

## **A Causal Role for Uric acid in Fructose-induced Metabolic Syndrome**

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**Running title: Fructose, uric acid and metabolic syndrome**

## **Abstract**

The worldwide epidemic of the metabolic syndrome correlates with an elevation in serum uric acid as well as a marked increase in total fructose intake (in the form of table sugar and high fructose corn syrup). Fructose raises uric acid, the latter which inhibits nitric oxide bioavailability. Since insulin requires nitric oxide to stimulate glucose uptake, we hypothesized that fructose-induced hyperuricemia may have a pathogenic role in the metabolic syndrome. Four sets of experiments were performed. First, pair feeding studies showed that fructose, and not dextrose, induced features (hyperinsulinemia, hypertriglyceridemia, and hyperuricemia) of the metabolic syndrome. Second, in rats receiving high fructose diet, the lowering of uric acid with either allopurinol (a xanthine oxidase inhibitor) or benzbromarone (a uricosuric agent) were able to prevent or reverse features of the metabolic syndrome. In particular, the administration of allopurinol prophylactically prevented fructose induced hyperinsulinemia (272.3 vs.160.8 pmol/L,  $p<0.05$ ), systolic hypertension (142 vs. 133 mmHg,  $p<0.05$ ), hypertriglyceridemia (233.7vs. 65.4 mg/dl,  $p<0.01$ ) and weight gain (455 vs. 425 g,  $p<0.05$ ) at 8 weeks. Neither allopurinol nor benzbromarone affected dietary intake of control diet in rats. Finally, uric acid dose-dependently inhibited endothelial function as manifested by a reduced vasodilatory response of aortic artery rings to acetylcholine.

These data provide the first evidence that uric acid may be a cause of the metabolic syndrome, possibly due to its ability to inhibit endothelial function. Fructose may have a major role in the epidemic of metabolic syndrome and obesity due to its ability to raise uric acid.

**Key Words:** Metabolic syndrome, uric acid, fructose, obesity, insulin resistance

The metabolic syndrome consists of a cluster of cardiovascular disease risk factors that include obesity, glucose intolerance, hyperinsulinemia, dyslipidemia, and hypertension (32). The prevalence of the metabolic syndrome is increasing and now affects 27% of the population in the USA (11). The epidemic correlates with pronounced changes in the environment, behavior and lifestyle, and is considered one of the main threats to human health worldwide. The metabolic syndrome confers a greater than three-fold increased risk for cardiovascular mortality (20). It is thus critical to identify mechanisms and strategies for preventing or treating this serious health problem.

As obesity and type 2 diabetes have escalated to epidemic proportions (28), the causal role of dietary components must be considered. The last 25 years have witnessed a marked increase in total per capita fructose intake, primarily in the form of sucrose (a disaccharide consisting of 50% fructose) and high fructose corn syrup (HFCS, 55% fructose content) (3). Fructose intake is linked with the epidemic of obesity and diabetes (22, 35). Soft drink intake (high in HFCS) is associated with an increased risk for obesity in adolescents (22) and for type 2 diabetes in young and middle-aged women (35). Excess fruit juice (also rich in fructose) is associated with the development of obesity in children (8). Fructose-fed rats also develop features of the metabolic syndrome (15). One distinction between fructose and glucose is that fructose raises serum uric acid (38). An elevated serum uric acid predicts the development of obesity and hypertension (23). This raised the possibility that uric acid may have a pathogenetic role in the metabolic syndrome. In the current study, we show that fructose-induced metabolic syndrome is partially prevented by lowering serum uric acid in the rat. The reduction of endothelial

nitric oxide bioavailability by uric acid may be a mechanism for insulin resistance and hypertension.

