Glucosamine Sulfate Use and Delay of Progression of Knee Osteoarthritis

A 3-Year, Randomized, Placebo-Controlled, Double-blind Study

Karel Pavelká, MD, PhD; Jindriska Gatterová, MD; Marta Olejarová, MD; Stanislav Machacek, MD; Giampaolo Giacovelli, PhD; Lucio C. Rovati, MD

Background: Conventional symptomatic treatments for osteoarthritis do not favorably affect disease progression. The aim of this randomized, placebo-controlled trial was to determine whether long-term (3-year) treatment with glucosamine sulfate can modify the progression of joint structure and symptom changes in knee osteoarthritis, as previously suggested.

Methods: Two hundred two patients with knee osteoarthritis (using American College of Rheumatology criteria) were randomized to receive oral glucosamine sulfate, 1500 mg once a day, or placebo. Changes in radiographic minimum joint space width were measured in the medial compartment of the tibiofemoral joint, and symptoms were assessed using the algo-functional indexes of Lequesne and WOMAC (Western Ontario and McMaster Universities).

Results: Osteoarthritis was of mild to moderate severity at enrollment, with average joint space widths of slightly less than 4 mm and a Lequesne index score of less than 9 points. Progressive joint space narrowing with placebo use was –0.19 mm (95% confidence interval, –0.29 to –0.09 mm) after 3 years. Conversely, there was no average change with glucosamine sulfate use (0.04 mm; 95% confidence interval, –0.06 to 0.14 mm), with a significant difference between groups ($P=.001$). Fewer patients treated with glucosamine sulfate experienced predefined severe narrowings (>0.5 mm): 5% vs 14% ($P=.05$). Symptoms improved modestly with placebo use but as much as 20% to 25% with glucosamine sulfate use, with significant final differences on the Lequesne index and the WOMAC total index and pain, function, and stiffness subscales. Safety was good and without differences between groups.

Conclusion: Long-term treatment with glucosamine sulfate retarded the progression of knee osteoarthritis, possibly determining disease modification.

Arch Intern Med. 2002;162:2113-2123

From the Department of Medicine and Rheumatology, Charles University (Dr Pavelká), and the Institute of Rheumatology (Drs Pavelká, Gatterová, Olejarová, and Machacek), Prague, Czech Republic; and the Department of Clinical Pharmacology, Rotta Research Laboratorium, Monza, Italy (Drs Giacovelli and Rovati).

OSTEOARTHRITIS IS the most common form of arthritis. Symptomatic disease in the knee occurs in approximately 6% of US adults 30 years and older,[1] and results of community-based surveys have shown that the general incidence and prevalence increases 2- to 10-fold from age 30 to 65 years, with further increases thereafter.[2] Overall, osteoarthritis of the knee is particularly common, and radiographic osteoarthritic changes of the tibiofemoral compartment occur in 5% to 15% of people aged 35 to 74 years in the Western world.[3] The impact on disability attributable to knee osteoarthritis is similar to that due to cardiovascular disease and greater than that caused by any other medical condition in the elderly.[4] Although pathologic and radiographic evidence of osteoarthritis is poorly correlated with symptoms, the most appropriate definition of osteoarthritis is one that combines the pathology of the disease with pain that occurs with joint use, as concluded in a recent National Institutes of Health conference that reviewed the disease and its risk factors.[5] Treatment approaches have also been reviewed,[6] and the American College of Rheumatology[7] and the European League Against Rheumatism[3] issued guidelines for the medical management of osteoarthritis. In the absence of a cure for the disease, current therapeutic modalities are primarily aimed at reducing pain and improving joint function by the use of nonspecific symptomatic agents. However, at least some conventional nonsteroidal anti-inflammatory drugs (NSAIDs), which are the most widely used nonspecific symptomatic agents, have been shown to negatively affect the progression of osteoarthritis.[8] Even the newest and possibly safer NSAIDs, the cyclooxygenase-2 selective inhibitors,[9] did not favorably modify the long-term progression of the disease in terms of joint structure changes compared with conventional NSAIDs.[10] For this reason, much attention

©2002 American Medical Association. All rights reserved.

is being given to more specific compounds that may affect some of the mechanisms underlying the disease, thus delaying progression of the disease and limiting disability in the long term.[3,7] Glucosamine sulfate, the pharmaceutical derivative of the naturally occurring aminomonosaccharide glucosamine, a constituent of glycosaminoglycans in cartilage matrix and synovial fluid,[11] was reviewed for its short-term effects on disease symptoms[12,13] and has recently been shown to delay the long-term progression of knee osteoarthritis in terms of joint structure changes and symptoms.[14] These results have been welcomed with enthusiasm by most of the scientific community[15] but in some cases with some reservations,[16] mainly because such impressive data need to be appropriately confirmed. The present trial was designed and conducted in parallel, to eventually confirm independently and possibly extend the results on the long-term (3-year) effects of glucosamine sulfate therapy on the progression of knee osteoarthritis structural modifications and symptoms.

PATIENTS AND METHODS

STUDY POPULATION

Outpatients of both sexes aged 45 to 70 years with primary knee osteoarthritis were eligible to participate in the study if they fulfilled the following predefined criteria. The diagnosis of knee osteoarthritis of the medial femorotibial compartment was based on the clinical and radiological criteria of the American College of Rheumatology.[17] Disease stage was based on the Kellgren and Lawrence radiographic system,[18] which grades osteoarthritis on a severity scale from 0 to 4 based on the assumed sequential appearance of osteophytes, joint space loss, subchondral sclerosis, and cyst formation. Minimum symptom severity was ensured by using a Lequesne algo-functional index[19] score of at least 4 points, whereas patients with an index value higher than 12 points were excluded so as not to jeopardize the knee full extension for the radiographic evaluation (see the "Assessment of Joint Structure Changes" subsection). Other principal exclusion criteria were a history of clinically significant articular and rheumatic diseases other than osteoarthritis, including inflammatory rheumatic diseases, or that may cause secondary osteoarthritis, including a history of traumas or lesions of the knee joint and severe articular inflammation as confirmed by physical examination (eg, a finding of severe joint effusion) at inclusion; evidence of rapidly progressive osteoarthritis obtained before the trial; overweight, defined as a body mass index (calculated as weight in kilograms divided by the square of height in meters) greater than 27; clinically significant alterations in hematologic variables and renal, hepatic, and metabolic functions in the opinion of the investigator (including a history of clinically evident diabetes mellitus); and systemic or intra-articular corticosteroid therapy in the previous 3 months.

STUDY DESIGN

The trial was conducted according to a randomized, placebo-controlled design in a single center at the Prague Institute of Rheumatology between June 29, 1995, and January 20, 1999. The protocol was approved by the institutional review board of the center, and patients provided written informed consent. Patients were screened at a baseline visit that included a physical examination, a knee radiograph according to a standardized method, a symptom questionnaire, and routine safety laboratory tests. After enrollment, patients were randomized to the study medication and were followed up until completion of a 3-year treatment course. Clinic visits were performed quarterly and included symptom assessment, and standardized knee radiographs and routine safety laboratory tests were performed at the end of each year.

TREATMENTS AND BLINDING AND RANDOMIZATION PROCEDURES

In this trial, we used crystalline glucosamine sulfate, that is, the original glucosamine sulfate described in most of the literature[12-14] and available as a prescription drug for osteoarthritis in several European and other countries and as a nutritional supplement in the United States (Dona, Viartril-S, or Xicil; Rotta Research/Rottapharm Group, Monza, and Rotta Pharmaceuticals Inc, Wall, NJ). The product was used in its once-a-day formulation (packets of powder for oral solution), with a net content equivalent to 1500 mg of glucosamine sulfate. Patients were randomized to double-blind treatment with either 1500 mg of glucosamine sulfate or placebo packets identical in external appearance and content consisting of the inactive excipients only. The study medication was taken once a day for 3 years. Compliance to the study medication was determined by asking the patients about missed doses and by counting unused packets.

