Title: The effect of prolonged glucosamine usage on HbA1c levels and new-onset diabetes mellitus in overweight and obese middle-aged women

Running head: The effect of prolonged glucosamine usage in overweight middle-aged women.

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Abstract

Objective. The aim of the present study was to evaluate the effect of a 2.5 year glucosamine sulphate intervention on haemoglobin A1c (HbA1c) levels and the incidence of new-onset diabetes mellitus over 6.5 years in middle-aged women with a BMI ≥27 kg/m^2.

Methods. In total, 407 women were randomized into either oral crystalline glucosamine sulphate or placebo. At baseline, 1 year, 2.5 years and 6.5 years a blood sample for the HbA1c level was drawn and a questionnaires were taken. After 6.5 years there was missing data for some variables, therefore multiple imputation was used. With the imputed data, a generalized estimating equation was performed to analyze the effect of glucosamine sulphate usage over 6.5 years. Finally, these analyses were rerun for the two subgroups of participants with and without high HbA1c level (≥42 mmol/mol) at baseline.

Results. There was no significant effect of a 2.5 year glucosamine sulphate intervention on mean HbA1c level or on obtaining a high HbA1c level and/or new-onset diabetes mellitus over 6.5 years. The subgroup analyses of participants with and without high HbA1c level at baseline were also not statistically significant. However, participants with a high HbA1c level at baseline had higher ORs compared with the participants with a normal HbA1c at baseline.

Conclusions. There was no effect of glucosamine sulphate on mean HbA1c level nor on obtaining a high HbA1c level and/or new-onset diabetes mellitus over 6.5 years, especially in participants with a normal HbA1c level at baseline.

Introduction

Glucosamine is a commonly used treatment for osteoarthritis (OA), although the evidence is limited.[1] It is an amino-monosaccharide derivative of glucose and a precursor of the

glycosaminoglycans and proteoglycans that make up articular cartilage. Glucosamine is metabolized by the hexosamine pathway, both glucose and glucosamine enter this pathway as glucosamine-6-phosphate.[2] It is a dietary supplement that is available over-the-counter and is considered to be safe; no fatal or serious adverse events have been reported.[3] However, there are concerns that it might adversely affect the glucose metabolism and causes insulin resistance, but this has only been proven in animal studies.[4] Recent studies suggest that it may also affect the glucose transport and insulin resistance in humans, especially among patients with impaired glucose tolerance.[5-7] However, all these studies had considerable heterogeneity in terms of dose, route and duration of glucosamine administration. This warrants further research, particularly in subjects with an impaired glucose tolerance or insulin resistance.[5]

To diagnose diabetes, haemoglobin A1c (HbA1c) levels can be used. It provides a reliable measurement of chronic glycaemia and is elevated when there is a long-term hyperglycaemia over the preceding two to three months.[8] It also correlates well with the risk of diabetes complications. Diabetes mellitus is diagnosed with a HbA1c level of ≥48 mmol/mol. Risk factors for diabetes development are elevated levels of triglycerides, blood pressure, BMI, and family history of diabetes. These risk factors have to be taken into account when considering treatment intervention at a HbA1c level of ≥42 mmol/mol.[9] The most prevalent risk factors for OA are overweight or obesity, a higher age, and the female gender.[10, 11] This means that some risk factors are identical for both diabetes mellitus and OA, and some people are therefore at increased risk for both diseases.

The main objectives of this study was to evaluate the effect of a 2.5 year glucosamine intervention on mean HbA1c levels and the risk of obtaining a high HbA1c level (≥42 mmol/mol) and/or new-onset diabetes mellitus over 6.5 years in middle-aged women with a BMI

≥27 kg/m^2. The second objective was the evaluation of the effect of the intervention on mean HbA1c level and the risk of obtaining a high HbA1c level (≥42 mmol/mol) and/or new-onset diabetes mellitus over 6.5 years in women with and without high HbA1c level (≥42 mmol/mol) at baseline.

