

# The role of hyperuricemia on vascular endothelium dysfunction

HAITAO ZHEN<sup>1</sup> and FEN GUI<sup>2</sup>

<sup>1</sup>Department of Internal Medicine, School of Medicine, Hubei University of Science and Technology;

<sup>2</sup>Xianning Central Hospital, Xianning, Hubei 437100, P.R. China

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**Abstract.** Hyperuricemia appears to be associated with an increased risk for cardiovascular disease and associated mortality. Population epidemiological data support a causal link between hyperuricemia and cardiovascular disease. Endothelium injury could be one of the potential mechanisms in hyperuricemia-induced cardiovascular disease. However, the specific role of uric acid (UA) in the impairment of vascular relaxation and its signal transduction pathway has not been examined. The authors investigated the role of UA on vascular relaxation, nitric oxide (NO) production and expression of proinflammatory cytokines. Brachial flow-mediated dilation and nitroglycerine-mediated dilation were measured by B-mode ultrasound with 10 megahertz linear-array transducer from 21 patients with hyperuricemia and 16 control subjects. Human umbilical vein endothelial cells (ECs) were incubated with UA (5-15 mg/dl) with or without nuclear factor (NF)- $\kappa$ B inhibitor II. Hyperuricemia inhibited brachial flow-mediated dilation. While UA significantly inhibited NO expression with time course- and dose- dependent manner in the cultured ECs, 10 mg/dl UA also increased expression of inflammation cytokine interleukin (IL)-6, IL-8 and tumor necrosis factor- $\alpha$  in vitro. These abnormalities were associated with UA-induced activation of transcription factor NF- $\kappa$ B. Furthermore, NF- $\kappa$ B inhibitor II prevented UA-induced reduction of NO and increased inflammation cytokines. These data suggested hyperuricemia-induced endothelium injury and vascular dysfunction by a reduction of NO and expression of inflammatory cytokines through the NF- $\kappa$ B pathway.

## Introduction

Hyperuricemia has been linked to cardiovascular disease (CVD) recent since it is regarded as an independent risk factors in the contribution of CVD including vascular disease, hypertension, metabolic syndrome and renal disease (1). Generally, uric acid (UA) levels >7 mg/dl in males and >6 mg/dl in females

are considered as hyperuricemia in a clinical aspect (2). Risk factors for hyperuricemia include alcohol consumption, intake of high fat diet and refined carbohydrate, and the medicines of diuretics and angiotensin converting enzyme antagonists (1,2). Population epidemiological studies support the notion that there is a causal link between hyperuricemia and CVD. For example, Rotterdam (3) and a NHANES I study (4) found that there is an association between high levels of UA and myocardial infarctions and cardiovascular death even after adjustment for related factors such as age, dyslipidemia and obesity. Multiple center studies also suggested that hyperuricemia prompts hypertension and coronary heart disease, as well as vascular diseases such as cerebrovascular disease, preeclampsia, vascular dementia and kidney disease (5,6). Some of the potential pathophysiological mechanisms have been investigated to explain the association between UA and vascular injury. One study suggested that high dose UA significantly increased angiotensin II and oxidative stress in cultured endothelial cells (ECs) and this further prompted EC senescence and apoptosis (7). To this point, increase inflammation and reduction of nitric oxide (NO) are the characteristics of endothelium dysfunction (8). However, few studies have been conducted to investigate the role of UA on vascular relaxation, NO production, inflammation and its signaling pathway on ECs. In the current study, the authors hypothesized that UA-induced impairment of vascular relaxation is associated with a reduction of NO and expression of inflammatory cytokines through the nuclear factor (NF)- $\kappa$ B pathway.

## Materials and methods

**Study population.** The study investigated 21 patients with hyperuricemia and 16 control subjects at The First Clinical Hospital of Hubei University of Science and Technology and Xianning Central Hospital in China, between February 2014 and March 2016. Blood samples were collected at 8:00 a.m. while subjects were regularly fasting. The study was approved by the institutional review board on human investigation at The First Clinical Hospital of Hubei University of Science and Technology and Xianning Central Hospital (Xianning, China). Informed consent was obtained from all individuals in the study.

