

**Table 2**  
Femur volume.

	Total volume (cm <sup>3</sup> )	Cortical volume (cm <sup>3</sup> )	Cancellous volume (cm <sup>3</sup> )	Trabecular volume (cm <sup>3</sup> )
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<sup>3</sup> (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  $\gamma$ -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  $1\alpha,25(\text{OH})_2\text{D}_3$  or prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), 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  $\gamma$ -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 xenobiotic receptor (SXR), as well as a cofactor for  $\gamma$ -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.

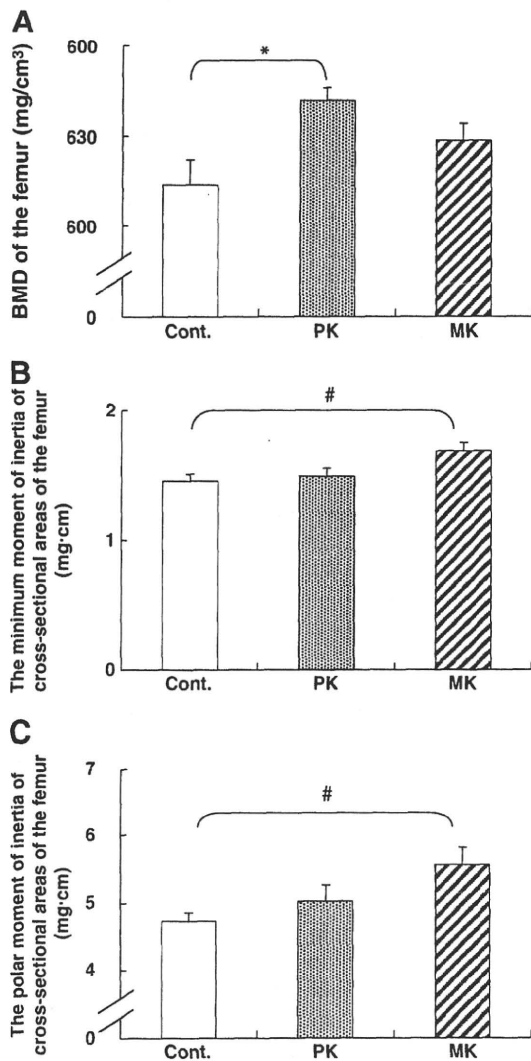
	Total BMC (mg)	Cortical BMC (mg)	Cancellous BMC (mg)	Trabecular BMC (mg)
Cont.	304.5 ± 7.2	248.1 ± 5.9	56.4 ± 1.6	48.1 ± 1.6
PK	326.3 ± 5.9 *	265.6 ± 4.8	60.7 ± 3.1	53.6 ± 3.1
MK	339.7 ± 7.9 ##	272.7 ± 5.0 #	66.9 ± 3.1 #	58.7 ± 3.1 #

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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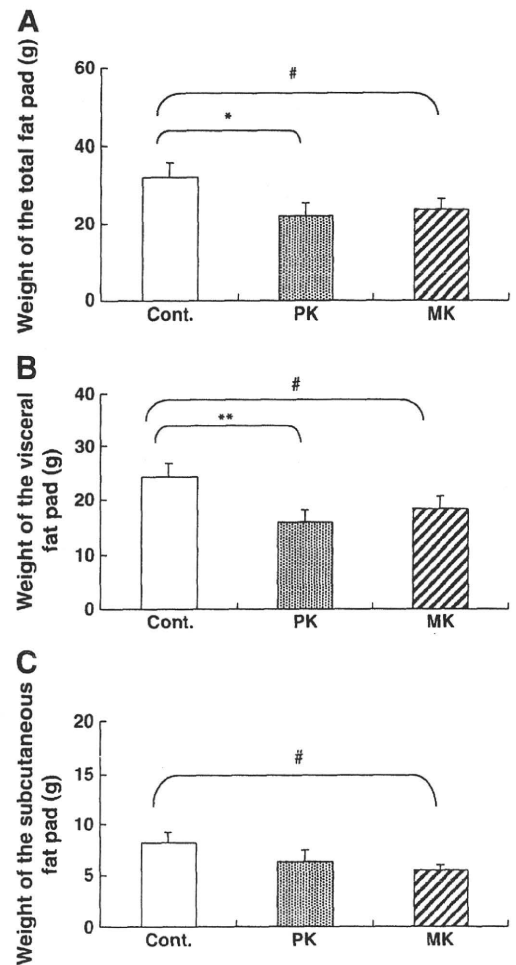


**Fig. 2.** Bone mineral density (BMD) and bone strength parameters of the femur in the control (Cont.), PK (PK), or MK (MK) diet group. Results are the mean  $\pm$  S.E. of 7 or 8 animals. Significant difference between the PK and Cont. groups (\*:  $p < 0.05$ ). Significant difference between the MK 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.

