

Oral Glucosamine for 6 Weeks at Standard Doses Does Not Cause or Worsen Insulin Resistance or Endothelial Dysfunction in Lean or Obese Subjects

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Glucosamine is a popular nutritional supplement used to treat osteoarthritis. Intravenous administration of glucosamine causes insulin resistance and endothelial dysfunction. However, rigorous clinical studies evaluating the safety of oral glucosamine with respect to metabolic and cardiovascular pathophysiology are lacking. Therefore, we conducted a randomized, placebo-controlled, double-blind, crossover trial of oral glucosamine at standard doses (500 mg p.o. t.i.d.) in lean ($n = 20$) and obese ($n = 20$) subjects. Glucosamine or placebo treatment for 6 weeks was followed by a 1-week washout and crossover to the other arm. At baseline, and after each treatment period, insulin sensitivity was assessed by hyperinsulinemic-isoglycemic glucose clamp (SI_{Clamp}) and endothelial function evaluated by brachial artery blood flow (BAF; Doppler ultrasound) and forearm skeletal muscle microvascular recruitment (ultrasound with microbubble contrast) before and during steady-state hyperinsulinemia. Plasma glucosamine pharmacokinetics after oral dosing were determined in each subject using a high-performance liquid chromatography method. As expected, at baseline, obese subjects had insulin resistance and endothelial dysfunction when compared with lean subjects (SI_{Clamp} [median {25th–75th percentile}] = 4.3 [2.9–5.3] vs. 7.3 [5.7–11.3], $P < 0.0001$; insulin-stimulated changes in BAF [% over basal] = 12 [–6 to 84] vs. 39 [2–108], $P < 0.04$). When compared with placebo, glucosamine did not cause insulin resistance or endothelial dysfunction in lean subjects or significantly worsen these findings in obese subjects. The half-life of plasma glucosamine after oral dosing was ~150 min, with no significant changes in steady-state glucosamine levels detectable after 6 weeks of therapy. We conclude that oral glucosamine at standard doses for 6 weeks does not cause or

significantly worsen insulin resistance or endothelial dysfunction in lean or obese subjects. *Diabetes* 55:3142–3150, 2006

Glucosamine (2-amino-2-deoxy-D-glucose), an amino monosaccharide derivative of glucose, is biosynthetically incorporated into glycosaminoglycans, proteoglycans, and collagen (components of articular cartilage) (1,2). Glucosamine is extensively marketed and used as an over-the-counter complementary medicine treatment for osteoarthritis (3). However, clinical intervention trials and meta-analyses examining the clinical efficacy of glucosamine, either alone or in combination with chondroitin, have failed to demonstrate convincing evidence of efficacy in the treatment of osteoarthritis (4–8). Two lengthy placebo-controlled glucosamine intervention trials (9,10) (daily dosing for 3 years in ~200 patients) demonstrated small, but statistically significant, improvement in radiographic joint space narrowing in the knees of patients with osteoarthritis treated with glucosamine. However, the clinical significance of these findings remains uncertain. More recently, the rigorous multicenter Glucosamine/Chondroitin Arthritis Intervention Trial concluded that “glucosamine and chondroitin sulfate alone or in combination did not reduce pain effectively in the overall group of patients with osteoarthritis of the knee” (8). Of note, no published clinical trial investigating the efficacy of glucosamine therapy has measured plasma glucosamine levels during glucosamine administration.

There are important potential safety concerns with glucosamine supplementation. Glucosamine and its acetylated derivative, *N*-acetylglucosamine, are rapidly synthesized by the amidation of glucose-6-phosphate via the hexosamine biosynthetic pathway (HBP) (11). Increased flux of substrates through the HBP secondary to hyperglycemia is one potentially important mechanism for acquired and/or genetic insulin resistance that may contribute to diabetes and cardiovascular diseases associated with insulin resistance (11–14). Incubation of muscle, adipose, or endothelial cells with high levels of glucosamine (1–10 mmol/l) causes impairment in metabolic actions of insulin as well as endothelial dysfunction (15–19). Indeed, glucosamine is often used in laboratory studies as a tool to investigate mechanisms of insulin resistance caused by increased flux through the HBP (11,15,20). Moreover, intravenous glucosamine administration causes metabolic insulin resistance and vascular

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AUC, area under the curve; BAF, brachial artery blood flow; HBP, hexosamine biosynthetic pathway; HPLC, high-performance liquid chromatography; MVI, microvascular imaging; QUICKI, quantitative insulin sensitivity check index; ROI, region of interest.

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endothelial dysfunction in both animals and humans (13,20–25). Thus, there is a significant safety concern that oral glucosamine therapy at standard over-the-counter doses may cause or worsen both insulin resistance and vascular endothelial dysfunction in patients. This concern is heightened by the fact that osteoarthritis is a disease commonly associated with and exacerbated by obesity, a condition characterized by insulin resistance and endothelial dysfunction that significantly increases the risk of developing diabetes and cardiovascular disease (26). Rigorous clinical studies evaluating the safety of oral glucosamine with respect to insulin resistance and endothelial dysfunction are lacking. Therefore, in the present study, we evaluated the effects of oral glucosamine at standard doses (1,500 mg/day for 6 weeks) in lean and obese subjects in a randomized, double-blind, placebo-controlled, crossover trial using state-of-the-art methods including glucose clamp, brachial artery ultrasound, and ultrasound with microbubble contrast to evaluate changes in insulin sensitivity, endothelial function, and insulin-stimulated skeletal muscle microvascular recruitment. Importantly, our intervention trial with oral glucosamine is the first to simultaneously evaluate the pharmacokinetics of glucosamine after single-dose oral administration.

RESEARCH DESIGN AND METHODS

This was a randomized, double-blind, placebo-controlled, crossover trial designed to evaluate the safety of glucosamine from a metabolic and vascular perspective. Study subjects between the ages of 22 and 65 years were recruited from the local community through newspaper advertisements. Thirty-two lean (BMI ≤ 25 kg/m²) healthy and 52 obese (BMI ≥ 30 kg/m²) otherwise healthy subjects were screened for inclusion in our study. Subjects were specifically excluded from enrolling if they were taking any medications; if they were pregnant or had diabetes, hypertension (blood pressure $\geq 130/85$ mmHg), liver disease, pulmonary disease, renal insufficiency, coronary heart disease, heart failure, peripheral vascular disease, coagulopathy, or any other severe systemic diseases; or if they were allergic to shellfish. Subjects were also excluded if they had actively smoked within the last 2 years, were under treatment for any form of cancer, or if they had positive tests for HIV or hepatitis B or C.