## Methods

*In vivo studies.*

*Experiment I; Treatment of fructose-induced hyperuricemia with allopurinol:* Male Sprague-Dawley rats (150-200g) were housed in standard conditions and fed control (n=7) or 60% fructose diet (Harlan, Madison, WI, n=14) for 10 weeks. "Control diet" contains 46% carbohydrate, which is mainly composed of starch whereas the fructose diet contained 60% fructose as the carbohydrate. The caloric content of these diets are 3.1 kcal/g and 3.6 kcal/g, respectively. At 4 weeks, blood sample were obtained at 11 am in the morning after 4h fasting. Half of the fructose-fed rats were administered allopurinol (AP, 150mg/L in the drinking water) (Sigma, St. Louis, MO) for an additional 6 weeks to lower serum uric acid. Fresh drinking water containing allopurinol was replaced every 2 days. Rats were divided into 3 groups: Control; Fructose (Fr); and Fr + AP. At 10 weeks an oral glucose tolerance test was performed, in which rats were fasted overnight (16 hours), and then administered 1.5g/kg OGTT (50% glucose solution) by gavage. Blood was sampled at 0, 30, 60, 120 min for blood glucose and serum insulin measurement. Rats were then sacrificed.

*Experiment II; Prevention of fructose-induced hyperuricemia with allopurinol:* To assess the effect of preventing hyperuricemia during the period of the study, allopurinol was initiated on the day when fructose diet was given (from week 0 to Week 8). Three groups (control, Fr, and Fr+AP; n=8 each) were designed for this prevention study. Body weight was measured every 2 weeks. Food consumption was measured for 3 days at 8 weeks.

*Experiment III; The effect of lowering of uric acid by either allopurinol or Benzbromarone (BZ) on body weight and food consumption:* In this experiment, the

effect of BZ, a uricosuric agent (150mg/L in the drinking water) (Sigma, St. Louis, MO), was also examined to confirm the effect of lowering of uric acid on body weight and food intake. Fresh drinking water containing Benzbromarone was replaced every 2 days. Three groups (control, AP, and BZ; n=8 each) were studied. All groups were fed with “Control diet” for 8 weeks. Body weight and the consumption of food were measured weekly for 8 weeks.

*Experiment IV; Comparison between 60% dextrose and 60% fructose on the development of metabolic syndrome and the effect of lowering uric acid with Benzbromarone:* Rats were pair-fed with 60% dextrose diet or 60% fructose diet for 4 weeks, which are isocaloric. Since Experiment II showed that each rat normally eats 25-30g/day, we administered 25g of diet to each rat every day. At 4 weeks, total food intake per animal was calculated from the food left over. Total food intake is the subtraction of the left-over food from total administered food (1425g/rat/28days). In addition to the above two groups, a third group of fructose fed rats were administered BZ. Body weight was measured weekly. At 4 weeks, after 5 h fasting, insulin, triglyceride and uric acid were measured.

All protocols were approved by the Animal Care Committee of the University of Florida.

*Measurements:* Systolic blood pressure was assessed as the mean value of 3 consecutive measurements obtained in the morning using a tail-cuff sphygmomanometer (Visitech BP2000, Visitech Systems, Inc., Apex, NC). All animals were preconditioned for blood pressure measurements 1 wk before each experiment. Serum uric acid was measured by uricase method. Blood glucose was measured with the ONE TOUCH system

(Johnson&Johnson, Milpitas, CA). Rat insulin was measured by ELISA (Crystal Chem. Inc., Chicago, IL). Insulin sensitivity index was calculated using the formula of Matsuda and DeFronzo ( $10,000/\text{square root of [fasting glucose X fasting insulin] X [mean glucose X mean insulin during OGTT]}$ ), which is highly correlated ( $r=0.73$ ,  $p<0.0001$ ) with rate of whole-body glucose disposal during the euglycemic insulin clamp (24). Serum lipids were measured with an autoanalyzer (VETAce, Alfa Wassermann Inc, West Caldwell, NJ) or Triglyceride-SL assay kit (Diagnostic chemicals Limited, Charlottetown, PE, Canada).

*Vasorelaxation of rat Aortic Artery (AA) segments:* Rat AA segments (1-0.5 mm diameter  $\times$  3-4 mm length) were isolated from the 2- to 3- month -old rats, AA segments were suspended in individual organ chambers (Radnoti Four-Unit Tissue Bath System) with 5 ml in Earl's solution, oxygenated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37°C. After 1hr equilibration of resting force of 1.5 g, vascular smooth muscle cell or endothelium integrity in this AA segment was confirmed by monitoring 0.5  $\mu$ M U-46619 (a thromboxane A<sub>2</sub> mimetic, sigma)-mediated AA contraction or acetylcholine (5  $\mu$ M)-mediated vasodilation, respectively. After washing several times, the segments were incubated with various concentration of uric acid (0-15mg/dl) in organ bath chamber for 30 min. Stable construction was induced by 0.5  $\mu$ M U-46619 for 10 min prior to acetylcholine-induced vasorelaxation. The vascular tensions were continuously monitored with an isometric force transducer (Harvard Apparatus, Holliston, MA). To standardize the data, U-46619-induced stable increase in vascular tone was set as 100%.

