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Effect of glucosamine supplementation on fasting and non-fasting plasma glucose and serum insulin concentrations in healthy individuals

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Summary

Objective: To test the hypothesis that glucose intolerance does not occur when healthy adults consume normal, recommended dosages of glucosamine sulfate.

Methods: Healthy adults (*N*=19*)* ingested 1500 mg of glucosamine sulfate or placebo (double blind) each day for 12 weeks. Three-hour oral glucose tolerance tests (OGTT) were performed using 75 g of dextrose. These occurred before the start of supplementation, at 6 weeks, and at the completion of supplementation (12 weeks).

Results: There were no significant differences between fasted levels of serum insulin or blood glucose. Glucosamine sulfate supplementation did not alter serum insulin or plasma glucose during the OGTT. There were no significant differences within or between treatments, ages or gender. Glycated hemoglobin measurements at the three time points showed no significant change over time, within or between treatments, ages or gender. The lack of significant changes may have been due to large standard deviations in the data.

Conclusion: The data suggests that glucosamine supplementation, with normal recommended dosages, does not cause glucose intolerance in healthy adults. This cannot be determined conclusively, however, until further studies are conducted using alternative types of testing.

Key words: Glucosamine sulfate, Glucose intolerance, Supplementation, Hexosamine pathway.

Introduction

Gluosamine sulphate is a widely consumed dietary supplement, particularly by osteoarthritic patients. Over three million Canadians use glucosamine[1,2]. Countless studies have shown glucosamine's effectiveness in alleviating osteoarthritis related joint pain[3]. However, there has been documented use of glucosamine for general joint pain[4]. This is of potential concern as some research suggests that glucosamine supplementation may cause glucose intolerance and potentially insulin resistance[5–8].

Glucosamine is an amino-polysaccharide, metabolized by the hexosamine pathway. Both glucose and glucosamine enter the hexosamine-biosynthetic pathway as glucosamine-6-phosphate. Some evidence suggests that the hexosamine biosynthesis pathway serves a regulatory function for glucose uptake by cells[7]. Thus, a flux of excess substance into the hexosamine pathway due to the routing of incoming glucose or glucosamine through the hexosamine pathway, and/or intracellular accumulation of glucosamine metabolites, has been suggested to cause insulin resistance[9]. Many studies have shown that this causes impairments in insulin stimulation of glycogen synthase, thereby limiting the flux of glucose in glycogen[7,10]. These studies suggest that glucosamine supplementation could cause glucose intolerance and insulin resistance. One human intervention trial noted that acute glucosamine infusion recapitulates some metabolic features of human diabetes[11].

Most of the studies mentioned above, have infused large amounts of glucosamine into cultured cells, or intravenously into rodents in short periods of time. This makes it difficult to extrapolate the data to the effects of normal recommended dosages of glucosamine in humans.

As reviewed by Rovanti *et al.*[12], many studies refute the hypothesis that glucosamine administration causes insulin resistance. Oral administration to dogs of twice the product label recommended amounts of glucosamine was reported to have no effect on fasting blood sugar[13]. Furthermore, studies that have investigated the effects of intravenous glucosamine administration in rodents found that glucose metabolism is not impaired by short-term glucosamine supplementation[12,14,15].

Currently, there is no conclusive evidence as to the effect of normal, recommended dosages of glucosamine supplementation on healthy adults' glucose metabolism, suggesting the need for human intervention trials with glucosamine aimed at measuring fasting insulin and fasting glucose levels. No studies to date have investigated the long-term effects of over-the-counter dosages of glucosamine sulfate in humans. The closest efforts were reported in two recent reports. An increase in fasting-insulin levels in a group of six human subjects orally administered 1500 mg glucosamine sulfate for 12 weeks found no corresponding increase in glucose, the diagnostic definition of diabetes[16]. Pouwels *et al.* used an euglycemic hyperinsulinemic clamp and