Of all subjects screened for the study, 23 lean and 30 obese subjects enrolled, and 20 lean and 20 obese subjects completed all phases of the study. Enrolled subjects were randomly assigned in a double-blind fashion (block randomization by the National Institutes of Health Clinical Center Pharmacy) to the initial arm of the study consisting of either glucosamine (500 mg p.o. t.i.d.) or matching placebo for 6 weeks. This was followed by a 1-week washout period. Subjects were then crossed over to the other treatment arm for an additional 6 weeks. Each enrolled subject underwent a hyperinsulinemic-isoglycemic glucose clamp study and forearm vascular studies at baseline and after each 6-week treatment period. In addition, during the beginning of the study day (at the end of each 6-week treatment period) blood samples were drawn following oral administration of glucosamine or placebo to estimate pharmacokinetics of glucosamine in plasma. Three lean and 10 obese subjects who were initially enrolled failed to complete the entire study for a variety of reasons, including family emergencies and other personal problems, difficulties with intravenous access, onset of hypertension, initiation of corticosteroid use, onset of pregnancy, and transient flank pain after administration of microbubble contrast agent. A research nurse counted glucosamine or placebo capsules at the end of each treatment period to help monitor subject compliance. To avoid drug interaction effects, subjects were not allowed to take additional medications during the study period. Informed consent was obtained from each subject. The study protocol was approved by the institutional review board of the National Heart, Lung, and Blood Institute, and all procedures followed were in accordance with institutional guidelines. All studies were conducted in the Clinical Center at the National Institutes of Health.

Glucosamine and placebo preparations. Glucosamine hydrochloride (250-mg capsules) and matching placebo capsules were purchased from the VA Cooperative Studies Program, Clinical Research Pharmacy Coordinating Center, Albuquerque, New Mexico. This center also provided glucosamine and placebo capsules for the National Center for Complementary and Alternative Medicine-sponsored multicenter Glucosamine/Chondroitin Arthritis Interven-

tion Trial (8). Our study was conducted under an investigational new drug application approved by the Food and Drug Administration. Glucosamine was tested for purity, potency, and quality, certificates of analysis were obtained, and drug master files were kept on file with the Food and Drug Administration. Capsules of glucosamine hydrochloride and matching placebo were manufactured, distributed, and placed on a shelf-life stability program throughout the study at the Clinical Research Pharmacy Coordinating Center.

Plasma glucosamine measurements and pharmacokinetics. Pharmacokinetics of oral glucosamine were assessed at the beginning of each glucose clamp study (conducted at the end of each 6-week treatment period). Subjects were given 500 mg glucosamine or placebo orally. Peripheral blood samples were then collected in EDTA-containing tubes at time 0 (before the dose) and 0.5, 1, 2, and 4 h after oral administration. Plasma was obtained from blood samples by centrifugation, and plasma samples were stored at -80°C before analysis. Plasma glucosamine concentrations were determined using reverse-phase high-performance liquid chromatography (HPLC) after derivatization of samples with phenylisothiocyanate using a method adapted from Liang et al. (27). D-Glucosamine hydrochloride and phenylisothiocyanate were purchased from Sigma Chemical (St. Louis, MO). D-Galactosamine hydrochloride was obtained from ICN Biochemical (Aurora, OH). Analyses were performed with an internal standard (20 μl galactosamine [100 $\mu\text{mol/l}$]) added to 200 μl plasma. Proteins were precipitated by adding 300 μl acetonitrile to each plasma sample. Samples were then centrifuged for 10 min at 10,000g, and 400 μl supernatant was removed and mixed with 50 μl N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate (50 mmol/l, pH 8.0) and 125 μl of 5% phenylisothiocyanate in acetonitrile. Derivatization reactions were allowed to proceed for 10 min at room temperature before solvent and unreacted phenylisothiocyanate were removed by evaporation under nitrogen. Dried reaction products were reconstituted in 200 μl of 20% acetonitrile. HPLC analysis was performed using a Waters model 717 autosampler, 626 pump, and 996 photodiode array detector (Waters, Milford, MA) along with a 250 \times 4.6-mm Spherex C18 column (Phenomenex, Torrance, CA). After injection of a 25- μl sample, separations were isocratically performed for 15 min at a flow rate of 0.7 ml/min of 10% methanol/0.09% acetic acid (by volume) in water. Between analyses, the column was washed with 75% methanol and allowed to reequilibrate to preanalysis conditions for 20 min. Analyses were calibrated using glucosamine standards of 1, 2, 4, 8, and 16 $\mu\text{mol/l}$ prepared in plasma. Detection of glucosamine was observed at 254 nm. Intra- and interassay variance was 2 and 15% for the 2- $\mu\text{mol/l}$ standard, respectively. The limit of detection of our HPLC assay was <0.3 $\mu\text{mol/l}$.

Pharmacokinetic parameters for glucosamine were derived from analysis of plasma glucosamine concentrations after oral dosing, assuming first-order kinetics (28). The elimination constant, K_e , was estimated from the slope of the linear regression of log-transformed concentration values plotted versus time in the terminal phase, assuming first-order kinetics and instantaneous mixing. The apparent elimination half-time ($t_{1/2}$) was calculated as $t_{1/2} = \log_e(2)/K_e$. (This assumes instantaneous mixing and no significant endogenous production of glucosamine over the examined time interval.) Time to peak plasma concentration (T_{max}) and peak plasma concentration (C_{max}) were estimated from the observed concentration versus time data, assuming instantaneous mixing. The area under the curve to the last measurable concentration (AUC_{0-240}) was calculated using the trapezoidal rule for the observed values from 0 h to the last measured time point (4 h).

