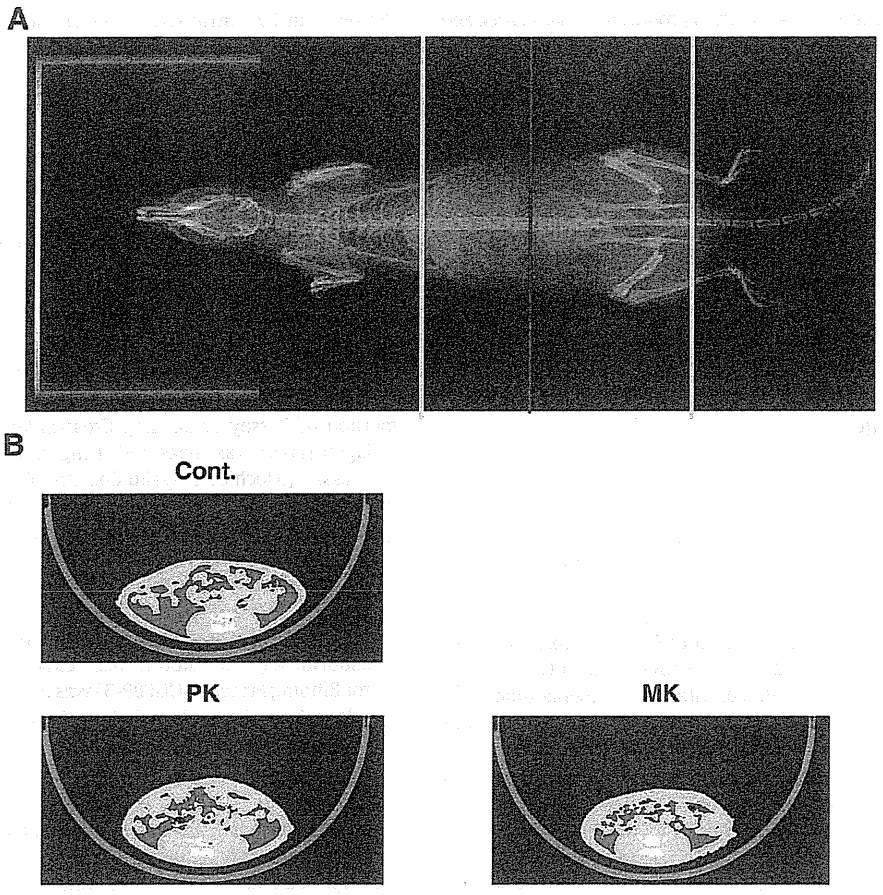

Fig. 1. X-ray computed tomography (CT) scanning after 82 days on the control (Cont.), PK (PK), or MK (MK) diet. (A) Representative images on X-ray CT scanning of the whole bodies of rats. For body composition measurements, tomographic images were acquired at 1.5-mm intervals in the measurement area presented as 2 yellow lines. (B) Cross-sectional appearance of rats in the Cont., PK, and MK groups. Tomographic X-ray CT images of the same 4th lumbar vertebral regions shown with a red line in panel A. The areas indicated in pink, yellow, and light-blue are visceral fat, subcutaneous fat, and muscle, respectively.

As shown in Table 3, the total bone mineral content (BMC) of the femur was higher in the PK and MK groups than that in the Cont. group ($p<0.05$ and $p<0.01$, respectively). The cortical, cancellous, and trabecular BMC in the MK group were significantly higher than those in the Cont. group ($p<0.05$, respectively, Table 3).

The BMD of the femur is shown in Fig. 2A. The BMD of the PK group was significantly higher than that of the Cont. group ($p<0.05$).

Bone strength

Two types of bone strength parameters: the minimum moment of inertia and polar moment of inertia of cross-sectional areas of the femur, are shown in Figs. 2B and C, respectively. The former parameter represents the flexural rigidity, and the latter torsional rigidity. Both of the minimum moment of inertia and polar moment of inertia of cross-sectional areas of the femur were significantly higher in the MK than in the Cont. group ($p<0.05$, respectively, Figs. 2B and C).

X-ray CT scanning of the fat area

Fig. 3 shows the results for the fat pad (g) after 82 days in the Cont., PK, and MK groups using an X-ray CT system for laboratory animals. The total fat weights were significantly lower in the PK and MK groups than in the Cont. group ($p<0.05$) (Fig. 3A). In the PK group, the weight of visceral fat was significantly lower than in the Cont. group ($p<0.01$, Fig. 3B). In the MK group, both the weights of visceral and subcutaneous fat were significantly lower than in the Cont. group ($p<0.05$, respectively, Figs. 3B and C). There was no significant difference in the amount of muscle among the Cont. (mean ± S.E., 97.9 ± 2.1 g), PK (101.4 ± 2.5 g), and MK (104.4 ± 2.2 g) groups.

Table 1
Length and weight of femur.