*Statistical analysis.* All values presented are expressed as mean  $\pm$  SD and analyzed by one-way analysis of variance (ANOVA) or by unpaired Student's *t* test. Significance was defined as  $p < 0.05$ .

## Results

### *In vivo study*

Serum uric acid levels, systolic blood pressure, and fasting insulin levels were elevated in fructose-fed rats compared to rats fed a control diet at 4 weeks (Table 1). In addition, the body weight of fructose-fed rats tended to increase compared to rats fed a normal diet (Table 1). These data demonstrate that fructose feeding induces early features of the metabolic syndrome in rats.

In order to examine the role of uric acid in this model, half of the fructose-fed rats were treated with allopurinol (a xanthine oxidase inhibitor) for 6 additional weeks. This treatment was effective at lowering uric acid, whereas the fructose-fed rats that did not receive treatment continued to be hyperuricemic (Figure 1A). In addition, we examined the urinary excretion of uric acid in these animals to clarify the mechanisms of hyperuricemia in fructose-fed rats. As shown in Figure 1B, fructose-fed rats had lower urinary excretion of uric acid. Interestingly, allopurinol prevented the reduced excretion of uric acid in fructose-fed rats.

Fructose-fed rats treated with allopurinol showed an improvement in the metabolic syndrome. Allopurinol significantly reduced systolic blood pressure in fructose-fed rats (Figure 1C), although pressures remained higher than that observed in control rats. Fructose-fed rats also developed marked hypertriglyceridemia that was abolished by allopurinol treatment (Figure 1D). The reduction in serum uric acid correlated directly with the decrease in triglyceride levels (Figure 1E). Fructose-fed rats also showed an increase in body weight compared to controls. Allopurinol prevented the

increase in body weight although this did not reach significance ( $522\pm 57$ g in Fr vs.  $470\pm 28$ g in control, and  $474\pm 37$ g in Fr+AP,  $p=NS$ ).

While no groups developed fasting or postprandial hyperglycemia (Figure 2A), fructose-fed rats developed fasting hyperinsulinemia that was reversed with allopurinol (Figure 2B). Postprandial hyperinsulinemia also occurred in fructose-fed rats administered an oral glucose tolerance test, and this was partially but significantly lower in allopurinol-treated rats (Figure 2B), resulting in improved insulin sensitivity (Figure 2C).

We also examined the effectiveness of allopurinol in preventing as opposed to treating rats with fructose-induced metabolic syndrome. Allopurinol was given simultaneously with the fructose diet from the starting point to avoid fructose-induced hyperuricemia. As shown in Figure 3A, the elevation of uric acid by fructose diet was prevented over the 6 week period in fructose-fed rats. Allopurinol treated rats had significantly lower fasting insulin levels compared to fructose-fed rats (Figure 3B) and the development of hypertriglyceridemia was completely prevented (Figure 3D). In addition, while fructose-fed rats gained weight compared to control rats ( $456\pm 24$  vs.  $414\pm 24$ g, final weights in Fr vs. control,  $p<0.01$ ), allopurinol treated rats had lower weight gain (final weight  $426\pm 26$ g,  $p<0.05$  vs. Fructose-fed rats). At 8 weeks, total food intake over 3 days in fructose-fed rats was slightly higher ( $92\pm 2$ g) compared to that of the Fructose+Allopurinol group ( $88\pm 4$  g), although this did not reach statistical significance.

The observation that administration of allopurinol to fructose fed rats prevented obesity led to additional studies to ensure that allopurinol did not have specific effects on food

intake or body weight. To address this possibility, allopurinol or benzbromarone ( a uricosuric) was administered to rats on control diets for 8 weeks. A third group received control diet alone. Total food consumption at 8 weeks and final body weight were not different among the three groups (Table 2).

