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Original article

Long-term effects of a lifestyle intervention and oral glucosamine sulphate in primary care on incident knee OA in overweight women

Bastiaan C. de Vos[1], Marieke L. A. Landsmeer[1], Marienke van Middelkoop[1], Edwin H. G. Oei[2], Marjolein Krul[1], Sita M. A. Bierma-Zeinstra[1,3,4] and Jos Runhaar[1,4]

Abstract

Objectives. The present study was designed to evaluate the effect of a lifestyle intervention aimed to reduce body weight and of oral glucosamine sulphate on the incidence of knee osteoarthritis (OA) after 6–7 years in a population of middle-aged, overweight women, without knee OA at baseline.

Methods. The Prevention of knee Osteoarthritis in Overweight Females study, ISRCTN42823086, was a randomized controlled trial with a 2 × 2 factorial design. Four hundred and seven women aged 50–60 years with a BMI of $\geqslant 27\,kg/m^2$ and free of knee OA were randomized.

Results. Four hundred and seventy-seven knees from 245 participants were available after a mean follow-up time of 6.6 years. Nineteen per cent of all knees showed incident knee OA. Both interventions showed no significant preventive effect on incident knee OA. Despite the fact that per protocol analyses showed greater differences between both groups for the lifestyle intervention, significance was not reached. A significant effect of losing $\geqslant 5\,kg$ or $\geqslant 5\%$ of baseline weight in the first 12 months on the incidence of knee OA according to the primary outcome was found (odds ratio = 0.10; 95% CI: 0.02, 0.41).

Conclusion. No significant preventive effect on incident knee OA of either the lifestyle intervention or the glucosamine intervention was found. As a proof of concept, the preventive effect of moderate weight loss in 1 year on the incidence of clinical knee OA is demonstrated. This trial provides important insights for future studies on the prevention of knee OA, which are currently lacking.

Trial registration. ISRTCN registry, http://www.isrctn.com, ISRCTN42823086.

Key words: knee osteoarthritis, obesity, lifestyle intervention, glucosamine, primary care

CLINICAL SCIENCE

Rheumatology key messages

- These are long-term results of the first randomized controlled trial on the prevention of knee OA.
- Neither a lifestyle intervention nor glucosamine resulted in long-term prevention of knee OA.
- A moderate amount of weight loss significantly lowered the odds of developing knee OA.

Introduction

The association between obesity and knee OA has been extensively described in the literature [1]. The majority of these studies have focused on obesity as a risk factor or weight loss as a treatment for knee OA in individuals with obesity [1–4]. Considering the increasing body of evidence stating that obesity is an important risk factor for knee OA, the options of primary prevention by weight loss should be investigated [5]. As early as 1992, results from

[1]Department of General Practice, [2]Department of Radiology, [3]Department of Orthopaedics, Erasmus MC University Medical Center, Rotterdam, The Netherlands and [4]D-BOARD Consortium, European Commission Framework 7 programme, UK; Bone and Cartilage Research Unit, Erasmus University Medical Center, Rotterdam, The Netherlands

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*Correspondence to: Bastiaan de Vos, Department of General Practice, Room NA 1923, Erasmus MC, PO Box 2040, 3000 CA, Rotterdam, The Netherlands.
E-mail: b.devos@erasmusmc.nl

The Framingham Study suggested a preventive approach to knee OA by weight loss [6]. Thereafter, few trials were specifically designed to study the preventive effect of weight loss on knee OA, despite recommendations in literature to design preventive trials [6–9]. Recent results of trials investigating the effect of weight loss on intermediate outcomes, such as cartilage thickness or chronic pain [10, 11], support the hypothesis that weight loss can prevent the development of knee OA, as suggested by Felson *et al.* [6]. Recommendations made in the literature regarding the design of a trial to investigate the preventive effect of weight loss on knee OA often include the following: a randomized design; a high-risk population of overweight, middle-aged participants without knee OA; a long follow-up period; and clinical and radiographic outcome measures [6–9, 12].