Methods

The present study used data of the PROOF study (PRrevention of knee Osteoarthritis in Overweight Females, IS RCTN 42823086), a randomized controlled trial funded by ZonMw, The Netherlands Organisation for Health Research and Development. In a 2x2 factorial design, the effects of glucosamine sulphate (double blind, placebo-controlled) and of a diet and exercise program on the development on knee OA were evaluated. The study was approved by the Medical Ethics Committee of Erasmus University Medical Centre Rotterdam. A full description of the study protocol can be found elsewhere.[12]

Study sample

All women between 50 and 60 years without major comorbidities and who were registered at a participating general practitioner (GP) in the region of Rotterdam, the Netherlands, were contacted. Interested women with a reported BMI ≥ 27 kg/m^2 were contacted by phone to check all inclusion criteria. Inclusion criteria were that all women had to be free of knee OA according to the ACR-criteria[13], were not under treatment for knee complaints, were free of MRI contraindications, were free of rheumatic diseases, were not using walking-aids, had mastered the Dutch language and had not used oral glucosamine in the past 6 months. All women who were willing to participate were invited for baseline measurements and randomization between

July 2006 and May 2009. Out of 6.691 women that were contacted, a total of 407 were interested in participating and fulfilled the inclusion criteria.[12] The participants were randomized to glucosamine or placebo one-on-one using a blocked randomization list with block size 20. Randomization for the diet and exercise program or control group was also done using block randomization with block size 20.[12]

Outcome measures

Height, weight and waist circumference were measured at baseline. Additionally, a blood sample was drawn for HbA1c level (mmol/mol) analysis, a SQUASH[14] (Short Questionnaire to Assess Health-enhancing physical activity) questionnaire was taken and there were questions to obtain information about their comorbidities.[12] After 1 year, 2.5 years and 6.5 years these measurements were repeated.

Crystalline glucosamine sulphate versus placebo

The study drugs were provided by Rottapharm Madaus in identical packaging. The participants and research staff were blinded for allocation throughout the whole study. Rottapharm Madaus was not involved in study design, data collection, or statistical analyses. All participants were asked to consume one sachet of 1500 mg of the study drug (powder) a day.[12] They only received the study drug in the first 2.5 years, after which they were observed for the remaining 4 years.

Analyses

The primary outcome measures were defined as mean HbA1c level and *High HbA1c/DM.* A normal HbA1c level was set at a level below 42 mmol/mol particularly because the participants

had risk factors for both diseases. The variable *High HbA1c/DM* was defined as having a high HbA1c level (≥ 42 mmol/mol) at a specific follow up moment or having a 'positive diabetes mellitus status'. A positive diabetes mellitus status was interpreted as having the diagnosis diabetes mellitus or receiving treatment for diabetes mellitus according to the questionnaire. Patients with a positive diabetes mellitus status at an early follow up moment and missing data at a later follow up moment were also defined as 'positive', considering the fact that diabetes mellitus is irreversible.

The baseline characteristics were presented as means ± standard deviation (SD) for numerical variables and percentages for categorical variables. Baseline differences were tested using t-tests for numerical variables and Fisher's exact test for categorical variables.

Multiple imputation was performed to estimate the missing data to improve the validity of the study.[15] Fifty imputed datasets were used, method was set to automated selection of linear regression or predictive mean matching, maximum iterations were set to 20 and a maximum of 150 parameters per variable was used.[16] Variables with more than 50% missing date were excluded from the multiple imputation, because of computational problems.[15, 17] This made it impossible to impute the variable about their diabetes mellitus status. In order to still use this variable in the analyses we only used the women with a positive diabetes mellitus status, meaning that missing data was filled in as a negative diabetes mellitus. The variables that were imputed by SPSS are shown in tables and graphs in the appendix.