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Table I
Physical characteristics of subjects in both treatment groups, reported as mean with standard deviations when appropriate

Characteristic	Placebo (*N*=8)	Glucosamine (*N*=11)
Females (n)	4	7
Males (n)	4	4
Age (year)	40.3±15	35.9±14
BMI (kg/m^2)	24.3±4.3	26.5±4.0
Compliance	0.94±0.08	0.91±0.07

infused glucosamine sulfate into healthy humans. Pouwel *et al*. concluded that glucosamine had no effect on insulin-induced glucose uptake[15]. These studies used a small number of patients, larger dosages of glucosamine than recommended in humans, and/or administered glucosamine intravenously, which results in an immediate surge of glucosamine in the blood stream. The experimental methods used in these two studies make it difficult to extrapolate directly to what is happening in the bodies of humans taking normal recommended oral doses of glucosamine supplementation. To date, there is insufficient evidence in the literature to determine whether the administration of normal, recommended doses of glucosamine sulfate as a dietary supplement has any detrimental effects on the glucose homeostasis of healthy adults. This study was the first to investigate the effect of supplementation with 1500 mg of glucosamine sulphate, for 12 weeks in healthy adults. We tested the hypothesis that glucose intolerance or insulin resistance does not occur when normal, recommended doses of glucosamine sulfate are consumed by healthy adults. We examined the serum insulin and plasma glucose responses in a group of healthy adults before, during and after supplementation with glucosamine sulfate. The subjects did not take glucosamine supplements prior to the study and daily activities and diets were maintained throughout the study.

Methods

SUBJECT SELECTION

Healthy, non-obese adults were recruited and screened for health problems including diabetes, other major health conditions, medication use, supplement use, caffeine intake, food allergies, smoking, alcohol abuse, drug use, pregnancy, exercise levels, body mass index (BMI) and weight changes greater than 10 lbs in the past 6 months. Subjects were excluded who had a BMI less than 18 or greater than 32, took supplements or medications that may interfere with glucose metabolism, had taken glucosamine in the last 6 months, had diabetes or were allergic to shellfish. Of the 20 subjects that began the study, one left for reasons unrelated to the supplementation. All statistics contain data of only those subjects who completed the study (*N*=19). The ages of the subjects in the study ranged between 22 and 58 years old (mean age=37.7) and the mean BMI was 25.5 (range 18.6–31.1). See Table I for subject characteristics. All subjects received both written and verbal explanation of the study, which received ethics approval from the University of Guelph Research Ethics Board. Informed consent was obtained from all subjects.

EXPERIMENT PROTOCOL

This study was a double-blinded, placebo controlled, human supplementation trial. Subjects were randomized by age and gender into two groups: placebo (dextrose), or supplement (glucosamine sulfate). The glucosamine sulfate (donated by Puresource Inc., Guelph, ON) was analysed to be 99% pure. Dosages of 500 mg were taken three times a day, for a 12-week period. Subjects were instructed to maintain their regular daily physical and medical activities for the duration of the trial. At baseline, after an overnight fast, an oral glucose tolerance test (OGTT) was performed to measure glucose tolerance. This was repeated at weeks 6 and 12. A catheter was inserted into the antecubital vein and kept open with 0.9% sodium chloride solution. Baseline samples of blood were taken (–15 min, 0 min) into blood collection tubes as previously indicated. A 10 oz dextrose solution containing 75 g of glucose was consumed less than 2 min after baseline samples were taken. Blood samples were then taken at 15, 30, 45, 60, 90, 120, 150 and 180 min post consumption of the dextrose solution into tubes containing nothing (for serum) or sodium heparin (for plasma). Subjects remained seated for the majority of the procedure either watching a video, conversing or reading. Adherence to the regimen of tablets was monitored by measuring pill counts in 6-week intervals and using a questionnaire at the end of the study.