Finally, we compared the effects of 60% Dextrose diet and 60% Fructose diet on the development of metabolic syndrome. In this experiment food intake was controlled so that each group received the same intake of calories and had the same weight gain. Nevertheless, only the fructose fed rats developed hyperuricemia, hypertriglyceridemia, and hyperinsulinemia (Table 3). Importantly, these effects observed in fructose fed rats were significantly improved by lowering uric acid levels with the uricosuric agent, benzbromarone (Table 3).

*In vitro studies.*

Endothelial dysfunction is common in metabolic syndrome. It is known that impaired nitric oxide response to insulin may be a mechanism for the development of insulin resistance (36). Previously, uric acid has been shown to potently reduce NO levels in cultured bovine endothelial cells (18). To further examine this relationship, we examined the acute effect of uric acid on acetylcholine-induced vasodilation of rat aortic artery rings. As shown in Figure 4, uric acid dose-dependently blocked the vasorelaxation of aortic arterial rings in response to acetylcholine.

## **Discussion**

In this study we examined the role of uric acid in the development of the insulin resistance syndrome (the metabolic syndrome) in fructose-fed rats. Fructose, but not dextrose, caused metabolic syndrome. Allopurinol, a xanthine oxidase inhibitor that lowers serum uric acid, was able to both prevent and reverse features of the metabolic syndrome in the fructose-fed rat. Allopurinol lowered systolic blood pressure, improved insulin sensitivity, and normalized triglyceride levels. Similar effects were observed with benzbromarone (a uricosuric agent). Allopurinol also prevented weight gain in fructose-fed rats, and this did not appear to be due to effects of allopurinol on diet, for rats on control diet fed allopurinol gained weight normally. Thus, these studies provide the first evidence that uric acid may have a causal role in the pathogenesis of fructose-induced metabolic syndrome.

There is supporting evidence that uric acid may have a pathogenic role in the metabolic syndrome. Hyperuricemia has been found to predict the development of both obesity and type2 diabetes (29). Hyperuricemia is also commonly observed in the metabolic syndrome (41), as well as in secondary insulin resistance syndromes such as that associated with gout (2), diuretic usage (21, 31), or preeclampsia (39). There are also older studies that showed that rats made chronically hyperuricemic with uricase inhibitors develop features of the metabolic syndrome (42). These data introduce the novel concept that uric acid may have a causal role in the metabolic syndrome.

Most authorities consider hyperuricemia in metabolic syndrome to be the consequence of elevated serum insulin levels which have been shown to stimulate renal reabsorption of uric acid (9). Consistent with this observation is the finding that

thiazolidinediones, which improve insulin sensitivity and lower insulin levels, also reduce the level of serum uric acid in diabetic patients (16, 40). On the other hand, our study demonstrated that lowering uric acid with either a xanthine oxidase inhibitor or a uricosuric agent also improves insulin sensitivity as well as other features of the metabolic syndrome, including hypertension, obesity, and hypertriglyceridemia. While multiple factors are known to drive the metabolic syndrome (6), these studies suggest uric acid may also have a contributory role in the development of insulin resistance.

Hypertriglyceridemia was completely blocked by lowering of uric acid with allopurinol in this study. Compatible with our findings, it has been shown that the association of elevated serum uric acid with hypertriglyceridemia is stronger than with insulin sensitivity (41). Interestingly, treatment of hypertriglyceridemia with fenofibrate or atorvastatin also reduces serum uric acid (1, 12, 27). Uricosuric agents such as benzbendazole also lower serum triglycerides (12). Although the role of uric acid on metabolism of triglyceride remains unknown, uric acid might be involved in either the overproduction or the reduction of clearance of triglycerides. A decrease in clearance of triglycerides in fructose-fed rats has been attributed to a reduction in lipoprotein lipase activity in endothelial cells (19, 30); whether this is mediated by uric acid remains to be determined. An alternative explanation is the possibility that the de novo increase in purine synthesis observed in fructose-fed rats may be pathogenetically linked to hepatic fatty acid synthesis, resulting in overproduction of triglycerides (25, 41).