Patients received randomization numbers sequentially from a secret randomization list that was computer generated in blocks of 4 by individuals who had no contact with the persons who assigned patients to study groups, performed any assessments on patients, evaluated the radiographs at the end of the study, or performed the statistical analysis. The block size was also masked from all investigators involved in the trial. The clinical research center was given a single-sealed, opaque envelope for each patient that contained the treatment code and was to be opened only in a medical emergency. Treatment assignment was thus concealed, and masking was successfully achieved during the study since no sealed envelope was opened voluntarily or accidentally or was tampered with during the study.

Acetaminophen in 500-mg tablets was provided for rescue analgesia as needed, and its use was recorded in a patient daily diary. No other pharmacologic treatments for osteoarthritis or other formulations containing analgesics were allowed. Among physical therapies, only hydrotherapy, exercise, and ultrasound, alone or in combination, were allowed if the patient was following a stable regimen.

ASSESSMENT OF JOINT STRUCTURE CHANGES

Joint structure changes were assessed on serial radiographs performed according to the standardized technique recommended by the current guidelines[20,21] and as Pavelká et al[22] described previously. Radiographs were taken for each patient at enrollment and after 1, 2, and 3 years of treatment by the same technician in the radiology unit of the center using a single x-ray machine (Siere graph C; Siemens, Erlangen, Germany). Anteroposterior, weightbearing radiographs were obtained with the patient's heels and toes together and the knees in full extension. The x-ray beam was horizontal, and the central ray was fluoroscopically directed to the center of the joint space at the level of the tibial tubercle. The x-ray cassette film was placed 115 cm from the tube. Repositioning of the patient for subsequent radiographs was guided by the original film, and the same radiographic settings (ie, kilovolts, milliamperes, and milliseconds) were used.

All radiographs in a patient set were read for all evaluations concomitantly by 2 trained independent readers masked to treatment assignment and to the sequence of assessments, which was randomized. The primary outcome measure was represented by joint space width changes in the narrowest medial compartment of the tibiofemoral joint,[20,21] that is, in the signal

©2002 American Medical Association. All rights reserved.

joint. Changes in joint space width in the contralateral knee of patients with bilateral disease were analyzed as a secondary variable. Joint space width was measured by visual reading according to a validated method[23] at the joint's narrowest point using an ×10 magnifying lens graduated in 0.1-mm intervals. When the 2 independent readings were within 0.3 mm, the mean of the 2 values was taken. In the case of a difference greater than 0.3 mm, the radiograph was reinterpreted separately by both readers until the difference was within 0.3 mm. The intraobserver error for each reader was estimated on 40 randomly selected radiographs measured 8 times in random order in 10 days: the coefficient of variation was 1.91% for one reader and 2.71% for the other. The interobserver error was estimated on all radiographs: the coefficient of variation was 2.64% before reinterpretation and 2.53% after reinterpretation.

Among secondary end points for joint structure modification, radiographic features of osteoarthritis other than joint space narrowing (marginal osteophytes and subchondral sclerosis, separately assessed for each medial and lateral femoral condyle and tibial plateau in both knees) were scored for severity on a scale from 0 to 3 according to a validated atlas[24]; the baseline and final (or last available) radiographs were taken for this evaluation.

ASSESSMENT OF SYMPTOM CHANGE

Symptoms of knee osteoarthritis were evaluated at clinic visits primarily by using the algo-functional severity index of Lequesne,[19] a validated, disease-specific questionnaire addressing in a single index knee pain (5 questions scored on a 0-2 scale, with 0 indicating absent and 2 indicating severe), function limitation (4 questions, same scale), and maximum distance walked (1 question scored on a 0-6 distance scale, with 0 indicating ability to walk unlimited distances and 6 indicating ability to walk <100 m); the worst possible total score is 24 points. In addition, we also used the WOMAC (Western Ontario and McMaster Universities) knee osteoarthritis index,[25] another validated and disease-specific questionnaire separately addressing severity of joint pain (5 questions), stiffness (2 questions), and limitation of physical function (17 questions) in the 48 hours before assessment. The Likert scale version of the WOMAC index was used, with each question scored on a scale from 0 to 5, with 0 indicating none and 5 indicating extreme; 25, 10, and 85 points, therefore, are the worst possible severity scores for pain, stiffness, and limitation of physical function, respectively.

SECONDARY EFFICACY AND SAFETY END POINTS

Consumption of acetaminophen for rescue analgesia was calculated from the patient daily dairy. Withdrawal rates were computed with the reason for dropout. Safety was investigated by recording the occurrence of adverse events and by results of routine yearly laboratory tests.

STATISTICAL ANALYSIS

The sample size was calculated on the basis of the data available for the technique used for measurement of joint space width, assuming a difference of 0.33 mm between groups in joint space narrowing after 3 years[26]; with type I error of .05 and type II error of .10 in a 2-tailed test and a high predicted coefficient of variation of the measurement (8%-10%), 86 (rounded to 100) patients per group were calculated to be necessary.[26]

The primary efficacy outcome measure was therefore the difference between groups in the change in joint space width, that is, in joint space narrowing after 3 years in the patient's narrowest medial compartment of the tibiofemoral joint at enrollment. The primary analysis was performed in the intent-to-treat population, which consisted of all randomized patients. The intent-to-treat approach was carried out according to a worst-case scenario analysis: patients who did not complete the treatment course or had not undergone the final 3-year radiograph were assigned a poor outcome, corresponding to the final average change recorded in the per-protocol completer population in the placebo group.[27] Results are expressed as the difference between final group means and 95% confidence intervals, with *P* values based on analysis of variance (ANOVA). The robustness of this approach was challenged by a second intent-to-treat analysis performed by the random sampling method to avoid repeatedly assigning the same value to a series of missing values.[28] With this method, missing end point values were replaced with values selected randomly from the distribution of all end point values in the 2 treatment groups combined. To lower the sampling error, 50 such datasets were constructed and analyzed independently using ANOVA, and the median of the significance values was taken.[28]

Similar methods were used for the secondary outcome, represented by the difference in joint space narrowing after the first and second years of treatment. In addition, we arbitrarily set a cutoff value of more than 0.5-mm joint space narrowing to represent severe joint structure damage progression, which is in the range of previous suggestions[29] and as recently reported in other studies[14,22]: we compared the proportion of all randomized patients reaching such a progression cutoff value by using the exact χ^2 test. We also calculated the number needed to treat, which is the number of patients who have to be treated with the active medication to prevent 1 event represented by such a progression: the number needed to treat is the reciprocal of the absolute risk reduction, consisting of the difference in event rates between groups.[30] The exact χ^2 test was also used to compare between groups the proportion of patients with changes of at least 1 point in the scale from 0 to 3 for the secondary radiographic features of osteoarthritis (osteophytes and subchondral sclerosis) at the end point (ie, after 3 years or on an earlier radiograph with such a change in case of early dropout).

For symptom modification, similar to structure modification, the final changes after 3 years in scores on the Lequesne index and the WOMAC index (the latter separately assessed for the total index and for the pain, stiffness, and physical function subscales) were selected as appropriate summary measures for the primary symptom outcome[31] and were analyzed as described for joint space narrowing. However, mainly for descriptive purposes, the behavior of the principal variable, represented by the mean change in the Lequesne index score, was also plotted on a time-response curve for the worst-case scenario intent-to-treat population, and the results were analyzed by using repeated-measures ANOVA.

To exclude that improvement in knee pain throughout the study might have improved a patient's ability to adopt the fully extended knee position required for radiography, consequently resulting in an artifactual, apparent increase in joint space width, the following posthoc analysis was performed. Three-year completers in both groups were selected on the basis of an improvement in the WOMAC pain subscale score equal to at least the average improvement in the best treatment group at the end of the study. The average improvement in the WOMAC pain subscale score and the change in joint space width were then calculated for this subset of patients in the placebo and glucosamine sulfate groups and are briefly presented for descriptive analysis purposes.

Other secondary analyses included comparison of the mean number of days with rescue medication intake using ANOVA. Withdrawal and dropout rates were compared between groups using the exact χ^2 or Fisher exact tests, as appropriate. The baseline group characteristics were compared using the exact χ^2 test

©2002 American Medical Association. All rights reserved.

and ANOVA for categorical and continuous variables, respectively. All reported *P* values are 2-sided.

