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The role of diet and exercise and of glucosamine sulfate in the prevention of knee osteoarthritis: Further results from the PRevention of knee Osteoarthritis in Overweight Females (PROOF) study

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ABSTRACT

Background and objectives: The PRevention of knee Osteoarthritis in Overweight Females (PROOF) study (ISRCTN 42823086) described a trend for a decrease in the incidence of knee osteoarthritis (OA) by a tailored diet and exercise program (DEP) or by oral glucosamine sulfate in women at risk for the disease, using a composite clinical and/or radiological outcome. The aim of this updated post-hoc analysis was to re-assess the results according to more precise techniques and take advantage of the 2×2 factorial design.
Methods: A total of 407 overweight (BMI $\geq$ 27 kg/m^2) women of 50–60 years of age with no diagnosis of knee OA were randomized to: (1) no DEP + placebo (Control, $N = 102$), (2) DEP + placebo (DEP, $N = 101$), (3) glucosamine sulfate + no DEP (GS, $N = 102$), and (4) DEP + glucosamine sulfate (DEP + GS, $N = 102$) and followed for 2.5 years, with standardized postero-anterior, semiflexed (MTP) view knee radiographs at baseline and end of the study. DEP consisted of a tailored low fat and/or low caloric diet and easy to implement physical activities. Glucosamine was given as oral crystalline glucosamine sulfate 1500 mg once daily, double-blinded vs. placebo. Incident knee OA was defined as radiographic progression of $\geq$1 mm minimum joint space narrowing (mJSN) in the medial tibiofemoral compartment, as previously assessed by the visual (manual) technique and by a new semi-automated method. Logistic regression analysis was used to calculate the odds ratio for the effect of the interventions.
Results: After 2.5 years, 11.8% of control subjects developed knee OA. This incidence was decreased with glucosamine sulfate, either alone or in combination with the DEP, but not by the DEP alone. Since there was no statistical interaction between treatments, the 2×2 factorial design allowed analysis of patients receiving glucosamine sulfate ($N = 204$) vs. those not receiving it ($N = 203$), similarly for those on the DEP ($N = 203$) or not ($N = 204$). Glucosamine sulfate significantly decreased the risk of developing knee OA: odds ratio (OR) = 0.41 (95% CI: 0.20–0.85, $P = 0.02$) by the manual JSN assessment method and OR = 0.42 (95% CI: 0.20–0.92, $P = 0.03$) by the semi-automated technique. Conversely, there was no decrease in risk with the DEP.
Conclusions: Glucosamine sulfate decreased the risk of developing radiographic knee OA over 2.5 years in overweight, middle-aged women at risk, as determined by medial mJSN progression. Conversely a tailored diet and exercise program exerted no preventive effect, possibly because of the lower than expected effect on weight loss.
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Introduction

Osteoarthritis (OA) is the most common joint disorder and is among the main causes of disability in the elderly [1]. Estimates in the Western world suggest that the incidence of OA may double by 2040 [2]. This is particularly worrying for the most prevalent OA localizations, such as OA of the knee joint, which today affects a minimum of 5% up to 24% of the general population in its symptomatic form [3]. It is therefore particularly important not only to develop new and more effective treatments for OA, but also to search for strategies to prevent the disease.

The PRevention of knee Osteoarthritis in Overweight Females (PROOF) study is probably the first preventive randomized controlled trial in knee OA [4] (ISRCTN registry number: 42823086). It was designed with the aim of assessing the effect of a tailored diet and exercise program (DEP) and/or of oral glucosamine sulfate on the incidence of knee OA in a group of women at high risk of developing the disease because of their age and being overweight or obese. In the first article relating to the PROOF study, we described a non-significant decrease in the incidence of knee OA either with the DEP or with glucosamine sulfate after a follow-up of 2.5 years [4]. However, the trial had been structured with a factorial design and a statistical interaction between treatments prevented us from analyzing the data taking full advantage of the factorial design features. This resulted in the requirement to analyze separately each of the four treatment groups, and the study was not sufficiently powered for this circumstance. Moreover, the primary outcome of incident knee OA was defined on the basis of a single combined outcome measure: the development of either radiographic OA according to the appearance of Kellgren–Lawrence (K–L) grade ≥ 2, or the combined American College of Rheumatology (ACR) criteria for knee OA, or according to radiographic progression defined as a minimum joint space narrowing (mJSN) of ≥ 1 mm. The latter was performed on both medial and lateral tibiofemoral compartments and by visual (manual) reading.