**Brachial flow-mediated dilation (FMD) and nitroglycerine-mediated dilation (NMD).** The diameter of the right

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*Correspondence to:* Dr Haitao Zhen, Department of Internal Medicine, School of Medicine, Hubei University of Science and Technology, 88 Xianning Road, Xianning, Hubei 437100, P.R. China  
E-mail: zht2020@yeah.net

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Table I. Clinical characteristic of the study population.

Variable	Control subjects (n=16)	Hyperuricemia patients (n=21)
Age (years)	65.2±7.28	68.5±6.6
Males in population (%)	50%	47.6%
Body mass index (kg/m <sup>2</sup> )	25.1±2.2	25.6±1.7
Systolic blood pressure (mmHg)	140±6	142±10
Diastolic blood pressure (mmHg)	85±4	86±4
Creatinine (mg/dl)	0.78±0.11	0.77±0.14
Plasma uric acid (mg/dl)	4.51±1.09	16.78±4.69 <sup>†</sup>
Plasma glucose (mg/dl)	98.65±11.35	100.47±9.69
Triglyceride (mg/dl)	99.46±37.33	128.44±51.22
Low-density lipoprotein	121.25±27.98	145.66±43.21
High-density lipoprotein (mg/dl)	53.15±6.23	62.22±8.09

Values are mean ± standard error of the mean. <sup>†</sup>P<0.05 vs. control subjects.

brachial arteries in all subjects were measured on B-mode ultrasound with 10 megahertz linear-array transducer (Beijing ADSS Development Co., Ltd., Beijing, China) by one investigator at rest and sublingual spray nitroglycerin (350 µg), respectively. A pressure cuff was placed and automatically inflated on the forearm in order to initial forearm ischemia, the pressure cuff was then released after 5 min so that blood flow in the forearm could be increased. The maximum diameters were recorded after cuff release (9,10). The percentage increase in FMD and NMD were calculated as the increased diameters in brachial arteries compared with the corresponding baseline at rest (9,10).

**EC culture.** Primary human umbilical vein ECs were purchased from (cat. no. 211-500, Shanghai Biotechnology Co., Ltd, Shanghai, China) and cultured at cell incubator with 37°C in 5% CO<sub>2</sub> atmosphere. The sub-cultured strains of ECs were used in experiments between passages 3-5. Prior to the experiments, ECs were serum-starved in Dulbecco's modified Eagle's medium for 24 h. UA and NF-κB inhibitor II were purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany).

**Measurement of NO production.** Nitrite, a stable metabolite of NO, is converted from nitrate by nitrate reductase and is an indicator of released NO in the media of cultured ECs (11). The NO productions in cultured ECs were measured using a nitrate/nitrite assay kit (cat. no. 23479; Sigma-Aldrich; Merck KGaA) following the protocol instructions (12).

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** The total RNA was isolated from the cultured human umbilical vein ECs using RNAzol (Sigma-Aldrich; Merck KGaA). After the first strand, cDNA was synthesized by using Improm II reverse transcription kit (Sigma-Aldrich; Merck KGaA), qPCR was done using 8 µl cDNA, 10 µl SYBR-Green PCR Master Mix and forward and reverse primers (10 pM/µl) using a qPCR system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The following primer sequences were used: interleukin (IL)-6 forward, 5'-ACAGTG TGAATCATATATGA-3' and reverse, 5'-GCGTCGTTATA

TGTTGAACGC-3'; IL-8 forward, 5'-CGTGGTTATGCTAC AAATGT-3' and reverse, 5'-ACGGGCAACCCGTGGAGAG C-3'; Tumor necrosis factor (TNF)-α forward, 5'-GCAGCCG TTAACGTAAACCT-3' and reverse, 5'-TGTGTTAGGGCCT GTGGCAC-3'; 18S forward, 5'-GACGGTTGCCATCAAGTT CGA-3' and reverse, 5'-TGCTGAGCATCGCTGGGAA-3'. The PCR cycling conditions were 2 min at 95°C for initial denaturation, 40 cycles of 45 sec at 95°C, 30 sec at 58°C and 45 sec at 72°C. The data were normalized to 18S ribosomal RNA that is a housekeeping gene.