A generalized estimating equation (GEE) with an unstructured correlation matrix was used to analyze the effect of glucosamine usage, using the 1 year, 2.5 year and 6.5 year measurements as repeated measurements. A linear GEE was used to analyze intervention effects on mean HbA1c level over 6.5 years. A binary logistic GEE was used to analyze the risk at obtaining a *High*

HbA1c/DM over 6.5 years. Both analyses were rerun in participants with (≥ 42 mmol/mol) and without elevated HbA1c level at baseline (< 42 mmol/mol). All analyses were adjusted for possible confounders (HbA1c level at baseline, smoking at baseline, education level, BMI, SQUASH at baseline and change over 6.5 years, change in waist circumference, ethnicity, cardiovascular diseases at baseline, and season change of HbA1c [18]) and the randomized groups of the diet and exercise program. A Pearson correlation matrix was calculated to measure any linear correlation between two variables, but there were none with a correlation under 0.7 and therefore there was no multi-collinearity.[19] When a GEE was impossible to run in an unstructured correlation matrix, variables that were the least significantly contributing to the GEE were removed using backward selection.

Using complete data (after imputation) of all 407 subjects, we had >90% power to detect a 1.0 mmol/mol difference between groups and 80% power to detect a difference of 11% (equals mean over time in placebo group) vs. 15.5% in proportion of subjects reaching *High HbA1c/DM*. A difference of 5 mmol/mol is considered clinically relevant.[20, 21]

All analyses were performed using SPSS 21, results from the GEE analyses are expressed as odds ratios (ORs) with associated 95% confidence intervals (CI) and significance level. Values of $p < 0.05$ were considered statistically significant.

Results

For the present study, all women with diabetes mellitus at baseline were excluded (N=29, 7.1%) from all statistical analyses, because the aim of the study was to analyze whether or not this diagnosis would develop during the follow up period. There was one outlier with a HbA1c level of 130.6 mmol/mol and was therefore excluded from all analyses. There were no significant

differences between the participants that were lost to follow up (drop-outs) and those that completed the study, as shown in Table 1.

Table 1 Baseline characteristics of the missing and available data (mean ± st.dev or percentage)

	Missing (N = 132)	**Available (N = 245)**	**P-values**
Age (years)	55.7 (SD±3.1)	55.7 (SD±3.2)	0.85
BMI (kg/m^2)	32.3 (SD±3.8)	32.1 (SD±4.1)	0.70
HbA1c (mmol/ml)	37.8 (SD±4.5)	38.2 (SD±3.9)	0.34
HbA1c ≥42 mmol/mol (%)	14.9%	14.2%	0.87
HbA1c ≥48 mmol/mol (%)	2.5%	1.7%	0.69
Total cholesterol (mmol/L)	6.0 (SD±1.2)	6.1 (SD±1.1)	0.45
Smoking (%)	20.6%	16.4%	0.32
Cardiovascular disease (%)	3.8%	3.3%	0.77
Systolic blood pressure (mmHg)	148.0 (SD±21.6)	106.1 (SD±21.7)	0.22
Diastolic blood pressure (mmHg)	95.2 (SD±11.7)	92.8 (SD±11.7)	0.07
Waist circumference (cm)	105.1 (SD±9.4)	106.1 (SD±9.8)	0.32
SQUASH*	6575.8 (SD±3799.4)	7049.6 (SD±3703.7)	0.24
Obesity (%)**	69.7%	63.3%	0.26

* SQUASH: validated physical activity questionnaire.

** Obesity: BMI ≥ 30.

Baseline characteristics

The study population consisted of 94.1% Caucasian women. At baseline there were no significant differences between the glucosamine and placebo group, as shown in Table 2.

