Original Article

Uric Acid Restores Endothelial Function in Patients With Type 1 Diabetes and Regular Smokers

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Endothelial dysfunction is a characteristic finding in both patients with type 1 diabetes and in regular smokers and is an important precursor to atherosclerosis. The urate molecule has antioxidant properties, which could influence endothelial function. The impact of acutely raising uric acid concentrations on endothelial function was studied in eight men with type 1 diabetes, eight healthy regular smokers, and eight age-matched healthy control subjects in a randomized, four-way, double-blind, placebo-controlled study. Subjects received 1,000 mg uric acid i.v. in vehicle, 1,000 mg vitamin C as a control antioxidant, vehicle alone, or 0.9% saline on separate occasions over 1 h. Forearm blood flow responses to intrabrachial acetylcholine and sodium nitroprusside were assessed using venous occlusion plethysmography. Responses to acetylcholine, but not sodium nitroprusside, were impaired in patients with diabetes ($P < 0.001$) and in smokers ($P < 0.005$) compared with control subjects. Administration of uric acid and vitamin C selectively improved acetylcholine responses in patients with type 1 diabetes ($P < 0.01$) and in regular smokers ($P < 0.05$). Uric acid administration improved endothelial function in the forearm vascular bed of patients with type 1 diabetes and smokers, suggesting that high uric acid concentrations in vivo might serve a protective role in these and other conditions associated with increased cardiovascular risk. *Diabetes* 55:3127–3132, 2006

Endothelial dysfunction is characterized by reduced bioavailability of nitric oxide (NO), which normally mediates local vasodilation, inhibits platelet aggregation, and reduces local vascular inflammation (1). Endothelial dysfunction is thought to be an important early step in the development of atherosclerosis (2) and is an independent predictor of increased cardiovascular risk in patients with hypertension and established atherosclerotic disease (3–5). Blood flow responses to endothelium-dependent vasodilators, including acetylcholine, are characteristically impaired in individuals with any one of a variety of major cardiovascular risk factors, including type 1 diabetes (6,7) and regular smoking (8,9). There is ongoing controversy as to whether the presence of type 1 diabetes itself is sufficient to cause development of endothelial dysfunction or whether other associated genetic and environmental factors are involved (10).

Increased free radical activity in the bloodstream or arterial intima appears to contribute to endothelial dysfunction (11,12), which can be ameliorated by administration of supplementary natural antioxidants, including ascorbic acid and tocopherol (13–15). In humans, uric acid is the most abundant aqueous antioxidant, accounting for up to 60% of serum free radical scavenging capacity (16) and is an important intracellular free radical scavenger during metabolic stress (17,18). Serum uric acid concentrations are reduced in patients with type 1 diabetes and in regular smokers (13,19), which could increase susceptibility to oxidative damage and account for the excessive free radical production characteristically found in both groups (21,21). In type 1 diabetes, low serum uric acid concentrations occur because of abnormally high uric acid renal clearance (22). At the time of first clinical presentation, children and young adults already have detectably low serum antioxidant defenses and increased plasma oxidizability (23).

Epidemiological studies have identified a strong association between raised serum uric acid concentrations and increased cardiovascular risk. There is considerable debate on the significance of this relationship, and it remains unclear whether uric acid is a causal, compensatory, or coincidental factor (24). There is a commonly held perception that raised serum uric acid concentrations play a causal role in the development of cardiovascular disease, although mechanisms that might be involved have not been established. This view has been reinforced by the observation that administration of allopurinol, to lower serum uric acid concentrations, improves endothelium-dependent vascular responses. However, inhibition of xanthine oxidase by allopurinol is likely to reduce the production of hydrogen peroxide and thereby ameliorate oxidative stress independent of effects on uric acid (25). Furthermore, allopurinol has antioxidant properties that are independent of its effects on xanthine oxidase activity (26). Therefore, cardiovascular effects of allopurinol require cautious interpretation and do not specifically address the question of a biological link between uric acid and mechanisms of endothelial dysfunction or atherosclerosis. The role of uric acid as an independent cardiovascular risk factor has not been proven.

In contrast, the possibility that uric acid confers protection against the development of atherosclerosis, in view of its antioxidant properties, has been recognized (27,28). The viability of administering uric acid in solution has only

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recently been established (29), and the potential effects of raising uric acid concentrations on endothelial function have not previously been investigated in groups exposed to oxidative stress. The purpose of the present study was to investigate the hypothesis that administration of uric acid or vitamin C, both powerful aqueous antioxidants, would improve endothelial dysfunction in patients with type 1 diabetes and in regular smokers, groups characteristically exposed to increased oxidative stress.