**Western blot analysis.** Human umbilical vein ECs were collected and lysed with RIPA protein lysis buffer (cat. no. 20-188, Sigma-Aldrich; Merck KGaA) and the protein concentration was investigated by Bio Rad protein assay (Bio Rad Laboratories, Inc.). Proteins were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes (Sigma-Aldrich; Merck KGaA). Membranes were incubated with antibody targeting NF-κB (cat. no. SAB4502609; 1:1,000 dilution; Sigma-Aldrich; Merck KGaA) and horseradish peroxidase (HRP) conjugated secondary antibody (cat. no. A9542; 1:5000 dilution; Sigma-Aldrich; Merck KGaA) at room temperature for 1 h. The HRP activity was examined by an enhanced chemiluminescence kit (Bio Rad Laboratories, Inc.). As a loading control, nuclear protein lamin B was probed in all the protein expression.

**Activation of transcription factor NF-κB.** Human umbilical vein ECs were transfected with 5 µg of pNF-κB-Luc (Clontech Laboratories, Inc., Mountainview, CA, USA). Transfection of human umbilical vein ECs was performed using the nucleofection device and solutions to deliver electrical stimuli to the ECs (Lonza Group, Basel, Switzerland) (13). Following 24 h transfection, 10 mg/dl UA was used to treat the cells for 3 h and luciferase activity was examined by using a Dual Luciferase Reporter Assay System and a luminometer (Promega Corporation, Madison, WI, USA) as per the manufacturer's protocol (14).

**Statistical analysis.** Results are calculated as the mean ± standard error of the mean. Data were analyzed by using SAS

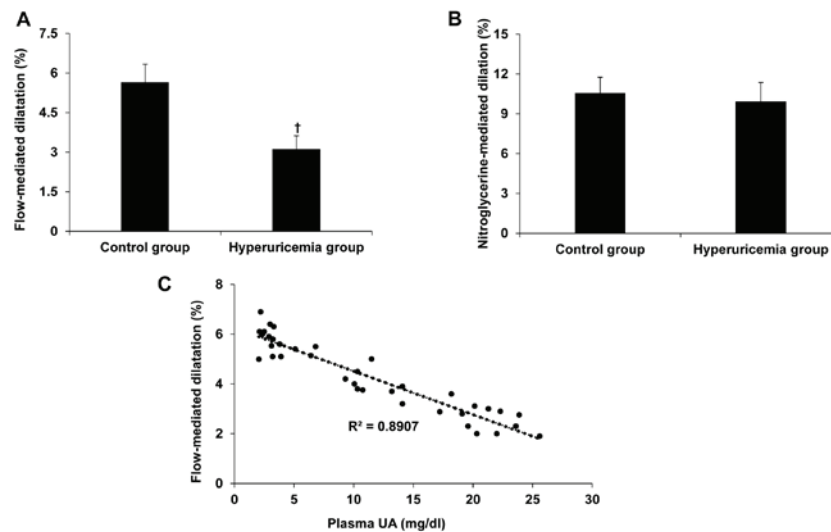


Figure 1. Effect of UA on human vascular endothelium function. Hyperuricemia reduced (A) brachial FMD, but not (B) nitroglycerine-mediated dilation. (C) There was a positive correlation between plasma UA and flow-mediated dilation in hyperuricemia patients ( $R^2=0.8907$ ).  $n=21$  in hyperuricemia patients;  $n=16$  in control subjects. Data are presented as mean  $\pm$  standard error of the mean. † $P<0.05$  vs. control group. UA, uric acid.