Endothelial dysfunction is a hallmark of the metabolic syndrome (7). Therefore, we investigated the role of uric acid on endothelial dysfunction as a mechanism for the insulin resistance. We showed that uric acid dose-dependently blocked acetylcholine-

mediated arterial dilation (Figure 4), suggesting that uric acid can impair endothelial function. In addition, we have found that uric acid potently reduces endothelial nitric oxide (NO) bioavailability in both cell culture and in experimental animal models (18). In turn, reducing endothelial NO levels are a known mechanism for inducing insulin resistance (33). Thus, eNOS deficient mice exhibit the features of the metabolic syndrome (5). The mechanism is due to a blockade of insulin action, as insulin stimulates glucose uptake in skeletal muscle by increasing blood flow to these tissues through an NO-dependent pathway (33). In this scenario, allopurinol or benzbromarone may be acting to prevent the metabolic syndrome by blocking hyperuricemia-induced endothelial dysfunction.

Unlike glucose, the oral ingestion of fructose results in a rapid increase in serum uric acid within 30 to 60 minutes in humans (38). The mechanism by which fructose raises serum uric acid has been previously studied. Fructose enters hepatocytes where it is rapidly phosphorylated by fructokinase to fructose-1-phosphate (13). During this reaction ATP donates the phosphate, resulting in the generation of ADP which is further metabolized to uric acid (13). This is aided by a fructose-mediated increase in AMP deaminase (37).

In addition to an effect of fructose to increase hepatic production of uric acid, we found that urinary excretion of uric acid was decreased in fructose fed rats. There are multiple potential explanations for this observation. First, we have found that experimental hyperuricemia causes endothelial dysfunction and renal vasoconstriction (18, 26, 34) which is known to impair urate excretion (10). Second, fructose results in lactate production which is a competitive inhibitor for urate excretion (14). Finally,

hyperinsulinemia itself can lead to an impairment in urate excretion (9). The observation that the impaired urate excretion was due in part to the hyperuricemia was shown by the observation that allopurinol could reverse this effect. This would support a uric acid induced endothelial dysfunction and/or hyperinsulinemia as the central mechanism for this effect.

While the above studies provide evidence supporting a role for uric acid in the development of the metabolic syndrome induced by fructose, allopurinol also blocks oxidants generated by the xanthine oxidase pathway. Oxidants are involved in the pathogenesis of diabetes and its complications (4). It is therefore possible that the beneficial effects of allopurinol may be attributed in part to the lowering of oxidants rather than an effect on uric acid per se. However, most studies suggest that the oxidants driving diabetic complications are generated as a consequence of mitochondrial dysfunction or activation of NADPH oxidase, neither of which would be blocked by a xanthine oxidase inhibitor. The observation that the uricosuric agent, benzbromarone, also prevented features of the metabolic syndrome further suggests that the mechanism by which allopurinol work likely includes lowering uric acid. While future studies will need to dissect out the mechanism by which allopurinol provides benefit, the observation that uric acid also impairs endothelial function provides a potential mechanism by which uric acid could have a pathogenic role in fructose-mediated metabolic syndrome. While speculative, we suggest that the worldwide epidemic in hypertension, obesity and the metabolic syndrome may have its roots in the marked increase in fructose intake, and in the progressive rise in the mean serum uric acid that has been observed in both developing and industrialized nations in the last century (17).

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**Conflict of Interest**

Dr Johnson is also a consultant for TAP pharmaceuticals.

Table1; Experiment I.  
General characteristics in Control and Fructose groups at 4 weeks

	Control (n = 7)	Fructose (n = 14)	
Initial body weight (g)	190±12	188±0.1	NS
Body Weight (g) at 4 weeks	357±15	375±22	P= 0.05
Systolic BP(mmHg) at 4 weeks	127±3	148±15	P<0.05
Uric acid (mg/dl) at 4 weeks	1.3±0.3	2.4±0.3	P<0.01
Insulin (pmol/L) at 4 weeks	121±64	176±51	P<0.05

Table 2. Experiment III  
The effect of lowering uric acid on body weight and food consumption for 8 weeks