RESEARCH DESIGN AND METHODS

Patients with type 1 diabetes were recruited from the Diabetes Outpatient Clinic of the Western General Hospital, Edinburgh, and age-matched regular smokers and control subjects were recruited from a community database of volunteers held at the Clinical Research Centre of the University of Edinburgh. The study protocol was approved by the local research ethics committee. Informed consent was obtained from all subjects after the nature of the procedure was explained, and the study was performed according to the principles outlined in the Declaration of Helsinki. Subjects refrained from alcohol and caffeine intake for 12 h, and studies were performed in the morning, in a quiet room maintained at 24–26°C. Men aged 18–45 years were included. Exclusion criteria were elevated blood pressure (>160/100 mmHg); clinical history of joint, kidney, or cardiovascular disease; serum creatinine >110 μmol/l; serum uric acid >400 μmol/l; and those receiving any regular medication (except insulin) or any over-the-counter medication in the week before the studies.

Intra-arterial drug administration. The brachial artery of the nondominant arm was cannulated with a 27–standard wire gauge steel needle (Cooper's Needle Works, Birmingham, U.K.) using aseptic technique and 1% lidocaine local anesthetic (Phoenix Pharma, Gloucester, U.K.). Vasoactive drugs were administered via a 16-gauge epidural catheter (Portex, Kent, U.K.) connected to an IVAC P1000 syringe pump (Alaris Medical, Hampshire, U.K.). The infusion rate was kept constant at 1 ml/min throughout.

Measurement of forearm blood flow. Blood flow was measured in both forearms by venous occlusion plethysmography, as previously described (2,30). Measurements were taken during the last 3 min of each 6 min-infusion and the last five recordings averaged to determine flow in each arm. Blood flow responses during vasoactive drug administration were determined from absolute blood flow in the infused forarm, expressed as $ml \cdot 100\ ml^{-1} \cdot min^{-1}$ (2,31).

Measurement of biochemical variables. Blood was collected in gel tubes (Sarstedt, Leicester, U.K.), allowed to clot, and centrifuged at 1,000*g* for 10 min. Serum was separated and uric acid concentration determined by an automated colorimetric dry-slide assay (Vitros; Ortho-Clinical Diagnostics, Amersham, U.K.). Antioxidant capacity was measured using the Total Antioxidant Status assay (Randox Laboratories, Antrim, U.K.). This assay is based on the interaction between a chromogen (2,2′-amino-di-[3-ethylbenzthiazole sulfonate]) and ferrylmyoglobin, which is a free radical formed by the reaction of metmyoglobin and hydrogen peroxide (32). The reaction forms a blue-green chromophore, and absorbance was determined at 600 nm using a Cobas Fara (Roche Diagnostics, West Sussex, U.K.), calibrated using Trolox (a water-soluble tocopherol analog), and expressed as micromoles per liter of Trolox equivalent. The intra-assay precision was 5.9%.

Drugs and reagents. Uric acid and lithium carbonate (Ultrapure preparations; Sigma Chemical, Poole, U.K.) were reconstituted in sterile dextrose solution (Baxter Healthcare, Norfolk, U.K.) and filtered before use (0.22-μm Millex filter; Millipore, Molsheim, France). Other drugs used were ascorbic acid (Medeva Pharma, Leatherhead, U.K.), acetylcholine (CIBAVision-Ophthalmics, Southampton, U.K.), and sodium nitroprusside (David Bull Laboratories, Warwick, U.K.), which were reconstituted in sterile 0.9% saline solution.

Study protocol. Eight patients with type 1 diabetes, eight regular smokers, and eight age-matched healthy control subjects were recruited to a four-way, randomized, placebo-controlled, crossover study that allowed at least 1 week between study visits. A needle was placed in the brachial artery of the nondominant forearm, as described above. An 18–standard gauge cannula was inserted into a vein in the antecubital fossa of each arm, using aseptic technique and local anesthetic. The cannula in the nondominant forearm allowed infusion of 1,000 mg uric acid in 500 ml 4% dextrose/0.1% lithium carbonate vehicle, 500 ml vehicle alone, 1,000 mg vitamin C in 500 ml 0.9% saline, or 500 ml saline alone over 1 h. Venous blood (5 ml) was drawn from the cannula in the dominant forearm for measurement of serum uric acid concentration and antioxidant capacity. Subjects underwent intra-arterial administration of saline for 30 min, to establish baseline blood flow, followed by acetylcholine (7.5, 15, and 30 μg/min) and sodium nitroprusside (2, 4, and 8 μg/min). The order of acetylcholine and sodium nitroprusside administration was randomized between subjects but invariate between visits. Drug infusions were administered for 6 min at each dose and separated by 20 min saline to allow restoration of basal blood flow (2).