RESULTS

Of 385 patients who underwent screening, 202 were randomized into the study (**Figure 1**). A larger proportion of patients did not complete the 3-year treatment course with placebo than with glucosamine sulfate, but the difference (46% vs 35%) was not statistically significant ($P=.15$), and there were no significant differences between groups in the reasons for dropping out. All randomized patients (101 in each treatment group) were included in the intent-to-treat population, whereas the per-protocol population consisted of all patients who completed the study only. **Table 1** gives the comparable baseline characteristics of the 2 groups. Knee osteoarthritis was long-standing (>10 years) and was of mild to moderate severity on average. Patients were similarly distributed between a Kellgren and Lawrence grading of 2 or 3, with a mean symptom severity score of less than 9 points on the Lequesne index and scores of similar magnitude on the WOMAC index scales. This was also reflected in the baseline values of the primary outcome measure, represented by joint space width in the signal joint, which was slightly below 4 mm and without differences between the glucosamine sulfate and placebo groups ($P=.24$ and $P=.50$ for the intent-to-treat and per-protocol populations, respectively).

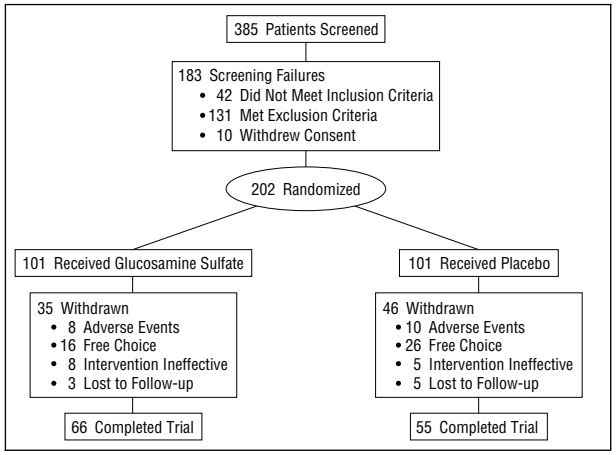

Figure 1. Flowchart of patient disposition.

Most patients in the 2 groups (77% and 66% taking placebo and glucosamine sulfate, respectively) had bilateral knee osteoarthritis at enrollment. Average symptom scores and signal joint space width were similar between these 2 subgroups and to the values of the overall patient population in Table 1 (data not shown). However, by definition, the average (SD) joint space width in the contralateral joint was larger than in the signal joint: 4.72 (1.53) mm and 4.90 (1.39) mm in the placebo and glucosamine sulfate subgroups, respectively.

A small proportion of patients, 27% taking placebo and 22% taking glucosamine sulfate, used any of the physical treatments allowed throughout the study (hydrotherapy, exercise, and ultrasound), alone or in combination, without any difference between groups. Compliance to the study medication was good, with at least 86% of patients reporting more than 90% drug intake at clinic visits in either group.

JOINT STRUCTURE CHANGES

In patients completing each year of treatment with placebo, there was progressive joint space narrowing, that is, a loss in joint space width (**Figure 2**). Conversely, there was no average joint space narrowing in patients receiving glucosamine sulfate, with a significant difference compared with the placebo group at each point. The final difference at 3 years between the 2 groups in the per-protocol population was 0.36 mm (95% confidence interval, 0.13-0.59 mm) (Figure 2). **Table 2** gives the changes in joint space width according to the intent-to-treat, worst-case scenario principal analysis, which confirmed the significant difference between the glu-

Table 1. Demographic and Baseline Clinical Characteristics of Intent-to-Treat (All Randomized) and per-Protocol Evaluable Patients*

	Intent-to-Treat Patients		Per-Protocol Patients	
Characteristic	Placebo (n = 101)	Glucosamine Sulfate (n = 101)	Placebo (n = 55)	Glucosamine Sulfate (n = 66)
Women, No. (%)	77 (76)	80 (79)	38 (70)	53 (80)
Age, y	63.5 (6.9)	61.2 (7.3)	63.0 (7.1)	61.2 (6.8)
Body mass index, kg/m^2	25.7 (1.8)	25.7 (2.1)	26.0 (1.8)	25.6 (2.3)
Duration of knee osteoarthritis, y	11.0 (6.8)	10.1 (8.1)	10.8 (6.3)	9.7 (6.2)
Kellgren and Lawrence grading, No. (%)				
Grade 2	54 (53)	55 (54)	32 (58)	35 (53)
Grade 3	47 (47)	46 (46)	23 (42)	31 (47)
Joint space width, mm	3.63 (1.57)	3.89 (1.48)	3.81 (1.56)	4.01 (1.54)
Lequesne index score, points	8.94 (2.27)	8.95 (2.30)	8.53 (2.30)	8.82 (2.39)
WOMAC index score, points				
Total	30.48 (14.43)	30.70 (14.40)	28.56 (14.12)	29.12 (13.90)
Pain	6.33 (3.13)	6.61 (3.45)	6.26 (3.19)	6.24 (3.09)
Function	22.00 (11.03)	21.84 (10.67)	20.30 (10.64)	20.68 (10.59)
Stiffness	2.15 (1.44)	2.25 (1.47)	2.00 (1.41)	2.20 (1.35)

*Data are given as mean (SD), except where indicated otherwise. WOMAC indicates Western Ontario and McMaster Universities.

©2002 American Medical Association. All rights reserved.

cosamine sulfate and placebo groups at each year of treatment. The statistical significance of the primary outcome after 3 years was confirmed for consistency by the random sampling intent-to-treat analysis, where the median significance was $P<.001$.

The number of patients experiencing severe joint space narrowing, according to the arbitrary cutoff value of greater than 0.5 mm, was 14 in the placebo group and 5 in the glucosamine sulfate group ($P=.05$) (**Figure 3**). The number of patients needed to be treated with glucosamine sulfate to prevent such a progression in joint structure deterioration is therefore 11. Also, the secondary radiographic features of osteoarthritis showed a favorable outcome in the glucosamine sulfate group in that there were only 4 of 66 patients with worsened atlas osteophyte scores at the end point in at least 1 joint compartment compared with 11 of 56 patients receiving placebo ($P=.03$) (**Figure 4**). Most worsenings occurred in the signal joint, although for 4 patients with bilateral disease (2 in each group), they occurred in the contralateral knee. The joint compartment most often affected by osteophyte worsening was the medial tibial plateau, followed by the medial femoral condyle. No patient in either group had a change in the subchondral sclerosis score.

In patients with bilateral knee osteoarthritis, the intent-to-treat signal joint space changes were of the same magnitude as those reported for the overall patient population in Table 2 (data not shown), with a similar statistically significant difference in favor of glucosamine sulfate therapy ($P=.004$). In the contralateral joint, patients receiving placebo underwent significant joint space narrowing (–0.13 mm; 95% confidence interval, –0.23 to –0.03 mm) that did not occur in those receiving glucosamine sulfate (–0.04 mm; 95% confidence interval, –0.12 to 0.05 mm), although the difference between the 2 subgroups was not statistically significant ($P=.17$).

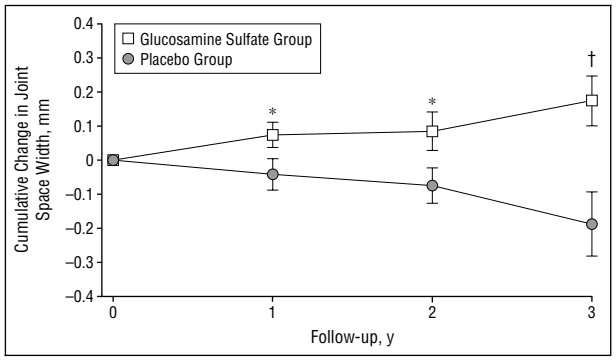

Figure 2. Joint space narrowing in patients completing each year of the study. The number of evaluable patients in the placebo and glucosamine sulfate groups, respectively, was 84 and 83 at year 1, 57 and 68 at year 2, and 55 and 65 at year 3. Error bars represent SEM. Asterisk indicates $P\leq.05$ vs placebo; dagger, $P<.01$ vs placebo.