At present, no guidelines are available for defining incident OA in preventive research. However, the scientific community as well as regulatory agencies such as the American Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require that structural progression of knee OA should be based on radiographic mJSN of the medial tibiofemoral compartment [5]. For this reason, we decided to perform a post-hoc analysis on the outcome of the PROOF study limiting the definition of incident knee OA to the development of mJSN ≥ 1 mm in the medial tibiofemoral compartment, thus avoiding the confounders of alternative OA definitions and the inherent larger variability of lateral JSN assessment [6]. In addition, recent recommendations for knee imaging in clinical OA trials advocate semi-automated measurement instead of manual measurement to improve precision, and thereby responsiveness [5]. We therefore based the current additional results and post-hoc analysis both on the original manual mJSN measurement of the medial compartment, as well as on the renewed and supposedly more responsive semi-automated mJSN measurement.

Methods

The methods of the PROOF study are described in details elsewhere [4]. In brief, women between 50 and 60 years of age with a BMI $\geq$ 27 kg/m^2, an absence of knee OA according to the ACR clinical criteria [7] and no treatment for knee complaints or history of rheumatic diseases, were selected based on the response to a reply card sent by 50 general practitioners in the Rotterdam region (The Netherlands) to all of their registered women without major co-morbidities. Inclusion and exclusion criteria, including no use of oral glucosamine over the last 6 months, were checked in a telephone interview and participants were invited to a clinic visit for baseline screening and assessments consisting of: medical history, physical examination, symptom questionnaires (consisting of the Western Ontario and McMaster Universities [WOMAC] index [8] and quality of life assessed by the EuroQoL questionnaire [9]), and knee radiographs. Patients were followed for up to 2.5 years (30 months), with home visits every 6 months to collect general medical information, carry out body weight measurements, retrieve unused study medications and provide the new supply. Clinic visits were performed at baseline and end of study, and included standardized knee radiographs.

Randomization and blinding

Following informed consent, baseline screening and assessments, subjects were randomized according to a computerized randomization scheme to the DEP or to a control group, and to glucosamine sulfate or placebo. While subjects were informed as to whether they had been randomized to the individually tailored DEP or not, there was full double-blindness with respect to glucosamine sulfate and placebo in a 2×2 factorial design. The treatment groups were as follows:

(1) no DEP + placebo (Control),
(2) DEP + placebo (DEP),
(3) glucosamine sulfate + no DEP (GS), and
(4) DEP + glucosamine sulfate (DEP + GS).

A block randomization was used, with a block size of 20.

Interventions

The tailored DEP is described in detail elsewhere [10]. In brief, nutritional and physical activity habits were recorded by specific questionnaires and discussed with a study dietician who set the goals for each individual subject using motivational interviewing techniques [11]. A tailored strategy was then given for a low fat and/or low caloric diet, with physical activities that were pleasing and easy to implement and maintain in the subject's daily routine, including low impact sports and exercise such as Nordic walking, aqua jogging and dancing. In this respect, subjects were invited to join weekly physical exercise classes (12–15 participants) of 1 h for 20 weeks, supervised by a local physical therapist. Subjects in the DEP control groups were not offered any advice.

Glucosamine sulfate was used in the original prescription formulation of patented crystalline glucosamine sulfate in sachets of powder for oral solution (Rottapharm, Monza, Italy), at the dose of 1500 mg glucosamine sulfate once daily. Placebo consisted of sachets identical in external appearance and content consisting of the inactive excipients only.