In addition, recommendations have been made to study the efficacy of pharmacological substances, such as glucosamine [13]. A large review found an overall significant beneficial effect of glucosamine on pain and function of the knee in participants with established knee OA [14]. However, the heterogeneity of the included studies was very high [14]. Literature suggests that in patients in an earlier stage of disease, larger effects could be found [15]. Furthermore, in some studies, glucosamine has been shown to modify disease progression, raising the question of whether it would be more effective as a preventive intervention rather than as a treatment [5, 15]. The above-mentioned review found the safety of glucosamine to be equal to placebo, making a trial to investigate the preventive effect of glucosamine on the development of knee OA feasible [14].

The objective of the present study was to evaluate the long-term effectiveness of a tailor-made weight-loss intervention, using diet and exercise, and of oral glucosamine on the incidence of knee OA in a high-risk population of overweight, middle-aged women without knee OA at baseline. Previously, short-term results of the trial were published, showing no significant preventive intervention effects on knee OA [16, 17]. It was hypothesized that prolongation of the follow-up time could possibly result in greater effects. The present study focuses on the long-term effectiveness 6–7 years after randomization.

Methods

The Prevention of knee Osteoarthritis in Overweight Females Study, of which the present study is a part, was approved by the Medical Ethics Committee of Erasmus MC in 2005 (Prevention of knee Osteoarthritis in Overweight Females, ISRCTN42823086). All participants provided informed consent according to the Declaration of Helsinki. The present manuscript was prepared according to the CONsolidated Standards of Reporting Trials (CONSORT) Statement guidelines [18]. A full description of the study protocol has been published elsewhere [17]. In short, in a 2×2 factorial design, the preventive effect of both a diet and exercise programme (DEP) and of oral glucosamine sulphate (OGS) on the incidence of knee OA was investigated. For the DEP, the study was open labelled, whereas the glucosamine intervention was double blind and placebo controlled. Inclusion took place between 2006 and 2009. Inclusion criteria were as follows: female gender; aged 50–60 years; BMI of $\geqslant 27\,kg/m^2$; free of clinical ACR criteria for knee OA [19]; no contraindications for MRI; no rheumatic diseases; not using a walking aid; not under treatment for knee complaints; mastering the Dutch language; and no use of oral glucosamine during the past 6 months. All women who were willing to participate and who met all inclusion criteria were invited for baseline measurements and randomization. For both interventions, participants were randomized 1:1 using block size 20 in block randomization.

Measurements

At baseline, body weight, body height, knee pain upon pressure at the joint margins, warmth and crepitation of both knees and Heberden's nodes in both hands were recorded. Also, semi-flexed posterior–anterior knee radiographs were taken according to the MTP protocol [20]. These measurements were repeated after 2.5 years of follow-up and after 6–7 years of follow-up. The radiographs were scored using Kellgren and Lawrence (K&L) criteria [21]. All radiographs were scored by a trained researcher, blinded for treatment assignment and clinical outcomes. Interobserver variability was determined by a second blinded researcher, who scored a subset of 20% of the radiographs. Digitally, medial knee alignment was measured, and varus alignment was defined as an angle of $<178^{\circ}$.

Participants filled out a questionnaire every 6 months for the first 2.5 years and one after 6–7 years, recording number of days with knee pain, physical activity, co-interventions and quality of life. Physical activity was measured using the validated Short Questionnaire to Assess Health-enhancing physical activity questionnaire [22, 23]. Quality of life was measured using the validated EQ-5 D EuroQol questionnaire [24]. In addition, participants filled in questions on knee complaints, menopausal status, comorbidities and filled in the Knee injury and Osteoarthritis Outcome Score questionnaire at baseline, 12 months and 2.5 and 6–7 years [25]. Mild knee symptoms were defined as having any knee pain in the past 12 months.