Groups	Length			Weight		
	Length (cm)	Width (cm)	Thickness (cm)	Fresh weight (g)	Dry weight (g)	Ash weight (g)
Cont.	3.48 ± 0.02	0.35 ± 0.01	0.28 ± 0.01	0.810 ± 0.018	0.534 ± 0.010	0.357 ± 0.008
PK	3.53 ± 0.03	0.35 ± 0.00 #	0.28 ± 0.01	0.830 ± 0.013	0.554 ± 0.010 #	0.374 ± 0.006 #
MK	3.51 ± 0.02	0.39 ± 0.01	0.29 ± 0.00	0.863 ± 0.019	0.576 ± 0.010	0.384 ± 0.007

Each value represents mean ± S.E.
#: Significant difference between the value of the control group and the MK group (#: $p<0.05$).

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Table 2
Femur volume.

	Total volume (cm^3)		Cortical volume (cm^3)		Cancellous volume (cm^3)		Trabecular volume (cm^3)	
Cont.	0.493 ± 0.010	##	0.367 ± 0.007	#	0.126 ± 0.004	##	0.090 ± 0.003	#
PK	0.507 ± 0.010		0.380 ± 0.006		0.127 ± 0.005		0.098 ± 0.005	
MK	0.533 ± 0.008		0.393 ± 0.005		0.141 ± 0.005		0.105 ± 0.005	

Each value represents mean ± S.E.
#: Significant difference between the value of the control group and the MK group (#: $p<0.05$, ##: $p<0.01$).

Biochemical analysis of serum parameters

There were no significant differences in the levels of serum total protein, calcium, inorganic phosphorus, ALP, NTx, GH, IGF-1, IGFBP-3, and glucose among the three groups (Tables 4 and 5, Fig. 4A). Interestingly, the levels of serum triglycerides were significantly lower in PK and MK groups than in the Cont. group ($p<0.05$ and $p<0.01$, respectively) (Fig. 4B). In addition, the level of serum total cholesterol was significantly lower in the MK group than in the Cont. group ($p<0.05$).

Discussion

We compared the effect of PK or MK-4 on BMD, bone strength, fat accumulation, and serum parameters *in vivo*. The total BMC and BMD of the femur were significantly increased after 82 days on the PK compared to the Cont. diet ($p<0.05$, respectively) (Table 3 and Fig. 2A). As shown in Tables 1 and 2, the width, dry or ash weight, and total volume of the femur in the MK group were significantly higher than those of the control group ($p<0.05$, $p<0.05$ and $p<0.001$, respectively). Further, significant increases in the BMC, minimum moment of inertia of cross-sectional areas, and polar moment of inertia of cross-sectional areas of femur were observed in the MK group ($p<0.05$, respectively) (Table 3 and Figs. 2B and C).

In the present study, we revealed the different effects of PK or MK-4 on femoral bone parameters (BMD, width, dry weight, ash weight, total volume, minimum moment of inertia of cross-sectional areas, and polar moment of inertia of cross-sectional areas). As shown in Fig. 2A, BMD of the femur was significantly higher in the PK group, whereas BMD of the femur was not significantly higher in the MK group. In the MK group, femoral bone parameters (dry weight, ash weight, total volume and BMC) were significantly increased (Tables 1, 2 and 3). Femoral BMD was calculated per cm^3 (bone volume), so we considered the significant increase of bone volume as the reason why femoral BMD was not significantly higher in the MK group. Moreover, it will be also understood that the significant increase of bone volume was one of the reasons why femoral bone strength parameters (minimum moment of inertia of cross-sectional areas, and polar moment of inertia of cross-sectional areas) were significantly higher in the MK group. In addition, the increase of bone volume seemed to be caused by the increase of bone width not by the increase of bone length (Table 1). These results suggested that PK has the beneficial effects on increasing both of femoral BMC and BMD, while MK has the beneficial effects of increasing femoral BMC, bone volume, width and bone strength parameters. It is interesting that the effect of MK in the growth process on the width of the bone was suggested during bone remodeling.

Several *in vitro* studies demonstrated that both PK and MK-4 have beneficial effects on bone formation [25–27]. It was reported that MK-4 suppressed bone resorption [28–30], but PK did not have such an effect [31]. Hara et al. reported that the inhibitory effect of MK-4 on bone resorption may not be due to γ-carboxylation and that the side chain of MK-4 may play an important role in this inhibitory effect [31]. MK-4 significantly inhibited calcium release from mouse calvaria treated with $1\alpha,25(OH)_2D_3$ or prostaglandin E_2 (PGE_2), and the inhibitory effect of MK-4 on calcium release from calvaria was not affected by the addition of warfarin, an inhibitor of the vitamin K cycle while PK at the same doses did not have these effects [31]. Therefore, the inhibitory effect of MK-4 on bone resorption does not seem to be via γ-carboxylation.

A previous study demonstrated that MK-4 inhibited decreasing bone strength measured by employing a 3-point bending test induced by ovariectomy [15]. Bone quality has become an important issue in the prevention of osteoporosis [32], because the BMD is not the only factor that affects the occurrence of fractures [33,34]. The NIH consensus meeting proposed that bone strength is related to many factors including bone mineralization, architecture, turnover, and the concentration of organic proteins [35]. Recent studies have revealed that vitamin K functions as a ligand for nuclear steroid and xenobitoic receptor (SXR), as well as a cofactor for γ-carboxylase [36]. Inoue et al. identified novel SXR target bone-related genes regulated by MK-4 in osteoblastic cells using microarray analysis. Among extracellular matrix-related genes, they revealed that a small leucine-rich repeat proteoglycan, tsukushi, contributed to collagen accumulation [37].