Hyperinsulinemic-isoglycemic glucose clamp. Insulin sensitivity was evaluated by glucose clamp as previously described (29). An insulin solution (regular Humulin; Eli Lilly, Indianapolis, IN) was infused at 120 $\text{mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ for 3 h using a calibrated syringe pump (model A-99; Razel Industries, Stamford, CT). A solution of potassium phosphate was simultaneously infused (0.23 $\text{mEq} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) to prevent hypokalemia. Blood glucose concentrations were measured at the bedside every 5–10 min using a glucose analyzer (YSI 2700 Select; YSI, Yellow Springs, OH), and an infusion of 20% dextrose was adjusted to maintain the blood glucose concentration at the fasting level. Blood samples were also collected every 20–30 min for determination of plasma insulin concentrations (DPC Immulite 2500; Diagnostic Products, Los Angeles, CA). The steady-state period of the clamp was defined as a ≥ 60 -min period (1–2 h after the beginning of the insulin infusion) during which the coefficient of variation for blood glucose, plasma insulin, and glucose infusion rate was $<5\%$. The glucose clamp-derived index of insulin sensitivity (SI_{Clamp}) was defined as $\text{M}/(\text{G} \times \Delta\text{I})$ corrected for body weight (where M is the steady-state glucose infusion rate [mg/min], G is the steady-state blood glucose concentration [mg/dl], and ΔI is the difference between basal and steady-state plasma insulin concentrations [$\mu\text{U/ml}$]).

Quantitative insulin sensitivity check index. Quantitative insulin sensitivity check index (QUICKI) was calculated as previously defined (29). $\text{QUICKI} = 1/[\log(I_0) + \log(G_0)]$, where I_0 = fasting insulin ($\mu\text{U/ml}$) and G_0 = fasting glucose (mg/dl). Since QUICKI is the reciprocal of the log-transformed

TABLE 1
Baseline clinical characteristics of study subjects

| Variables | Lean | Obese | <i>P</i> value |
|--|----------------|---------------|----------------|
| <i>n</i> | 20 | 20 | — |
| Age (years) | 40 ± 3 | 49 ± 3 | 0.02 |
| Sex (male/female) | 9/11 | 6/14 | — |
| BMI (kg/m ²) | 24.3 ± 0.6 | 34.2 ± 1.4 | 0.0001 |
| Fasting glucose (mg/dl) | 81 ± 2 | 88 ± 2 | 0.01 |
| Fasting insulin (μU/ml) | 3.9 ± 0.4 | 10.9 ± 1.5 | 0.0001 |
| SI _{Clamp} [10 ⁻⁴ dl · kg ⁻¹ · min ⁻¹ / (μU/ml)] | 7.3 (5.7–11.3) | 4.3 (2.9–5.3) | 0.0001 |
| QUICKI | 0.410 ± 0.008 | 0.346 ± 0.007 | 0.0001 |
| A1C (%) | 5.4 ± 0.1 | 5.6 ± 0.1 | 0.03 |
| Systolic blood pressure (mmHg) | 116 ± 2 | 124 ± 2 | 0.006 |
| Diastolic blood pressure (mmHg) | 67 ± 2 | 68 ± 1 | 0.70 |
| Mean blood pressure (mmHg) | 84 ± 2 | 87 ± 1 | 0.16 |
| Total cholesterol (mg/dl) | 192 ± 8 | 187 ± 10 | 0.70 |
| LDL cholesterol (mg/dl) | 116 ± 6 | 121 ± 7 | 0.58 |
| HDL cholesterol (mg/dl) | 58 ± 5 | 47 ± 2 | 0.03 |
| Triglycerides (mg/dl) | 123 ± 13 | 176 ± 37 | 0.21 |

Data are means ± SE or median (25th–75th percentile).

product of fasting glucose and insulin, it is a dimensionless index without units.

Brachial artery blood flow. At the beginning of each glucose clamp study and 2 h after initiation of the insulin infusion (during steady-state period), brachial artery diameter and blood flow were assessed by Doppler ultrasound as previously described (30). Briefly, the right brachial artery was visualized on the anterior aspect of the arm, 2–15 cm proximal to the antecubital fossa using a high-resolution ultrasound probe (HDI-5000 ultrasound machine with a 12-MHz linear array transducer; Philips Ultrasound, Bothell, WA). The position of the transducer on the arm was marked to facilitate visualization of the same portion of the artery throughout the study. Brachial artery diameter was measured from the anterior to the posterior “m” line (the interface between media and adventitia) using video calipers at end-diastole, coincident with the R wave on the electrocardiogram. Brachial artery blood flow (BAF) was estimated from blood velocity (*V*), arterial diameter (*D*), and heart rate measurements using the equation $BAF = \pi \times (D/2)^2 \times V \times 60$.

Forearm skeletal muscle microvascular perfusion. Insulin-stimulated microvascular perfusion in the deep flexor muscles of the forearm was assessed using real-time microbubble contrast-enhanced ultrasonography. We compared estimates of microvascular blood flow before insulin infusion and in a period of steady-state hyperinsulinemia during the glucose clamp. Immediately after each BAF determination, skeletal muscle microvascular perfusion was estimated using the microvascular imaging (MVI) technique (Philips Ultrasound, Bothell, WA). MVI is a low-energy real-time scanning technique based on maximum intensity projection that offers better vessel visualization under conditions of both high and low vascularity (31). A suspension of echogenic microbubbles with a similar size and rheology to erythrocytes (Optison; Mallinckrodt Medical, St. Louis, MO or Definity; Bristol Myers Squibb Medical Imaging, North Billerica, MA) was intravenously infused at a constant rate of 1.4–1.6 ml/min (Optison, 0.04–0.07 ml/kg; Definity, 39 μl/min) for 6 min (2 min preceding data acquisition and then during 4 min of data acquisition) using a model A-99 infusion pump (Razel Industries, Stamford, CT). The first nine subjects studied received Optison, whereas Definity was administered to the remaining participants. Ultrasound imaging of the deep flexor muscles of the forearm was performed in a transaxial plane 5 cm distal to the antecubital fossa (L7–4 transducer, HDI-5000; Phillips Ultrasound, Bothell, WA). Gain settings were kept constant throughout each study. MVI uses proprietary image-processing software to suppress background signals from surrounding tissue in order to capture and track images of microbubbles as they traverse the microvasculature. Two minutes after initiating infusion of microbubbles (sufficient to achieve systemic steady-state distribution of microbubbles), a brief high-energy ultrasound pulse (“burst” imaging) was used to destroy microbubbles in the region of interest (ROI). Immediately after bursting, MVI imaging under low-power conditions was continued and microbubble replenishment visualized over 15 s. Images were quantitatively analyzed offline using a commercially available software tool (Q-Lab; Philips Medical Systems). Mean video contrast intensity in the ROI after the “burst” was quantified. Larger conduit, recurrent, interosseous, and muscular arteries were excluded from the ROI. Plots of video contrast intensity (*y*) versus time (*t*) were fit to the first-order exponential equation $y = \alpha (1 - e^{-\beta t})$ (32). The parameter α represents the maximal

signal intensity measured after complete refilling and is proportional to microvascular blood volume in the ROI. The parameter β is proportional to the initial microvascular blood flow velocity.