Participants were visited at home every 6 months for the first 2.5 years to measure body weight, to check the questionnaire for unanswered questions and to replace the batch of study drugs with a new one. The retrieved batch was used for objective calculation of compliance.

Interventions

Both interventions are described in detail elsewhere [16, 17]. In short, participants randomized to the DEP were referred to a local dietitian, agreements were made on frequency of visits, and personal goals regarding nutritional patterns and physical activity were set, using motivational interviewing [26]. In addition, participants were invited to participate in a series of 20 weekly physical exercise classes. These 1 h classes were supervised by a

physiotherapist, were offered near participants' homes and were conducted in small groups of 12–15 participants. The goal of these classes was to regain pleasure in physical exercise and to find activities suited for long-term continuation. A wide variety of low-impact sports were offered. Participants in the control group did not receive this intervention, but were free to take any actions to improve their health independently.

Participants randomized to OGS were prescribed 1500 mg of oral crystalline glucosamine sulphate per day for 2.5 years. Participants in the control group received placebo. All study drugs were provided by Rottapharm Madaus (Monza, Italy). There was no involvement of Rottapharm Madaus in study design, data collection or statistical analyses. All participants and research staff were blinded for allocation during these 2.5 years. After the intervention ended, observation of participants continued for 4 years.

Statistical analyses

The primary outcome measure for the present study was the incidence of knee OA after 6–7 years, according to the combined clinical and radiological ACR criteria [19]. The secondary outcome measure was the incidence of knee OA after 6–7 years, defined as K&L grade 2 or higher. Analyses were performed at the knee level. OA was considered an irreversible process. Therefore, all knees that met ACR criteria at 2.5 or 6–7 years of follow-up were considered positive for knee OA for the primary outcome. Given that initial screening of inclusion criteria was done by telephone, it was expected that there would be a proportion of participants that met ACR criteria or showed K&L grade $\geqslant 2$ for one or two knees at baseline already. These knees were excluded from the analysis.

Intention-to-treat (ITT) analysis served as the primary analysis. The intervention effect on the primary and secondary outcome measures was tested using generalized estimating equations (GEE), because this method takes the correlation of both knees of one participant into account. Effects were reported as odds ratios (ORs) with 95% CIs. First, the associations between known prognostic variables and the outcome were tested with univariate GEE analyses. Age, K&L grade $\geqslant 1$ *vs* 0, varus alignment, mild knee symptoms, BMI, a history of knee injury, Heberden's nodes and postmenopausal status were tested accordingly. Next, all variables with a P-value <0.2 were analysed using multivariate GEE analysis. All variables with a P-value <0.05 in the multivariate model were adjusted for in the analyses testing the intervention effects. This was done separately for the primary and secondary outcome measures. Additionally, all analyses were adjusted for follow-up duration in months, because follow-up time was not equal for all participants, owing to the long period of recruitment (July 2006 to May 2009). Using the GEE model, interaction between both interventions was assessed. In the event of significant interaction, all four groups would be assessed separately.

For the predefined per protocol (PP) analyses, participants who were compliant to the intervention were compared with the participants who were randomized to the control group. Compliance to the DEP intervention was defined as having visited the dietitian at least six times and having attended at least seven physical activity classes. Regarding the OGS intervention, an objective compliance calculation of $\geqslant 75\%$ was used, which was assessed using the retrieved batches of study drugs.

As an explanatory analysis, incident knee OA was compared between participants who lost 5 kg or 5% of their baseline weight at 1 year of follow-up and participants who did not meet this predefined goal in the first year of the study. This outcome served as the primary outcome of the weight-loss intervention and was chosen for its associations with improvement of cardiovascular risk factors [16]. We hypothesized that achieving this goal in the period of 1 year could possibly be an easily achievable goal to recommend to patients in primary care in the context of preventing knee OA. Adjusting for follow-up duration and confounding factors, as in the ITT and the PP analyses, GEE was used for this analysis.