Data analysis and statistics. Subject numbers were determined to provide >80% power to detect a 10% difference in response to acetylcholine. Blood flow responses to vasoactive drugs were compared between patient groups and between infusions by two-way ANOVA. Baseline and postinfusion measures of serum uric acid concentration were compared using paired Student's *t* tests. Data are presented as means ± SE, and statistical significance is accepted at P values <0.05 in all cases.

TABLE 1
Baseline characteristics in regular smokers, patients with type 1 diabetes, and control subjects

	Regular smokers	Patients with type 1 diabetes	Control subjects
n (men)	8 (8)	8 (8)	8 (8)
Age (years)	30 ± 2	30 ± 2	30 ± 2
Systolic blood pressure (mmHg)	120 ± 2*	130 ± 2†	111 ± 2
Diastolic blood pressure (mmHg)	70 ± 1†	77 ± 2†	64 ± 2
Heart rate (bpm)	60 ± 3	70 ± 2†	60 ± 1
Height (cm)	176 ± 2	174 ± 2	177 ± 3
Weight (kg)	80 ± 5	74 ± 4	78 ± 2
BMI (kg/m^2)	26 ± 1	24 ± 1	25 ± 1
Creatinine (μmol/l)	79 ± 2	63 ± 5*	82 ± 3
Glucose (mmol/l)	4.7 ± 0.1	8.5 ± 1.1†	4.6 ± 0.1
Cholesterol (mmol/l)	4.2 ± 0.1*	4.2 ± 0.4*	3.6 ± 0.2
Uric acid (μmol/l)	315 ± 11*	245 ± 18†	336 ± 11

Data are means ± SE. *$P < 0.05$, †$P < 0.005$ compared with control subjects.

RESULTS

Baseline characteristics of the study population are shown in Table 1. Baseline serum uric acid concentrations were significantly lower in patients with type 1 diabetes (245 ± 18 μmol/l, $P < 0.005$) and in regular smokers (315 ± 11 μmol/l, $P < 0.05$) than in healthy control subjects (336 ± 11 μmol/l). Uric acid administration significantly increased serum concentrations by 270 ± 20, 286 ± 17, and 262 ± 18 μmol/l, respectively, in each group (Fig. 1). Serum antioxidant capacity was significantly lower in patients with type 1 diabetes and in regular smokers than in healthy control subjects and became higher in all three groups after administration of uric acid and vitamin C (Table 2).

After systemic saline administration, the forearm blood flow responses to intrabrachial acetylcholine and sodium nitroprusside were taken to represent baseline vascular function in each group. Vasodilator responses to acetylcholine were significantly reduced in patients with type 1 diabetes ($P < 0.001$) and in regular smokers ($P < 0.005$) compared with healthy subjects (Fig. 2). Uric acid and vitamin C significantly increased blood flow responses to acetylcholine, but not those to sodium nitroprusside, in patients with diabetes ($P < 0.01$ and $P < 0.01$) and in regular smokers ($P < 0.05$ and $P < 0.05$) (Fig. 3). Neither uric acid nor vitamin C had any effect on blood flow responses to acetylcholine or sodium nitroprusside in healthy subjects. Analyses of blood flow responses expressed as percentage change from baseline gave similar results to analyses of absolute blood flow responses.

Intrabrachial drug administration had no effect on blood

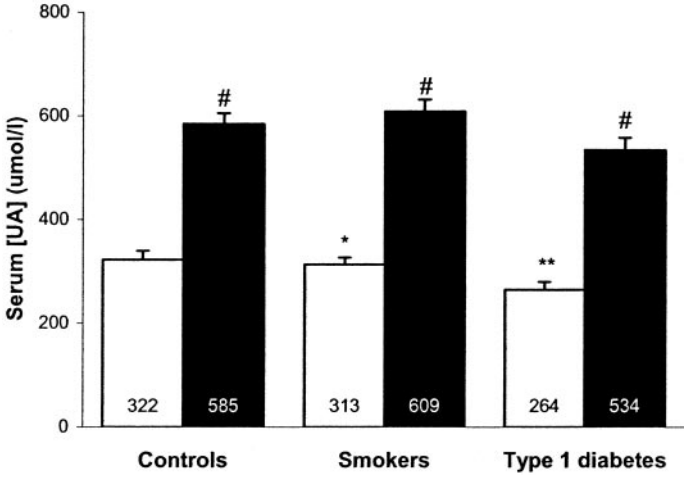

FIG. 1. Serum uric acid (UA) concentration before (□) and after (■) 1,000 mg uric acid i.v. in regular smokers, patients with type 1 diabetes, and control subjects. *P < 0.05, **P < 0.005 compared with control subjects; #P < 0.001 compared with baseline.

flow in the noninfused forearm and no effects on systemic hemodynamics. Systemic administration of vehicle had no effect on vascular responses in patients with type 1 diabetes, in regular smokers, or in healthy subjects. No adverse effects were encountered in any subject.