Laboratory assays. Routine assays for lipids, glucose, and HbA_{1c} (A1C) were performed in the Department of Laboratory Medicine at the Clinical Center, National Institutes of Health.

Statistical analyses. The presence of skewed data was evaluated by visual inspection of Q-Q plots, stem and leaf plots, or box plots and verified by the Shapiro-Wilk test for normal distribution. After testing data for normality, we used Student’s unpaired *t* or Mann-Whitney *U* tests for evaluation of differences between lean and obese groups as appropriate. Differences among outcome measures SI_{Clamp}, BAF, and capillary recruitment for the three evaluation points (baseline, after treatment with placebo, after treatment with glucosamine) were evaluated by Friedman’s test or by repeated-measures ANOVA. A post hoc Dunn’s test (also known as the Bonferroni-Dunn test) was used for comparisons between groups without adjustment for multiple comparisons (compares the difference in the sum of ranks between two groups with the expected average difference based on the number of groups and size). Change in insulin sensitivity, as measured by glucose clamp, was prospectively designated as the primary end point of the study. All other comparisons were considered secondary. Based on mean values of SI_{Clamp} that we obtained for lean and obese subjects in previous studies (29), we calculated that 20 subjects in each group (lean or obese) would provide >90% power for detecting a 10% difference in insulin sensitivity caused by glucosamine treatment when compared with placebo treatment. This power calculation is extremely conservative because it is based upon the sample size needed for a two-sided *t* test with $\alpha = 0.05$ rather than our present crossover design of equivalent size (29). To assess the possibility of a carryover effect from the initial treatment period to the final treatment period, we compared changes in principal outcomes between subgroups who received placebo in the first treatment arm with those of subgroups who received glucosamine in the first treatment arm in both lean and obese subject groups relative to respective baseline values by using Student’s unpaired *t* or Mann-Whitney test. Values of *P* < 0.05 were considered to represent statistical significance.

RESULTS

Study subjects. Baseline clinical characteristics of the 20 lean and 20 obese subjects who completed our study are reported in Table 1. The ages of the subjects ranged between 22 and 65 years. The mean age of the lean group was significantly younger than that of the obese group. In the lean group, there were 15 Caucasians, 2 African Americans, and 3 Asians, while in the obese group there were 15 Caucasians and 5 African Americans. When compared with the lean group, the obese group had significantly higher mean systolic blood pressure, fasting blood glucose, fasting plasma insulin, and A1C levels. Both the mean glucose clamp index of insulin sensitivity (SI_{Clamp})

TABLE 2
Vascular parameters of lean subjects before and after treatment with placebo or glucosamine

| Vascular parameters | Baseline | Placebo | Glucosamine | <i>P</i> value (overall; glucosamine vs. placebo) |
|--|-----------------|------------------|-----------------|---|
| BAF (ml/min) | | | | |
| Baseline | 75 (38–121) | 105 (62–150) | 94 (77–119) | |
| During steady-state insulin infusion | 147 (92–165) | 111 (87–209) | 120 (71–194) | |
| Insulin-induced Δ (% over basal) | 39 (2–142) | 66 (–7 to 122) | 20 (–6 to 80) | 0.28; >0.05 |
| Microvascular volume, parameter α | | | | |
| Baseline | 3.3 (1.7–4.9) | 2.3 (1.8–3.7) | 3.1 (2.1–4.7) | |
| During steady-state insulin infusion | 3.2 (2.0–4.4) | 2.9 (1.9–4.5) | 3.1 (2.2–4.9) | |
| Insulin-induced Δ (% over basal) | –4 (–21 to 48) | 13 (–6 to 56) | –10 (–21 to 81) | 0.11; >0.05 |
| Microvascular flow velocity, parameter β | | | | |
| Baseline | 0.6 (0.3–2.7) | 0.5 (0.2–1.1) | 0.4 (0.2–3.5) | |
| During steady-state insulin infusion | 0.7 (0.3–2.1) | 0.7 (0.5–2.4) | 1.8 (0.5–3.0) | |
| Insulin-induced Δ (% over basal) | 42 (–33 to 170) | 158 (–48 to 378) | 108 (–6 to 444) | 0.52; >0.05 |

Data are median (25th–75th percentile). Parameters α and β for microvascular recruitment (proportional to microvascular volume and initial flow velocity, respectively) were estimated as described in RESEARCH DESIGN AND METHODS. The overall *P* value is derived from a Friedman statistic that compares within-group changes between baseline and after treatment with placebo or glucosamine. *P* value for pairwise posttreatment comparisons (placebo and glucosamine) is also shown.

and QUICKI were significantly and substantially lower in the obese group when compared with the lean group, consistent with the expected insulin resistance of obesity. HDL cholesterol levels were significantly lower in the obese group when compared with the lean group, but no other significant differences in fasting lipid profiles were observed. The observed differences between the groups remained significant even after adjusting for age. When compared with baseline values, hyperinsulinemia during the glucose clamp caused a significant increase in BAF, microvascular blood volume, and microvascular blood flow velocity in both lean and obese groups when all three periods were pooled (Tables 2 and 3; pretreatment, postglucosamine, postplacebo, *P* < 0.02). With respect to endothelial function at baseline before placebo or glucosamine treatment, the median insulin-induced increase in BAF was lower in the obese than in the lean group, consistent with the expected endothelial dysfunction of obesity (% increase over basal [25th–75th percentile] = 12% [–6 to 84] vs. 39% [2–108], *P* = 0.04). Along these same lines, insulin-stimulated increases in microvascular blood

flow velocity tended to be lower in the obese than in the lean group (cf., Tables 2 and 3, *P* < 0.07). However, insulin-stimulated increases in parameters related to forearm skeletal muscle microvascular volume were not significantly different when the obese and lean groups were compared. In all study subjects, baseline plasma glucosamine levels before placebo or glucosamine treatment were below the limit of detection of our assay (0.3 μ mol/l). **Glucosamine pharmacokinetics.** We measured plasma glucosamine levels over 4 h in all study subjects after a single oral dose (500 mg) of either placebo or glucosamine given at the beginning of each glucose clamp study (conducted after each 6-week intervention with placebo or glucosamine) (Fig. 1). As expected, placebo administration was not associated with any significant increases in plasma glucosamine. By contrast, in both obese and lean subjects, we observed a significant increase in plasma glucosamine levels 30 min after an oral dose of glucosamine. The plasma glucosamine profile over time had a trend to be slightly lower in obese subjects when compared with lean subjects (*P* < 0.09 by ANOVA), presum-