Knee radiographs and their assessments

Knee radiographs were taken at baseline and end of study (30 months) by the standardized postero-anterior, semiflexed (MTP) view [12]. Medial knee alignment angle was assessed by digitally determining the angle between the line from the center of the tibial spine through the center of the femoral shaft at approximately 10 cm from the joint margin and the matching line through the tibia [13]. All radiographs were scored by the K–L staging criteria [14]. In the PROOF primary analysis [4], minimum joint space width (mJSW) was measured by visual reading with the use of a digital ruler [15] within the whole width of the medial and lateral tibiofemoral compartments by two trained readers, blinded for group allocation and clinical outcomes, but with

sequence known. Scores with a difference ≥2.0 mm between readers were re-assessed. mJSN was defined as the difference between the average follow-up minimum mJSW of the two readers minus the average baseline mJSW of the two readers. The intra-class correlations of 0.67–0.76 of this methodology suggested that a re-analysis performed by a computer-assisted, semi-automated technique and limited to the medial compartment might provide more precise measures [5]. We therefore used a previously validated semi-automated method of measuring mJSW on digital radiographs [6] with good intra- and inter-observer variability represented by intra-class correlation coefficients up to 0.97 and 0.94, respectively [16]. In this method, an observer places a horizontal line at the inferior margins of the femur. Next, the software generates two vertical lines at the margins of the femur. After that, two additional parallel vertical lines are created—one 10 mm from the first vertical condyle line and the other 25 mm from this line. Within this 15 mm area, the observer manually delineated the tibial and femur bone edges. Minimal JSW corresponds to the smallest possible circle crossing these lines (automatically calculated). Since external calibration (e.g., a graduated ruler) was missing on several individual radiographs, we improved this technique by using internal calibration, i.e., the size of the bone within a delimited location of the radiograph, like the femoral intercondylar distance. Reproducibility of this method was very good with an intra-class correlation coefficient between measurements of 0.94 (95% CI: 0.87–0.97) [17]. This post-hoc measurement was performed by a single reader in a blinded fashion for group allocation, clinical outcomes, and randomizing the sequence of radiographs.

Statistical analysis

The original trial was sized (one-sided α = 5% and 80% statistical power) to observe a decrease in the incidence of knee OA from 20% in one group (DEP or glucosamine sulfate, respectively) to 10% in the respective control according to the factorial design analysis plan. This hypothesis led to a calculation of 176 subjects in each of the two large groups involving DEP or glucosamine sulfate. The final sample size was increased to 200 subjects in each of the two large groups (i.e., 100 in each of the four single intervention groups) to account for a 10% drop-out rate.

In this updated post-hoc analysis, the incidence of knee OA was intended (as originally foreseen by the protocol) per subject, i.e., knee OA was considered to occur if one or the other knee had met the primary outcome in one subject.

The primary outcome in the first report [4] was OA in each knee joint (in order to increase the study power) if one of the following had occurred: K–L grade ≥2, diagnosis of knee OA according to the ACR combined clinical and radiographic criteria, or radiologic mJSN ≥1.0 mm in either the medial or lateral tibiofemoral compartment. Since the present analysis only concerned the radiographic progression with the measurement of mJSW, only the definition of incident knee OA based on a mJSN ≥1.0 mm was retained and limited to the medial tibiofemoral compartment according to current recommendations [5].

The primary analysis was performed in the intention-to-treat (ITT) population, i.e., including all randomized patients. According to the factorial design approach, at first we assessed the presence of a statistical interaction between interventions. The analyses were conducted by means of a Binomial Model for analysis of variance. If no statistical interaction occurred, the effect of glucosamine sulfate (or of the DEP) could be analyzed taking full advantage of the 2 × 2 factorial design, i.e., pooling the two groups with or without that specific intervention [18]. Otherwise, in the case of a significant statistical interaction, each of the single interventions should be analyzed against controls [4]. Odds ratios (OR) (with 95% CI) were also calculated.