DISCUSSION

As expected, baseline uric acid concentrations and serum antioxidant capacity were low in patients with type 1 diabetes and in regular smokers (16,19,22). Consistent with previous studies (6,9), forearm blood flow responses to intrabrachial acetylcholine, but not sodium nitroprusside, were significantly impaired in patients with type 1 diabetes and in smokers. In the setting of endothelial dysfunction, vasodilator responses to acetylcholine are diminished, and vasoconstriction may be observed due to a direct effect on vascular smooth muscle. Our findings indicate impaired endothelium-dependent NO-mediated vasodilator responses, but intact endothelium-independent NO-mediated vasodilatation, in patients with type 1 diabetes and in regular smokers. As anticipated from earlier studies, vitamin C improved acetylcholine-mediated blood flow responses in patients with type 1 diabetes and in smokers but had no effect on responses to sodium nitroprusside. Importantly, for the first time we have shown that uric acid also improved endothelial function in these groups. Therefore, both uric acid and vitamin C allow restoration of dynamic endothelium-dependent NO bioavailability but do not alter underlying endothelium-independent vascular responses to NO stimulation.

TABLE 2
Serum antioxidant capacity (micromoles per liter trolox equivalent) before and after 1,000 mg uric acid i.v. and 1,000 mg vitamin C in regular smokers, patients with type 1 diabetes, and control subjects

	Regular smokers	Patients with type 1 diabetes	Control subjects
Vitamin C administration			
Pre	1,203 ± 46*	1,089 ± 46*	1,404 ± 57
Post	1,316 ± 50†	1,240 ± 48†	1,532 ± 66†
Change	120 ± 12‡	148 ± 17‡	131 ± 13‡
Uric acid administration			
Pre	1,224 ± 54	1,106 ± 48*	1,341 ± 60
Post	1,402 ± 66†	1,349 ± 52‡	1,578 ± 67‡
Change	203 ± 22‡	221 ± 19‡	235 ± 30‡

Data are means ± SE. *P < 0.05 compared with control subjects; †P < 0.05, ‡P < 0.01 compared with baseline.

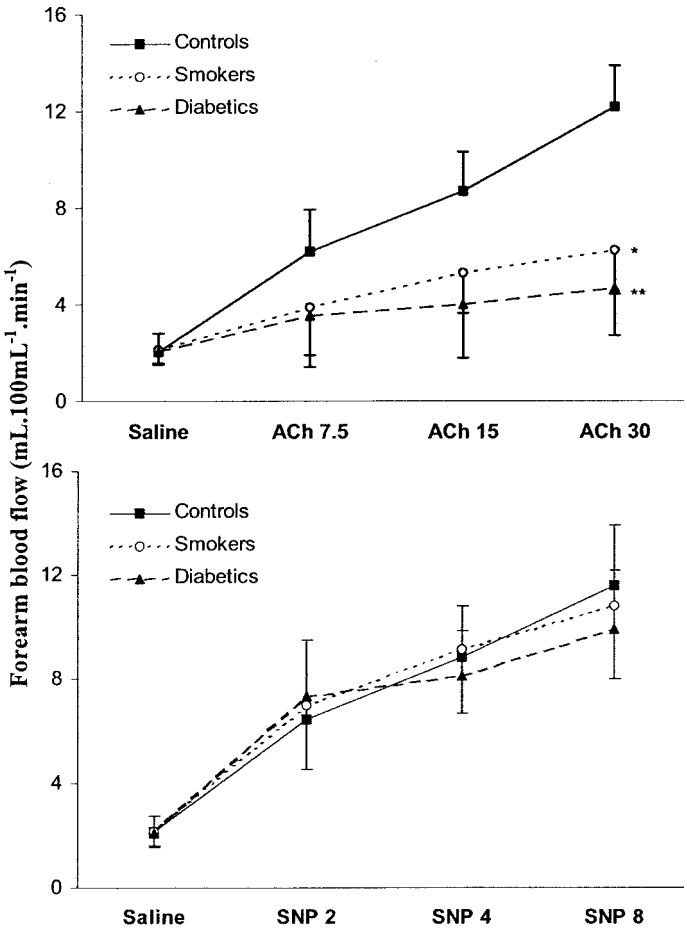

FIG. 2. Forearm blood flow responses to intrabrachial acetylcholine (ACh; 7.5, 15, and 30 μg/min) and sodium nitroprusside (SNP; 2, 4, and 8 μg/min) in patients with type 1 diabetes, in regular smokers, and in control subjects. *P < 0.005, **P < 0.001 compared with control subjects by ANOVA.