SYMPTOM CHANGES

Pain and function limitation decreased in completers in both treatment groups according to the Lequesne index (**Figure 5**) and WOMAC index (**Figure 6**) scores. However, the improvements were significantly larger in patients receiving glucosamine sulfate, with score reductions of 20% to 25% compared with baseline. The final change in the WOMAC index joint stiffness subscale, although of smaller size, showed a significant difference in favor of glucosamine sulfate use too (Figure 6D). These results were confirmed by the principal analysis according to the intent-to-treat worst-case scenario approach (**Table 3**), where the magnitude of the improvements was again larger in the glucosamine sulfate group: approximately 20% on the Lequesne index and approximately 15% on the WOMAC total index and pain or function subscales (with the smaller changes in the stiffness subscale being nevertheless again statistically significant). The median significance between groups in the intent-to-treat analysis performed according to the random sampling approach for consistency was also statistically significant ($P<.001$ and $P=.002$ for the Lequesne index and the WOMAC total index, respectively).

The intent-to-treat pattern of the Lequesne index at the quarterly clinic visits is shown in **Figure 7**. The glucosamine sulfate group had progressive and constant improvement compared with the placebo group, especially during the first year and maintained during the second and third years ($P=.004$ between groups on the ANOVA for repeated measures). Symptom changes followed the same pattern in patients with bilateral disease (data not shown).

There were no statistically or clinically significant differences between treatment groups in the consumption of acetaminophen for rescue analgesia, which was minor and variable in most patients. Approximately 30% to 40% of patients in both groups consistently took acetaminophen at or above the average of once every 3 days, without an apparent relationship with joint structure or symptom outcomes.

EFFECT OF SYMPTOM IMPROVEMENT ON JOINT STRUCTURE CHANGES

To exclude that the significantly higher symptom improvement achieved with glucosamine sulfate use at the end of the study could have biased the joint space width

Table 2. Intent-to-Treat Cumulative Joint Space Narrowing at Each Year of Treatment*

Year	Placebo Group (n = 101)	Glucosamine Sulfate Group (n = 101)	Difference	*P* Value
1	–0.04 (–0.12 to 0.03)	0.05 (–0.007 to 0.12)	0.097 (0.0006 to 0.19)	.049
2	–0.08 (–0.14 to –0.02)	0.03 (–0.05 to 0.11)	0.11 (0.01 to 0.20)	.03
3	–0.19 (–0.29 to –0.09)	0.04 (–0.06 to 0.14)	0.23 (0.09 to 0.37)	.001

*Data are given as mean (95% confidence interval) change in joint space width, in millimeters, for each group and the difference in means between groups.

©2002 American Medical Association. All rights reserved.

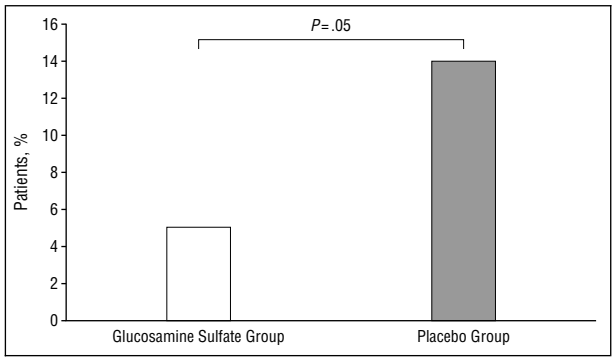

Figure 3. Proportion of all randomized patients (n=101 in each group) with joint space narrowing greater than 0.5 mm throughout the study.

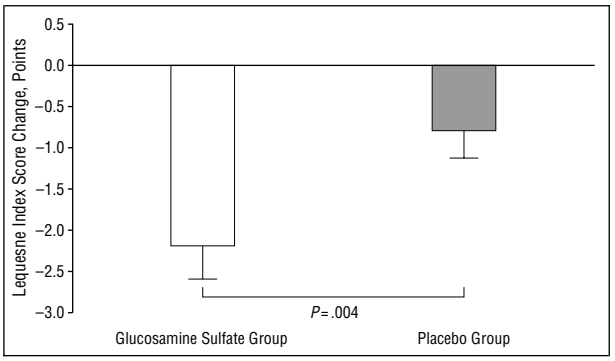

Figure 5. Per-protocol analysis of mean change in the Lequesne index score after 3 years. Error bars represent SEM.

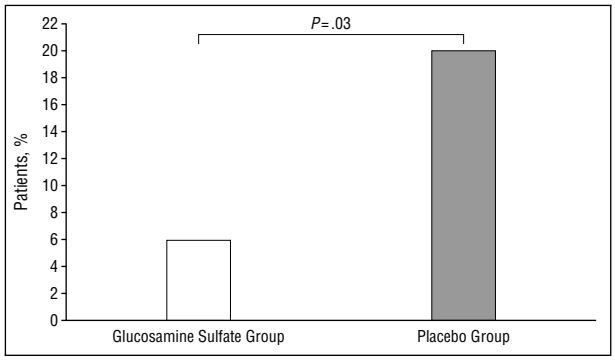

Figure 4. Proportion of patients reaching the end point who had worsening osteophyte atlas scores (n=56 receiving placebo and n=66 receiving glucosamine sulfate).

radiological assessment, completers in the 2 groups were selected according to a threshold of improvement of at least 2 points on the WOMAC index pain subscale (corresponding to the average intent-to-treat improvement with glucosamine sulfate use). There were more patients above this threshold in the glucosamine sulfate group (41 vs 27), but the 2 patient subsets had comparable baseline WOMAC index pain scores and joint space widths (data not shown) and comparable mean pain improvement after 3 years (**Figure 8**A) of almost 4 points on the WOMAC index pain subscale, that is, of more than 50% compared with their mean baseline scores. Notwithstanding this major pain relief, the placebo patient subset underwent definite joint space narrowing compared with the glucosamine sulfate subset (Figure 8B), with a difference that was at the limits of statistical significance ($P=.06$) despite the small sample size in this subanalysis and in the same range as that observed in the overall study population.

TREATMENT SAFETY

Overall, 64% of patients receiving placebo and 66% receiving glucosamine sulfate reported at least 1 adverse event during the 3 years of study. **Table 4** gives the proportion of patients reporting adverse events according to body system; there were no statistically significant differences between groups in the proportion or pattern of adverse events. The most frequently reported complaints were attributable to the gastrointestinal tract and liver systems and consisted predominantly of transient episodes of abdominal pain and dyspeptic symptoms: 3 patients in each group dropped out of the study for abdominal pain, dyspepsia, or nausea; 2 additional patients in the placebo group dropped out for diarrhea or cholecystitis. Musculoskeletal reports were mainly for osteoarthritis-related symptoms or back pain; however, 1 patient in each group was withdrawn for developing episodes of possible gout arthritis, and, in 2 patients taking glucosamine sulfate, probable rheumatoid arthritis was diagnosed after treatment started and was classified as an adverse event, although it was most likely preexisting; finally, 1 patient taking placebo dropped out because of a hip fracture. Cardiovascular events consisted predominantly of episodes of increased blood pressure or recurrent manifestations of preexisting ischemic heart disease in this elderly population: 1 patient receiving placebo was withdrawn because of a stroke, and in the glucosamine sulfate group 1 male patient with a 6-year history of ischemic heart disease with previous myocardial infarctions, cardiac failure, and recent coronary bypass died of a fatal myocardial infarction before completing 3 months of treatment with the study medication. Skin and appendage disorders were represented mainly by cutaneous rash episodes: 1 patient receiving placebo was withdrawn because of an allergic exanthema and 1 receiving glucosamine sulfate because of eczema. As expected, a high proportion of patients reported seasonal upper respiratory tract infections. Reports of urinary tract infections were also common in both groups. Among metabolic and nutritional problems, 4 patients developed clinically evident diabetes mellitus during the study (3 were taking placebo [1 dropout] and 1 was taking glucosamine sulfate). Routine safety laboratory test results did not show significant differences between groups. No patient underwent knee joint replacement during the study, but 2 patients in the placebo group underwent hip replacement for osteoarthritis or a traumatic lesion.

