# Allopurinol alleviates hypertension and proteinuria in high fructose, high salt and high fat induced model of metabolic syndrome

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**Metabolic syndrome (MetS) is a global epidemic associated with great socioeconomic and public health impact. Prevalence of the MetS has been consistently associated with cardiorenal mortality. The objective of this study was to investigate the effect of allopurinol treatment on various components of an established MetS in rats. In a first group, MetS was induced in male Wistar rats by the addition of 10% fructose to drinking water and placing the rats on high-fat and high-salt diet for 12 weeks (M). In the second group, MetS was induced for 12 weeks plus allopurinol administration (20 mg/kg/d) orally for 4 weeks starting at week 9 (MA). The third group was control (C) group that received a normal diet. The M group had higher blood pressure (BP) (85.5 ± 3.17 vs 66.1 ± 3.3 mm Hg) and proteinuria (1.8 ± 0.3 vs 0.59 ± 0.13 g/d) compared with the C group. Allopurinol reversed the BP and proteinuria in MA rats to the control level. Allopurinol administration suppressed the low-grade inflammation associated with MetS and reversed the increases in kidney transforming growth factor beta and urine 8-isoprostane acid observed in the MA group to control levels. In addition, allopurinol reduced angiotensin II and angiotensin receptor type 1 levels in the kidney of MA rats compared with the M group. The administration of allopurinol for short term in an established MetS model reduced features of the MetS especially hypertension and proteinuria. Addition of allopurinol to the therapy of MetS may provide superior means to alleviate hypertension and proteinuria associated with MetS. (Translational Research 2015;165:621–630)**

**Abbreviations:** ACE = Angiotensin-Converting Enzyme; AngII = angiotensin II; $AT_1$ = angiotensin receptor type 1; ATP = adenosine triphosphate; BSA = bovine serum albumin; MetS = metabolic syndrome; NADPH = reduced nicotinamide adenine dinucleotide phosphate; NAG = N-acetyl-$\beta$-D-glucosaminidase; NO = nitric oxide; PBS = phosphate buffer saline; ROS = reactive oxygen species; TF$\beta$1 = transforming factor beta1; TNF$\alpha$ = tumor necrosis factor alpha; XO = xanthine oxidase

## INTRODUCTION

The metabolic syndrome (MetS) is a disease condition characterized by variable coexistence of obesity, hyperuricemia, hyperinsulinemia, hypertension, and dyslipidemia. The pathogenesis of the syndrome includes multiple organs involving the cardiorenal system.[1-3] Subjects with MetS have a greater risk for cardiovascular mortality with microalbuminuria correlated with the strongest risk of cardiovascular death.[4] This is particularly important as

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## AT A GLANCE COMMENTARY

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### Background

Allopurinol has been shown to prevent the development of hypertension and insulin resistance in animal models of metabolic syndrome (MetS) when given from the first day of induction of MetS by fructose, which took 6–9 weeks to develop hypertension. The present study determined if treatment with allopurinol after the development of MetS for 4 weeks will reverse symptoms of an established high fructose, high fat and salt–induced MetS in male Wistar rats.

### Translational Significance

The study suggests that addition of allopurinol to the therapeutic regimen of MetS may provide superior means to alleviate hypertension and proteinuria associated with MetS.

the cardiovascular disease represents the leading cause of death in the US and worldwide with 1.4 million deaths in the developed world and 5.7 million deaths in developing regions in the year 2013.[5]

This rise in the prevalence of MetS and cardiovascular disease is correlated with the great increase in fast food consumption with high diet content of fat and salt[6] and the dramatic increase of fructose intake in the last 100 years. Unlike other sugars, fructose rapidly causes adenosine triphosphate depletion and results in nucleotide turnover with generation of uric acid.[7] In the late 1990s, more evidence from animal models supports the pathogenetic role of hyperuricemia in the progression of hypertension and renal disease and its role as a causal risk factor for cardiovascular disease.[8] Recently, it was shown in human that serum uric acid predicted the development of cardiovascular complications in patients suffering from type 1 diabetes.[9]

In addition to uric acid production, xanthine oxidase (XO) enzyme generates reactive oxygen species (ROS). The XO-derived ROS further contribute to various forms of inflammation, ischemia, and vascular injuries.[10] Allopurinol, a standard inhibitor of XO, has been widely used for treatment of gout for decades but recently got more attention for potential therapeutic benefits in cardiovascular disease.[11] A combination of allopurinol and captopril has been found to block the development of MetS in fructose-fed model.[12]

Therefore, the aim of the present study was to examine if treatment with allopurinol alone will correct the pathologic changes in an established MetS induced by high-fructose, high-fat, and high-salt diet and to illustrate the possible mechanisms involved in this protection.

## MATERIALS AND METHODS

Studies were carried out in strict accordance with institutional animal care and use guidelines. Male Wistar rats (age 6 weeks; King Abdulaziz University, Jeddah, KSA) were used in the present study. The rats were housed in standard light-dark cycles with access to standard rat diet and water ad libitum.

**Study protocol.** The experimental protocol was approved by the Unit of Biomedical Ethics Research Committee, King Abdulaziz University, KSA. Animals were randomly divided into 3 experimental groups (8 animals each) of control (C), MetS (M), and allopurinol-treated MetS (MA). MetS was induced by adding fructose (10%) to every day drinking water[13] and feeding rats on high-fat and high-salt diet (16% crude protein, 28.2% crude fat, 2.8% crude fiber, 4.8% ash, and 3.4% salt; prepared in our laboratories) for 12 weeks. High fat and high salt were added to the diet to enhance the speed of development and magnitude of MetS. Control animals received tap water and standard diet (20% crude protein, 4% crude fat, 3.5% crude fiber, 6% ash, and 0.5% salt). Allopurinol (20 mg/kg/d) was daily administered by dissolving it in drinking water (100–120 mg/L) and adjusting the concentration based on water consumption. Allopurinol administration started after 8 weeks of fructose feeding and lasted for 4 weeks. At the end of the 12-week study, 24-hour urine was collected individually using metabolic cages for determination of urine volume and levels of protein, albumin, N-acetyl-$\beta$-D-glucosaminidase (NAG), creatinine, and 8-isoprostane. A tail capillary droplet was used to determine the 8-hour fasting blood glucose level. Rats were then anesthetized via urethane (1.5 g/kg, intraperitoneal) injection.[14] A microtip catheter was then inserted into the ascending aorta through an opening in the right carotid artery to measure the arterial blood pressure (BP). After 10 minutes of BP recording, 2 mL of venous blood was withdrawn from the inferior vena cava and allowed to coagulate for 30 minutes at 4°C. The left kidney was quickly dissected and cut transversely into 3 parts. Two parts were snap-frozen and stored at −80°C. One part was homogenized in tissue extraction reagent I (50 mM Tris, pH 7.4; 250 mM NaCl; 5 mM EDTA; 2 mM $Na_3VO_4$; 1 mM NaF;