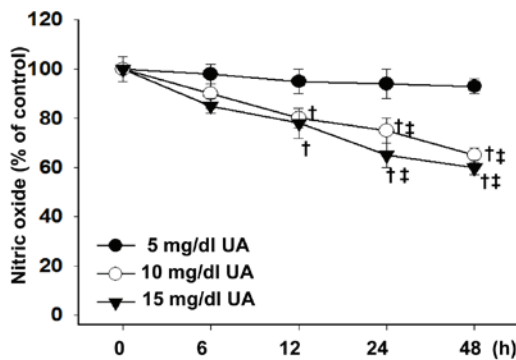


Figure 2. High dose UA represses NO production. Human umbilical vein endothelial cells were treated with UA in the dose of 5, 10 and 15 mg/dl. The nitric oxide production was measured at 6, 12, 24 and 48 h ( $n=5$  per group). † $P<0.05$  vs 0 h group; ‡ $P<0.05$  vs. 5 mg/dl UA group. Data are presented as mean  $\pm$  standard error of the mean. UA, uric acid.

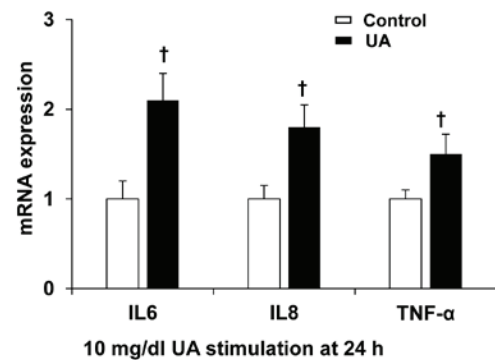


Figure 3. High dose UA increases inflammation cytokine expression. Human umbilical vein endothelial cells were treated by 10 mg/dl for 24 h. Expression of the inflammatory transcripts in IL-6, IL-8 and TNF- $\alpha$  were examined by reverse transcription-quantitative polymerase chain reaction ( $n=5$  per group). † $P<0.05$  vs. control group. Data are presented as mean  $\pm$  standard error of the mean. UA, uric acid; IL, interleukin; TNF, tumor necrosis factor.

software (version, 9.4; SAS Institute Inc., Cary, NC, USA). Differences in data were determined using one-way analysis of variance or paired Student's  $t$  tests and  $P<0.05$  was considered to indicate a statistically significant difference.

## Results

**Clinical characteristics of the subjects.** There was no significant difference in age, sex, body mass index, blood pressure, plasma levels of creatinine, glucose, triglyceride and low/high-density lipoprotein between health control subjects and hyperuricemia patients (Table I). However, the plasma UA level is significantly higher in hyperuricemia patients than in control group (Table I).

**Hyperuricemia induces arterial endothelium dysfunction.** FMD and NMD are applied to access the endothelium-dependent and -independent arterial dilation, respectively and thus arterial endothelial function can be evaluated using FMD and NMD (9,10). In current study, the authors explored

arterial endothelial function using FMD in hyperuricemia patients and control subjects to disclose whether an increase in serum UA is associated with arterial endothelial dysfunction. Hyperuricemia inhibited brachial FMD but not NMD (Figs. 1A and B). Furthermore, there was a positive correlation between hyperuricemia and impairment of FMD ( $R^2=0.8907$ ; Fig. 1C), suggesting that hyperuricemia impairs endothelium-dependent arterial dilation.

**UA reduced NO production in cultured human umbilical vein ECs.** The authors further investigated the effect of high dose of UA on NO production in cultured human umbilical vein ECs with various concentrations of UA treatments and different time points. As presented in Fig. 2, 5 mg/dl UA (normal dose) did not alter NO production. However, 10 and 15 mg/dl UA (high dose) significantly inhibited NO expression from 12-48 h. Compared to the 5 mg/dl group, both 10 and 15 mg/dl UA also significantly inhibited NO expression at 24 and 48 h (Fig. 2).