TABLE 3
Vascular parameters of obese subjects before and after treatment with placebo or glucosamine

| Vascular parameters | Baseline | Placebo | Glucosamine | <i>P</i> value (overall; glucosamine vs. placebo) |
|--|-----------------|-----------------|----------------|---|
| BAF (ml/min) | | | | |
| Baseline | 110 (42–152) | 66 (30–138) | 64 (37–130) | |
| During steady-state insulin infusion | 130 (55–152) | 101 (73–147) | 109 (58–172) | |
| Insulin-induced Δ (% over basal) | 12 (–7 to 84) | 32 (–5 to 186) | 31 (–2 to 109) | 0.76; >0.05 |
| Microvascular volume, parameter α | | | | |
| Baseline | 2.4 (1.8–3.3) | 1.2 (1.0–2.1) | 1.1 (0.5–2.2) | |
| During steady-state insulin infusion | 1.9 (1.4–2.9) | 1.9 (0.7–2.6) | 2.1 (1.2–3.2) | |
| Insulin-induced Δ (% over basal) | –18 (–43 to 33) | 22 (–36 to 156) | 31 (–2 to 157) | 0.12; >0.05 |
| Microvascular flow velocity, parameter β | | | | |
| Baseline | 0.7 (0.4–1.3) | 0.4 (0.2–0.8) | 0.4 (0.3–1.4) | |
| During steady-state insulin infusion | 0.4 (0.3–1.8) | 0.4 (0.3–0.7) | 0.5 (0.4–1.0) | |
| Insulin-induced Δ (% over basal) | –34 (–56 to 91) | –15 (–48 to 59) | 3 (–51 to 83) | 0.70; >0.05 |

Data are median (25th–75th percentile). Parameters α and β for microvascular recruitment (proportional to microvascular volume and initial flow velocity, respectively) were estimated as described in RESEARCH DESIGN AND METHODS. The overall *P* value is derived from a Friedman statistic that compares within-group changes between baseline and after treatment with placebo or glucosamine. *P* value for pairwise posttreatment comparisons (placebo and glucosamine) is also shown.

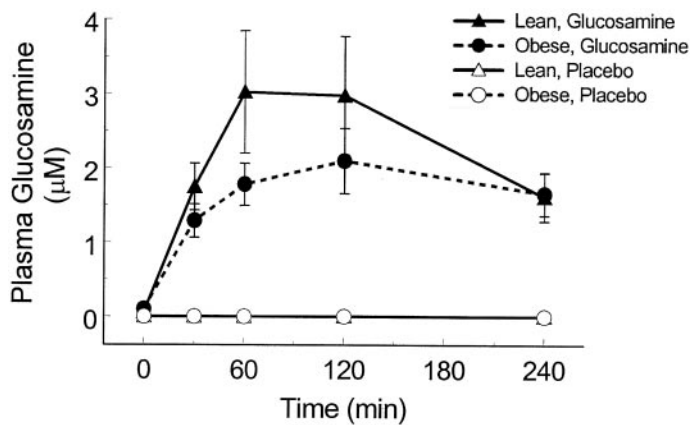


FIG. 1. Plasma glucosamine concentrations were measured after oral administration of glucosamine hydrochloride (500 mg) in lean (triangles) and obese (circles) subjects. Plasma glucosamine measurements after oral administration of placebo are shown in the open symbols. The limit of detection of our HPLC assay is ~0.3 µmol/l. Values shown are mean ± SE.

ably because of a larger volume of distribution or slower absorption in obese subjects. The pharmacokinetic parameters for plasma glucosamine estimated from our data are reported in Table 4. The mean apparent elimination half-life of plasma glucosamine estimated from lean study subjects was 148 min. In obese subjects, the peak glucosamine levels achieved were not high enough to determine a reliable elimination half-life.

Effects of placebo or glucosamine treatment on metabolic parameters. We did not observe any significant differences among SI_{Clamp} , QUICKI, fasting blood glucose, or fasting plasma insulin levels in lean (Table 5) or obese (Table 6) subjects. Likewise, there were no significant differences between either treatment arm for A1C, fasting lipids, or blood pressure in either lean or obese subjects (Tables 5 and 6). When results were analyzed and compared among subgroups that started with the placebo treatment arm (12 lean and 9 obese subjects) versus the glucosamine treatment arm (8 lean and 11 obese subjects), we did not find any significant differences (data not shown). Taken together with the relatively short half-life of plasma glucosamine that we observed, this suggests that the 1-week washout period in our study was sufficient to eliminate any significant carry-over effects on metabolic parameters in our crossover design. Moreover, even when results from lean and obese subjects were pooled together, neither placebo nor glucosamine treatment had

TABLE 4
Pharmacokinetic parameters of plasma glucosamine in lean and obese subjects determined after oral administration of glucosamine hydrochloride (500 mg)

| Pharmacokinetic parameters | Lean | Obese | P value |
|------------------------------|-----------|-----------|---------|
| n | 20 | 20 | |
| C_{max} (µmol/l) | 3.8 ± 0.8 | 2.5 ± 0.4 | 0.15 |
| T_{max} (min) | 110 ± 17 | 129 ± 18 | 0.42 |
| AUC_{0-240} (µmol/l × min) | 540 ± 112 | 403 ± 70 | 0.31 |

Data are means ± SE. AUC_{0-240} , AUC from time 0 to 240 min. The mean half-life of plasma glucosamine estimated from the lean study subjects was ~148 min. In obese subjects, the peak glucosamine levels were not high enough to determine a reliable half-life. C_{max} , maximum plasma concentration; T_{max} , time to reach maximum concentration.

any significant effects on metabolic parameters measured. Thus, our data strongly suggests that oral glucosamine at standard doses for 6 weeks does not cause insulin resistance in lean subjects or worsen insulin resistance in obese subjects.