Increased antioxidant capacity alone is insufficient to explain our findings because this was similar in all three groups. In the setting of excess free radical activity, aqueous antioxidants may be consumed more rapidly and endothelium-derived NO is subjected to abnormally rapid degradation and reduced bioavailability (33). It is possible that uric acid and vitamin C, by virtue of enhanced antioxidant capacity, protect NO against oxidative degradation in the setting of oxidative stress. This pathophysiological mechanism might account for similarity between the effects of uric acid and vitamin C on acetylcholine responses in patients with type 1 diabetes and in smokers. This hypothesis is supported by a lack of influence of uric acid and vitamin C on responses in healthy control subjects. Other mechanisms may be important. Peroxynitrite is a potentially harmful oxidant that provides a source of free radicals and may contribute to vascular dysfunction in the setting of oxidative stress. Uric acid quenches per-

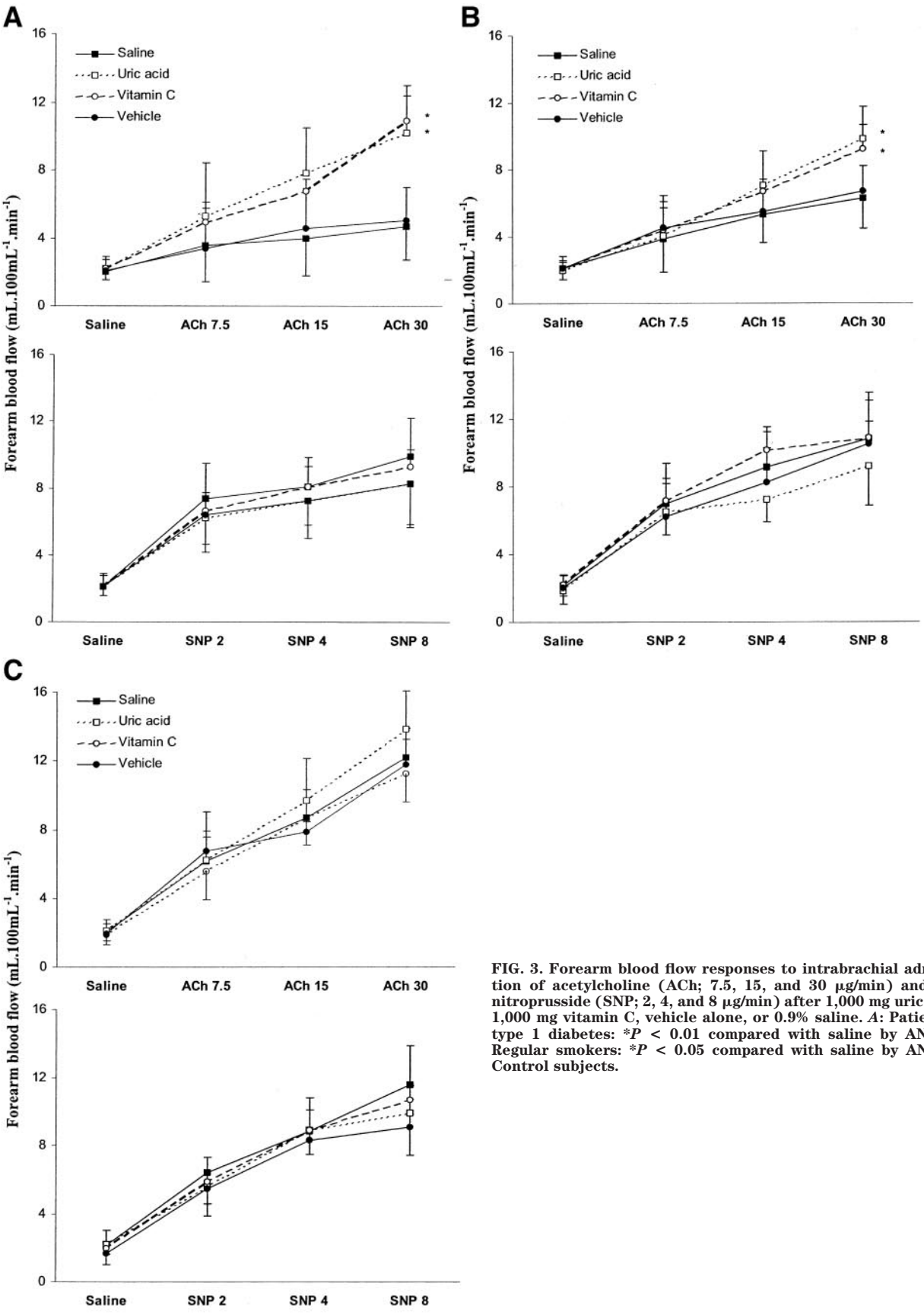

FIG. 3. Forearm blood flow responses to intrabrachial administration of acetylcholine (ACh; 7.5, 15, and 30 μg/min) and sodium nitroprusside (SNP; 2, 4, and 8 μg/min) after 1,000 mg uric acid i.v., 1,000 mg vitamin C, vehicle alone, or 0.9% saline. *A*: Patients with type 1 diabetes: **P* < 0.01 compared with saline by ANOVA. *B*: Regular smokers: **P* < 0.05 compared with saline by ANOVA. *C*: Control subjects.