	Control (n=8)	Allopurinol (n=8)	Benzbromarone (n=8)
Initial body weight (g)	162±7	164±3	160±8
Final body weight at 8 weeks (g)	469±69	504±37	468±43
Total food intake (g) for 8 weeks	1452±68	1562±118	1494±90
Uric acid (mg/dl) at 8 weeks	1.5±0.5	0.5±0.4	1.1±0.3

Table 3. Experiment IV

Pair-feeding with 60% Dextrose diet and 60% Fructose diet at 4 weeks on development of metabolic syndrome and the effect of lowering uric acid by Benzbromarone

	60% Dextrose (n=8)	60% Fructose (n=8)	60% Fructose +Benzbromarone (n=8)
Initial body weight (g)	144±7	144±5	144±7
Final body weight at 4 weeks (g)	353±14	364±17	360±20
Total food intake (g) for 4 weeks	700±10	709±6	698±15
Uric acid (mg/dl) at 4 weeks	1.4±0.3	2.1±0.9 <sup>a</sup>	1.1±0.4 <sup>b</sup>
Triglyceride (mg/dl) at 4 weeks	112±28	419±60 <sup>c</sup>	293±86 <sup>d</sup>
Insulin (pmol/L) at 4 weeks	112±43	204±62 <sup>c</sup>	147±42 <sup>d</sup>

a, p<0.05 vs. Dextrose. b, p<0.01 vs. Fructose. c, p<0.01 vs. Dextrose. d, p<0.05 vs. Fructose

## FIGURE LEGENDS

**Figure 1. Effects of allopurinol treatment for hyperuricemia on the metabolic parameters in Fructose-fed Rats.** (A) Fructose-fed rats (Fr) are hyperuricemic at 9 weeks and this is prevented by allopurinol (AP; 150mg/L). (\* $p < 0.01$  vs. control; # $p < 0.05$  vs. Fr.) (B) Fructose reduced urinary excretion of uric acid at 9 weeks and this is prevented by allopurinol. (\* $p < 0.01$  vs. Fr; # $p < 0.05$  vs. control.) (C) Hypertension develops in fructose-fed rats, which is significantly reduced by allopurinol. (\* $p < 0.01$  vs. control; # $p < 0.05$  vs. Fr.) (D) Serum triglycerides are increased in fructose-fed rats, and this is completely prevented by allopurinol (# $p < 0.01$  vs. control, and Fr+AP). (E) The serum triglyceride level correlates directly with the serum uric acid. Data are mean  $\pm$  SD.

**Figure 2. Effect of allopurinol treatment on glucose metabolism in Fructose-fed rats.** (A) Glucose tolerance test at 10 weeks. Similar blood glucose levels were observed in all groups. (B) Plasma insulin levels following the glucose tolerance test. Fructose ingestion was associated with fasting and postprandial hyperinsulinemia. Allopurinol (AP; 150mg/L) prevented basal hyperinsulinemia and significantly reduced postprandial hyperinsulinemia. (\* $p < 0.01$  vs. control; # $p < 0.05$  vs. Fr.) (C) Insulin sensitivity index (ISI). Insulin sensitivity was reduced with fructose diet and improved by allopurinol. All data are means  $\pm$  SD. Statistical analysis among three groups were analyzed by ANOVA with Bonferoni correction in Figure B. (\* $p < 0.01$  vs. control; # $p < 0.05$  vs. Fr.). Comparison was done between Fr and Fr+AP using unpaired  $t$  test in Figure C.

**Figure 3. Blocking of hyperuricemia in fructose-fed rats with allopurinol prevents features of the metabolic syndrome.** (A) Allopurinol (AP; 150mg/L) prevented the rise in uric acid in fructose-fed rats. (#,  $p < 0.05$  vs con, Fr+AP) (B) Allopurinol treatment was associated with significantly lower fasting insulin levels compared to fructose-fed rats at 8 weeks. (C) Allopurinol also prevented the increase in BW induced with fructose. Statistical analysis among three groups was analyzed by ANOVA with Bonferoni correction.