Recently, we reported for the first time that both PK and MK-4 as nutritional factors enhance intestinal alkaline phosphatase (ALP) activity [38]. The high activity of intestinal ALP, which localizes at the brush border of the intestinal epithelium cells, suggests the participation of this enzyme in the transport of nutrients. In humans and rodents, a diet with a high fat content or the fat-feeding had elevated serum levels of intestinal ALP activity [39,40]. It was reported that intestinal ALP knockout mice showed no difference from the wild-type controls under the normal chow, however, when maintained long-term on a high-fat diet, the intestinal ALP knockout mice showed faster body weight gain

Table 3
Bone mineral measurements of femur.

	Total BMC (mg)		Cortical BMC (mg)		Cancellous BMC (mg)		Trabecular BMC (mg)	
Cont.	304.5 ± 7.2	*	248.1 ± 5.9		56.4 ± 1.6		48.1 ± 1.6	
PK	326.3 ± 5.9		265.6 ± 4.8		60.7 ± 3.1		53.6 ± 3.1	
MK	339.7 ± 7.9	##	272.7 ± 5.0	#	66.9 ± 3.1	#	58.7 ± 3.1	#

Each value represents mean ± S.E.
: Significant difference between the value of the control group and the PK group (: $p<0.05$).
#: Significant difference between the value of the control group and the MK group (#: $p<0.05$, ##: $p<0.01$).

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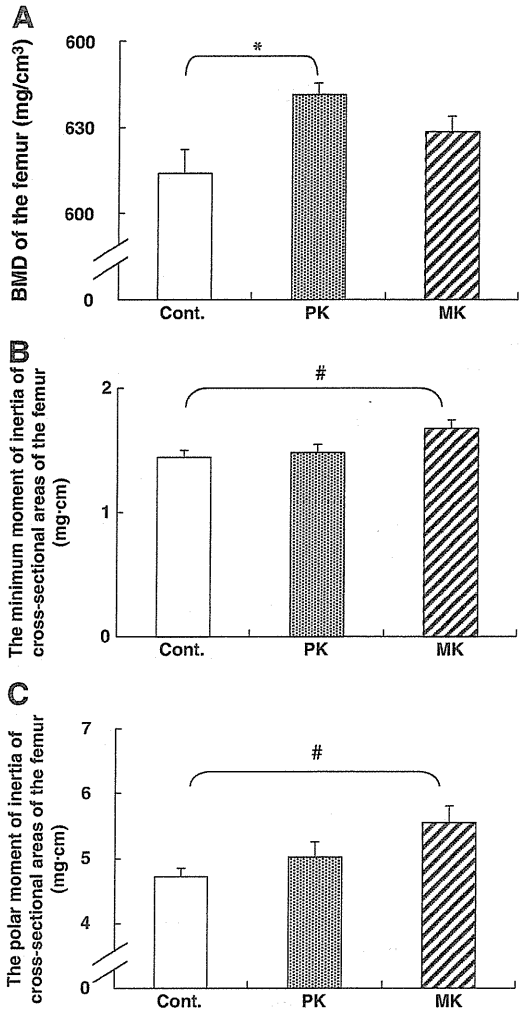

Fig. 2. Bone mineral density (BMD) and bone strength parameters of the femur in the control (Cont.), PK (PK), or MK (MK) diet group. Results are the mean ± S.E. of 7 or 8 animals. Significant difference between the PK and Cont. groups (*: $p<0.05$). Significant difference between the MK and Cont. groups (#: $p<0.05$). (A) BMD of the femur. (B) The minimum moment of inertia of cross-sectional areas of the femur. (C) The polar moment of inertia of cross-sectional areas of the femur.