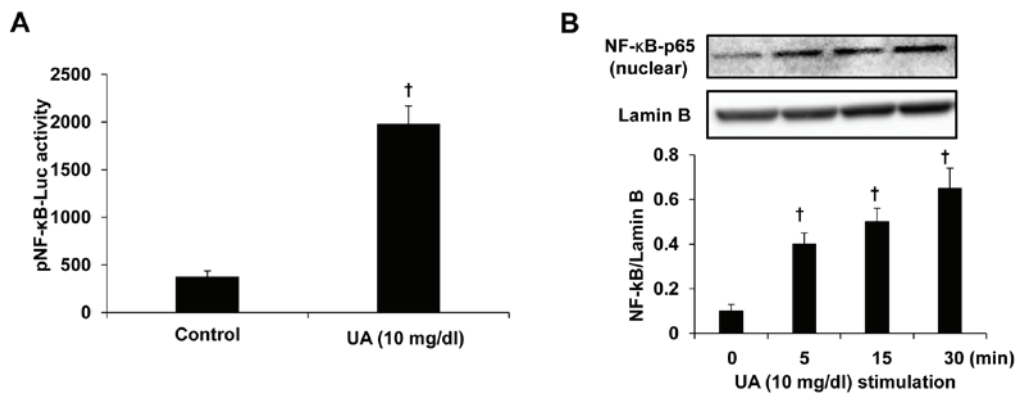


Figure 4. High dose UA increases activation of transcription factor NF-κB in cultured human umbilical vein ECs. (A) UA (10 mg/dl) induced activation of transcription factor NF-κB luciferase activity. (B) UA (10 mg/dl) induced NF-κB nuclear translocation at 5, 15 and 30 min in ECs (n=5 per group). <sup>†</sup>P<0.01 vs control group. Data are presented as mean ± standard error of the mean. UA, uric acid; NF-κB, nuclear factor-κB; ECs, endothelial cells.

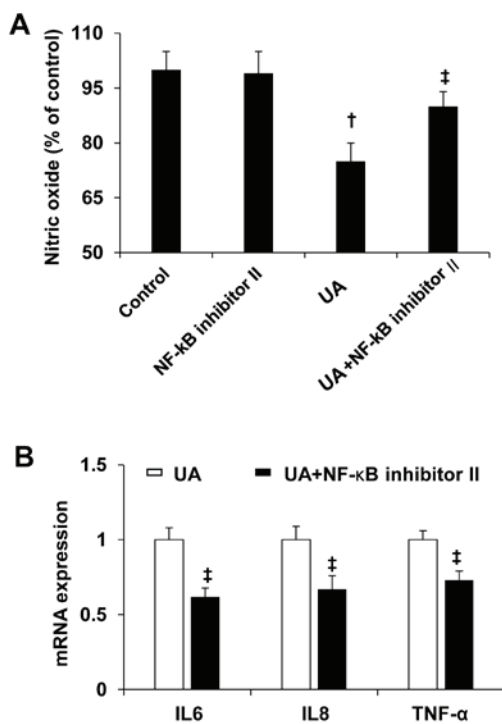


Figure 5. NF-κB antagonist prevents UA-induced reduction of NO and increase in inflammation cytokines. Human umbilical vein endothelial cells were treated by 10 mg/dl UA with or without NF-κB inhibitor II (20 nmol/l) for 24 h. (A) NF-κB inhibitor II prevented UA-induced reduction of NO. (B) NF-κB inhibitor II prevented UA-induced increases in IL-6, IL-8 and TNF-α (n=5 per group). <sup>†</sup>P<0.05 vs. control group; <sup>‡</sup>P<0.05 vs UA group. Data are presented as mean ± standard error of the mean. NF-κB, nuclear factor-κB; UA, uric acid; NO, nitric oxide; IL, interleukin; TNF, tumor necrosis factor.

**UA increased inflammation cytokine expression in cultured human umbilical vein ECs.** To further confirm the roles of UA on the expression of inflammation cytokines, human umbilical vein ECs were incubated with 10 mg/dl UA for 24 h. UA significantly induced IL-6, IL-8, TNF-α expression (Fig. 3), suggesting that UA induces endothelium inflammation.