oxynitrite, resulting in formation of a stable NO donor in vitro (34). The beneficial effects of uric acid on endothelial function could be explained if a similar mechanism operates in vivo. Vitamin C prevents superoxide-mediated NO degradation but only at concentrations significantly higher than physiological values (35). Instead, vitamin C increases

endothelial NO synthase activity by enhancing intracellular concentrations of tetrahydrobiopterin in a reduced state (36). Such a mechanism might also be relevant to the effects of increased uric acid concentrations.

The present findings lend further support to the view that reduced antioxidant defenses might contribute to endothelial dysfunction in patients with type 1 diabetes and in regular smokers. We have previously shown that systemic uric acid administration increases in vivo serum antioxidant defenses to at least the same extent as vitamin C (29) and that raising serum uric acid concentrations protects against oxidative damage in the setting of acute oxidative stress (37). The present study shows that administration of uric acid and vitamin C increased circulating antioxidant defenses and allowed restoration of endothelial function in conditions normally associated with chronic oxidative stress. This protective effect of uric acid appears to conflict with earlier reports of allopurinol administration in patients with diabetes, which lowered uric acid and restored endothelial function. However, allopurinol is capable of exerting a number of effects in addition to uric acid lowering, which might allow restoration of endothelial function such as lowered xanthine oxidase–dependent hydrogen peroxide liberation and direct antioxidant properties (25,26).

Endothelial dysfunction is a characteristic finding in conditions associated with increased cardiovascular risk, and the degree of functional impairment is predictive of increased risk of cardiovascular mortality (38). Previous studies (39,40) have found that vitamin C administration is capable of restoring endothelial function in certain high-risk groups characterized by oxidative stress, and ongoing research may establish whether this treatment can reduce cardiovascular risk in a clinical setting.

A limitation of this series of acute, mechanistic studies is that they do not address the effects of chronic exposure to elevated serum uric acid concentrations, which may be a more important determinant of future cardiovascular risk. A limitation of the study is that we do not know if the acute effects can be translated into expected long-term cardiovascular benefits. Short-term vascular benefits of antioxidant administration have not consistently translated into improved clinical outcomes after long-term administration. This might, at least in part, be due to important distinctions between various antioxidants, i.e., their ability to quench free radical activity, and their localization within the vascular wall. However, mechanistic study of chronic uric acid elevation may prove more of a challenge, and the current study design appears to be a valid approach because acute elevation of established major cardiovascular risk factors causes impaired endothelial function in healthy individuals, i.e., after ingestion of a meal rich in saturic acid fats (41) or after raising circulating homocysteine concentrations by oral methionine administration (42). Assessment of endothelial function as a risk marker appears valid because of correlations between short- and long-term effects of interventions that are known to be effective in reducing cardiovascular risk, i.e., blood pressure lowering and statin therapy (43,44). Therefore, restoration of endothelial function after acute uric acid administration is likely to be mechanistically important and indicates that uric acid does not operate in a similar manner to established cardiovascular risk factors.

Another limitation is that the study design did not incorporate measurement of vitamin C concentrations or circulating markers of oxidative stress and free radical activity. Further studies are required to more fully explore the mechanisms underlying the present findings. Both uric acid and vitamin C are known to be powerful aqueous antioxidants, and administration caused increased circulating antioxidant defenses in patients with diabetes, in smokers, and in healthy control subjects. It appears likely that the increase in serum antioxidant capacity by uric acid and vitamin C was responsible for restoration of endothelial function in patients with diabetes and in smokers because both groups have consistently been shown to be exposed to greater oxidant activity than healthy individuals and have higher circulating markers of vascular oxidative damage.

Hypercholesterolemia and raised blood pressure were present in the patient group and could be potential confounding factors. The patients with diabetes were selected from a real-life population and representative of patients seen in the everyday clinic situation. Endothelial dysfunction has been consistently reported in patients with type 1 diabetes and in regular smokers, irrespective of lipid profile. Although retrospective analyses did not identify any relationship between blood pressure and blood flow responses, vitamin C has previously been shown to reverse endothelial dysfunction in patients with hypertension (45). Further work is now required to explore whether uric acid restores endothelial function in patients with other risk factors for endothelial dysfunction and cardiovascular disease.