COMMENT

Results of the present trial show that long-term oral administration of glucosamine sulfate for 3 years can delay the natural progression of knee osteoarthritis. Symptoms of joint pain and limitation of function significantly improved throughout the study in the glucosamine sulfate group compared with the placebo group, and, espe-

©2002 American Medical Association. All rights reserved.

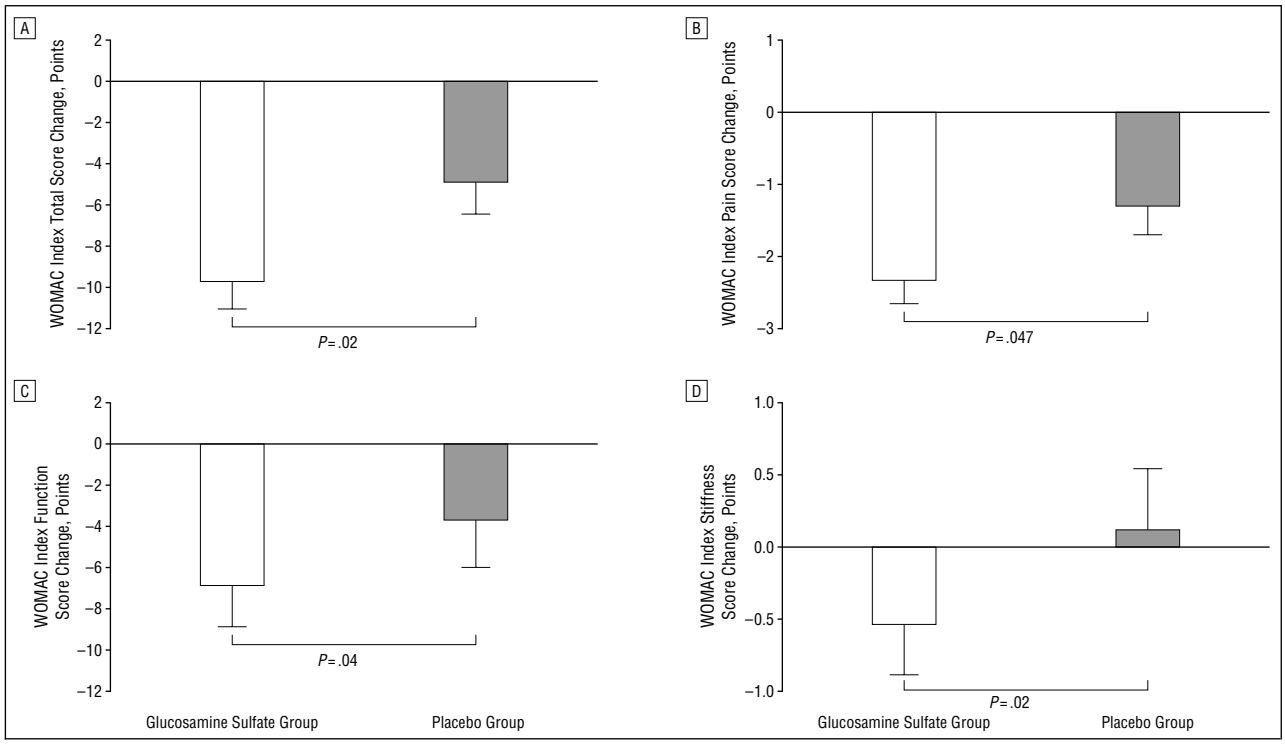

Figure 6. Per-protocol analysis of mean change in the WOMAC (Western Ontario and McMaster Universities) total index (A) and pain (B), function (C), and stiffness (D) subscale scores after 3 years. Error bars represent SEM.

Table 3. Intent-to-Treat Change in Symptom Scores After 3 Years Compared With Baseline Scores*

Measure	Placebo Group (n = 101)	Glucosamine Sulfate Group (n = 101)	Difference	*P* Value
Lequesne index	−0.82 (−1.1 to −0.51)	−1.7 (−2.2 to −1.2)	0.91 (0.34 to 1.5)	.002
WOMAC index				
Total scale	−4.9 (−6.5 to −3.2)	−8.0 (−9.8 to −6.3)	3.1 (0.77 to 5.5)	.01
Pain subscale	−1.3 (−1.7 to 0.88)	−2.0 (−2.4 to −1.5)	0.7 (0.06 to 1.3)	.03
Function subscale	−3.7 (−4.9 to −2.5)	−5.8 (−7.1 to −4.4)	2.1 (0.28 to 3.9)	.02
Stiffness subscale	0.11 (−0.12 to 0.34)	−0.31 (−0.55 to 0.07)	0.42 (0.09 to 0.75)	.01

*Data are given as mean (95% confidence interval) change in the score (in points) for each group and the difference in means between groups.
WOMAC indicates Western Ontario and McMaster Universities.

cially, patients receiving glucosamine sulfate did not undergo, on average, the progressive joint structure changes radiologically observed in patients taking placebo. These results are of particular relevance in that they independently confirm and extend the results of another recent study.[14] Glucosamine sulfate, therefore, is the first agent that meets the current requirements to be classified as a symptom- and structure-modifying drug in osteoarthritis, according to the definition of scientific organizations,[20,21] as acknowledged by regulatory agencies.[32,33]

Structural effects have been evaluated on standardized radiographs by measuring the change in width of medial tibiofemoral joint space as the primary outcome, as recommended by expert consensus.[20,21,32,33] Measurement was performed at the joint's narrowest point by visual inspection with the aid of a magnifying glass, that is, using the gold standard, accepted method.[21,23] A sound protocol with 2 independent, masked readers, resulting in low interobserver and intraobserver variability, has been

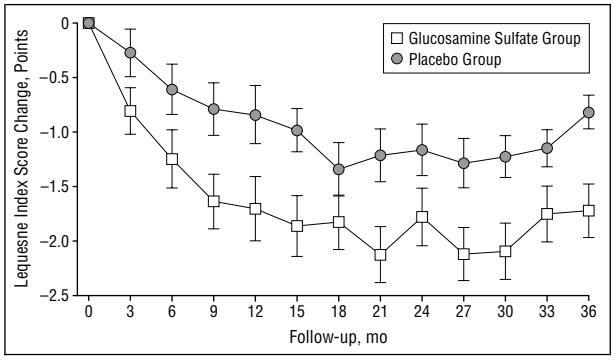

Figure 7. Intent-to-treat Lequesne index score mean change at clinic visits throughout the study. Analysis of variance for repeated measures: *P*=.004 between treatments. Error bars represent SEM.

preferred to one of the different methods of digital image analysis that measure either mean joint space or a specific distance in the joint and that have also been sug-

©2002 American Medical Association. All rights reserved.

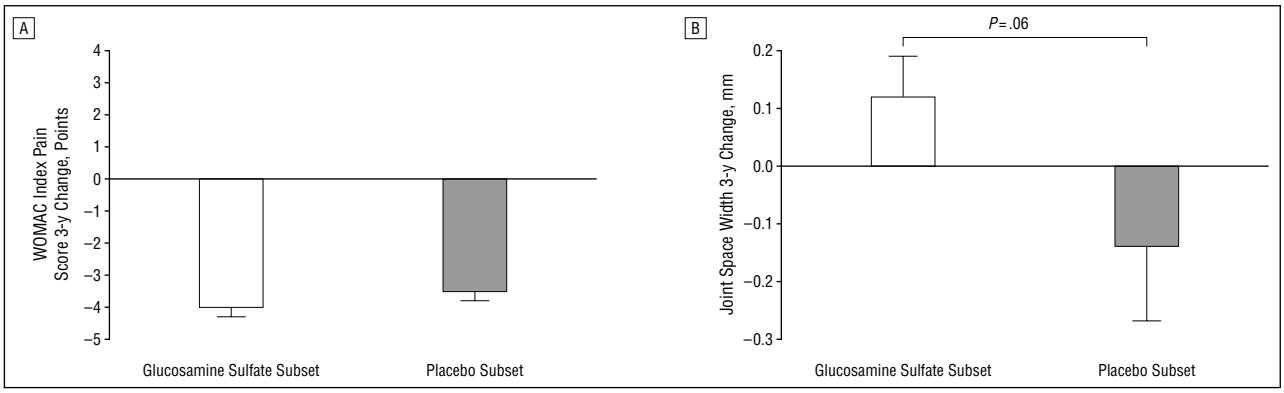

Figure 8. Pain improvement (A) and joint space narrowing (B) in the subset of patients reporting major pain relief (27 patients receiving placebo and 41 receiving glucosamine sulfate). Error bars represent SEM. WOMAC indicates Western Ontario and McMaster Universities.

