Oral Administration of Glucosamine Improves Vascular Endothelial Function by Modulating Intracellular Redox State

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Summary
Glucosamine, used to treat osteoarthritis, has been shown to have anti-inflammatory and anti-atherosclerotic effects in experimental studies. A recent cohort study has demonstrated that the use of glucosamine was significantly associated with decreased total mortality. Vascular endothelial function is a potent surrogate marker of atherosclerosis and cardiovascular mortality where oxidative stress could participate. Therefore, we investigated whether glucosamine improves vascular endothelial function and intracellular redox state. We examined the effects of oral glucosamine administration (3000 mg/day) for 4 weeks on flow-mediated vasodilation (FMD) and intraerythrocyte glutathione parameters in 20 volunteers. Nineteen age-matched volunteers served as controls. Glucosamine administration significantly increased FMD (from 7.0 ± 2.3 to 8.7 ± 2.3%, P = 0.022). In the control group, FMD did not change. Glucosamine administration significantly increased intraerythrocyte total glutathione levels (from 212.9 ± 46.2 to 240.6 ± 49.4 μmol/L, P = 0.006), intraerythrocyte reduced form of glutathione (GSH) levels (from 124.7 ± 42.6 to 155.2 ± 47.7 μmol/L, P = 0.004) and intraerythrocyte GSH/oxidized form of glutathione (GSSG) ratios (from 3.18 ± 1.64 to 3.88 ± 1.61, P = 0.04). In the control group, any glutathione parameters did not change. Moreover, a stepwise multivariate analysis revealed percent change of GSH/GSSG is the only independent predictor for those of FMD (standardized β = 0.58, P = 0.007) in the glucosamine group. Glucosamine administration improved FMD in association with amelioration of intraerythrocyte GSH/GSSG ratios. These results suggest that oral glucosamine administration might improve vascular endothelial function by modulating intracellular redox state.

Key words: Glutathione, Flow-mediated vasodilation

Glucosamine, a naturally occurring amino monosaccharide, is present in the connective and cartilage tissues, and contributes to maintaining the strength, flexibility, and elasticity of these tissues. Indeed, glucosamine has been widely used to treat osteoarthritis in humans. Glucosamine has been also shown to have anti-inflammatory and anti-atherosclerotic properties in experimental studies. Recently, the vitamin and lifestyle cohort study has demonstrated that the use of glucosamine was significantly associated with decreased total mortality. Taken together, glucosamine might decrease total mortality through controlling the atherosclerotic process.

Endothelial cells from the inner lining of all blood vessels play a central role in vascular homeostasis. Disruption of the normal homeostatic endothelial condition is identified as endothelial dysfunction. Endothelial dysfunction is related to the development of atherosclerosis and mortality.

Therefore, the purpose of this study is to investigate the effects of oral administration of glucosamine on vascular endothelial function, an established surrogate marker for atherosclerosis.

Methods

Study subjects and protocols: This clinical study began in May 2010 and finished in May 2011. Forty-one male volunteers were divided into the two groups by turns in order of application. The study groups consisted of 20 male volunteers with 3000 mg of glucosamine administration for 4 weeks as the glucosamine group, and 19 age-matched male volunteers without glucosamine administration for 4 weeks observation served as controls. Flow-mediated vasodilation (FMD) measurement and blood sampling were performed in the afternoon before and after 4-week observation. The subjects were instructed to eat only a light meal in the morning and fast for at least 4 hours before the study. They were also instructed not to exercise and not to ingest substances that might affect FMD, such as caffeine, high-fat foods, and vitamin C, or...
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Figure 1. A- and B-mode images of FMD measurement. A representative case of measuring brachial artery diameter. FMD indicates flow-mediated vasodilation.

use tobacco for at least 6 hours before initiating the protocol. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mmHg, on at least 3 different occasions in a sitting position, or currently taking antihypertensive medication. Dyslipidemia was identified by the third report of the National Cholesterol Education Program. Diabetes mellitus was identified by American Diabetes Association criteria. Hyperuricemia was defined as serum urate level ≥ 7.0 mg/dL. Smoker was defined as those who were current smokers.

The study was designed and conducted according to the ethical principles for medical research stated in the Declaration of Helsinki, and it has been registered in UMIN Clinical Trials Registry (UMIN000020586). Written informed consent was obtained from all subjects.