Effects of placebo or glucosamine treatment on vascular parameters. We used Doppler ultrasound of the brachial artery and ultrasound with microbubble contrast of forearm skeletal muscle microvasculature to evaluate endothelial function in each study subject before and after 6-week administration of placebo or glucosamine. When data obtained after placebo and glucosamine treatments were compared, we did not observe any significant differences among insulin-stimulated BAF or parameters of insulin-stimulated microvascular recruitment in lean (Table 2) or obese (Table 3) subjects. When results for insulin-stimulated increases in BAF and microvascular recruitment were analyzed and compared among subgroups that started with the placebo treatment arm (12 lean and 9 obese subjects) versus the glucosamine treatment arm (8 lean and 11 obese subjects), we did not find any significant differences (data not shown). This suggests that the 1-week washout period in our study was sufficient to eliminate any significant carry-over effects on endothelial function in our crossover design. In addition, as with metabolic parameters, even when results from lean and obese subjects were pooled together, neither placebo nor glucosamine treatment had any significant effects on vascular outcomes measured. Thus, our data suggests that oral glucosamine at standard doses for 6 weeks does not cause endothelial dysfunction in lean subjects or worsen endothelial dysfunction in obese subjects.

Correlations among study parameters. Simple regression analyses revealed significant, direct correlations between SI_{Clamp} and QUICKI ($r = 0.62, P < 0.0001$). There were no significant relationships among insulin-induced changes in BAF, microvascular blood volume, or microvascular blood flow velocity and SI_{Clamp} or QUICKI when values for these parameters for all three treatment periods were analyzed together.

DISCUSSION

Glucosamine is a popular nutritional supplement often taken by patients for treatment of osteoarthritis. Increases in the aging population in the U.S. as well as the current epidemic of obesity are likely to elevate the prevalence of osteoarthritis, already a major and significant public health problem (26,33). Therefore, glucosamine usage is also likely to substantially increase in the near future. Aging and obesity are both associated with metabolic abnormalities, including insulin resistance, as well as with increased risk for cardiovascular diseases characterized by vascular endothelial dysfunction (26,34). Endogenously synthesized glucosamine is a product of the HBP that is important for modulating posttranslational modifications of proteins including glycosylation (11). Approximately 5% of glucose-6-phosphate is metabolized to N-acetylglucosamine (GlcNAc) by glutamine:fructose-6-phosphate amidotransferase (the rate-limiting enzyme in the HBP). In addition, extracellular glucosamine is transported into muscle and adipose tissue via specific glucose transporters (i.e., GLUT1 and GLUT4) where it is subsequently phosphorylated and enters the HBP downstream from glutamine:fructose-6-phosphate amidotransferase. Increased flux through the HBP has been implicated as one

TABLE 5
Blood pressure and metabolic parameters of lean subjects before and after treatment with placebo or glucosamine

| Variables | Baseline | Placebo | Glucosamine | ΔP | ΔG | <i>P</i> value (glucosamine vs. placebo) |
|--|----------------|---------------|----------------|----------------------|----------------------|--|
| <i>n</i> | 20 | 20 | 20 | — | — | — |
| BMI (kg/m ²) | 24.3 ± 0.6 | 24.8 ± 0.7 | 24.7 ± 0.7 | 0.4 ± 0.2 | 0.4 ± 0.2 | 0.88 |
| Systolic blood pressure (mmHg) | 116 ± 2 | 116 ± 3 | 115 ± 3 | -0.9 ± 2.0 | -1.6 ± 2.0 | 0.70 |
| Diastolic blood pressure (mmHg) | 67 ± 2 | 67 ± 2 | 67 ± 2 | -0.3 ± 1.8 | -0.9 ± 1.7 | 0.71 |
| Mean blood pressure (mmHg) | 84 ± 2 | 84 ± 2 | 83 ± 2 | -0.5 ± 1.6 | -1.1 ± 1.6 | 0.67 |
| Fasting glucose (mg/dl) | 81 ± 2 | 83 ± 2 | 82 ± 1 | 2.4 ± 1.2 | 0.8 ± 0.9 | 0.11 |
| Fasting insulin (μU/ml) | 3.9 ± 0.4 | 5.0 ± 0.7 | 4.4 ± 0.5 | 1.1 ± 0.4 | 0.4 ± 0.3 | 0.10 |
| SI _{Clamp} [10 ⁻⁴ dl · kg ⁻¹ · min ⁻¹ / (μU/ml)] | 7.3 (5.7–11.3) | 6.9 (5.7–9.3) | 7.6 (5.4–10.9) | -0.43 (-1.9 to 0.46) | -0.18 (-2.0 to 1.47) | 0.25*; >0.05 |
| QUICKI | 0.410 ± 0.008 | 0.396 ± 0.008 | 0.404 ± 0.008 | -0.014 ± 0.005 | -0.006 ± 0.006 | 0.16 |
| A1C (%) | 5.4 ± 0.1 | 5.3 ± 0.1 | 5.4 ± 0.1 | -0.01 ± 0.1 | 0.01 ± 0.05 | 0.84 |
| Total cholesterol (mg/dl) | 192 ± 8 | 171 ± 6 | 179 ± 7 | -21 ± 5 | -12 ± 4 | 0.08 |
| LDL cholesterol (mg/dl) | 116 ± 6 | 101 ± 6 | 110 ± 5 | -15 ± 4 | -5 ± 3 | 0.01 |
| HDL cholesterol (mg/dl) | 58 ± 5 | 54 ± 4 | 53 ± 3 | -5 ± 2 | -5 ± 2 | 0.69 |
| Triglycerides (mg/dl) | 123 ± 13 | 105 ± 13 | 113 ± 15 | -18 ± 11 | -9 ± 10 | 0.46 |

Data are means ± SE or median (25th–75th percentile). *The overall *P* value is derived from a Friedman statistic that compares within-group changes between baseline and after treatment with placebo or glucosamine. *P* value for pairwise posttreatment comparisons (placebo and glucosamine) is also shown. ΔG (postglucosamine – baseline), ΔP (postplacebo – baseline).

potential mechanism for hyperglycemia to contribute to development of both insulin resistance and endothelial dysfunction (13,15–21,23,24,35–38). Indeed, treatment of cells in vitro with glucosamine (1–10 mmol/l) causes both insulin resistance and endothelial cell dysfunction. More importantly, intravenous infusions of glucosamine that achieve plasma concentrations of 0.5–1.8 mmol/l in both animals and humans causes insulin resistance and endothelial dysfunction (20,25). Thus, there are significant potential safety concerns associated with glucosamine therapy for osteoarthritis. For these reasons, we undertook a rigorous randomized, double-blind, placebo-con-

trolled, crossover trial to evaluate the safety of oral glucosamine at standard doses from both metabolic and vascular perspectives. Importantly, in addition to evaluating safety of glucosamine, our study represents the first clinical intervention study with oral glucosamine to evaluate glucosamine pharmacokinetics.