To estimate the effect of the missing data, multiple imputation was performed, as recommended in the literature [27]. Fifty imputed data sets were used. The method was set to automated selection of linear regression or predictive mean matching, maximal iterations were set to 20, and a maximum of 150 parameters per variable was used. All variables used in the GEE, including the outcome variables, were imputed and used as predictors. Both baseline characteristics and follow-up data were used as auxiliary variables.

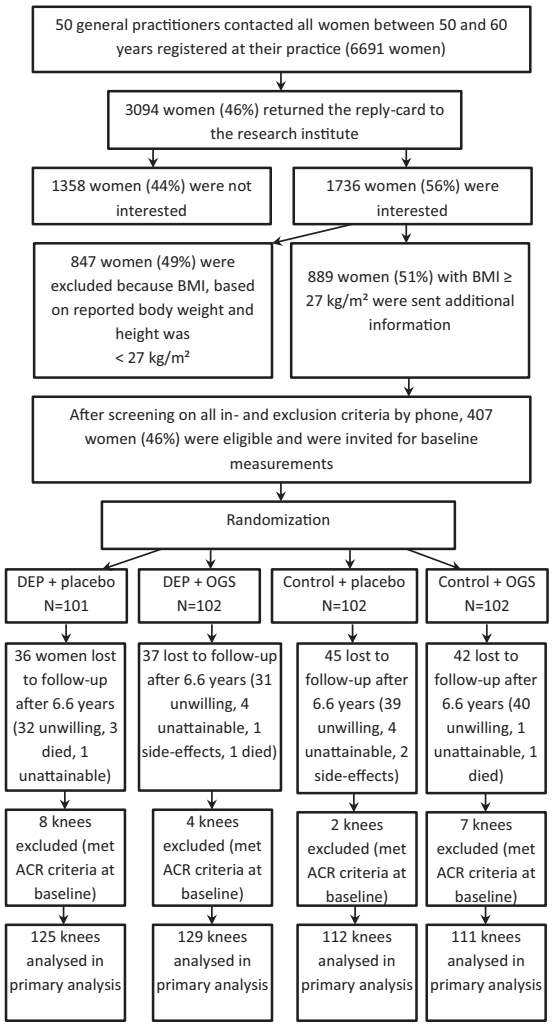
As a sensitivity analysis, a worst-case scenario was explored according to literature recommendations [28]. We hypothesized that no intervention effect at all would be the worst-case scenario. Therefore, in all participants with missing data, the outcome was imputed evenly distributed over the two groups that were compared, resulting in an equal incidence of knee OA in both groups in all participants with missing data. The incidence found in the completers' analysis was used to impute these variables. Missing values in covariates used in the GEE model were imputed by the average value of the completers. The results of the sensitivity analysis were used to check the plausibility of the results produced by the multiple imputation model.

All analyses were performed using IBM SPSS statistics version 21 (SPSS Inc., Chicago, IL, USA). In all analyses, a value of $P < 0.05$ was defined as statistically significant.

Results

Four hundred and seven women were randomized after 50 general practitioners contacted 6691 women. A full description of the selection process is published elsewhere [17]. Figure 1 shows the selection process and participants lost to follow-up with reasons. At baseline, knee OA data were available for 405 participants with 810 knees (99.5%). After 2.5 years, there were 356 participants for whom knee OA data were available on 712 knees (87.5%). After 6.6 years, 260 participants supplied knee OA data on 508 knees (62.4%). At baseline, 32 knees (4.0%) met ACR criteria and were excluded

Fig. 1 Flowchart of recruitment process



from analyses concerning the primary outcome. In addition, 51 knees (6.3%) showed K&L grade $\geqslant 2$ and were excluded from analyses concerning the secondary outcome. As a result, knee OA data were available on 477 knees (58.9%) for the primary outcome measure, and for the secondary outcome measure knee OA data on 452 knees (55.8%) were available. Attrition rates were similar between both randomized groups of the DEP intervention: 27% in the intervention group *vs* 31% in the control group. For the OGS intervention, attrition rates were higher in the placebo group: 35%, *vs* 18% in the intervention group. Participants who completed the long-term follow-up had a lower baseline BMI (32.0 *vs* 33.0 kg/m^2) and more Heberden's nodes (18 *vs* 15%). Baseline knee OA incidence figures were similar between participants who completed the follow-up time and those who did not: 4 *vs* 4% for the primary outcome and 5 *vs* 8% for the secondary outcome.