ANALYSIS

For insulin analysis, non-treated blood was allowed to clot for at least 30 min and then centrifuged at 2400 rpm for 10 min. Serum was removed and stored at –20°C for insulin analysis ('Coat-a-count' RIA kit, Diagnostic Products Corporation, Los Angeles, California). Glycated hemoglobin was analyzed at weeks 0, 6 and 12, as an indicator of 2–3 month glucose metabolism, using an affinity chromatography column (Glycated Hemoglobin Kit, Sigma Diagnostics, St. Louis, MO). For glucose analysis, blood was collected in sodium heparin tubes and 200 µl was transferred to 1 ml of 0.6 M PCA. Following centrifugation, the supernatant was stored at –20°C until blood glucose analysis[17].

STATISTICAL ANALYSIS

Serum insulin and plasma glucose for each subject and OGTT were converted to an area under the curve (AUC) using the trapezoid method. The resulting AUC data was analyzed for differences between the supplement and placebo, as well as across age and gender, using a repeated measures analysis of variance (SAS 1998). Statistical analysis of glycated hemoglobin values were also done using a repeated measures analysis of variance. Differences were accepted as statistically significant if $P<0.05$.

Results

SERUM INSULIN

Fig. 1 summarizes the serum insulin data. There were no significant differences in fasting serum insulin (–15 min and 0 min) between treatments across trials ($P=0.99$), (Table II). There were no time ($P=0.09$) or treatment ($P=0.39$) effects between 15 min and 180 min. There were no significant interactions between treatment and age over-time or between treatment and gender over-time for insulin. The insulin levels rose after ingestion of dextrose, but at no