Study subjects. The 20 lean and 20 obese individuals who completed our study had expected clinical and biochemical characteristics. In particular, lean subjects were healthy, with normal metabolic and hemodynamic parameters, while obese subjects were insulin resistant and had impaired insulin-stimulated BAF, characteristics represen-

TABLE 6
Blood pressure and metabolic parameters of obese subjects before and after treatment with placebo or glucosamine

| Variables | Baseline | Placebo | Glucosamine | ΔP | ΔG | <i>P</i> value (glucosamine vs. placebo) |
|--|---------------|---------------|---------------|----------------------|-----------------------|--|
| <i>n</i> | 20 | 20 | 20 | — | — | — |
| BMI (kg/m ²) | 34.2 ± 1.4 | 34.5 ± 1.2 | 34.6 ± 1.2 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.61 |
| Systolic blood pressure (mmHg) | 124 ± 2 | 123 ± 2 | 121 ± 2 | -2.0 ± 2.1 | -3.9 ± 1.8 | 0.41 |
| Diastolic blood pressure (mmHg) | 68 ± 1 | 68 ± 2 | 70 ± 2 | -1.1 ± 1.0 | 1.5 ± 1.4 | 0.06 |
| Mean blood pressure (mmHg) | 87 ± 1 | 86 ± 2 | 87 ± 2 | -1.4 ± 1.2 | -0.3 ± 1.2 | 0.44 |
| Fasting glucose (mg/dl) | 88 ± 2 | 87 ± 2 | 87 ± 2 | -0.4 ± 1.2 | -0.4 ± 0.9 | 0.98 |
| Fasting insulin (μU/ml) | 10.9 ± 1.5 | 11.4 ± 1.30 | 11.3 ± 1.8 | 0.5 ± 0.8 | 0.3 ± 0.76 | 0.88 |
| SI _{Clamp} [10 ⁻⁴ dl · kg ⁻¹ · min ⁻¹ / (μU/ml)] | 4.3 (2.9–5.3) | 5.4 (3.4–6.3) | 4.1 (3.1–5.4) | 0.31 (-0.25 to 1.69) | -0.25 (-0.74 to 0.42) | 0.08*; >0.05 |
| QUICKI | 0.346 ± 0.007 | 0.341 ± 0.005 | 0.345 ± 0.007 | -0.005 ± 0.005 | -0.001 ± 0.006 | 0.45 |
| A1C (%) | 5.6 ± 0.1 | 5.6 ± 0.1 | 5.6 ± 0.1 | -0.03 ± 0.03 | -0.04 ± 0.04 | 0.76 |
| Total cholesterol (mg/dl) | 187 ± 10 | 170 ± 7 | 178 ± 8 | -14 ± 9 | -9 ± 8 | 0.51 |
| LDL cholesterol (mg/dl) | 121 ± 7 | 110 ± 6 | 116 ± 7 | -10 ± 5 | -6 ± 4 | 0.54 |
| HDL cholesterol (mg/dl) | 47 ± 2 | 48 ± 3 | 46 ± 2 | -1 ± 2 | -1 ± 1.5 | 0.56 |
| Triglycerides (mg/dl) | 176 ± 37 | 121 ± 17 | 120 ± 17 | -53 ± 28 | -56 ± 27 | 0.94 |

Data are means ± SE or median (25th–75th percentile). *The overall *P* value is derived from a Friedman statistic that compares within-group changes between baseline and after treatment with placebo or glucosamine. *P* value for pairwise posttreatment comparisons (placebo and glucosamine) is also shown. ΔG (postglucosamine – baseline), ΔP (postplacebo – baseline).

tative of healthy lean and obese populations at large. Therefore, our study subjects are appropriate to use for investigating whether oral glucosamine causes insulin resistance and endothelial dysfunction in lean subjects or worsens these abnormalities in obese subjects.

Glucosamine preparation and pharmacokinetics. Commercially available glucosamine supplements sold over the counter to patients are available as the sulfate, hydrochloride, *N*-acetyl, or chlorhydrate salt. The content and purity of commercially available glucosamine supplements varies widely (39). In the present study, we chose a preparation of glucosamine hydrochloride that was identical to that used in the recently reported Glucosamine/Chondroitin Arthritis Intervention Trial (conducted under pharmaceutical rather than dietary supplement regulations to maximize purity, quality, and potency) (8). Since bioavailability of glucosamine from sulfate and hydrochloride salts is similar (40), and the stability and purity of our preparation was optimized, it is unlikely that metabolic and vascular outcome measures evaluated in our study would be significantly affected by use of a glucosamine sulfate preparation instead of the glucosamine hydrochloride preparation we utilized.

The present study is the first clinical intervention study with oral glucosamine to evaluate glucosamine pharmacokinetics. Baseline plasma glucosamine levels in healthy humans ($\sim 0.15 \mu\text{mol/l}$) are slightly below the lower limit of detection of our HPLC assay ($< 0.3 \mu\text{mol/l}$) (28). Thus, it is not surprising that we were unable to detect significant amounts of plasma glucosamine in any of our study subjects at baseline (before glucosamine administration) or in any of the placebo-treated subjects. Upon oral ingestion, glucosamine hydrochloride is rapidly absorbed as free glucosamine in the intestine. A significant fraction of this is catabolized by first-pass metabolism in the liver resulting in a bioavailability of $\sim 25\%$ (28). Of note, when our subjects were given a single dose of glucosamine orally (500 mg), we detected significant increases in plasma glucosamine levels (peak concentrations at ~ 60 min) that were 10- to 20-fold higher than basal levels in healthy humans reported in other studies (28,41). Although we did not observe statistically significant differences in C_{max} , T_{max} , or AUC between lean and obese groups, C_{max} and AUC tended to be slightly lower in obese than in lean subjects. This may be the result of a larger volume of distribution or slower absorption in obese subjects. The half-life estimated from glucosamine disappearance curves in our lean subjects was ~ 150 min, consistent with previous studies in humans (28,41). Glucosamine is a highly hydrophilic, fully ionized compound predominantly distributed in aqueous space (42). Given the aqueous distribution and relatively short half-life of glucosamine, it is unlikely that dosing three times daily would result in an increase in steady-state levels of plasma glucosamine. Indeed, basal glucosamine levels were undetectable in all subjects when measured at the end of either the first or the second arm of our crossover intervention study.