Measurement of intraerythrocyte glutathiones: Intraerythrocyte GSH (the reduced form of glutathione) and GSSG (the oxidized form of glutathione) levels were determined with a quantification kit (Dojindo Molecular Technologies Inc., Kumamoto, Japan) according to the manufacturer’s instructions. Briefly, venous blood samples (5 mL) were taken and were collected in vacutainer tubes containing disodium EDTA (1.5 mg/mL). Samples were immediately centrifuged (1000xgf for 10 minutes at 4°C) and the supernatant was discarded. 20 μL of 5% 5-sulfosalycylic acid was added to the pellet. Then the mixture was centrifugated (8000xgf for 10 minutes at 4°C). The supernatant was determined according to the manufacturer’s protocol by measuring absorbance at 405 nm with a microtiter plate ELISA reader. We calculated the GSH/GSSG ratio as the marker of the oxidative stress.

Measurement of flow-mediated vasodilation: Endothelium-dependent vasodilation was assessed by FMD of the brachial artery according to the guidelines for the ultrasound assessment of endothelium-dependent FMD of the brachial artery by skilled examiners blinded to the subjects’ profiles. Brachial artery diameter was measured by B-mode ultrasound imaging (UNEX EF 18G, UNEX Co., Aichi, Japan) with a 7.5-MHz linear artery transducer while an electrocardiogram was simultaneously recorded. The right brachial artery was scanned in longitudinal sections 1~10 cm above the elbow after at least 5 minutes of rest in a supine position; the skin surface was marked, and the arm was kept in the same position during the study. After baseline measurement of the brachial artery diameter (average of 10 times measurements), FMD was determined by scans during reactive hyperemia. A pneumatic cuff placed around the forearm was inflated to 50 mmHg above systolic pressure and was deflated after 5 minutes. The brachial artery diameter was scanned and recorded continuously from 30 seconds before to 2 minutes after cuff deflation to obtain a maximal diameter. The diameter of the artery was measured by the A-mode waves as a signal of the intima-media complex to the other at end-diastole, coincident with the R-wave on a continuously recorded electrocardiogram (Figure 1). The FMD was expressed as maximal percentage change in arterial diameter from the baseline diameter after the release of occlusion.

Statistical analysis: Values are presented as means ± standard deviation. The Fisher exact test was used to illustrate heterogeneity between the groups. Statistical comparisons between two groups were performed by Mann-Whitney U test or Wilcoxon signed-rank test. The relationship between two variables was analyzed by use of Spearman rank correlation coefficient. A stepwise multivariate regression analysis was used for detecting the independent predictors. Differences were considered statistically significant at $P < 0.05$.

Results

Clinical characteristics of the study subjects: The clinical characteristics of the study subjects are shown in Table I. There were no significant differences in the concomitant ratio of smoking, dyslipidemia, hypertension, and hyperuricemia between the groups. There were no diabetic patients.

Atherosclerotic risk factors before and after observation: As shown in Table II, the two groups did not differ in levels of blood pressure, LDL cholesterol, HDL cholesterol, triglyceride, blood sugar, HbA1C, and uric acid be-
creased (from 124.7 ± 42.6 to 155.2 ± 47.7 μmol/L, \( P \) significantly increased (from 3.18 ± 1.64 to 3.88 ± 1.61, \( P = 0.004 \)). Moreover, the intraerythrocyte GSH/GSSG ratio significantly correlated with those of any factors including other factors:

**Flow-mediated vasodilation:** Groups.

Before observation was similar between the control and glucosamine groups. Although FMD did not change in the two groups. As shown in Figure 2, FMD change in the two groups. As shown in Figure 2, FMD and intracellular glutathione levels, such as systolic and diastolic blood pressure, cholesterol profiles, blood sugar, HbA1C, and uric acid were observed in the control and glucosamine groups.

**Flow-mediated vasodilation:** The baseline brachial diameter was similar before observation between control and glucosamine group (3.9 ± 0.4 versus 4.0 ± 0.3 mm, respectively), and after observation (3.9 ± 0.3 versus 3.9 ± 0.3 mm, respectively). Baseline brachial diameter did not change in the two groups. As shown in Figure 2, FMD before observation was similar between the control and glucosamine groups. Although FMD did not change in the control group, FMD significantly increased by glucosamine administration (from 7.0 ± 2.3 to 8.7 ± 2.3 %, \( P = 0.022 \)).