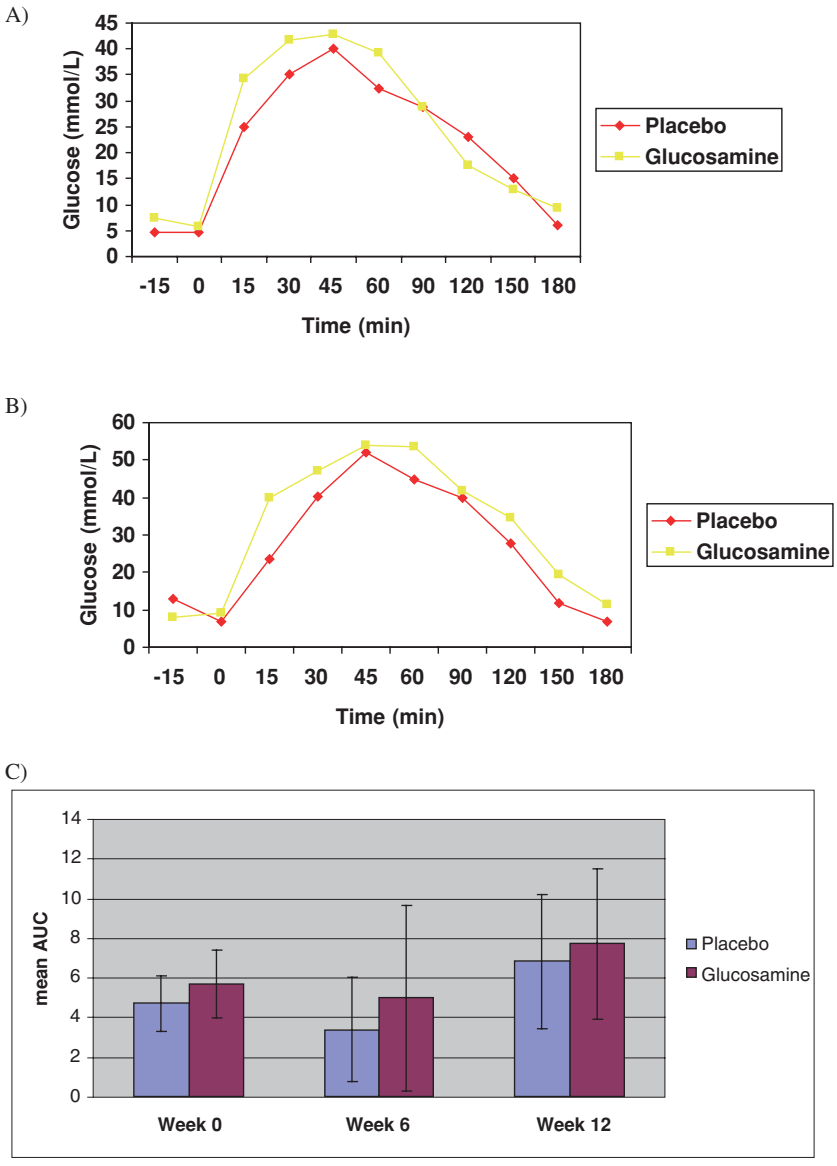

Fig. 1. Serum insulin response before and during an OGTT. A represents the placebo treatment group, while B represents the treatment group receiving glucosamine. Ingestion of dextrose occurred just after time 0. (A) Illustrates mean insulin values for each group during an OGTT in week 0. (B) Illustrates mean insulin values for each group during an OGTT in week 12. (C) Illustrates mean AUC for insulin.

point in time was there a treatment effect. These values were not significantly different.

PLASMA GLUCOSE

Fig. 2 summarizes the plasma glucose data. There were no significant differences in fasting plasma glucose (–15 min and 0 min) between treatments across trials (P=0.94), (Table III). Over time, there was a significant difference (P=0.02) across both groups. There were no treatment (P=0.4) effects between 15 min and 180 min. There were no significant interactions between treatment and age over-time or between treatment and gender over-time for glucose. The plasma glucose levels rose accordingly after ingestion of dextrose, but at no point in time was there a treatment effect. These values were not significantly different.

Table II
Fasting serum insulin (uIU/ml) for both groups, for all three trials. Values are reported as mean with standard deviations

Treatment	Week 0	Week 6	Week 12
Placebo	4.68±1.4	3.38±2.6	6.8±3.4
Glucosamine	5.69±1.7	4.96±4.7	7.7±3.8

GLYCATED HEMOGLOBIN

Fig. 3 summarizes the glycated hemoglobin data. There were no significant changes in glycated hemoglobin levels between treatments, and across time (P<0.80). There were no significant differences in glycated hemoglobin levels between treatment and age (P<0.39) over-time, or between treatment and gender (P<0.37) over-time.