Table 4. Patients Reporting at Least 1 Adverse Event During the 3-Year Study, by Body System*

System Organ Class (WHO Coding)	Placebo Group, % (n = 101)	Glucosamine Sulfate Group, % (n = 101)
Gastrointestinal tract and liver	28	25
Musculoskeletal	22	30
Cardiovascular	20	23
Skin and appendages	15	10
Respiratory tract	7	17
Urinary tract	11	12
Metabolic and nutritional	6	7
Other†	14	14

*Each patient may have reported >1 adverse event in the same or other organ classes. WHO indicates World Health Organization.

†Includes isolated adverse events on the following systems: nervous, psychiatric, blood cell disorders, neoplasms, endocrine disorders, reproductive (male/female), vision disorders, hearing, and vestibular.

gested to decrease variability.[21] By this method, we found a natural rate of joint space narrowing in the placebo group that was below 0.1 mm/y, that is, in the same range detected by large, observational, community-based studies[34,35] or clinic-based populations[36] and slower than proposed in earlier studies possibly biased by the small number of patients included or by short follow-up.[37] In addition, the rate of joint space narrowing in our control group was similar to that recently reported in other long-term randomized controlled trials.[14,22] Glucosamine sulfate therapy prevented this naturally occurring slow joint space narrowing. This effect was evident after the first and second years of treatment, although the loss in space width in the placebo group was not linear and increased especially during the third year, thus confirming an observation that had already been proposed.[14] The final difference in the change in joint space width between the glucosamine sulfate and placebo groups in patients completing 3 years of treatment was similar to the figure we used for our sample size calculation and that was suggested by experts to define an effective treatment.[26] This per-protocol analysis result was then confirmed by an intent-to-treat approach on all randomized patients that adopted 2 different methods to evaluate the robustness of the findings.

In addition, treatment with glucosamine sulfate dramatically decreased the proportion of patients with clinically substantial joint space loss, as defined by an arbitrary cutoff value previously suggested.[14,29] Patients who experience such structural damage may be more subject to future disability: the reduction in this absolute risk with glucosamine sulfate use allowed calculation of a sufficiently low number needed to treat of 11. This means that 11 patients have to be treated with glucosamine sulfate to prevent 1 from experiencing clinically substantial joint space loss. This way of presenting joint space narrowing data, besides being more easily interpretable, has been suggested to be more relevant in clinical terms.[38] A similar trend in favor of glucosamine sulfate therapy for retarding joint space narrowing was found in the less affected knee in patients with bilateral osteoarthritis, which would support the opportunity for early intervention with this compound. This aspect needs further evaluation.

Precise measurement of joint space width depends on correct standardization of the radiographic technique.[21] In the present study, we used the state-of-the-art technique available at the time of study design and still advised by the current recommendations,[21] that is, with the knee in full extension. However, recent evidence[39] suggests that different radiographic views, for example, with the semiflexed knee, may improve precision and avoid having the presence of pain or functional limitation impair the possibility of knee full extension. How much this theoretical limitation translates into real bias is unclear,[15] but the discussion seems to be mostly academic and may affect any long-term clinical study that uses techniques that are state-of-the-art at the time of study design but that inexorably undergo substantial refinement by the time of study completion. In any case, in our study, this did not seem to represent a bias on the final results. In fact, pain and function limitation were of mild to moderate severity at enrollment and did not prevent knee full extension. Moreover, they improved in both treatment groups (although with a clinically and statistically significant difference in favor of glucosamine sulfate therapy), thus excluding that joint space narrowing observed in the placebo group with this radiographic technique should be mainly an artifact of symptom worsening. Furthermore, several studies have repeatedly shown that symptom and structure changes are poorly corre-

©2002 American Medical Association. All rights reserved.

lated in osteoarthritis,[40] and even the long-term effects of glucosamine sulfate use on symptoms occur irrespective of the outcome on joint space width.[14] However, the ultimate evidence that knee pain and its improvement were not confounders of the radiographic assessment of joint space narrowing progression came from our analysis of the subgroup of patients who experienced major pain relief regardless of treatment with placebo or glucosamine sulfate. Indeed, this did not prevent joint space narrowing with placebo compared with glucosamine sulfate use.

In addition, and for the first time, to our knowledge, in a randomized, controlled, intervention trial, we used a validated atlas[24] to score secondary radiographic features of osteoarthritis, that is, features other than joint space width. Although the results are preliminary and should be interpreted with caution because this was not a primary outcome measure, substantially more patients taking placebo vs glucosamine sulfate worsened their osteophyte score. It is unlikely that this finding is biased by the radiographic technique adopted, and it would therefore confirm an overall beneficial effect of glucosamine sulfate therapy in the progression of joint structure changes. This preliminary finding also loosens the pressure on the ongoing discussion about the relevance of radiographic joint space width as the only determinant of joint structure modification in knee osteoarthritis. Although this variable is currently advised as the primary outcome in trials of disease modification,[20,21,32,33] and its relevance remains of primary importance, results of recent studies suggest that loss of joint space in the early stages of osteoarthritis may be due in part to meniscal extrusion and not only to articular cartilage erosion.[41] Moreover, plain radiography may not be the ideal tool to measure tibiofemoral joint space,[42] unless careful protocols that include use of fluoroscopy are used to standardize the radioanatomic position of the knee,[42] as in our study. Therefore, other radiographic features of osteoarthritis might be favorably affected by treatment with glucosamine sulfate. In particular, osteophytes have been indicated to be better associated with pain in knee osteoarthritis.[43]

In the present study, symptoms improved with glucosamine sulfate use to a significantly larger extent than in the placebo group. The fact that symptoms tended to slightly improve also with placebo therapy is not surprising in a long-term trial in which the principal effort is to keep the patient in the study, with several clinic visits and assessments being performed. Actually, there was a statistically nonsignificant trend for a higher proportion of withdrawals in the placebo than in the glucosamine sulfate group, which may reflect lower treatment satisfaction in the control group. Other large, prospective studies[36] on the natural history of the disease also did not show a worsening in pain severity after 3 years, notwithstanding the fact that most patients reported an overall worsening of their condition. In any case, symptom improvement with placebo therapy was limited to a few percentage points, whereas patients receiving glucosamine sulfate reported an average 20% to 25% improvement in pain and function according to the per-protocol analysis and 15% to 20% by the intent-to-treat approach adopted, which is in agreement with the effect size reported in similar studies.[14] Such results were obtained by assessment using the Lequesne index[19] and were confirmed for consistency using the WOMAC index,[25] that is, the 2 most widely used algo-functional indexes of the severity of knee osteoarthritis.[20,21] Besides reporting the final symptom score, we analyzed the development of the symptomatic effect of glucosamine sulfate during treatment and found a steadily developing effect over the first year that then remained constant until completion of the study after 3 years. These results on osteoarthritis symptoms have already attracted much attention,[15] and one should consider that they have been obtained in patients with mainly mild to moderate disease and in a long-term study that may not be optimal to fully appreciate the development and effect size of the improvement. Indeed, short-term studies specifically designed with such an aim have already described the pattern of the symptomatic effect of glucosamine sulfate use and have recently been reviewed.[12,13] These studies showed a significantly better effect than placebo therapy and at least similar to that of conventional NSAID use in the first 4 weeks of treatment,[44,45] with improvement in pain and function up to 40% to 50% relative to basal conditions within 12 weeks,[46] that is, a moderate to large effect size[12,13] and a carryover effect at drug withdrawal.[46] The present study, together with other similar experiences,[14] completes the pattern of the effects of glucosamine sulfate for long-term chronic treatment, as demanded lately by the scientific community.[47,48] As a possible limitation in this regard, although the patient population in our study is largely representative of the general population with knee osteoarthritis, we excluded obese patients and those with metabolic diseases that may be responsible for secondary osteoarthritis. In addition, as expected in a placebo-controlled, long-term trial, we included a limited number of patients with severe disease.