Table 2 Baseline characteristics between intervention groups (mean ± st.dev or percentage)

	Placebo (N=185)	**Glucosamine (N=192)**	**P-values**

Age (years)	55.7 (SD±3.2)	55.7 (SD±3.1)	0.99
BMI (kg/m^2)	32.3 (SD±4.3)	32.1 (SD±3.7)	0.52
Caucasian origin (%)	95.6%	92.7%	0.35
High education level (%)	31.0%	34.5%	0.49
HbA1c (mmol/mol)	37.8 (SD±3.8)	38.3 (SD±4.4)	0.23
HbA1c ≥42 mmol/mol (%)	14.8%	14.0%	0.88
HbA1c ≥48 mmol/mol (%)	0.6%	3.4%	0.12
Total cholesterol (mmol/L)	6.0 (SD±1.1)	6.1 (SD±1.1)	0.30
Smoking (%)	14.8%	20.8%	0.14
Cardiovascular disease (%)	3.8%	3.1%	0.78
Systolic blood pressure (mmHg)	145.4 (SD±20.1)	146.8 (SD±23.2)	0.55
Diastolic blood pressure (mmHg)	93.5 (SD±11.1)	93.8 (SD±12.3)	0.80
Waist circumference (cm)	105.5 (SD±9.6)	105.5 (SD±9.8)	0.65
SQUASH*	6946.0 (SD±3905.5)	6823.6 (SD±3581.0)	0.75
Obesity (%)**	65.4%	65.6%	1.00

* SQUASH: validated physical activity questionnaire.

** Obesity: BMI ≥ 30.

HbA1c levels during follow up

Mean HbA1c levels were available for 354 women (94%) at baseline, 313 (83%) after 1 year, 325 (86%) after 2.5 years and 209 (55%) after 6.5 years. Mean HbA1c levels in the placebo group were 37.8 (SD±3.8) at baseline, 38.2 (SD±3.4) after 1 year, 38.1 (SD±3.7) after 2.5 years and 38.3 (SD±4.2) after 6.5 years. In the glucosamine group mean HbA1c levels this was respectively 38.3 (SD±4.4), 39.1 (SD±5.5), 39.1 (SD±6.2) and 38.7 (SD±5.4). These results are shown in Table 3.

High HbA1c/DM was available for 355 women (94%) after 1 year, 330 (88%) after 2.5 years and 245 (65%) after 6.5 years. Table 3 also shows that the percentage of participants with *High*

HbA1c/DM in the placebo group was 12.6% after 1 year, 11.0% after 2.5 years and 8.3% after 6.5 years. For the glucosamine group this was 18.3%, 18.6% and 15.2% respectively.

Table 3 HbA1c levels and participants with *High HbA1c/DM* during follow up (mean ± st.dev or percentage)

		Total	**Placebo**	**Glucosamine**
Baseline	N	354	176	178
	HbA1c (mmol/mol)	38.1 (SD±4.1)	37.8 (SD±3.8)	38.3 (SD±4.4)
	N	354	176	178
	High HbA1c* (%)	14.4%	14.8%	14.0%
1 year	N	313	155	158
	HbA1c (mmol/mol)	38.6 (SD±4.6)	38.2 (SD±3.4)	39.1 (SD±5.5)
	N	355	175	180
	*High HbA1c/DM*** (%)	15.5%	12.6%	18.3%
2.5 years	N	325	161	164
	HbA1c (mmol/mol)	38.6 (SD±5.1)	38.1 (SD±3.7)	39.1 (SD±6.2)
	N	330	163	167
	*High HbA1c/DM*** (%)	14.8%	11.0%	18.6%
6.5 years	N	209	106	103
	HbA1c (mmol/mol)	38.5 (SD±4.8)	38.3 (SD±4.2)	38.7 (SD±5.4)
	N	245	120	125
	*High HbA1c/DM*** (%)	11.8%	8.3%	15.2%

* HbA1c level ≥ 42 mmol/mol; at baseline all women with diabetes mellitus were excluded.

** HbA1c ≥ 42 mmol/mol at specific follow-up moment and/or positive diabetes mellitus status.

Generalized estimating equation

Table 4 and 5 show all ORs obtained from the multiple imputation sets with corresponding 95% confidence interval (95% CI) and significance level. The glucosamine intervention showed

higher mean HbA1c levels over 6.5 years and a higher risk of obtaining *High HbA1c/DM* over 6.5 years among all subjects, but these differences did not reach statistical significance. Both analyses were rerun for the subgroups with and without elevated HbA1c level at baseline. These ORs also did not show significant differences between the glucosamine and placebo group over 6.5 years. However, the participants with a normal HbA1c level at baseline had an OR closer to one compared with the participants with a high HbA1c level at baseline.