Results

A total of 407 women were enrolled after the screening process and were randomized to the four intervention groups (Fig.). There

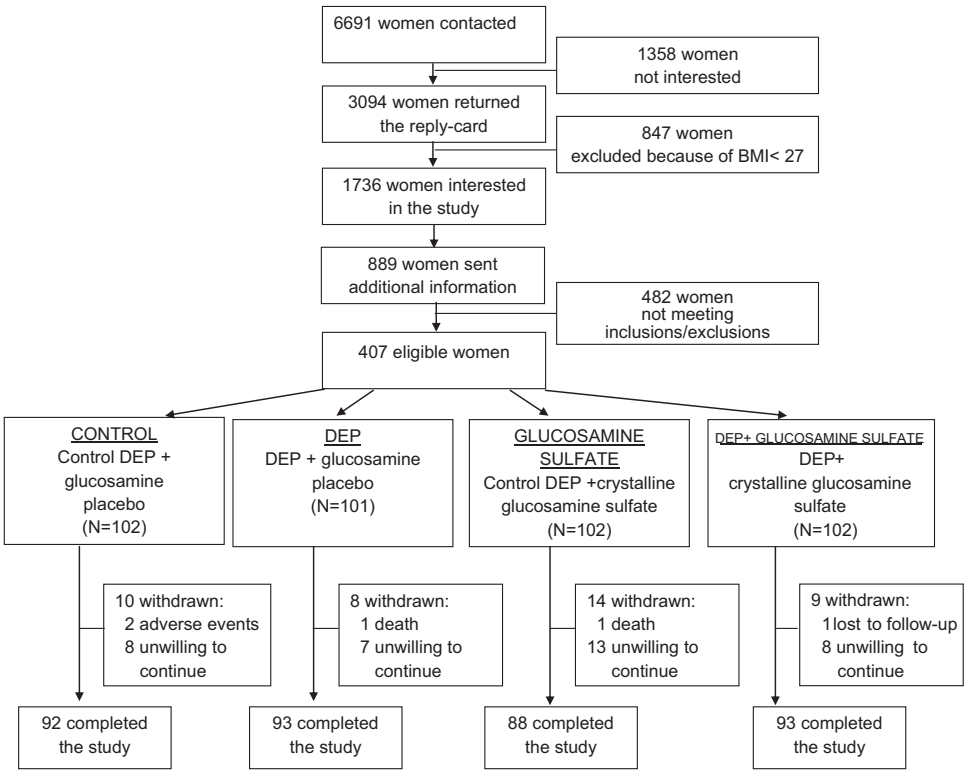

Fig. PROOF study flow diagram.

Table 1
Baseline characteristics and prognostic features in the four intervention groups

	Control[a] (N = 102)	DEP[a] (N = 101)	Glucosamine sulfate[a] (N = 102)	DEP + glucosamine sulfate (N = 102)
Age (years)	55.7 (3.3)	55.7 (3.2)	55.7 (3.1)	55.7 (3.1)
Postmenopausal status (%)	70	66	68	67
BMI (kg/m^2)	32.6 (4.3)	32.3 (4.5)	32.4 (4.6)	32.1 (3.7)
EuroQoL, 0–1[b]	0.90 (0.12)	0.88 (0.14)	0.88 (0.13)	0.90 (0.12)
Heberden's nodes (%)	25	32	30	21
History of knee injury (%)	14	10	12	13
Varus malalignment (%)	46	38	38	37
Mild knee symptoms (%)	29	36	30	27
WOMAC, 0–100				
Pain	5.1 (8.5)	8.1 (13.3)	7.1 (11.7)	6.6 (11.4)
Function	5.3 (8.7)	7.7 (12.2)	7.1 (12.2)	5.9 (10.4)
Kellgren–Lawrence				
Grade 0–1 (%)	92	89	92	85
Grade ≥2 (%)	8	11	8	15
Medial mJSW reading				
Semi-automated (mm)	4.1 (0.8)	4.1 (0.9)	4.1 (0.8)	4.1 (1.0)
Manual (mm)	4.8 (0.8)	4.7 (0.8)	4.7 (0.8)	4.7 (0.9)

Data are mean (SD) or % of available data.
BMI, body mass index; DEP, diet and exercise program; mJSW, minimum joint space width; WOMAC, Western Ontario and McMaster Universities questionnaire.
[a] Control is no DEP and placebo; DEP is DEP and placebo; Glucosamine sulfate is no DEP and glucosamine sulfate.
[b] Higher scores represent better quality of life.

was good retention in the study, with an average withdrawal rate of 10.1%. Most of the withdrawals were unwilling to remain in the study for its full duration of 2.5 years, without any study-related complaints. There were only two withdrawals because of adverse events and both were in the control group. In all, two women died in the course of the study, one in the DEP group and one in the glucosamine sulfate group, but none of these events were related to the study interventions.