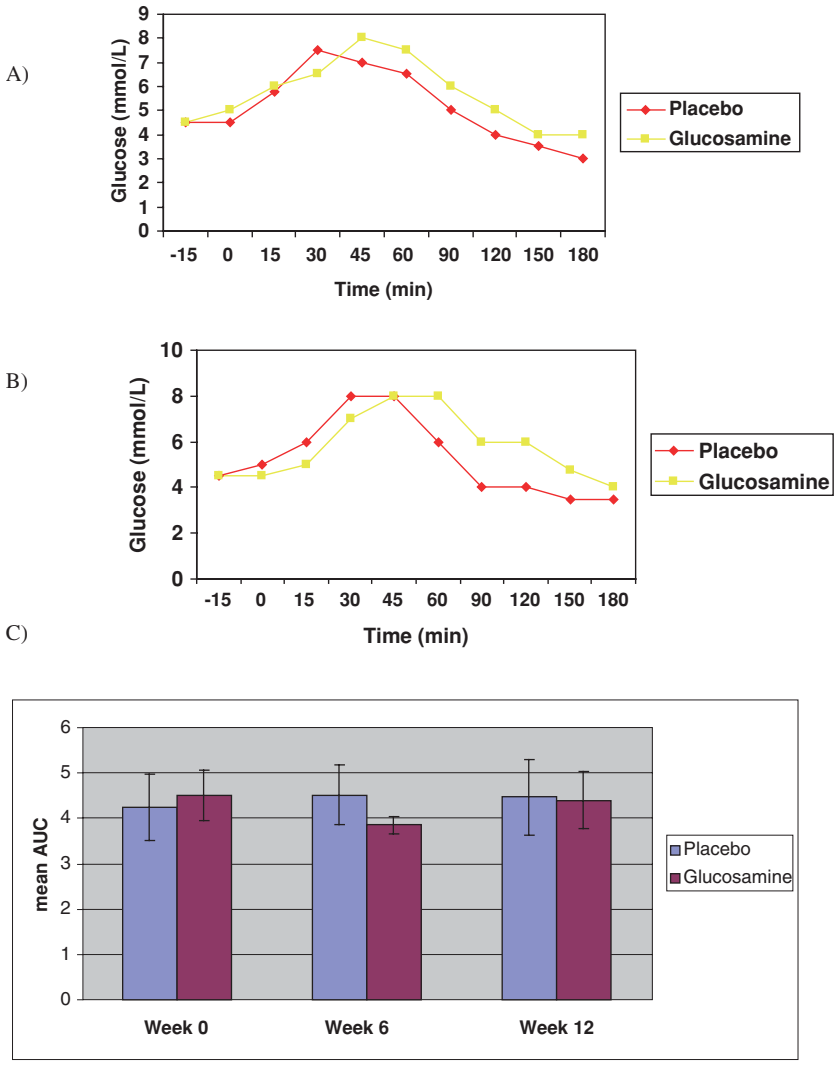

Fig. 2. Plasma glucose response before and during an OGTT, for both placebo and glucosamine groups. Ingestion of dextrose occurred just after time 0. (A) Illustrates mean glucose values for each group during an OGTT in week 0. (B) Illustrates mean glucose values for each group during an OGTT in week 12. (C) Illustrates mean AUC for glucose.

Discussion

As glucosamine supplements become more popular, there will be increased concern of their safety, in particular, whether glucosamine supplementation plays a role in insulin resistance. This study examined whether glucose intolerance occurs when normal, recommended dosages of glucosamine sulfate are consumed by healthy adults. The hexosamine pathway appears to have a regulatory role in human glucose homeostasis[18]. An increased flux of fructose-6-phosphate into the hexosamine pathway has been shown to cause insulin resistance[9]. Since glucosamine is metabolized through the hexosamine pathway, increasing dietary intake of glucosamine sulfate is hypothesized to over-load the system. Studies have shown that over-loading the hexosamine pathway inhibits basal and insulin stimulated glucose transport[7]. Some animal and cell culture data has shown that glucosamine could cause insulin resistance by inhibiting glucose transport[5–7]. Human studies have not agreed[15].

Table III
Fasting plasma glucose (mmol/l) both groups, for all three trials. Values are reported as means with standard deviations

Treatment	Week 0	Week 6	Week 12
Placebo	4.24±0.73	4.52±0.65	4.47±0.84
Glucosamine	4.57±0.55	3.85±0.20	4.40±0.63

Of note, glucosamine may impair insulin secretion by causing apoptosis of the insulin secreting beta cells[19]. This may be caused by beta cells having a high affinity for glucosamine transporters. This apoptosis may inhibit insulin secretion thereby blunting a compensatory insulin response to the induction of insulin making it appear that glucosamine does not cause insulin resistance, when it may in fact do so. Detection methods of this study and the use of non-diabetic patients limit the ability of this study to determine if this process occurred. The data, however, add to the growing number of publications investigating the effect of glucosamine on glucose tolerance.