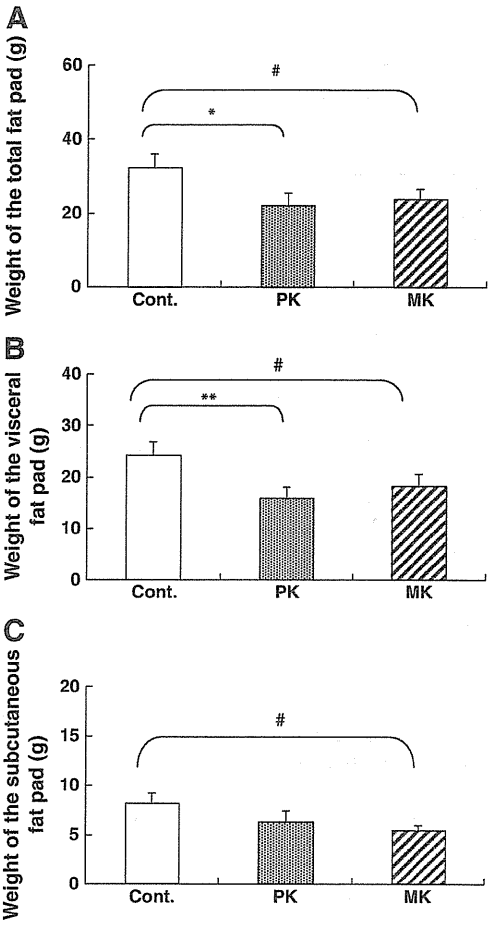

Fig. 3. Weight of the fat pad. (A) Weight of the total fat pad (g), (B) weight of the visceral fat pad (g), and (C) weight of the subcutaneous fat pad (g) after 82 days on the control (Cont.), PK (PK), or MK (MK) diet. Results are the mean ± S.E. of 7 or 8 animals. Significant difference between the PK and Cont. groups (*: $p<0.05$, **: $p<0.01$). Significant difference between the MK and Cont. groups (#: $p<0.05$).

compared with the wild-type animals [41]. These finding suggests the possibility that intestinal ALP may regulate not only phosphate metabolism but also fat metabolism.

In order to examine whether the effect of MK-4 on the bone volume was via GH secretion, we investigated the level of GH in serum [42–44]. Although the level of GH in the MK group tended to be higher than in the Cont. group, there was no significant difference, as shown in Table 5. In addition, we measured the levels of IGF-1 and IGFBP-3 in serum, which are markers of bone-related growth. As the results, IGF-1 and IGFBP-3 were similar among the three groups (Cont., PK, and MK) (Table 5), and the supplementation of MK-4 did not influence these growth factors affecting bone metabolism.

Interestingly, the addition of both PK and MK-4 to the Cont. diet may regulate not only bone strength but also fat deposition. Body weight gain (g/day), food intake (g/day) and food efficiency (body weight gain/food intake) were not significantly different among the three groups (Cont., PK, and MK). As shown in Fig. 3A and B, the weights of total and visceral fat in both the PK and MK groups were significantly lower than in the Cont. group. A previous *in vitro* study reported that MK-4 but not PK inhibited the formation of adipocytes in bone marrow cells [45]. It demonstrated that MK-4 inhibited the expression of osteoclast differentiation factor (ODF)/RANK ligand and the formation of osteoclast-like cells induced by $1\alpha,25(OH)_2D_3$, and that MK-4 specifically influenced the differentiation and functions of bone marrow cells. The present study revealed that both PK and MK-4 had effects on the reduction of fat deposition *in vivo*. Structural differences in the isoprenoid side chain may influence vitamin K metabolism, including the way it is transported, taken up by target tissues, and subsequently excreted. In the post-prandial state, PK is transported mainly by triglyceride-rich lipoproteins (TRL) and long-chain MKs mainly by low-density lipoproteins (LDL) [46]. As shown in Fig. 4B, the levels of triglycerides in both PK and MK groups were significantly decreased ($p<0.05$ and $p<0.001$, respectively). PK is converted into MK-4 and accumulates in extrahepatic tissues [47], and so we suggest that the regulation of fat deposition might be

Table 4
Biochemical parameters of serum.

Groups	Total protein (g/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline phosphatase (U/l)	NTx (nmol/l)
Cont.	7.1 ± 0.1	10.6 ± 0.1	5.4 ± 0.6	118.7 ± 6.6	11.9 ± 0.5
PK	6.9 ± 0.1	10.4 ± 0.1	5.6 ± 0.5	136.1 ± 11.2	12.9 ± 0.8
MK	7.0 ± 0.2	10.5 ± 0.1	6.6 ± 0.4	125.6 ± 13.9	12.4 ± 0.9

Each value represents mean ± S.E.

Table 5
Hormone and cytokine parameters of serum.

Groups	GH (ng/ml)	IGF-1 (ng/ml)	IGFBP-3 (μg/ml)
Cont.	0.009 ± 0.001	1.92 ± 0.23	0.145 ± 0.007
PK	0.006 ± 0.002	1.69 ± 0.13	0.144 ± 0.003
MK	0.041 ± 0.017	1.43 ± 0.18	0.158 ± 0.006

Each value represents mean ± S.E.

mediated by not only dietary vitamin K, but also MK-4 converted from PK.

Some recent studies proposed that osteocalcin of undercarboxylated form is involved with a hormone in the endocrine regulation of energy homeostasis [48], and that picomolar amount of undercarboxylated osteocalcin regulates the expression of insulin genes and beta-cell proliferation markers whereas nanomolar amounts of osteocalcin affects adiponectin expression [49].

The effect of vitamin K on fat mass could be mediated through adiponectin regulation which itself has been found to be associated with fat mass. There is also another recent published work in humans that vitamin K supplementation with a daily dose of 0.5 mg of phylloquinone for 3 years had a protective effect on the progression of insulin resistance in older men [50]. These data indicate the need for further research and better understanding of the relationship among osteocalcin, its carboxylation, and vitamin K intakes.

The amount of vitamin K intake from the experiment diets is massive compared to nutritional requirements for vitamin K, and further dose–response studies are required to investigate whether long-term supplementation with doses in a more nutritional range would deliver the changes seen in this study.