Participating subjects (Table 1) were representative of a population of mostly postmenopausal, middle-aged women, overweight to obese (average BMI approximately, 32 kg/m^2), with good quality of life. Few subjects (10–15%) had a history of knee injury, but 35–45% had varus malalignment and around 30% complained of mild knee symptoms not necessitating treatment. WOMAC scores were on average well below 10 on a 0–100 scale, for both pain and functional limitation. Although all knees had K–L grades 0 or 1 at the baseline screening, approximately 10% of subjects had developed a K–L grade of at least two in one or the other knee at the post-randomization central K–L reading. Mean average baseline medial JSW was 4.1 ± 0.8 mm and 4.7 ± 0.8 mm according to the semi-automated measurement, or to the manual reading, respectively.

Primary outcome: Incidence of knee OA

Table 2 reports the incidence of knee OA, as determined by medial mJSN ≥ 1.0 mm according to both measurement techniques.

After the 2.5 year observation period, 11.8% of subjects developed radiographic knee OA in the control group, irrespective of the measurement method. The incidence was decreased in those subjects receiving glucosamine sulfate or the combination of glucosamine sulfate and the DEP, with only small differences between the two mJSN assessment techniques. Results were more variable for subjects receiving only the DEP. No statistically significant interaction between the interventions (P = 0.44 for semi-automated, and P = 0.79 for the manual method) was found and it was therefore possible to perform the treatment comparison taking full advantage of the 2 × 2 factorial design. As reported in Table 3, the incidence of knee OA was lower in the subjects receiving glucosamine sulfate compared with those not receiving this treatment. Such a difference was evident with both assessment methods, with an OR of 0.42 (95% CI: 0.20–0.92), and 0.41 (95% CI: 0.20–0.85) for the semi-automated and the manual mJSN assessment methods, respectively.

Conversely, no significant difference was observed when comparing patients receiving the DEP with those not receiving it, with corresponding ORs of 1.01 (95% CI: 0.49–2.08) and 1.20 (95% CI: 0.61–2.35), for the semi-automated and manual mJSN methods, respectively.

Compliance and safety

Compliance with glucosamine sulfate was reasonably good with 57% women taking at least 75% of the study medication. Compliance with the DEP (defined as attendance at at least six dietary consultations and at least seven exercise classes) was present in only 28% women randomized to the DEP, with or without glucosamine sulfate. The proportion of women on the DEP who reached the target of 5 kg or 5% weight loss was 14% at 6 months (vs. 6% in the other two groups; P = 0.01), 17% at 12 months (vs. 10%; P = 0.04), and 15% at the end of the study (at 30 months) without differences between groups. Women that were compliant on the DEP had an average weight loss of 1.4 ± 5.2 kg compared with 0.0 ± 6.7 kg in the other two groups.

The safety of glucosamine sulfate vs. placebo was good, with no difference in the number of women reporting adverse events (P = 0.23) or serious adverse events (P = 0.26); none of the adverse events reported were considered related to the study medication [4].