Table 1 shows baseline characteristics on the 508 knees from 260 participants that were available after complete follow-up. Mean follow-up time was 6.6 (0.7) years. There were no significant differences in baseline characteristics between both groups for both interventions. In the multivariate analysis, three baseline characteristics were associated with the primary outcome: BMI; K&L grade $\geqslant 1$ *vs* 0; and mild knee symptoms. Regarding the secondary outcome, BMI, K&L grade $\geqslant 1$ *vs* 0 and a history of knee injury were associated with the outcome in a multivariate model. Consequently, adjustment of these variables was performed in all analyses. Of the participants randomized to the DEP, 32% were compliant to that intervention. For the OGS intervention, 65% of all participants were considered compliant.

Intervention effects

After 6.6 years, the overall incidence of knee OA according to the primary outcome was 19%. No significant interaction between both interventions was found. ITT analysis showed no significant difference in knee OA between randomized groups, for both the DEP intervention (OR = 0.86, 95% CI: 0.47, 1.54) and the OGS intervention (OR = 1.58, 95% CI: 0.86, 2.89). Regarding the secondary outcome, knee OA incidence was 14%, also with no differences between both groups for both interventions.

Per protocol analysis showed greater intervention effects for the DEP than the ITT analysis, but they did not reach statistical significance, with ORs of 0.55 (95% CI: 0.23, 1.33) for the primary outcome and 0.39 (95% CI: 0.12, 1.29) for the secondary outcome. For the OGS intervention, effects in the PP analysis were not consistently greater. The intervention effect on the primary outcome did not change significantly, whereas the intervention effect on the secondary outcome changed in direction. ORs for the secondary outcome were 0.96 (95% CI: 0.48, 1.92) for the PP analysis and 1.26 (0.67, 2.39) for the ITT analysis. All incidence numbers and ORs obtained from ITT and PP analyses are presented in Table 2.

Exploratory analysis

Sixty-nine participants achieved the goal of losing 5 kg or 5% of their baseline body weight after 1 year of follow-up. These participants showed a lower incidence of knee OA after 6.6 years than participants who did not achieve this goal at 1 year of follow-up (7 *vs* 21%). Adjusted OR for the primary outcome was 0.10 (95% CI: 0.02, 0.41) and for the secondary outcome 0.28 (95% CI: 0.08, 0.94). Table 2 shows these ORs.

Multiple imputation

Pooled ORs obtained from the imputed data sets showed no significant intervention effects. Incidence numbers were markedly higher than in the original data. In these analyses, both interventions showed greater effects in the PP analyses compared with the ITT analyses. The association between losing 5 kg or 5% baseline weight became less strong and non-significant. Table 3 shows all incidence numbers and ORs obtained from the multiple imputation data sets.