Further studies on the effects of vitamin K on the regulation of the body composition would provide useful data on the prevention of lifestyle-related disorders, including osteoporosis.

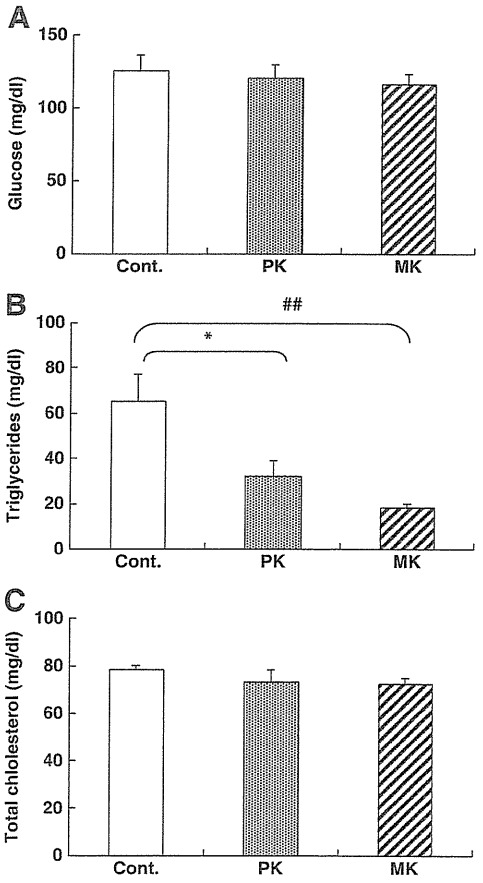

Fig. 4. Levels of serum glucose (A), serum triglycerides (B), and serum total cholesterol (C) after 85 days on the control (Cont.), PK (PK), or MK (MK) diet. Results are the mean ± S.E. of 7 or 8 animals. Significant difference between the PK and Cont. groups (*: $p<0.05$). Significant difference between the MK and Cont. groups (##: $p<0.01$).

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ORIGINAL ARTICLE

Hip fracture incidence in Japan: estimates of new patients in 2007 and 20-year trends

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Abstract
Summary We estimated the number of hip fracture patients in 2007 in Japan and investigated the trends in incidence during a 20-year period from 1987 to 2007. Despite the increasing number of new patients, the incidence of hip fracture in some age groups showed the possibility of decline.
Purpose The aims of this study were to estimate the number of hip fracture patients in 2007, to investigate the trends in incidence during a 20-year period from 1987 to 2007, and to show the regional differences in Japan.
Methods Data were collected through a nationwide survey based on hospitals by the mailing method. Hip fracture incidences by sex and age and standardized incidence ratios by region were calculated.
Results The estimated numbers of new hip fracture patients in 2007 were 148,100 in total (95% CI, 144,000–152,200), 31,300 (30,500–32,100) for men, and 116,800 (113,900–119,700) for women. The incidence rate in men aged 60–69 years and that in women aged 60–79 years were the lowest in the 15-year period from 1992 to 2007. The incidence was higher in western areas of Japan than in eastern areas in both men and women.
Conclusions Despite the increasing number of new patients, the incidence of hip fracture in some age groups for both men and women showed the possibility of decline. The exact reasons for this are unknown, but drug therapy for osteoporosis and fall prevention programs might have influenced the results. Some nutrient intakes might explain the regional differences not only in Japan but also in some other countries.

Keywords Hip fracture · Incidence · Osteoporosis · Drug therapy · Time trends · Aging

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Introduction

Hip fracture is the most serious outcome of osteoporosis and is becoming more frequent as the age of the world's population increases [1]. It is estimated that the number of hip fractures worldwide, which was about 1.7 million in 1990, will increase to 6.3 million in 2050 even if age-adjusted incidence rates for hip fracture remain stable [2].

From a different perspective, osteoporotic fractures worldwide accounted for 0.83% of the global burden of non-communicable diseases. In Europe, osteoporotic fractures accounted more for the Disability Adjusted Life Years lost than did common cancers with the exception of lung cancer [3].

In Japan, hip fracture, along with cerebrovascular disease, is a major cause of becoming bedridden, markedly

decreasing quality of life in the elderly [4]. Hence, countermeasures against hip fracture are urgent medical and social issues in the rapidly aging Japanese population.

A nationwide survey in Japan has been implemented every 5 years since 1987, and the trend in hip fracture incidence has been reported. The first nationwide survey was carried out in 1987 to clarify the sex-specific and age-specific incidences of hip fractures, and the estimated number of new patients in 1987 was about 53,200 [5]. The second nationwide survey, using a different method to improve accuracy, was performed in 1992, and the number of new patients was estimated to be 76, 600 [6]. In the third nationwide survey, carried out in 1997, the estimated number of new patients was 92,400 [7]. In the fourth survey, in 2002, the estimated number was 117,900 [8]. The number of new patients with hip fracture increased 2.2-fold in the 15-period from 1987 to 2002.

The aims of this study were to estimate the number of hip fracture patients in 2007, to investigate the trends in incidence during the 20-year period from 1987 to 2007, and to show the regional distribution of hip fracture incidence in Japan.