**UA increased activation of transcription factor NF-κB in cultured human umbilical vein ECs.** In order to investigate the role of UA on the activation of transcription factor

NF-κB, human umbilical vein ECs were co-transfected with pNF-κB-Luc and 10 mg/dl UA. UA significantly induced activation of transcription factor NF-κB luciferase activity (Fig. 4A). Meanwhile, UA also induced NF-κB nuclear translocation at 5, 15 and 30 min in ECs (Fig. 4B), suggesting that UA increased transcription factor NF-κB activity.

**NF-κB antagonist prevented UA-induced reduction of NO and increased inflammation cytokines.** To determine the role of NF-κB in UA-induced EC dysfunction, human umbilical vein ECs were treated by 10 mg/dl UA with or without NF-κB inhibitor II (20 nmol/l) for 24 h. NF-κB inhibitor II prevented UA-induced reduction of NO (Fig. 5A). In addition, NF-κB inhibitor II prevented UA-induced increases in expression of IL6, IL8, and TNF-α in human umbilical vein ECs.

## Discussion

Hyperuricemia is an independent risk factor in the pathogenesis of vascular disease, hypertension and metabolic syndrome (15). The current study further found that hyperuricemia inhibited endothelium-dependent arterial dilation and brachial FMD. While UA significantly inhibited NO expression with time course and dose dependent manner in the cultured HUVECs, high dose UA also increased expression of inflammation cytokine IL-6, IL-8 and TNF-α in vitro. These abnormalities were associated with UA-induced activation of transcription factor NF-κB. However, NF-κB inhibitor II, an antagonist of NF-κB, prevented UA-induced reduction of NO and increased inflammation cytokines. Thereby, these data implicated that activated NF-κB plays an important role in UA-induced endothelial inflammation and injury.

Hyperuricemia is a common clinical finding in patients with obesity, dyslipidemia, hypertension and other CVDs (16). Epidemiological studies have confirmed the association of hyperuricemia and CVD. Recent data from cross-sectional, interventional, and cohort studies have found that hyperuricemia is an independent risk factor for hypertension (17,18). To this point, endothelium injury and dysfunction is regarded as the first step to develop the hypertension, coronary heart disease and other CVDs (19). Thus, high dose UA has ability to initial the endothelium injury to development of CVD



by increases in platelet aggregation and pro-inflammatory activity and inhibition of EC NO production (20). In the present study, FMD is used for assessing the vascular NO response and endothelial function in patients with hyperuricemia that have similar clinical characteristics with control individuals in age, body mass index, blood pressure, plasma levels of creatinine, glucose, triglyceride and low/high-density lipoprotein. Interestingly, hyperuricemia inhibited brachial FMD but not NMD and there is a positive correlation between hyperuricemia and impairment of FMD. Therefore, hyperuricemia induces the impairment of endothelium-dependent arterial dilation.

Inhibition of UA through targeting of xanthine oxidase has been found to decrease vascular dysfunction and improve cardiovascular function recent (21). The current study further found that high dose UA directly impaired NO generation in cultured human umbilical vein ECs, suggesting that hyperuricemia impairs vascular function by a mechanism of inhibition of NO production. In this regard, high dose may increase the activity of nicotinamide adenine dinucleotide phosphate oxidase that prompts oxidative stress and decreases the bioavailability of NO (22). Further, hyperuricemia impairs vascular function by increasing inflammation cytokine expression in IL-6, IL-8 and TNF- $\alpha$ . Therefore, UA-induced inflammatory immune response is as an important mechanism in UA's vascular effects.

The nuclear factor transcription factor NF- $\kappa$ B pathway is regarded as a proinflammatory signaling pathway since activated NF- $\kappa$ B mediates the expression of proinflammatory cytokines, chemokines, and adhesion molecules (23,24). In the current study, UA increased NF- $\kappa$ B activity and its nuclear protein translocation that further prompted the maladaptive immune response. Inhibition of NF- $\kappa$ B reduced the UA-induced inflammation cytokine expression of IL-6, IL-8 and TNF- $\alpha$ . Interestingly, inhibition of NF- $\kappa$ B also inhibited UA-induced reduction of NO. Indeed, NF- $\kappa$ B is a negative regulator of endothelial NO synthase expression via upregulation of microRNA155 under inflammatory conditions (25). Sustained activation of NF- $\kappa$ B decreases NO bioavailability in response to shear and endothelial inflammation (26). Therefore, activated NF- $\kappa$ B mediates UA-induced reduction of NO and increased inflammation response.