Table 1 Means and distribution of prognostic variables

	Diet and exercise programme		Oral glucosamine sulphate	
	Control group	Intervention group	Placebo	Glucosamine
Baseline characteristics				
Subjects, n	122	138	130	130
Age, mean (S.D.), years	55.9 (3.2)	55.6 (3.2)	55.7 (3.2)	55.8 (3.1)
BMI, mean (S.D.), kg/m^2	32.1 (4.1)	31.9 (3.9)	32.4 (4.2)	31.6 (3.6)
Postmenopausal status, %	73.7	65.4	70.4	68.3
Heberden's nodes, %	29.4	27.0	31.2	25.0
Knees, n	238	270	253	255
ACR[a], %	3.8	4.5	4.0	4.3
K&L grade, %				
Grade 0	49.6	48.5	49.4	48.6
Grade 1	47.0	44.8	44.6	47.1
Grade $\geqslant$2	3.4	6.7	6.0	4.3
Minimal JSW				
Medial, mean (S.D.), mm	4.8 (0.7)	4.7 (0.8)	4.7 (0.8)	4.8 (0.8)
Lateral, mean (S.D.), mm	6.2 (1.0)	6.2 (1.1)	6.1 (1.1)	6.2 (1.0)
Varus alignment, %	44.4	39.5	43.0	40.6
Mild symptoms[b], %	32.4	32.3	32.8	31.9
History of knee injury, %	15.0	10.9	12.1	13.5

[a]Knee OA according to the ACR criteria. [b]Mild symptoms defined as any pain in the concerned knee in the past 12 months. JSW: Joint Space Width; K&L: Kellgren and Lawrence.

Table 2 Incidence figures and odds ratios on knee OA from intention-to-treat and per protocol analyses

	Incident knee OA, %	Incident knee OA intervention group, %	Incident knee OA control group, %	OR (adjusted)[a] (95% CI)
Intention-to-treat analyses				
Diet and exercise programme (n = 477: 254 *vs* 223)				
ACR criteria[b]	19	18	19	0.86 (0.47, 1.54)
K&L grades[c]	15	14	16	0.91 (0.48, 1.72)
Oral glucosamine sulphate (n = 477: 240 *vs* 237)				
ACR criteria	19	20	17	1.58 (0.86, 2.89)
K&L grades	15	15	14	1.26 (0.67, 2.39)
Per protocol analyses				
Diet and exercise programme (n = 305: 82 *vs* 223)				
ACR criteria	18	13	19	0.55 (0.23, 1.33)
K&L grades	14	8	16	0.39 (0.12, 1.29)
Oral glucosamine sulphate (n = 413: 176 *vs* 237)				
ACR criteria	18	19	17	1.64 (0.86, 3.14)
K&L grades	13	12	14	0.96 (0.48, 1.92)
Exploratory analysis[d]				
Lost 5 kg or 5% in 1 year (n = 477: 69 *vs* 408)				
ACR criteria	19	7	21	0.10 (0.02, 0.41)
K&L grades	15	6	16	0.28 (0.08, 0.94)

Numbers are numbers of knees. [a]Generalized estimating equations adjusted for baseline differences and confounding factors. [b]Knee OA according to the ACR criteria. [c]Knee OA, defined as K&L grade $\geqslant$2. [d]Comparing the incidence of knee OA between participants who lost 5 kg or 5% of their baseline weight in the first year of follow-up *vs* all participants who did not lose this amount of body weight in the first year of follow-up. K&L: Kellgren and Lawrence; OR, odds ratio.

Table 3 Incidence figures and odds ratios after multiple imputation

	Incident knee OA, %	Incident knee OA intervention group, %	Incident knee OA control group, %	OR (adjusted)[a]	95% CI
Intention-to-treat analyses					
Diet and exercise programme					
ACR criteria[b]	31	29	33	0.84	0.49, 1.44
K&L grades[c]	28	26	31	0.83	0.45, 1.53
Oral glucosamine sulphate					
ACR criteria	31	32	30	1.07	0.63, 1.81
K&L grades	28	28	29	0.92	0.56, 1.52
Per protocol analyses					
Diet and exercise programme					
ACR criteria	30	22	33	0.57	0.27, 1.22
K&L grades	27	18	31	0.47	0.19, 1.19
Oral glucosamine sulphate					
ACR criteria	28	25	30	0.81	0.44, 1.51
K&L grades	25	20	29	0.61	0.34, 1.10
Exploratory analysis[d]					
Lost 5 kg or 5% in 1 year					
ACR criteria	31	22	32	0.54	0.23, 1.31
K&L grades	28	20	30	0.56	0.22, 1.43

[a]Generalized estimating equations adjusted for baseline differences and confounding factors. [b]Knee OA according to the ACR criteria. [c]Knee OA, defined as K&L grade $\geqslant 2$. [d]Comparing the incidence of knee OA between participants who lost 5 kg or 5% of their baseline weight in the first year of follow-up *vs* all participants who did not lose this amount of body weight in the first year of follow-up. K&L: Kellgren and Lawrence; OR, odds ratio.