Discussion

Oral glucosamine sulfate, with or without a personalized diet and exercise program (DEP), decreased the incidence of knee OA as defined by joint structural damage progression over 2.5 years of observation in a group of overweight and obese women with no diagnosis of knee OA and without treatment for knee symptoms at

Table 2
Incidence of knee OA in subjects receiving the four interventions during 2.5 years of observation, in the intention-to-treat population

	Control[a] (N = 102)	Diet and exercise program[a] (DEP) (N = 101)	Glucosamine sulfate[a] (N = 102)	Glucosamine sulfate + DEP (N = 102)
Semi-automated mJSN ≥ 1.0 mm, n (%)	12 (11.8%)	10 (9.9%)	4 (3.9%)	6 (5.9%)
Manual mJSN ≥ 1.0 mm, n (%)	12 (11.8%)	14 (13.9%)	5 (4.9%)	7 (6.9%)

[a] Control is no DEP and placebo; DEP is DEP and placebo; glucosamine sulfate is no DEP and glucosamine sulfate; DEP, diet and exercise program; mJSN, minimum joint space narrowing.

Table 3
Incidence of knee OA in subjects receiving glucosamine sulfate compared with those not receiving it during 2.5 years of observation, in the intention-to-treat population

	No glucosamine sulfate (± DEP) ($N = 203$)	Crystalline glucosamine sulfate (± DEP) ($N = 204$)
Semi-automated mJSN ≥ 1.0 mm, *n* (%)	22 (10.8%) Odds ratio = 0.42 (95% CI: 0.20–0.92) $P = 0.03$	10 (4.9%)
Manual mJSN ≥ 1.0 mm, *n* (%)	26 (12.8%) Odds ratio = 0.41 (95% CI: 0.20–0.85) $P = 0.02$	12 (5.9%)

DEP, diet and exercise program; mJSN, minimum joint space narrowing.

baseline. In comparison, a customized DEP did not lead to significant changes in incident knee OA. These data derive from further data and a post-hoc analysis of the PRevention of knee Osteoarthritis in Overweight Females (PROOF) study [4].

In the original report of the pre-defined primary outcome measure of this trial [4], it was not possible to analyze the data taking full advantage of the 2×2 factorial design features, since there was unforeseen statistical interaction between the interventions. This interaction forced us to analyze each intervention group individually against controls, with an ample loss of power. Nevertheless, the analysis suggested a trend for the efficacy of either glucosamine sulfate or the DEP to decrease the incidence of knee OA [4]. We hypothesized that the statistical interaction observed might be due, at least in part, to the lack of sufficient precision in the method of assessing radiographic mJSW, and thus mJSN, that was performed in both the medial and lateral tibiofemoral compartments and consisted of visual (manual) reading of the knee radiographs [15]. We therefore decided to re-read, post-hoc but in a blinded fashion, the digital radiographic images also according to a semi-automated technique [6,16,17] in agreement with current guidelines to increase precision [5], and to limit the analysis to the medial tibiofemoral compartment [5], in order to better substantiate the previous results. Actually, there was no statistical interaction between treatments with either the semi-automated or the visual (manual) measurement of mJSN in the medial compartment. Indeed, the results were very comparable for the two methods, with a significant decrease of 58–59% in the risk of developing knee OA in the women who had received glucosamine sulfate with or without DEP.

The DEP showed no effect on incident knee OA as defined by radiographic progression of medial mJSN with both measurement techniques. A possible explanation was that the effect of DEP on weight loss, the main reason for adopting such a strategy, was slightly lower than expected. Actually, compliance on the DEP was suboptimal, with only 28% women showing reasonable compliance and, indeed, only around 15% of women achieved the target weight loss of 5 kg or 5%, with a significant difference between DEP and control groups only observed over the first 12 months of the study. Even in compliant women, the mean weight loss was less than 2 kg over the 2.5 years of observation, which may not be sufficient to exert a significant preventive effect on structural knee OA. Based on the available evidence [19] and more recent studies [20,21], the new algorithm recommendations by the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) strongly advise to target at least 10% weight loss to achieve significant symptom benefit and to improve the quality and thickness of the medial tibiofemoral compartment cartilage [1]. It is therefore entirely possible that this weak effect of the DEP on weight loss was sufficient to induce a trend on the prevention of knee OA, but not to further improve the already good result achieved by glucosamine sulfate alone when the two interventions were combined. On the other hand, despite solid evidence supporting the efficacy of weight loss for symptom improvement, a recent study failed to show any improvement on knee joint structure of diet with or without exercise even when achieving weight loss up to 10% [22]. Future studies are therefore needed to better explore whether diet and exercise strategies have a role in the prevention of structural knee OA.