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



**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  $\pm$  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 (#:  $p < 0.05$ ).

expression of osteoclast differentiation factor (ODF)/RANK ligand and the formation of osteoclast-like cells induced by  $1\alpha,25(\text{OH})_2\text{D}_3$ , 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 $\pm$ 0.1	10.6 $\pm$ 0.1	5.4 $\pm$ 0.6	118.7 $\pm$ 6.6	11.9 $\pm$ 0.5
PK	6.9 $\pm$ 0.1	10.4 $\pm$ 0.1	5.6 $\pm$ 0.5	136.1 $\pm$ 11.2	12.9 $\pm$ 0.8
MK	7.0 $\pm$ 0.2	10.5 $\pm$ 0.1	6.6 $\pm$ 0.4	125.6 $\pm$ 13.9	12.4 $\pm$ 0.9

Each value represents mean  $\pm$  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

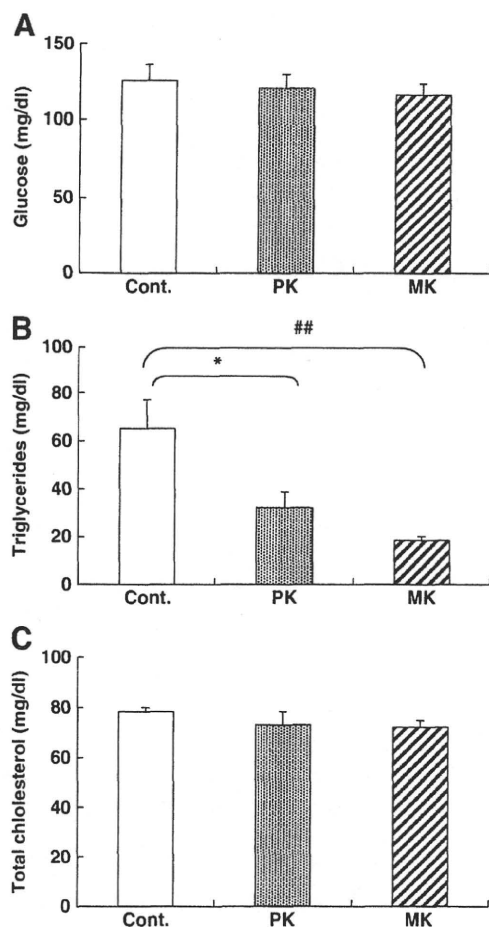
Each value represents mean ± S.E.

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



**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 (##:  $p < 0.01$ ).

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.

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# 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-marketing 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 <sup>a</sup>	
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 <sup>b</sup>	
Exclusion criteria	
Metabolic bone diseases other than osteoporosis <sup>c</sup>	
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	

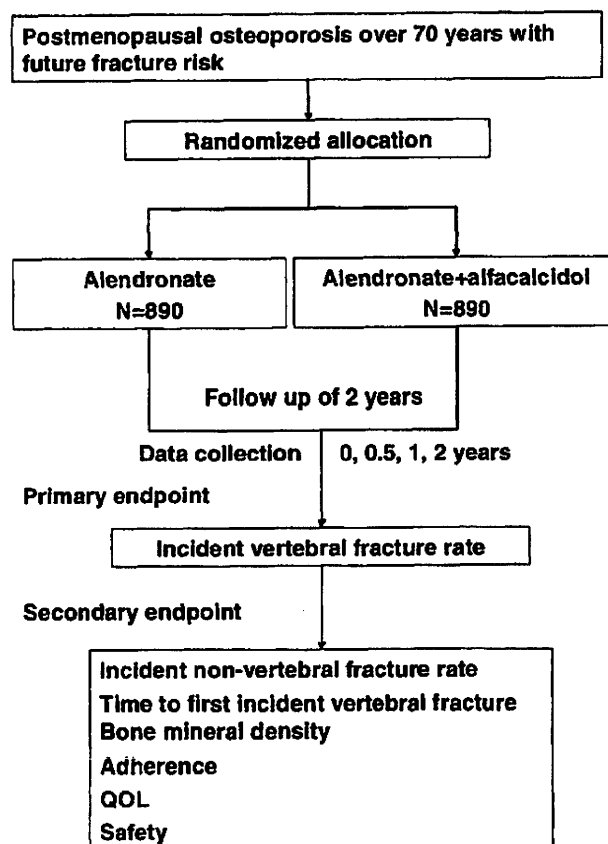
These thresholds were decided by risk analysis by the A-TOP research group

<sup>a</sup> Over 1 year after menopause

<sup>b</sup> Pre-existing vertebral fracture number  $\geq 1$ ; BMD  $\leq$  Young Adult Mean  $-3$  SD; Urinary DPD  $\geq 7.6$  nmol/mmol Cr; or NTX  $\geq 54.3$  nmol BCE/mmol Cr

<sup>c</sup> Hyperparathyroidism and hyperthyroidism were excluded

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

**Fig. 1** Study design and outcomes

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 metacarpal 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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