In conclusion, these data reveal that the NF- $\kappa$ B pathway mediates hyperuricemia-induced endothelium impairment and vascular dysfunction by a reduction of NO and expression of inflammatory cytokines. The link between activated NF- $\kappa$ B, reduction of NO, maladaptive immune responses and vascular dysfunction offers possibilities for identification of UA-induced vascular dysfunction and allows for development of novel therapeutic strategies for protection of cardiovascular disease.

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## References

- Mallat SG, Al Kattar S, Tanios BY and Jurjus A: Hyperuricemia, Hypertension, and Chronic Kidney Disease: An Emerging Association. *Curr Hypertens Rep* 18: 74, 2016.
- Essex MN, Hopps M, Bienen EJ, Udall M, Mardekian J and Makinson GT: Evaluation of the relationship between serum uric acid levels and cardiovascular events in patients with gout: A retrospective analysis using electronic medical record data. *J Clin Rheumatol* 23: 160-166, 2017.
- Bos MJ, Koudstaal PJ, Hofman A, Witteman JC and Breteler MM: Uric acid is a risk factor for myocardial infarction and stroke: The Rotterdam study. *Stroke* 37: 1503-1507, 2006.
- Fang J and Alderman MH: Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. *National Health and Nutrition Examination Survey. JAMA* 283: 2404-2410, 2000.
- Ford ES, Li C, Cook S and Choi HK: Serum concentrations of uric acid and the metabolic syndrome among US children and adolescents. *Circulation* 115: 2526-2532, 2007.
- Lehto S, Niskanen L, Rönnemaa T and Laakso M: Serum uric acid is a strong predictor of stroke in patients with non-insulin-dependent diabetes mellitus. *Stroke* 29: 635-639, 1998.
- Li P, Zhang L, Zhang M, Zhou C and Lin N: Uric acid enhances PKC-dependent eNOS phosphorylation and mediates cellular ER stress: A mechanism for uric acid-induced endothelial dysfunction. *Int J Mol Med* 37: 989-997, 2016.
- Cai W, Duan XM, Liu Y, Yu J, Tang YL, Liu ZL, Jiang S, Zhang CP, Liu JY and Xu JX: Uric Acid Induces Endothelial Dysfunction by Activating the HMGB1/RAGE Signaling Pathway. *BioMed Res Int* 2017: 4391920, 2017.
- Wong CK, Chen Y, Ho LM, Zhen Z, Siu CW, Tse HF and Yiu KH: The effects of hyperuricaemia on flow-mediated and nitroglycerin-mediated dilatation in high-risk patients. *Nutr Metab Cardiovasc Dis* 24: 1012-1019, 2014.
- Naka KK, Papathanassiou K, Bechlioulis A, Kazakos N, Pappas K, Tigas S, Makriyannis D, Tsatsoulis A and Michalis LK: Determinants of vascular function in patients with type 2 diabetes. *Cardiovasc Diabetol* 11: 127, 2012.
- Calvert JW: Cardioprotective effects of nitrite during exercise. *Cardiovasc Res* 89: 499-506, 2011.
- Wang R, Zhang Y, Xu L, Lin Y, Yang X, Bai L, Chen Y, Zhao S, Fan J, Cheng X, *et al*: Protein Inhibitor of Activated STAT3 Suppresses Oxidized LDL-induced Cell Responses during Atherosclerosis in Apolipoprotein E-deficient Mice. *Sci Rep* 6: 36790, 2016.
- Slanina H, Schmutzler M, Christodoulides M, Kim KS and Schubert-Unkmeir A: Effective plasmid DNA and small interfering RNA delivery to diseased human brain microvascular endothelial cells. *J Mol Microbiol Biotechnol* 22: 245-257, 2012.
- Zhang D, Atlasi SS, Patel KK, Zhuang Z and Heaney AP: False responses of Renilla luciferase reporter control to nuclear receptor TR4. *Mol Cell Biochem* 430: 139-147, 2017.
- Ding XH, Wang X, Cao R, Yang X, Xiao W, Zhang Y, Bai Y, Wu H and Ye P: A higher baseline plasma uric acid level is an independent predictor of arterial stiffness: A community-based prospective study. *Medicine (Baltimore)* 96: e5957, 2017.
- Volterrani M, Iellamo F, Sposato B and Romeo F: Uric acid lowering therapy in cardiovascular diseases. *Int J Cardiol* 213: 20-22, 2016.
- Huang J, Sun Y, Niu K, Wan Z, Yao W, Gao Y, Zhang W, Li Y, Zhao H and Wu X: Does elevated serum uric acid level predict the hypertension incidence? A Chinese prospective cohort study. *Clin Exp Hypertens* 37: 498-504, 2015.
- Yang TY, Fang CY, Chen JS, Po HL, Chou LP, Chiang CY and Ueng KC: Association of Serum Uric Acid with Cardiovascular Disease in Taiwanese Patients with Primary Hypertension. *Acta Cardiol Sin* 31: 42-51, 2015.
- McCarthy CG, Gouloupoulou S, Wenceslau CF, Spitler K, Matsumoto T and Webb RC: Toll-like receptors and damage-associated molecular patterns: Novel links between inflammation and hypertension. *Am J Physiol Heart Circ Physiol* 306: H184-H196, 2014.
- Verdoia M, Barbieri L, Schaffer A, Cassetti E, Nardin M, Bellomo G, Aimaretti G, Marino P, Sinigaglia F and De Luca G; Novara Atherosclerosis Study Group (NAS): Impact of diabetes on uric acid and its relationship with the extent of coronary artery disease and platelet aggregation: A single-centre cohort study. *Metabolism* 63: 640-646, 2014.
- Borghesi C and Desideri G: Urate-lowering drugs and prevention of cardiovascular disease: The emerging role of xanthine oxidase inhibition. *Hypertension* 67: 496-498, 2016.
- Kaneko C, Ogura J, Sasaki S, Okamoto K, Kobayashi M, Kuwayama K, Narumi K and Iseki K: Fructose suppresses uric acid excretion to the intestinal lumen as a result of the induction of oxidative stress by NADPH oxidase activation. *Biochim Biophys Acta* 1861: 559-566, 2017.