The maximal concentrations of plasma glucosamine we observed after administration of the standard 500-mg oral dose ($\sim 3 \mu\text{mol/l}$) is $\sim 1,000$ - to $10,000$ -fold less than glucosamine concentrations used in human, animal, and cell-based studies reporting effects of glucosamine to cause insulin resistance and endothelial dysfunction (12,13,15,18,20,25,43). Moreover, the peak concentrations of glucosamine we observed after 500 mg oral dosing is

also $\sim 1,000$ -fold below the K_m of GLUT4 for glucosamine transport (44). Thus, based solely upon pharmacokinetic considerations and what is known about glucosamine distribution, it seems unlikely that oral glucosamine administration at standard doses would pose safety problems from either a metabolic or vascular perspective.

Effects of glucosamine treatment on insulin sensitivity. We did not observe any significant effects of either placebo or glucosamine to cause insulin resistance in healthy lean subjects or to worsen insulin resistance in obese subjects. Similarly, we did not observe any significant changes in either lean or obese subjects in any other measured parameters related to insulin sensitivity including lipid profiles, blood pressure, or A1C. These results are consistent with our measurements of glucosamine pharmacokinetics and plasma glucosamine levels. We used the glucose clamp technique to assess insulin sensitivity since this method is considered the reference standard for direct measurement of insulin sensitivity in humans. Our study was abundantly powered to detect small changes in insulin sensitivity. Although QUICKI is an excellent surrogate index of insulin sensitivity, we do not consider it to be equivalent to glucose clamp measurements. We report QUICKI in addition to glucose clamp results because the concordance between these methods may be relevant to applying QUICKI in larger studies where application of the glucose clamp may not be practical. Results from our study with respect to the lack of effect of oral glucosamine on insulin sensitivity are consistent with previous less rigorous studies (45–47) that were all limited by the use of indirect measures of insulin sensitivity, inadequate study design, small sample size, and lack of assessment of plasma glucosamine levels.

Effects of glucosamine treatment on endothelial function. We did not observe any significant effects of either placebo or glucosamine to cause endothelial dysfunction in healthy lean subjects or to worsen endothelial dysfunction in obese subjects. We assessed endothelial function by measuring vasodilator responses to insulin in the brachial artery (a large conduit artery) as well as in nutritive microvascular beds of forearm skeletal muscle using sensitive ultrasound techniques. We chose to examine these two types of vasculature because they have distinct, but related, physiological functions implicated in regulation of insulin sensitivity, hemodynamics, and development of atherosclerosis (48–50). The effects of insulin to increase total limb blood flow are somewhat controversial and technique dependent. Although flow-mediated dilation or intra-arterial infusion of nitric oxide-dependent vasodilators are widely used measures of endothelial function, our study was focused on evaluating whether oral glucosamine would impair metabolic and vascular actions of insulin. With our methods, we were able to observe significant insulin-mediated increases in BAF in lean and obese subjects. It is possible that this may be due, in part, to actions of insulin on the vascular smooth muscle in addition to endothelial actions. The absolute values for BAF we observed are high. One possible explanation for this may be the effects of warming the contralateral arm (done to arterialize venous samples for the purposes of the clamp studies). Our measurements of microvascular blood volume and flow velocity had substantial variability that may limit our ability to detect small differences in these parameters. Nevertheless, with our methods, we were able to observe significant insulin-mediated increases in microvascular blood flow velocity in lean and obese subjects.

This is in contrast to a recent study by Clerk et al. (51) that demonstrated insulin significantly increased skeletal muscle capillary volume without changing capillary flow velocity in lean (but not obese) individuals. This discrepancy with our study may be due to technical differences including lower sensitivity and precision of the MVI technique we used compared with the Power imaging technique used by Clerk et al. (51) and inclusion of small arteries (diameter <350 μm) in our analysis. The increase in microvascular flow velocity we observed may reflect flow dynamics in small arteries consistent with insulin-enhanced blood flow in the proximal brachial artery. Although, microcirculation typically refers to vessels <150 μm in diameter, an alternative definition based on arterial vessel physiology rather than diameter has been proposed such that arterial vessels that respond to increasing pressure by a myogenic reduction in lumen diameter along with capillaries would constitute microcirculation. Such a definition includes the smallest arteries and arterioles in the microcirculation and is in line with the recent suggestion that small arterial and arteriolar components should be considered a continuum rather than distinct sites of resistance control (52).

Change in insulin sensitivity as measured by the glucose clamp was prospectively designated as the primary end point of our study. All other outcome measures, including measures of endothelial function, were considered secondary. Therefore, power calculations for measures of endothelial function were not carried out a priori. The coefficient of variation for brachial artery ultrasound is comparable to that of the glucose clamp (~10%), while the coefficient of variation for capillary recruitment measurements is ~25%. Given the fact that our study had abundant power to detect changes in insulin sensitivity based on extremely conservative power calculations that did not factor in the substantial additional power of our crossover design, it seems likely that our study was sufficiently powered to detect changes in endothelial function. Moreover, since our pharmacokinetic data demonstrated that peak plasma concentrations of glucosamine were low and steady-state concentrations of glucosamine were undetectable, it seems unlikely that increasing sample size would result in any significant differences in outcome measures or conclusions.

Summary. From a metabolic and vascular perspective, oral glucosamine therapy at standard doses for 6 weeks appears safe. Moreover, consistent with glucosamine pharmacokinetics measured in this study, and the known distribution of glucosamine, steady-state levels of glucosamine did not increase with the standard dosing regimen over 6 weeks. However, definitive conclusions regarding long-term safety cannot be made without longer-term studies. The relatively short half-life of glucosamine after oral administration taken together with the lack of change in steady-state plasma glucosamine levels observed during 6-week glucosamine therapy may have important implications for interpreting results from glucosamine efficacy studies.

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