Table 4 Incidence figures and odds ratios after worst-case scenario

	Incident knee OA, %	Incident knee OA intervention group, %	Incident knee OA control group, %	OR (adjusted)[a] (95% CI)
Intention-to-treat analyses				
Diet and exercise programme				
ACR criteria[b]	19	19	19	0.97 (0.65, 1.45)
K&L grades[c]	14	14	14	0.95 (0.60, 1.49)
Oral glucosamine sulphate				
ACR criteria	19	20	18	1.07 (0.71, 1.60)
K&L grades	14	15	14	1.03 (0.67, 1.60)
Per protocol analyses				
Diet and exercise programme				
ACR criteria	18	14	19	0.68 (0.35, 1.33)
K&L grades	13	10	14	0.55 (0.24, 1.27)
Oral glucosamine sulphate				
ACR criteria	19	21	18	1.19 (0.74, 1.89)
K&L grades	13	12	13	0.99 (0.57, 1.69)
Exploratory analysis[d]				
Lost 5 kg or 5% in 1 year				
ACR criteria	19	11	20	0.45 (0.23, 0.90)
K&L grades	14	8	15	0.43 (0.20, 0.93)

[a]Generalized estimating equations adjusted for baseline differences and confounding factors. [b]Knee OA according to the ACR criteria. [c]Knee OA, defined as K&L grade $\geqslant 2$. [d]Comparing the incidence of knee OA between participants who lost 5 kg or 5% of their baseline weight in the first year of follow-up *vs* all participants who did not lose this amount of body weight in the first year of follow-up. K&L: Kellgren and Lawrence; OR, odds ratio.

Sensitivity analysis

ORs obtained from the worst-case scenario were very similar to the completers' analysis (Table 4). Naturally, all effects decreased; all ORs moved closer to one. Regarding the DEP intervention, the OR for the primary outcome changed from 0.86 (0.47, 1.54) to 0.97 (0.65, 1.45) and

OR for the secondary outcome changed from 0.91 (0.48, 1.72) to 0.95 (0.60, 1.49). Regarding the OGS intervention, OR for the primary outcome changed from 1.58 (0.86, 2.89) to 1.07 (0.71, 1.60) and OR for the secondary outcome changed from 1.26 (0.67, 2.39) to 1.03 (0.67, 1.60). None of the associations changed in direction, and none of the confidence intervals that included one in the completers' analysis became significantly different from one, or vice versa.

Discussion

The present study presents the long-term results of the first preventive randomized controlled trial in knee OA. ITT analyses showed no significant effects of either the DEP or the glucosamine sulphate on the long-term incidence of knee OA according to ACR criteria. Also, no effects were found on the incidence of knee OA, defined as K&L grade $\geqslant 2$. Per protocol analyses showed greater effects for the DEP, but significance was not reached. As a proof of concept, the present study demonstrated the preventive effect of losing 5 kg or 5% of baseline body weight in the first year of the study on the incidence of knee OA after 6.6 years.