**Intraerythrocyte redox status of glutathiones:** Before observation, intraerythrocyte total glutathione levels, GSH levels, GSSG levels, and the GSH/GSSG ratio did not differ between the control and glucosamine groups (Figure 3 A, B, C, and D). After observation, intraerythrocyte total glutathione levels (from 212.9 ± 46.2 to 240.6 ± 49.4 μmol/L, \( P = 0.006 \)) and GSH levels significantly increased (from 124.7 ± 42.6 to 155.2 ± 47.7 μmol/L, \( P = 0.004 \)). Moreover, the intraerythrocyte GSH/GSSG ratio significantly increased (from 3.18 ± 1.64 to 3.88 ± 1.61, \( P = 0.04 \)). However, any glutathione parameters in the control group did not change (Figure 3A, B, C, and D).

**Relationship between flow-mediated vasodilation and other factors:** As shown in Table III, in all groups and the control group, percentage changes of FMD were not significantly correlated with those of any factors including intraerythrocyte glutathione levels, glucosamine administration, age, changes of BMI, blood pressures, lipid profiles, glucose levels, HbA1C levels, creatinine levels, and urate levels. On the other hand, in the glucosamine group, percentage changes in FMD significantly correlated with those of intraerythrocyte GSH levels (\( r = 0.46, P = 0.04 \)), GSSG levels (\( r = -0.49, P = 0.03 \)) and GSH/GSSG ratio (\( r = 0.64, P = 0.002 \)). A stepwise multivariate regression analysis revealed a percentage change of GSH/GSSG is the only independent predictor for that of FMD (standardized \( \beta = 0.58, P = 0.007 \) in glucosamine group (Table III).

### Discussion

In the present study, we found for the first time that oral administration of glucosamine significantly improved FMD. Intraerythrocyte GSH levels and GSH/GSSG ratios increased by oral administration of glucosamine. Moreover, the percentage change of FMD was significantly correlated with that of intraerythrocyte GSH/GSSG. Furthermore, the percentage change of GSH/GSSG was the only independent predictor for that of FMD. There were no changes in subjects’ characteristics that might affect FMD and intracellular glutathione levels, such as systolic and diastolic blood pressure, cholesterol profiles, blood sugar, HbA1C, and uric acid between baseline and after 4 weeks glucosamine administration. No changes in FMD and intracellular glutathione levels were observed in the control group at baseline and after 4 weeks of observation.

### Table I. Comparison of Clinical Characteristics between the Control and the Glucosamine Groups

|                         | Control Group | Glucosamine Group | \( P \) |
|-------------------------|---------------|-------------------|--------|
| Number of subjects \( n \) | 19            | 20                |        |
| Age (years; Mean ± SD)   | 34.8 ± 6.3    | 36.4 ± 7.5        | 0.55   |
| Hypertension \( n \)     | 1             | 2                 | 1.00   |
| Dyslipidemia \( n \)     | 4             | 5                 | 1.00   |
| Diabetes Mellitus \( n \) | 0            | 0                 | 1.00   |
| Hyperuricemia \( n \)    | 2             | 3                 | 1.00   |
| Smoker \( n \)           | 4             | 6                 | 0.72   |

SD indicates standard deviation; and \( n \), number.