Although we standardized the consumption of a rescue medication to acetaminophen only, contrary to the study by Reginster et al,[14] in which selected NSAIDs were also used, we did not observe a difference between groups in its consumption either. These data seem to confirm that consumption of rescue analgesics is not a valid outcome measure in osteoarthritis trials, possibly being subject to different confounding factors.[21]

Several experimental studies have now elucidated the mechanism of action of glucosamine sulfate in osteoarthritis. First, a wide and recently reviewed pharmacokinetic experience[49] has shown that after oral administration, glucosamine sulfate is bioavailable and reaches the articular cartilage. Glucosamine is preferentially incorporated by the chondrocytes into the components of the glycosaminoglycan chains in the intact cartilage,[50] stimulates the synthesis of physiological proteoglycans,[51-53] and decreases the activity of catabolic enzymes, including metalloproteases.[52-54] In addition, there is increasing evidence[54-56] that the compound reverses some of the negative effects of interleukin 1 on cartilage metabolism. Such activities on cartilage should be responsible for the long-term effects of glucosamine sulfate, especially those on joint structure changes.[16] Conversely, the rapid effects on symptoms observed for shorter

©2002 American Medical Association. All rights reserved.

treatment courses[12,13,44-46] are better explained by the mild anti-inflammatory effects exerted by the suppression of superoxide-radical generation[57] or the inhibition of inducible nitric oxide synthesis and, selectively, of the cyclooxygenase-2 pathway.[58]

The mechanism of action of glucosamine sulfate supports the good safety of the compound, largely described during short-term treatment[12,13] as being without differences from placebo[44,46] but significantly better than with conventional NSAIDs.[45,46] Our study confirmed another observation[14] of a similarly good treatment tolerance over long-term administrations, with no differences from placebo and no signal for peculiar toxic effect patterns being identified. In this respect, it was suggested by the results of animal experimental studies[48] that glucosamine may determine insulin resistance, although this hypothesis was not confirmed by recent human experiences,[59] and that its long-term administration might unmask clinically overt diabetes mellitus.[48] In our study, 4 patients were diagnosed as developing diabetes mellitus during the study, but only 1 was receiving glucosamine sulfate, whereas the remaining 3 were receiving placebo, thus confirming the long-term safety of the substance also on glucose metabolism.[14]

Glucosamine derivatives are popular dietary supplements in the United States and other countries, exploiting the opportunity provided by the American Dietary Supplement Health and Education Act and the clinical research data obtained with glucosamine sulfate approved as a prescription drug for the treatment of osteoarthritis in Europe and elsewhere. The latter was used in our study and in most of the previous clinical experiences[12-14]; at present, it is difficult to generalize these results to the highly variable and uncontrolled formulations of the other nutritional products claiming a glucosamine content.[15]

In conclusion, this study demonstrates that glucosamine sulfate is the first pharmacologic intervention that retards the progression of osteoarthritis during long-term treatment, according to the current scientific and regulatory recommendations.[20,21,32,33] The compound merits further attention as a possible disease-modifying agent for the treatment of osteoarthritis.

Accepted for publication February 13, 2002.

This study was funded by the Rotta Research/ Rottapharm Group.

This study was presented at the annual meeting of the American College of Rheumatology, Philadelphia, Pa, November 1, 2000, and at the annual meeting of the European Congress of Rheumatology, Prague, Czech Republic, June 14, 2001.

Corresponding author and reprints: Karel Pavelká, MD, PhD, Institute of Rheumatology, Na Slupi 4, 12850 Praha 2, Czech Republic (e-mail: pavelka@revma.cz).

REFERENCES

1. Felson DT, Zhang Y. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. *Arthritis Rheum.* 1998;41:1343-1355.
2. Oliveria SA, Felson DT, Reed JI, Cirillo PA, Walker AM. Incidence of symptomatic hand, hip and knee osteoarthritis among patients in a health maintenance organization. *Arthritis Rheum.* 1995;38:1134-1141.
3. Pendleton A, Arden N, Dougados M, et al. EULAR recommendations for the management of knee osteoarthritis: report of a task force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). *Ann Rheum Dis.* 2000;59:936-944.
4. Guccione AA, Felson DT, Anderson JJ, et al. The effects of specific medical conditions on the functional limitations of elders in the Framingham Study. *Am J Public Health.* 1994;84:351-358.
5. Felson DT, Lawrence RC, Dieppe PA, et al. Osteoarthritis: new insights, part 1: the disease and its risk factors. *Ann Intern Med.* 2000;133:635-646.
6. Felson DT, Lawrence RC, Hochberg MC, et al. Osteoarthritis: new insights, part 2: treatment approaches. *Ann Intern Med.* 2000;133:726-737.
7. American College of Rheumatology Subcommittee on Osteoarthritis Guidelines. Recommendations for the medical management of osteoarthritis of the hip and knee. *Arthritis Rheum.* 2000;43:1905-1915.
8. Rashad S, Revell P, Hemmingway A, Low F, Rainsford K, Walker F. Effect of non-steroidal anti-inflammatory drugs on the course of osteoarthritis. *Lancet.* 1989; 2:519-522.
9. Hawkey CJ. COX-2 inhibitors. *Lancet.* 1999;353:307-314.
10. Van Kuijk C, Cheng X, Hottya G, et al. The effects of rofecoxib and diclofenac on knee osteoarthritis articular cartilage: the results from one-year prospective clinical trials [abstract]. *Arthritis Rheum.* 2000;43(suppl):924.
11. Hamerman D. The biology of osteoarthritis. *N Engl J Med.* 1989;320:1322-1330.
12. McAlindon TE, La Valley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. *JAMA.* 2000;283:1469-1475.
13. Towheed TE, Anastassiades TP, Shea B, Houpt J, Welch V, Hochberg MC. Glucosamine therapy for treating osteoarthritis [Cochrane Review on CD-ROM]. Oxford, England: Cochrane Library, Update Software; 2001;issue 2.
14. Reginster JY, Deroisy R, Rovati LC, et al. Long-term effects of glucosamine sulfate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. *Lancet.* 2001;357:251-256.
15. McAlindon T. Glucosamine for osteoarthritis: dawn of a new era? *Lancet.* 2001; 357:247-248.
16. Chard J, Dieppe P. Glucosamine for osteoarthritis: magic, hype or confusion? *BMJ.* 2001;322:1439-1440.
17. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. *Arthritis Rheum.* 1986;29:1039-1049.
18. Kellgren JK, Lawrence JS. Radiological assessment of osteoarthritis. *Ann Rheum Dis.* 1957;16:494-501.
19. Lequesne MG, Mery C, Samson M, Gerard P. Indexes of severity for osteoarthritis of the hip and knee: validation-value in comparison with other assessment tests. *Scand J Rheumatol.* 1987;65(suppl):85-89.
20. Dougados M, for the Group for the Respect of Ethics and Excellence in Science. Recommendations for the registration of drugs used in the treatment of osteoarthritis. *Ann Rheum Dis.* 1996;55:552-557.
21. Altman R, Brandt K, Hochberg M, Moskowitz R. Design and conduct of clinical trials of patients with osteoarthritis: recommendations from a task force of the Osteoarthritis Research Society. *Osteoarthritis Cartilage.* 1996;4:217-243.
22. Pavelká K, Gatterová J, Gollerova V, Urbanova Z, Sedlackova M, Altman RD. A 5-year randomised controlled, double-blind study of glycosaminoglycan polysulphuric acid complex (Rumalon) as a structure modifying therapy in osteoarthritis of the hip and knee. *Osteoarthritis Cartilage.* 2000;8:335-342.
23. Lequesne M. Quantitative measurements of joint space width during progression of osteoarthritis: chondrometry. In: Kuetter KE, Goldberg VM, eds. *Osteoarthritic Disorders.* Rosemont, Ill: American Academy of Orthopaedic Surgeons; 1995:427-444.
24. Altman RD, Hochberg M, Murphy WA, Wolfe F, Lequesne M. Atlas of individual radiographic features in osteoarthritis. *Osteoarthritis Cartilage.* 1995;3(suppl A): 3-70.
25. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol.* 1988;15:1833-1840.
26. Lequesne M, Brandt K, Bellamy N, et al. Guidelines for testing slow acting drugs in osteoarthritis. *J Rheumatol.* 1994;21:65-73.
27. Hollis S, Campbell F. What is meant by intention to treat analysis? survey of published randomised controlled trials. *BMJ.* 1999;319:670-674.
28. Herxenberg LA, Moore WA, De Rosa SC. Estimation of missing values [letter]. *Lancet.* 1999;354:686.
29. Ravaud P, Girardeau B, Auleley GR, et al. Variability in knee radiographing: implication for definition of radiological progression in medial knee osteoarthritis. *Ann Rheum Dis.* 1998;57:624-629.
30. Laupacis A, Sackett DL, Roberts RS. An assessment of clinically useful mea-