The primary analysis of the present study is a completers' analysis. As a result, under- or overestimation of the intervention effect could have occurred. Weight-loss studies often suffer from high dropout rates, resulting in a wide variety of methods used to handle missing data [27]. Multiple imputation was recommended in the literature as the best method for handling missing data in obesity randomized controlled trials [27]. In the present study, however, multiple imputation led to markedly higher incidence numbers of knee OA. In the original data, the incidence was 19 and 14% for the primary and secondary outcome, respectively. In the multiple imputation data sets, these incidence numbers were 31 and 28%. These numbers are markedly higher than the range found in population-based cohorts [29–32]. Incidence numbers found in the completers' analyses were much more comparable to the incidence numbers reported in the literature, giving reason to question the reliability of the multiple imputation. When looking only at the participants with missing data on the outcome, the imputed incidence numbers were 50% for the primary and secondary outcome; more than double the incidence numbers in the completers' analysis. Moreover, some of the ORs obtained from the multiple imputation sets were outside of the range of the ORs found in the completers' analysis and the worst-case analysis. For instance, in the exploratory analyses, ORs from multiple imputation were closer to one than ORs from the worst-case scenario. Given that the worst-case scenario simulated the scenario of no intervention effect at all in participants with missing data, this would indicate that the preventive effect of losing 5 kg or 5% baseline weight reversed in the participants with missing data, and increased the risk of incident knee OA. To our knowledge, an association between weight loss and knee OA in this direction has not been found before. Therefore, results from the multiple imputation model were considered unreliable. A possible reason why the multiple imputation model did not result in more plausible incidence rates is the possibility that not all of the assumptions underlying multiple imputation were met, such as the missing data mechanism being random [33]. Additionally, large amounts of missing data, especially on the outcome variable, can result in unreliable results and can introduce bias not present in a completers' analysis [34].

The present study pioneered in the prevention of knee OA and is, to our knowledge, the first to investigate the prevention of knee OA with incidence of knee OA as the primary outcome [17]. Results presented from trials investigating the preventive effect of weight loss on intermediate outcomes indicated a high possibility of a preventive effect of weight loss on knee OA [10, 11]. The present study, however, failed to find a significant intervention effect. Two possible mechanisms could have caused underestimation of the intervention effect. First, weight loss in the control group was considerably higher than expected, possibly caused by a high baseline motivation to participate in a DEP [35]. As a result, the difference in weight loss between both groups was smaller than expected. Second, compliance rates were lower than expected. A mere 32% of all participants randomized to the intervention group were compliant to the intervention. Considering these possible reasons for underestimation of the intervention effect, in addition to the fact that per protocol analyses showed greater effects than ITT analyses, a true preventive effect of weight loss on incident knee OA should be considered, despite the lack of significant findings in the present study. For this reason, as a proof of concept, the exploratory analysis was undertaken, which did show a significant effect of losing 5 kg or 5% of baseline body weight on incident knee OA in the completers' analysis. This finding is consistent with intervention effects found on weight loss and physical activity [35].

In conclusion, no long-term effectiveness in preventing incident knee OA of the DEP or of the OGS was found in the present study. However, the PP effects of the DEP intervention were greater than the ITT effects, indicating a possibility of a significant effect, if there had been higher compliance rates and a more representative control group. Exploratory analyses showed an association between losing 5 kg or 5% baseline weight and a considerable decrease in incident knee OA. This association indicates that weight loss could be a successful strategy in preventing knee OA in an overweight population, but needs further study. However, these conclusions should be interpreted with caution, because the large amount of missing data resulted in high uncertainty of the results. As illustrated in the present study, this problem cannot always be mitigated reliably through multiple imputation.

The present study provides important insights in the possibilities of preventing knee OA. A follow-up time of 6.6 years seems to be sufficient to study the development of knee OA, given the large differences in knee OA incidence between groups in the exploratory analyses. Future research should investigate further the preventive effect of

weight loss on incident knee OA. Adherence rates should be of the utmost importance when designing trials to investigate prevention of knee OA. Further individualization of the content of the lifestyle intervention offered could add to this cause. The present study illustrates the large consequences of missing data, resulting in high uncertainty about the validity and usefulness of conclusions drawn. Additionally, higher compliance rates should be given high priority, in order to achieve a clinically significant amount of weight loss in a considerable proportion of the study population. Weight loss remains challenging in the present population, but this study provides proof that the concept of preventing knee OA through weight loss is viable.

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