Results of this study suggest that there was no difference between placebo and treatment groups in terms of

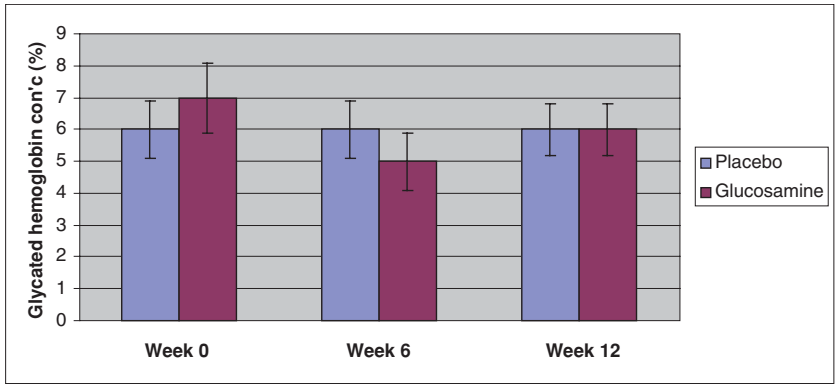

Fig. 3. Mean glycated hemoglobin levels at week 0, 6 and 12 for both treatments.

circulating insulin or glucose prior to the ingestion of the dextrose drink of the first OGTT, nor prior to dextrose ingestion in any of the subsequent trials. Furthermore, no significant differences within or between treatments in these measures in the succeeding 3-h post-ingestion period of the OGTT was found. As results of the OGTT showed no significant change, then insulin resistance was most likely also not significantly changed. Although this data suggests that the use of normal, recommended doses of glucosamine sulfate does not cause glucose intolerance or insulin resistance in healthy adults, the small number of subjects and the large standard deviations which occurred in this study mean that we cannot conclusively determine this and results from future studies are needed.

A lack of change in levels of glycated hemoglobin, a measure of 2–3 month glucose metabolism, supports our hypothesis that chronic administration of normal, recommended doses of glucosamine sulfate does cause large alterations in glucose metabolism in healthy adults. Furthermore, the lack of alteration in insulin levels in association with glucosamine sulfate administration observed in this study, might suggest that this procedure did not administer sufficient glucosamine to overload the hexosamine pathway. Again definitive conclusions must await the results of future trials using larger subject pools and different methodologies.

These findings are difficult to compare to other studies as most previous research has involved the administration of large amounts of glucosamine in short periods of time into cultured cells or intravenously to rodents[6,20]. These studies cannot be extrapolated to humans supplemented orally with normal recommended dosages of glucosamine. There have been two human studies in which healthy adults were supplemented with glucosamine. The first noted an increase in fasting-insulin levels without a corresponding increase in glucose[16], suggesting insulin resistance. These results differ from this study. This may be due to subjects used, and/or other variables such as the different methods used to measure insulin sensitivity. The second was noted in a recently published letter in Diabetes Care by Yu *et al.*[21] concerning a study that was similar to this one. They found no difference between placebo and supplemented groups after 6 weeks of glucosamine treatment.

This study does not provide a definitive conclusion to the debate surrounding the issue of glucosamine supplementation and insulin resistance. Limitations to this study include the small number of subjects and the short duration period. Also, the use of insulin and glucose levels to detect changes in insulin sensitivity is a surrogate approach to measure insulin action and is not the gold standard for measuring insulin sensitivity. A euglycemic insulin clamp technique would be a superior method to measure insulin sensitivity in future studies. Future studies should investigate the effects of longer periods of glucosamine sulfate supplementation, ideally up to 2 years, to determine the effects on glucose metabolism in prolonged users, such as osteoarthritis patients. Also, higher dosages of glucosamine sulfate should be tested, to take into consideration users who take more than the recommended dosage. Most importantly, future studies should investigate subject groups with more sensitive glucose metabolism such as the elderly and diabetics. This may shed light on the extent of any potential alterations in glucose metabolism caused by glucosamine supplementation. Measurement of secondary indicators of metabolism including C-peptide and serum glycerol may also be useful in future studies.

In summary, this investigation of 12 weeks of supplementation with 1500 mg of glucosamine sulfate in healthy adults, the dose most often recommended to osteoarthritis patients, suggests that changes in glucose homeostasis does not occur. Definitive conclusions await more studies in this area.

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