The binary logistic GEE analysis of the subgroup with a high HbA1c at baseline was impossible to run in an unstructured correlation and backward selection was used. This resulted in an OR of 2.65 (95% CI 0.81-8.66, P=0.11).

Because of the relatively high percentage of participants with *High HbA1c/DM* after 2.5 years in the glucosamine group, as showed in Table 3, additional GEEs were performed to evaluate the effect of the glucosamine sulphate intervention on the mean HbA1c level and on obtaining *High HbA1c/DM* over 2.5 years. These analyses had approximately the same ORs and significance level compared with the analyses over 6.5 years. The results of these analyses over 2.5 years can be found in tables added to the Appendix.

Table 4 Linear generalized estimating equation on mean HbA1c level after multiple imputation over 6.5 years

		Glucosamine versus placebo group		
	N	OR[*]	95% CI	Sig.
All participants	377	1.40	1.77-3.46	0.47
Participants with a normal HbA1c at baseline	309-319[†]	1.06	0.44-2.56	0.89
Participants with a high HbA1c at baseline	58-68[†]	24.36	0.02-27805.80	0.37

[*] Adjusted for lifestyle intervention group, HbA1c level at baseline, season change of HbA1c, cardiovascular diseases at baseline, ethnicity, smoking at baseline, education level, BMI at

baseline, age at baseline, SQUASH at baseline, change in SQUASH after 6.5 years, change in waist circumference after 6.5 years.

[†]Number of participants depending on imputation number.

Table 5 Binary logistic generalized estimating equation on *High HbA1c/DM* after multiple imputation over 6.5 years

		Glucosamine versus placebo group		
	N	OR[*]	95% CI	Sig.
Total participants	377	1.28	0.79-2.07	0.31
Participants with a normal HbA1c at baseline	309-319[†]	1.24	0.71-2.14	0.45
Participants with a high HbA1c at baseline	58-68[†]	2.65[**]	0.81-8.66	0.11

[*] Adjusted for lifestyle intervention group, HbA1c level at baseline, season change of HbA1c, cardiovascular diseases at baseline, ethnicity, smoking at baseline, education level, BMI at baseline, age at baseline, SQUASH at baseline, change in SQUASH after 6.5 years, change in waist circumference after 6.5 years.

[**] Adjusted for lifestyle intervention group, HbA1c level at baseline, season change of HbA1c, ethnicity, smoking at baseline, BMI at baseline, change in SQUASH after 6.5 years and change in waist circumference after 6.5 years.

[†]Number of participants depending on imputation number.

Discussion

This study evaluated the effects of glucosamine sulphate on mean HbA1c levels and the risk of obtaining *High HbA1c/DM* over 6.5 years in overweight and obese middle-aged women. During the last four years of the study the participants did not use glucosamine sulphate, making it possible to observe any long term effects of glucosamine sulphate on the glucose metabolism after discontinuation of the study drug.

Although there was a trend towards a higher mean HbA1c level and a higher risk of obtaining *High HbA1c/DM* over 6.5 years for the glucosamine sulphate group, there was no statistically significant effect of the intervention on either mean HbA1c level or on the risk of obtaining *High HbA1c/DM* over 6.5 years. In the subgroup analyses, in participants with and without elevated HbA1c level, there was no statistically significant effect of glucosamine sulphate on mean HbA1c level or on the risk of obtaining *High HbA1c/DM* over 6.5 years. However, the subgroup analysis in participants with a high HbA1c at baseline had a high OR in both the linear and binary logistic GEE, respectively 24.36 (95% CI 0.02-27805.80, P=0.37) and 2.65 (95% CI 0.81-8.66, P=0.11). These results have a broad 95% CI interval and this could be due to the small number of participants in this subgroup, approximately 58-68 participants depending on the imputation number, leading to lack of statistical power and this makes it impossible to state with certainty whether there is no truly intervention effect.