©2002 American Medical Association. All rights reserved.

sures of the consequences of treatment. *N Engl J Med*. 1988;318:1728-1733.
31. Matthews JSN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *BMJ*. 1990;300:230-235.
32. Committee for Proprietary Medicinal Products. *Points to Consider on Clinical Investigation of Medicinal Products Used in the Treatment of Osteoarthritis*. London, England: The European Agency for the Evaluation of Medicinal Products; 1998.
33. Center for Drug Evaluation and Research. *Clinical Development Programs for Drugs, Devices and Biological Products Intended for the Treatment of Osteoarthritis*. Rockville, Md: US Food and Drug Administration; 1999.
34. Lethbridge-Cejku M, Hochberg MC, Scott WW Jr, Plato CC, Tobin JD. Longitudinal change in joint space of the knee: data from the Baltimore Longitudinal Study of Aging [abstract]. *Arthritis Rheum*. 1995;38(suppl):S262.
35. Neuhauser KB, Anderson JJ, Felson DT. Rate of joint space narrowing in normal knees and knees with osteoarthritis [abstract]. *Arthritis Rheum*. 1994;37(suppl): S423.
36. Dieppe PA, Cushnaghan J, Shepstone L. The Bristol "OA500" Study: progression of osteoarthritis (OA) over 3 years and the relationship between clinical and radiographic changes at the knee joint. *Osteoarthritis Cartilage*. 1997;5:87-97.
37. Mazzucca SA, Brandt KD, Katz BP. Is conventional radiography suitable for evaluation of a disease-modifying drug in patients with knee osteoarthritis? *Osteoarthritis Cartilage*. 1997;5:217-226.
38. Ravaud P, Ayral X, Dougados M. Radiologic progression of hip and knee osteoarthritis. *Osteoarthritis Cartilage*. 1999;7:222-229.
39. Buckland-Wright JC, Wolfe F, Ward RJ, Flowers N, Hayne C. Substantial superiority of semiflexed (MTP) views in knee osteoarthritis: a comparative radiographic study, without fluoroscopy, of standing extended, semiflexed (MTP), and schuss views. *J Rheumatol*. 1999;26:2664-2674.
40. Creamer P, Lethbridge-Cejku M, Hochberg MC. Factors associated with functional impairment in symptomatic knee osteoarthritis. *Rheumatology*. 2000;39: 490-496.
41. Adams JG, McAlindon T, Dimasi M, Carey J, Eustace S. Contribution of meniscal extrusion and cartilage loss to joint space narrowing in osteoarthritis. *Clin Radiol*. 1999;54:502-506.
42. Mazzuca SA, Brandt KD. Plain radiography as an outcome measure in clinical trials involving patients with knee osteoarthritis. *Rheum Dis Clin North Am*. 1999; 25:467-480.
43. Creamer P, Lethbridge-Cejku M, Hochberg MC. Determinants of pain severity in knee osteoarthritis: effect of demographic and psychosocial variables using 3 pain measures. *J Rheumatol*. 1999;26:1785-1792.
44. Noack W, Fischer M, Förster KK, Rovati LC, Setnikar I. Glucosamine sulfate in osteoarthritis of the knee. *Osteoarthritis Cartilage*. 1994;2:51-59.
45. Müller-Faβbender H, Bach GL, Haase W, Rovati LC, Setnikar I. Glucosamine sulfate compared to ibuprofen in osteoarthritis of the knee. *Osteoarthritis Cartilage*. 1994;2:61-69.
46. Rovati LC. The clinical profile of glucosamine sulfate as a selective symptom modifying drug in osteoarthritis: current data and perspectives [abstract]. *Osteoarthritis Cartilage*. 1997;5(suppl A):72.
47. Towheed TE, Anastassiades TP. Glucosamine and chondroitin for treating symptoms of osteoarthritis: evidence is widely touted but incomplete. *JAMA*. 2000; 283:1483-1484.
48. Adams ME. Hype about glucosamine. *Lancet*. 1999;354:353-354.
49. Setnikar I, Rovati LC. Absorption, distribution, metabolism and excretion of glucosamine sulfate: a review. *Arzneimittelforschung*. 2001;51:699-725.
50. Noyszewski EA, Wroblewski K, Dodge GR, et al. Preferential incorporation of glucosamine into the galactosamine moieties of chondroitin sulfates in articular cartilage explants. *Arthritis Rheum*. 2001;44:1089-1095.
51. Bassleer C, Rovati LC, Franchimont P. Glucosamine sulfate stimulates proteoglycan production in human chondrocytes in vitro. *Osteoarthritis Cartilage*. 1998; 6:427-434.
52. Piperno M, Reboul P, Helio Le Graverand MP, et al. Glucosamine sulfate modulates dysregulated activities of human osteoarthritic chondrocytes in vitro. *Osteoarthritis Cartilage*. 2000;8:207-212.
53. Dodge GR, Hawkins DF, Jimenez SA. Modulation of aggrecan, MMP1, and MMP3 production by glucosamine sulfate in cultured human osteoarthritis articular chondrocytes [abstract]. *Arthritis Rheum*. 1999;42(suppl):S253.
54. Sandy JD, Gamett D, Thompson V, Verscharen C. Chondrocyte-mediated catabolism of aggrecan: aggreganase-dependent cleavage induced by interleukin-1 or retinoic acid can be inhibited by glucosamine. *Biochem J*. 1998;335: 59-66.
55. Yaron I, Shirazi I, Judovich R, Yaron M. Glucosamine sulfate inhibits nitric oxide and stromelysin production in cartilage cultures and reverses IL-1 inhibition of osteoarthritic articular cartilage synthesis [abstract]. *Ann Rheum Dis*. 2001;60 (suppl):50.
56. Gouze JN, Bordji K, Gulberti S, et al. Interleukin-1β down-regulates the expression of glucuronosyltransferase I, a key enzyme priming glycosaminoglycan biosynthesis: influence of glucosamine on interleukin-1β–mediated effects in rat chondrocytes. *Arthritis Rheum*. 2001;44:351-360.
57. Setnikar I, Cereda R, Pacini MA, Revel L. Antireactive properties of glucosamine sulfate. *Arzneimittelforschung*. 1991;41:157-161.
58. Shikhman AR, Kuhn K, Alaaeddine N, Lotz M. N-acetylglucosamine prevents IL-1 β-mediated activation of human chondrocytes. *J Immunol*. 2001;166:5155-5160.
59. Pouwels MJ, Jacobs JR, Span PN, Lutterman JA, Smits P, Tack CJ. Short-term glucosamine infusion does not affect insulin sensitivity in humans. *J Clin Endocrinol Metab*. 2001;86:2099-2103.

©2002 American Medical Association. All rights reserved.