### Table II. Comparison of Atherosclerotic Risk Factors before and after Observation in the Two Groups

|                         | Control Group \( n = 19 \) | Glucosamine Group \( n = 20 \) | \( P \) for intergroup |
|-------------------------|----------------------------|-------------------------------|-----------------------|
|                         | Before 4-week observation | After 4-week observation | Before 4-week observation | After 4-week observation | Before 4-week observation | After 4-week observation |
| Systolic blood pressure (mmHg) | 122 ± 8 | 120 ± 9 | 0.16 | 124 ± 13 | 121 ± 10 | 0.11 | 0.96 | 0.89 |
| Diastolic blood pressure (mmHg) | 70 ± 6 | 70 ± 8 | 0.75 | 71 ± 10 | 71 ± 8 | 0.98 | 0.71 | 0.99 |
| Heart rate (beats/minute) | 68 ± 9 | 65 ± 8 | 0.067 | 69 ± 10 | 69 ± 10 | 0.41 | 0.92 | 0.19 |
| Total cholesterol (mg/dL) | 198 ± 34 | 193 ± 28 | 0.18 | 201 ± 34 | 202 ± 27 | 0.90 | 0.62 | 0.36 |
| Triglyceride (mg/dL) | 158 ± 60 | 168 ± 74 | 0.46 | 158 ± 76 | 176 ± 131 | 0.63 | 0.99 | 0.58 |
| HDL cholesterol (mg/dL) | 58 ± 11 | 56 ± 11 | 0.14 | 61 ± 12 | 60 ± 11 | 0.68 | 0.54 | 0.29 |
| LDL cholesterol (mg/dL) | 115 ± 29 | 110 ± 29 | 0.15 | 111 ± 33 | 111 ± 33 | 0.50 | 0.98 | 0.70 |
| Blood glucose (mg/dL) | 102 ± 15 | 99 ± 13 | 0.46 | 103 ± 13 | 96 ± 11 | 0.073 | 0.75 | 0.60 |
| HbA1c (%) | 4.9 ± 0.2 | 5.0 ± 0.2 | 0.097 | 5.0 ± 0.2 | 5.0 ± 0.2 | 0.90 | 0.72 | 0.58 |
| Creatinin (mg/dL) | 0.89 ± 0.11 | 0.91 ± 0.12 | 0.29 | 0.92 ± 0.16 | 0.89 ± 0.19 | 0.13 | 0.74 | 0.18 |
| Uric acid (mg/dL) | 6.3 ± 1.4 | 6.4 ± 1.2 | 0.35 | 6.3 ± 1.6 | 6.3 ± 1.4 | 0.39 | 0.97 | 0.78 |

Values are expressed average ± standard deviation. HDL indicates high density lipoprotein; and LDL, low density lipoprotein.
Glucosamine, a constituent of articular glycosaminoglycans, is being marketed extensively as a therapeutic agent for osteoarthritis. Recent studies demonstrated the anti-inflammatory and anti-atherosclerotic effects of glucosamine. Glucosamine decreased superoxide anion generation, surface expression of CD11b of human peripheral neutrophils induced by formyl-Met-Leu-Phe, inhibited the release of granule enzyme lysozyme, and suppressed neutrophil chemotaxis toward zymosan-activated serum. Glucosamine suppressed human platelet aggregation, the extracellular release of granule contents, and production of thromboxane A2 by adenosine diphosphate stimulation. Glucosamine inhibited smooth muscle cell proliferation and reduced the atherosclerotic lesion in the aortic root of ApoE-null mice. Moreover, a recent cohort study provides the evidence that glucosamine decreases total mortality. Experimental studies suggested that glucosamine impedes the progression of cardiovascular disease. Accordingly, we examined the effects of oral glucosamine administration on the atherosclerotic process.

Endothelial dysfunction is an initial event in the development of atherosclerosis and ischemic heart disease and an independent predictor of cardiovascular diseases. In the present study, we evaluated the endothelial function by FMD. FMD refers to the arterial dilation occurring within a minute of the increase in shear stress, which is endothelium dependent. This response is completely abolished with nitric oxide inhibition following application of N^\text{\textdagger}-nitro-L-arginine methyl ester. In human brachial artery Doshi, et al. found that the FMD response was almost fully blocked using N^\text{\textdagger}-monomethyl-L-arginine. Thus, NO bioavailability is the most important factor of FMD. FMD of the brachial artery is an accepted technique to quantify endothelial function and has shown to have prognostic value for future cardiovascular disease. Thus, FMD was used to evaluate the efforts of glucosamine administration on endothelial function.