In literature, there are two studies that also investigated the long term effects of glucosamine on glucose metabolism.[7] Both studies, by Pavelká *et al.* [22] and Reginster *et al.* [23], monitored the fasting plasma glucose level as secondary outcome measure. After three years of glucosamine supplementation there was no effect of glucosamine on the fasting plasma glucose level in either study. However, the participants in both these studies were not at an increased risk for diabetes mellitus compared to the population of the present study. The systematic review of Simon *et al.* also pointed out that there was no study that specifically addressed the long-term safety of glucosamine in diabetic or pre-diabetic subjects.[7]

Studies that evaluated the effect of glucosamine on the HbA1c level, instead of fasting plasma glucose, had a variation in this measurement making it hard to compare the HbA1c levels.[6, 24, 25]

In the United States the HbA1c level is already used to diagnose diabetes mellitus and it can be

used in studies to evaluate the glucose status.[20, 21] Considering this variance a new variable was made, in contrast with previous studies, to analyze the effect of glucosamine on obtaining a high HbA1c level and/or new-onset diabetes mellitus over 6.5 years.

Strengths of this study includes the repeated measures that were used in the analyses, while this gives a reliable indication of the intervention effect over 6.5 years instead of after 6.5 years. Other strengths were the large number of participants, the HbA1c levels that were measured during glucosamine sulphate usage and the specific high risk group of participants that used the study drugs.

On the contrary, a limitation of the present study was the impossibility to impute the variable about the participants diabetes mellitus status. As a result, we might have underestimated the effect while participants with a normal HbA1c level can still have diabetes mellitus. Secondly, there was a slight variation in the course of mean HbA1c level and *High HbA1c/DM* during follow up between the original data and imputed data. These differences could have been due to the large amount of missing data after 6.5 years, the withdrawing of participants not at random or due to unknown or unavailable predictors for the analysis.[15]

Taking all results into consideration it is important that future studies will be carried out to investigate whether there is a significant effect of glucosamine on the glucose metabolism, especially in participants with a high risk for developing diabetes mellitus such as having a high HbA1c level at baseline. Although there was no statistically significant effect of glucosamine sulphate on mean HbA1c nor on the risk on obtaining *High HbA1c/DM*, a possible effect was seen in participants with a high HbA1c level at baseline. These analyses had high ORs that were not statistically significant, but this subgroup was too small to draw definitive conclusions due to lack of power.

In conclusion, there is no significant effect of a 2.5 year glucosamine sulphate intervention on mean HbA1c level or on obtaining a high HbA1c level and/or new-onset diabetes mellitus over 2.5 nor over 6.5 years in obese and overweight middle-aged women. More specifically, there was no intervention effect of glucosamine sulphate in the subgroup with participants with a normal HbA1c level at baseline. We cannot rule out a possible effect in the subgroup of participants with a high HbA1c level at baseline, although these results were also not statistically significant. However, this subgroup lacked power to state with certainty that there is no effect of the intervention. Future studies should therefore focus on subjects with an increased risk of developing diabetes mellitus. This indicates that for now glucosamine sulphate is safe to use in terms of its safety profile, certainly in participants with a normal HbA1c level at baseline.

In conclusion, there is no significant effect of a 2.5 year glucosamine sulphate intervention on mean HbA1c level or on obtaining a high HbA1c level and/or new-onset diabetes mellitus over 2.5 nor over 6.5 years in obese and overweight middle-aged women. More specifically, there was no effect of glucosamine sulphate in the subgroup with participants with a normal HbA1c level at baseline. We cannot rule out a possible effect in the subgroup of participants with a high HbA1c level at baseline, although these results were also not statistically significant. However, this subgroup lacked power to state with certainty that there is no effect. Future studies should therefore focus on subjects with an increased risk of developing diabetes mellitus. This indicates that glucosamine sulphate is safe to use, certainly in participants with a normal HbA1c level at baseline.

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