In summary, the relationship between elevated serum uric acid concentration and increased cardiovascular disease risk has been subject to considerable debate. The present study shows that in patients with type 1 diabetes and in regular smokers, groups usually characterized by excess free radical activity and endothelial dysfunction, have low baseline serum uric acid concentrations and that administration of uric acid raises circulating antioxidant defenses and allows restoration of endothelium-dependent vasodilation. Therefore, high serum uric acid concentrations might be protective in situations characterized by increased cardiovascular risk and oxidative stress. Further work is required to explore whether similar effects on endothelial function might be observed in the coronary circulation.

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REFERENCES

1. Rabelink TJ, Luscher TF: Endothelial nitric oxide synthase: host defense enzyme of the endothelium? *Arterioscler Thromb Vasc Biol* 26:267–271, 2006
2. Landmesser U, Hornig B, Drexler H: Endothelial function: a critical determinant in atherosclerosis? *Circulation* 109 (Suppl. 1):II27–II33, 2004
3. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G: Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 104:191–196, 2001
4. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, Vita JA: Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol* 41:1769–1775, 2003
5. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A: Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 101:948–954, 2000

6. Calver A, Collier J, Vallance P: Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J Clin Invest* 90:2548–2554, 1992
7. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA: Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 88:2510–2516, 1993
8. Barnoya J, Glantz SA: Cardiovascular effects of secondhand smoke: nearly as large as smoking. *Circulation* 111:2684–2698, 2005
9. Noma K, Goto C, Nishioka K, Hara K, Kimura M, Umemura T, Jitsuiki D, Nakagawa K, Oshima T, Chayama K, Yoshizumi M, Higashi Y: Smoking, endothelial function, and Rho-kinase in humans. *Arterioscler Thromb Vasc Biol* 25:2630–2635, 2005
10. Schalkwijk CG, Stehouwer CD: Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clin Sci* 109:143–159, 2005
11. Cai H, Harrison DG: Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 87:840–844, 2000
12. Madamanchi NR, Vendrov A, Runge MS: Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol* 25:29–38, 2005
13. Timimi FK, Ting HH, Haley EA, Roddy MA, Ganz P, Creager MA: Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 31:552–557, 1998
14. Motoyama T, Kawano H, Kugiyama K, Hirashima O, Ohgushi M, Yoshimura M, Ogawa H, Yasue H: Endothelium-dependent vasodilation in the brachial artery is impaired in smokers: effect of vitamin C. *Am J Physiol* 273: H1644–H1650, 1997
15. Heitzer T, Yla Herttuala S, Wild E, Luoma J, Drexler H: Effect of vitamin E on endothelial vasodilator function in patients with hypercholesterolemia, chronic smoking, or both. *J Am Coll Cardiol* 33:499–505, 1999
16. Maxwell SR, Thomason H, Sandler D, LeGuen C, Baxter MA, Thorpe GH, Jones AF, Barnett AH: Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *Eur J Clin Invest* 27:484–490, 1997
17. Mathru M, Dries DJ, Barnes L, Tonino P, Sukhani R, Rooney MW: Tourniquet-induced exsanguination in patients requiring lower limb surgery: an ischemia-reperfusion model of oxidant and antioxidant metabolism. *Anesthesiology* 84:14–22, 1996
18. Hellsten Y, Tullson PC, Richter EA, Bangsbo J: Oxidation of urate in human skeletal muscle during exercise. *Free Radic Biol Med* 22:169–174, 1997
19. Tsuchiya M, Asada A, Kasahara E, Sato EF, Shindo M, Inoue M: Smoking a single cigarette rapidly reduces combined concentrations of nitrate and nitrite and concentrations of antioxidants in plasma. *Circulation* 105: 1155–1157, 2002
20. Davi G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C: In vivo formation of 8-iso-prostaglandin F2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* 99:224–229, 1999
21. Reilly M, Delanty N, Lawson JA, FitzGerald GA: Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation* 94:19–25, 1996
22. Golembiewska E, Ciechanowski K, Safranow K, Kedzierska K, Kabat-Koperska J: Renal handling of uric acid in patients with type 1 diabetes in relation to glycemic control. *Arch Med Res* 36:32–35, 2005