Subjects and methods

Sampling method

For a nationwide estimate

To estimate the number of new hip fracture patients nationwide, in the fifth survey in 2007, hospitals and clinics including or specializing in orthopedics throughout Japan were divided into 13 strata according to the number of beds, maintaining comparability with previous surveys. In Japan, small hospitals with 19 or fewer beds are defined as clinics. All hospitals with 200 beds or more were included, and hospitals with 199 or fewer beds were randomly selected by Neyman's allocation method [9] to minimize standard error. In this survey, among 8,234 orthopedic institutions in Japan, 4,500 institutions were selected as sites to investigate a nationwide estimate, using the same sample size as the previous surveys (Table 1). The number of new patients with hip fracture was estimated by the following formula:

$$\text{Number of patients} = \sum \frac{N_i}{n_i} \cdot P_i,$$

where N_i is the number of surveyed institutions in each stratum, n_i is the number of responding institutions in each stratum, and P_i is the summation of the number of new patients in each stratum [7].

For regional estimates

Data from 5,613 institutions, all hospitals with 20 beds or more and 479 clinics, were used for regional estimates to improve estimation accuracy. Data from clinics that had been randomly selected for the nationwide estimate were used (Table 1).

To evaluate the regional differences in incidence of hip fracture, Japan was divided into 12 regions based on the National Health and Nutrition Survey in Japan.

The standardized incidence ratio was calculated as follows:

$$\text{Standardized incidence ratio} = \frac{B}{\sum (I \times P)}$$

where B is the estimated number of patients in each region, I is the nationwide incidence of hip fracture by sex and age groups, and P is the regional population by sex and age groups.

Incidences by sex and age groups were calculated on the basis of the estimated number of new patients. This incidence was multiplied by the population by the sex and age groups in each region to obtain the expected number of patients. The ratio of estimated number of patients to expected number was calculated as the standardized incidence ratio.

Population figures from the 2005 national census were used to calculate the incidence of hip fracture and the standardized incidence ratio by region.

Questionnaire

A questionnaire was sent by mail to all participating or selected hospitals and clinics based on administrative hospital data from the Ministry of Health, Labour, and Welfare. We asked for information on the number of new patients with hip fracture between January 1 and December 31 in 2007 and information on each patient's sex and age. Patients that underwent surgery for hip fracture at other institutions or patients for rehabilitation were excluded to avoid double counting of new hip fracture patients.

Results

Response rates

For a nationwide estimate, replies were obtained from 2,997 institutions, a response rate of 66.6%. The response rate was highest (76.2%) in hospitals with 900 beds and more and was lowest (58.7%) in hospitals with 500–599 beds. For regional estimates, replies were obtained from 3,778 institutions, a response rate of 67.3% (Table 2). The

Table 1 Number of institutions and sampling numbers for a nationwide estimate and regional estimates by stratum

Stratum number	Number of beds	Number of institutions	Sampling number for nationwide estimate (%)	Sampling number for regional estimates (%)
1	−19	3,100	479 (15.5)	479 (15.5)
2	20–49	453	226 (50.0)	453 (100)
3	50–99	1,321	830 (62.8)	1,321 (100)
4	100–149	834	598 (71.7)	834 (100)
5	150–199	804	645 (80.2)	804 (100)
6	200–299	580	580 (100)	580 (100)
7	300–399	501	501 (100)	501 (100)
8	400–499	250	250 (100)	250 (100)
9	500–599	150	150 (100)	150 (100)
10	600–699	101	101 (100)	101 (100)
11	700–799	47	47 (100)	47 (100)
12	800–899	30	30 (100)	30 (100)
13	900+	63	63 (100)	63 (100)
Total		8,234	4,500 (54.7)	5,613 (68.2)

response rates by region were 78.4% in Hokuriku, 74.9% in Tohoku, 73.1% in Minamikyushu, 72.6% in Chugoku, 72.6% in Kitakyushu, 70.7% in Hokkaido, 67.0% in Kinki II, 66.1% in Shikoku, 65.4% in Kanto II, 65.3% in Tokai, 63.2% in Kinki I, and 59.4% in Kanto I.

Nationwide estimate

The estimated numbers of new hip fracture patients in 2007 were 148,100 in total (95% CI, 144,000–152,200), 31,300 (30,500–32,100) for men, and 116,800 (113,900–119,700) for women. As shown in Table 3, the number of new patients in the 2007 survey was 1.26 times higher than that in the 2002 survey [8, 10], and the number of new patients in the 2007 survey was 2.78 times higher than that in the 1987 survey [5]. The number of new female patients increased from 39,700 in 1987 to 116,800 in 2007.