The mechanisms of effects on endothelial function by glucosamine administration remain to be elucidated. Much evidence has emerged showing that oxidative stress would participate in mechanisms of endothelial dysfunction. To further address this issue, we measured intraerythrocyte levels of GSH and GSSG (oxidized form of GSH) and obtained the ratio of GSH/GSSG as a marker of intraerythrocyte oxidative stress. GSH serves as a free radical scavenger. Moreover, GSH not only regulates the intracellular redox status but also modulates the action and metabolism of NO. Consistent with the results of FMD, glucosamine administration significantly elevated the intraerythrocyte ratios of GSH/GSSG and the intraerythrocyte GSH levels. These results consist with the previous reports that demonstrated the antioxidant activity of glucosamine. Glucosamine has been shown to possess antioxidant capacity manifested by scavenging superoxide and hydroxyl radicals and chelating effect on ferrous ions. Recently, glucosamine also has been shown to have a protective property against free radical-induced erythrocytes damage. Furthermore, the percentage changes in FMD significantly...
III. Univariate and Multivariate Analysis for Percentage Change of FMD

Table III. Univariate and Multivariate Analysis for Percentage Change of FMD

| Covariate                          | All Subjects (n = 39) | Control (n = 19) | Glucosamine (n = 20) | Multivariate analysis | Standardized β | P      |
|------------------------------------|----------------------|------------------|---------------------|----------------------|----------------|--------|
|                                    | r        | P       | r       | P       | r        | P       | r       | P       | r       | P       |
| Age                                | 0.05     | 0.77    | -0.15   | 0.54    | 0.23     | 0.33    |
| Glucosamine administration         | 0.23     | 0.16    |         |         |          |         |
| % change of systolic blood pressure| -0.46    | 0.78    | 0.05    | 0.85    | -0.14    | 0.57    |
| % change of diastolic blood pressure| -0.20    | 0.22    | -0.14   | 0.58    | -0.24    | 0.31    |
| % change of total cholesterol level| 0.09     | 0.57    | 0.08    | 0.74    | 0.11     | 0.63    |
| % change of LDL cholesterol level  | 0.17     | 0.29    | 0.04    | 0.88    | 0.33     | 0.33    |
| % change of HDL cholesterol level  | -0.08    | 0.61    | 0.10    | 0.87    | -0.23    | 0.34    |
| % change of triglyceride level     | 0.01     | 0.97    | 0.03    | 0.92    | 0.02     | 0.95    |
| % change of creatinin level        | 0.04     | 0.83    | 0.25    | 0.30    | -0.07    | 0.78    |
| % change of urate level            | -0.02    | 0.91    | 0.01    | 0.96    | -0.01    | 0.98    |
| % change of fasting glucose level   | 0.21     | 0.19    | 0.21    | 0.40    | 0.32     | 0.17    |
| % change of HbA1C level            | -0.07    | 0.67    | -0.10   | 0.98    | -0.10    | 0.68    |
| % change of total glutathione level| 0.10     | 0.57    | 0.16    | 0.52    | 0.02     | 0.93    |
| % change of HDL level              | 0.24     | 0.15    | 0.08    | 0.75    | 0.46     | 0.04    |
| % change of HDL level              | -0.11    | 0.51    | 0.03    | 0.92    | -0.49    | 0.03    |
| % change of GSSG level             | -0.01    | 0.96    | 0.01    | 0.96    | -0.04    | 0.89    |

FMD indicates flow-mediated vasodilation; HDL, high density lipoprotein; LDL, low density lipoprotein; GSH, reduced form of glutathione; and GSSG, oxidized form of glutathione.
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correlated with those in the intraerythrocyte ratios of GSH/GSSG and those in the intraerythrocyte GSH levels. The percentage change of GSH/GSSG was the independent predictor for that of FMD. Taken together, our results indicate that glucosamine administration increases FMD by modulating the intracellular oxidative stress.

We acknowledge that there are some limitations in this study. First, the objectives number was small in this study. However, glucosamine significantly increased FMD with improving intraerythrocyte redox status in spite of the small number. Second, we cannot exclude the possibility that glucosamine administration increased FMD by enhancing vascular smooth muscle relaxation. But a recent meta-analysis reveals that up to 72% of the FMD is mediated by nitric oxide in healthy subjects. Therefore, glucosamine administration attributes to the improvement of FMD, at least in part.

In conclusion, the present study provides the first demonstration in humans that glucosamine improves vascular endothelial function by modulating intracellular redox balance. Thus, glucosamine might have antiatherosclerotic properties, possibly through the improvement of endothelial function induced by the antioxidant capacity. Further randomized studies with a large number of participants for a longer duration should be performed to determine whether long-term administration of glucosamine would prevent cardiovascular events.

Disclosures

Conflicts of interest: There are no conflicts of interest to declare.

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