23. Spiga R, Marini MA, Mancuso E, Di Fatta C, Fuoco A, Perticone F, Andreozzi F, Mannino GC and Sesti G: Uric acid is associated with inflammatory biomarkers and induces inflammation via activating the NF- $\kappa$ B Signaling Pathway in HepG2 Cells. *Arterioscler Thromb Vasc Biol* 37: 1241-1249, 2017.
24. Luo C, Lian X, Hong L, Zou J, Li Z, Zhu Y, Huang T, Zhang Y, Hu Y, Yuan H, *et al*: High Uric Acid Activates the ROS-AMPK Pathway, Impairs CD68 Expression and Inhibits OxLDL-Induced Foam-Cell Formation in a Human Monocytic Cell Line, THP-1. *Cell Physiol Biochem* 40: 538-548, 2016.
25. Lee KS, Kim J, Kwak SN, Lee KS, Lee DK, Ha KS, Won MH, Jeoung D, Lee H, Kwon YG, *et al*: Functional role of NF- $\kappa$ B in expression of human endothelial nitric oxide synthase. *Biochem Biophys Res Commun* 448: 101-107, 2014.
26. Grumbach IM, Chen W, Mertens SA and Harrison DG: A negative feedback mechanism involving nitric oxide and nuclear factor kappa-B modulates endothelial nitric oxide synthase transcription. *J Mol Cell Cardiol* 39: 595-603, 2005.