23. Martin-Gallan P, Carrascosa A, Gussinye M, Dominguez C: Estimation of lipoperoxidative damage and antioxidant status in diabetic children: relationship with individual antioxidants. *Free Radic Res* 39:933–942, 2005
24. Waring WS, Webb DJ, Maxwell SRJ: Uric acid as a risk factor for cardiovascular disease. *QJM* 93:707–713, 2000
25. Desco MC, Asensi M, Marquez R, Martinez-Valls J, Vento M, Pallardo FV, Sastre J, Vina J: Xanthine oxidase is involved in free radical production in type 1 diabetes: protection by allopurinol. *Diabetes* 51:1118–1124, 2002
26. Ricardo SD, Bertram JF, Ryan GB: Podocyte architecture in puromycin aminoglycoside-treated rats administered tungsten or allopurinol. *Exp Nephrol* 3:270–279, 1995
27. Becker BF: Towards the physiological function of uric acid. *Free Radic Biol Med* 14:615–631, 1993
28. Nieto FJ, Iribarren C, Gross MD, Comstock GW, Cutler RG: Uric acid and serum antioxidant capacity: a reaction to atherosclerosis? *Atherosclerosis* 148:131–139, 2000
29. Waring WS, Webb DJ, Maxwell SR: Systemic uric acid administration increases serum antioxidant capacity in healthy volunteers. *J Cardiovasc Pharmacol* 38:365–371, 2001
30. Witherow FN, Dawson P, Ludlam CA, Webb DJ, Fox KA, Newby DE: Bradykinin receptor antagonism and endothelial tissue plasminogen activator release in humans. *Arterioscler Thromb Vasc Biol* 23:1667–1670, 2003
31. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, Lerman A, Mancia G, Oliver JJ, Pessina AC, Rizzoni D, Rossi GP, Salvetti A, Schiffrin EL, Taddei S, Webb DJ: Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 23:7–17, 2005
32. McLemore JL, Beeley P, Thorton K, Morrisroe K, Blackwell W, Dasgupta A: Rapid automated determination of lipid hydroperoxide concentrations and total antioxidant status of serum samples from patients infected with HIV. *Am J Clin Pathol* 109:268–273, 1998
33. Maytin M, Leopold J, Loscalzo J: Oxidant stress in the vasculature. *Curr Atheroscler Rep* 1:156–164, 1999
34. Skinner KA, White CR, Patel R, Tan S, Barnes S, Kirk M, Darley-Usmar V, Parks DA: Nitrosation of uric acid by peroxynitrite: formation of a vasoactive nitric oxide donor. *J Biol Chem* 273:24491–24497, 1998
35. Jackson TS, Xu A, Vita JA, Keaney JF Jr: Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. *Circ Res* 83:916–922, 1998
36. Huang A, Vita JA, Venema RC, Keaney JF Jr: Ascorbic acid enhances endothelial nitric-oxide synthase activity by increasing intracellular tetrahydrobiopterin. *J Biol Chem* 275:17399–17406, 2000
37. Waring WS, Convery A, Mishra V, Shenkin A, Webb DJ, Maxwell SR: Uric acid reduces exercise-induced oxidative stress in healthy adults. *Clin Sci* 105:425–430, 2003
38. Hollenberg SM, Klein LW, Parrillo JE, Scherer M, Burns D, Tamburro P, Bromet D, Satran A, Costanzo MR: Changes in coronary endothelial function predict progression of allograft vasculopathy after heart transplantation. *J Heart Lung Transplant* 23:265–271, 2004
39. Hamabe A, Takase B, Uehata A, Kurita A, Ohsuzu F, Tamai S: Impaired endothelium-dependent vasodilation in the brachial artery in variant angina pectoris and the effect of intravenous administration of vitamin C. *Am J Cardiol* 87:1154–1159, 2001
40. Teramoto K, Daimon M, Hasegawa R, Toyoda T, Sekine T, Kawata T, Yoshida K, Komuro I: Acute effect of oral vitamin C on coronary circulation in young healthy smokers. *Am Heart J* 148:300–305, 2004
41. Williams MJ, Sutherland WH, McCormick MP, de Jong SA, Walker RJ, Wilkins GT: Impaired endothelial function following a meal rich in used cooking fat. *J Am Coll Cardiol* 33:1050–1055, 1999
42. Chambers JC, McGregor A, Jean-Marie J, Kooner JS: Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: an effect reversible with vitamin C therapy. *Circulation* 99:1156–1160, 1999
43. Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, Kang MH, Ahn TH, Choi IS, Shin EK: Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. *Circulation* 110:3687–3692, 2004
44. Ceriello A, Assaloni R, Da Ros R, Maier A, Piconi L, Quagliaro L, Esposito K, Giugliano D: Effect of atorvastatin and irbesartan, alone and in combination, on postprandial endothelial dysfunction, oxidative stress, and inflammation in type 2 diabetic patients. *Circulation* 111:2518–2524, 2005
45. Ulker S, McKeown PP, Bayraktutan U: Vitamins reverse endothelial dysfunction through regulation of eNOS and NAD(P)H oxidase activities. *Hypertension* 41:534–539, 2003