The combination of glucosamine sulfate and the DEP did not further improve the preventive effects of glucosamine sulfate alone that were observed in the present study. However, combining diet and exercise with an early background treatment such as glucosamine sulfate, should not be discouraged. First of all, an adequate diet and exercise program with weight loss provides overall benefit during health and disease. Secondly, even when limited to the treatment or prevention of OA and in particular knee OA, exercise and weight loss are in the core set of interventions according to all current treatment guidelines [1,23–25]. In the PROOF study, there was no difference in weight loss when glucosamine sulfate was added to the DEP; this is similar to what was shown in the short-term with a combination of glucosamine hydrochloride and chondroitin sulfate (GH/CS) in patients with established knee OA, with no negative effect of such combination on knee strength in both short-term [26] and long-term studies [27]. Other studies showed no effect of glucosamine sulfate addition on short-term improvement of joint magnetic resonance imaging parameters by exercise alone [28], but significant additive effects on cartilage metabolism as assessed by biomarkers [29,30], with slightly better improvement on muscle strength [31].

The PROOF study used the patented prescription preparation [32] of 1500 mg crystalline glucosamine sulfate once daily, i.e., the only preparation recommended by current guidelines for the treatment of knee OA [1]. Indeed this is the only glucosamine formulation that has been proven effective in high-quality clinical trials of knee OA symptoms [33,34], as also described in a recent Cochrane Review where over-the-counter glucosamine sulfate formulations or glucosamine hydrochloride were not effective in contrast to prescription crystalline glucosamine sulfate [35]. It is therefore unlikely that the results of the PROOF study can be transferred to other glucosamine formulations, including combinations of glucosamine with chondroitin sulfate where the latter decreased the already poor bioavailability of glucosamine hydrochloride, which is, per se, not effective in OA [36].

Crystalline glucosamine sulfate 1500 mg once daily was also shown to be effective as a structure-modifying agent in established knee OA, particularly in mild disease [37,38]; this was a good basis for the hypothesis that the compound might be effective in a preventive setting.

In the current study, incident knee OA was defined as radiological progression of mJSN in the medial tibiofemoral compartment. In all, two different methods were used for assessing mJSN, i.e., the standard manual method, or a semi-automated method as recommended by current guidelines [5]. As noted above, the results were very comparable for the two methods. Thus, while semi-automated methods may be recommended in trials of interventions for progression of established OA, it is postulated here that either method might be used in OA prevention trials when a definite threshold of mJSN is pre-determined to define incident OA. Conversely, lateral compartment mJSN was not used in this analysis since, according to current guidelines [5], the medial compartment is the most common site of involvement of

knee OA and it is strongly related to future total knee replacement. The role of lateral mJSN in preventive trials should be further investigated, similar to the statistical interaction we found in our previous report [4].

While mJSW assessment is still considered by regulatory agencies such as the FDA and EMA as the gold standard to assess joint structure modification, recent reports suggest that fixed-location radiographic JSN measures may show better responsiveness than mJSW locations [39]. On the other hand, magnetic resonance imaging-based cartilage thickness measures might even show the best responsiveness [39]. It is therefore suggested that future intervention studies should evaluate these more advanced imaging techniques.

Conclusion

Treatment with oral crystalline glucosamine sulfate for 2.5 years with or without a customized diet and exercise program prevents knee OA as defined by structural progression of mJSN in the knee medial compartment, in a group of overweight or obese middle-aged women at risk for future knee OA development. Further studies should investigate to what extent such an effect is maintained over long-term observations after treatment withdrawal, whether it might be favorably affected by longer treatment periods, and whether this eventually leads to lower incident rates of established clinical knee OA. Conversely, there was no significant effect of diet and exercise alone. Possibly, this was due to the mild effect of the diet and exercise program on weight loss. Further studies with stronger diet and exercise programs targeting greater weight loss are warranted with or without the addition of glucosamine sulfate in the OA prevention setting.

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