The crude annual incidence rate (per 10,000) of hip fracture in 2007 was calculated by sex and age (Table 4). The incidence rates of hip fracture in men and women by age were 0.32 and 0.15 under 40 years, 0.92 and 0.70 in 40s, 2.03 and 2.95 in 50s, 4.81 and 8.11 in 60s, 18.12 and 39.71 in 70s, 61.03 and 157.14 in 80s, and 146.62 and 313.58 over 90 years, respectively. The incidence rates of

Table 2 Number of responding institutions and response rates for a nationwide estimate and regional estimates by stratum

Stratum number	Number of beds	Sampling number for nationwide estimate	Number of responding institutions	Response rate (%)	Sampling number for regional estimates	Number of responding institutions	Response rate (%)
1	−19	479	320	66.8	479	320	66.8
2	20–49	226	150	66.4	453	311	68.7
3	50–99	830	580	69.9	1,321	927	70.2
4	100–149	598	410	68.6	834	565	67.7
5	150–199	645	474	73.5	804	592	73.6
6	200–299	580	350	60.3	580	350	60.3
7	300–399	501	299	59.7	501	299	59.7
8	400–499	250	153	61.2	250	153	61.2
9	500–599	150	88	58.7	150	88	58.7
10	600–699	101	75	74.3	101	75	74.3
11	700–799	47	31	66.0	47	31	66.0
12	800–899	30	19	63.3	30	19	63.3
13	900+	63	48	76.2	63	48	76.2
Total		4,500	2,997	66.6	5,613	3,778	67.3

Table 3 Trends in estimated number of new hip fracture patients per year, 1987–2007

	1987	1992	1997	2002	2007
Total point estimation	53,200 people	76,600	92,400	117,900	148,100
95% confidence interval		69,000–84,000	89,900–94,900	114,700–121,100	144,000–152,200
5-year increase (% rate of increase)		23,400 (+44.0%)	15,800 (+20.6%)	25,500 (+27.6%)	30,200 (+25.6%)
Men point estimation	13,500	18,700	20,800	25,300	31,300
95% confidence interval		17,000–21,000	20,100- 21,400	24,500–26,000	30,500–32,100
5-year increase (% rate of increase)		5,200 (+38.5%)	2,100 (+11.2%)	4,500 (+21.6%)	6,000 (+23.7%)
Women point estimation	39,700	57,900	71,600	92,600	116,800
95% confidence interval		52,000–64,000	69,600–73,600	90,000–95,200	113,900–119,700
5-year increase (% rate of increase)		18,200 (+45.8%)	13,700 (+23.7%)	21,000 (+29.3%)	24,200 (+26.1%)

hip fracture were lowest for men aged 60–69 years and for women aged 60–79 years in the 15-year period from 1992 to 2007. As for the incidence of hip fracture overall, it kept increasing in each 5-year period in both men and women. The overall incidences in the 1992 survey were 3.08 in men and 9.20 in women, and those in 2007 were 5.11 in men and 18.14 in women. The overall incidences in the 2007 survey were 1.66 times higher in men and 1.97-times higher in women than those in 1992.

Regional estimates

Crude incidences of hip fracture per 10,000 by region were high in men in the western areas of Japan (Shikoku, 7.46 per 10,000; Minamikyushu, 7.28 per 10,000; and Chugoku, 7.03 per 10,000) compared with those in the eastern areas (Kanto I, 3.77 per 10,000; Tohoku, 4.28 per 10,000; and Tokai, 4.97 per 10,000). The pattern in women was similar, with the highest rates in the western areas of Minamikyushu (27.37 per 10,000), Chugoku (24.98 per 10,000), and Shikoku (24.64 per 10,000) and lower rates in the eastern areas of Kanto I (13.59 per 10,000), Tohoku (13.66 per 10,000), and Kanto II (17.1 per 10,000).

Figure 1 shows the east–west regional differences expressed by standardized incidence ratios. Similarity in the patterns for both men and women is noteworthy.

Table 4 Incidence rate of hip fracture per 10,000, 1992–2007

	1992	1997	2002	2007
Male, age				
−39	0.36	0.30	0.30	0.32
40+	1.03	0.91	0.84	0.92
50+	2.21	2.00	1.82	2.03
60+	5.74	5.12	5.26	4.81
70+	19.13	17.29	17.49	18.12
80+	56.02	57.41	58.61	61.03
90+	124.96	128.89	141.39	146.62
All ages	3.08	3.38	4.08	5.11
Female, age				
−39	0.16	0.13	0.12	0.15
40+	0.61	0.60	0.58	0.70
50+	2.82	2.39	2.41	2.95
60+	9.69	9.07	9.11	8.11
70+	44.32	40.85	41.07	39.71
80+	139.60	147.79	156.10	157.14
90+	264.66	281.04	315.52	313.58
All ages	9.20	11.19	14.43	18.14

Discussion

The results of our study showed the incidence of hip fracture in 2007 and the trend in hip fracture over a 20-year period. According to data obtained every 5 years, the number of new hip fracture patients has continued to increase. The total number of hip fracture patients was 53,200 in 1987, and the number increased markedly to 148,100 in 2007. The number of female hip fracture patients was about 3.7 times larger than the number of male hip fracture patients in 2007, indicating a disparity in sex. It is clear that the number of new patients increases yearly because of changes in the national demographic structure. Between 1985 and 2005, the proportion of people 65 years of age or over in Japan increased from 10.3% (12.5 million) to 20.1% (25.7 million; 8.6% to 17.4% in men and 12.0% to 22.6% in women). The numbers of men and women 60 years of age or over in 2000 were 13 million and 16.8 million, respectively, and those in 2005 were 15 million and 19.2 million, respectively. Increases in number of people 60 years of age or over in 2005 were approximately two million (rate of increase of 15.9%) for men and 2.4 million (rate of increase of 14.4%) for women compared to the numbers in the year 2000. Japan is now facing an unprecedented situation, an aging society.

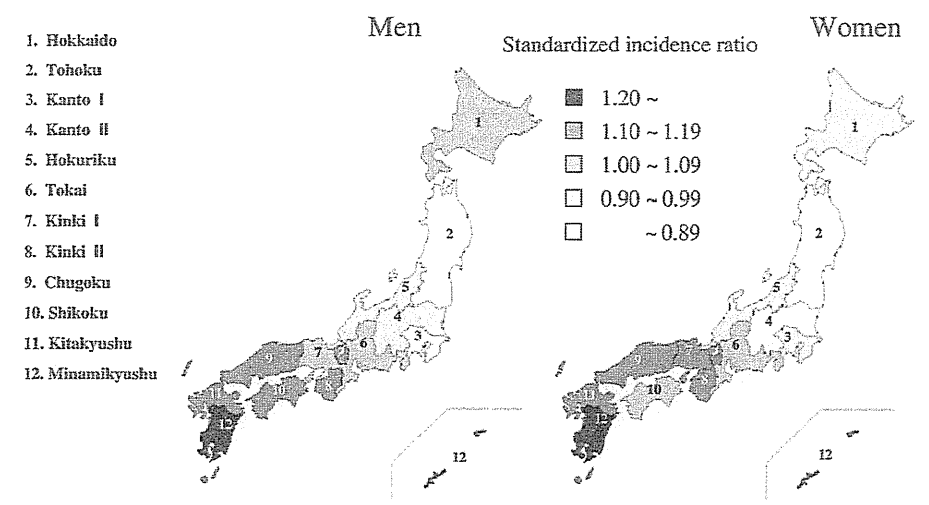

Fig. 1 Standardized incidence ratio of hip fracture

The aging population has become a major issue not only in Japan but also in many other countries. The World Population Prospects based on the 2006 Revision Population Database show the proportions of people aged 60 years of age or over in each country. The most remarkable difference among countries is the speed of population aging [11].

A study in Korea, based on data from the Health Insurance Review Agency, showed that the incidence rate of hip fractures in women, but not that in men, increased from 2001 to 2004 in Korea [12]. The reasons for the increase were suggested to be due to an increase in the number of frail elderly women and a substantial increase in chronic disease related to frailty. The proportions of people aged 60 years of age or over in Korea were 11.4% in 2000 and 13.7% in 2005. A study in Taiwan, based on an inpatient database of the National Health Insurance Program, showed a modest increase in hip fracture incidence and a steady increase in the actual number of hip fractures for both men and women from 1996 to 2000 in Taiwan [13]. The reasons for this trend were suggested to be inadequate nutrition for the elderly in Taiwan in past decades, high probability of falls, and insufficient geriatric care. Another study in Taiwan, a nationwide 7-year trend of hip fractures in the elderly population aged 65 years or over, showed that overall incidence increased by 30% from 1996 to 2002 and that there were dramatic increases in incidences for people aged older than 85 years in both males and females [14]. Although the percentage of people aged 60 years or over in both of these Asian countries was not so high during the study period, the percentage is predicted to be much higher in the near future.

According to a study on trend of hip fracture in Germany during the period from 1995 to 2004, using data obtained from the national discharge register, age and sex-adjusted annual incidence ratios increased statistically significantly, but only slightly [15]. The proportions of people aged 60 years or over in Germany were 20.9% in 1995 and 25.1% in 2005 [16]. These percentages are similar in those in Japan. However, the prospect of the speed of population aging after 2005 is quite different. In Germany, it is estimated that a further increase will not greatly exceed that expected from the demographic aging of the population. A study in southeastern Norway during the period from 1998 to 2003 showed a high incidence of hip fracture, but did not indicate any increase [17]. The proportions of people aged 60 years or over in Norway were 20.5% in 1995 and 19.6% in 2005 [16].

While some studies have shown an increase in the incidence of hip fractures, one nationwide study in Finland suggested a declining trend in fracture rates. That study showed that the crude incidence of hip fracture decreased between 1997 and 2004, following a constant rise between 1970 and 1997 [18]. Although the reasons for the secular change in risk of hip fracture incidence were unknown, the authors provided possible explanations, such as a cohort effect toward healthier elderly populations, increase in average body weight and body mass index, specific actions to prevent and treat osteoporosis, and effects of programs and interventions to prevent falling by strength and balance training.

In addition to the nationwide study in Finland, two recent small studies in Canada and Denmark have also suggested the decline in the incidence of hip fracture. The study in Ontario, Canada, showed the possibility of decline in hip fracture rate because of increased patterns of diagnosis through the bone mineral density testing and treatment for osteoporosis, introduction of the bisphosphonate family [19]. According to the study conducted in Funen, Denmark, between 1996 and 2003, the incidence