**Figure 4. Uric Acid Inhibits Acetylcholine-Mediate Vasodilation in Rat Aortic Artery Segments.** Acetylcholine (5  $\mu\text{M}$ )-induced vasorelaxation was assessed in the presence of various concentration of uric acid for 10 min after stable construction by U-46619 (0.5  $\mu\text{M}$ ).  $n=4$ , \* $p < 0.01$  vs. control, # $p < 0.05$  vs. 0.7mg/dl, ## $p < 0.01$  vs. 0.7mg/dl

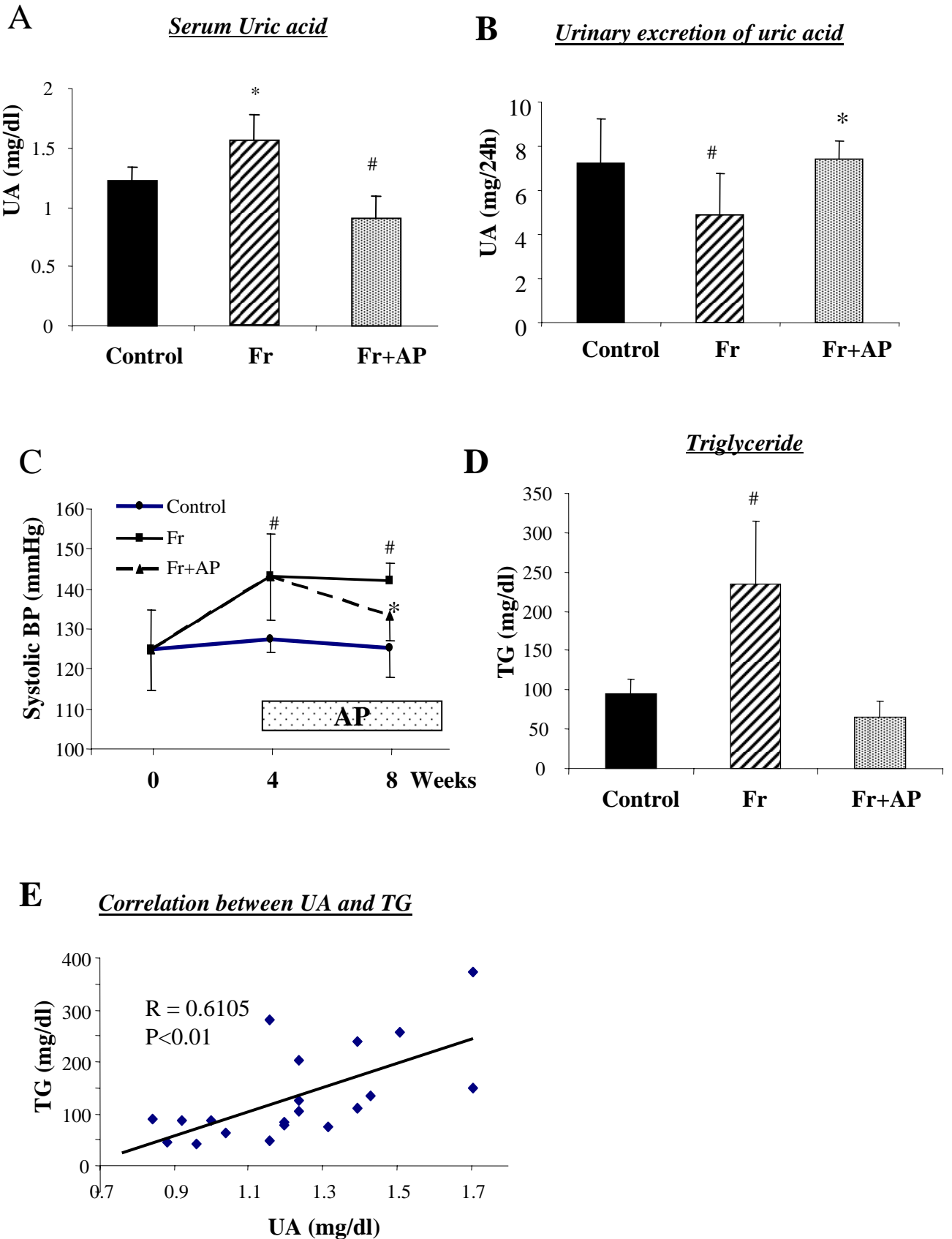
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# Figure 1



**Figure 2**

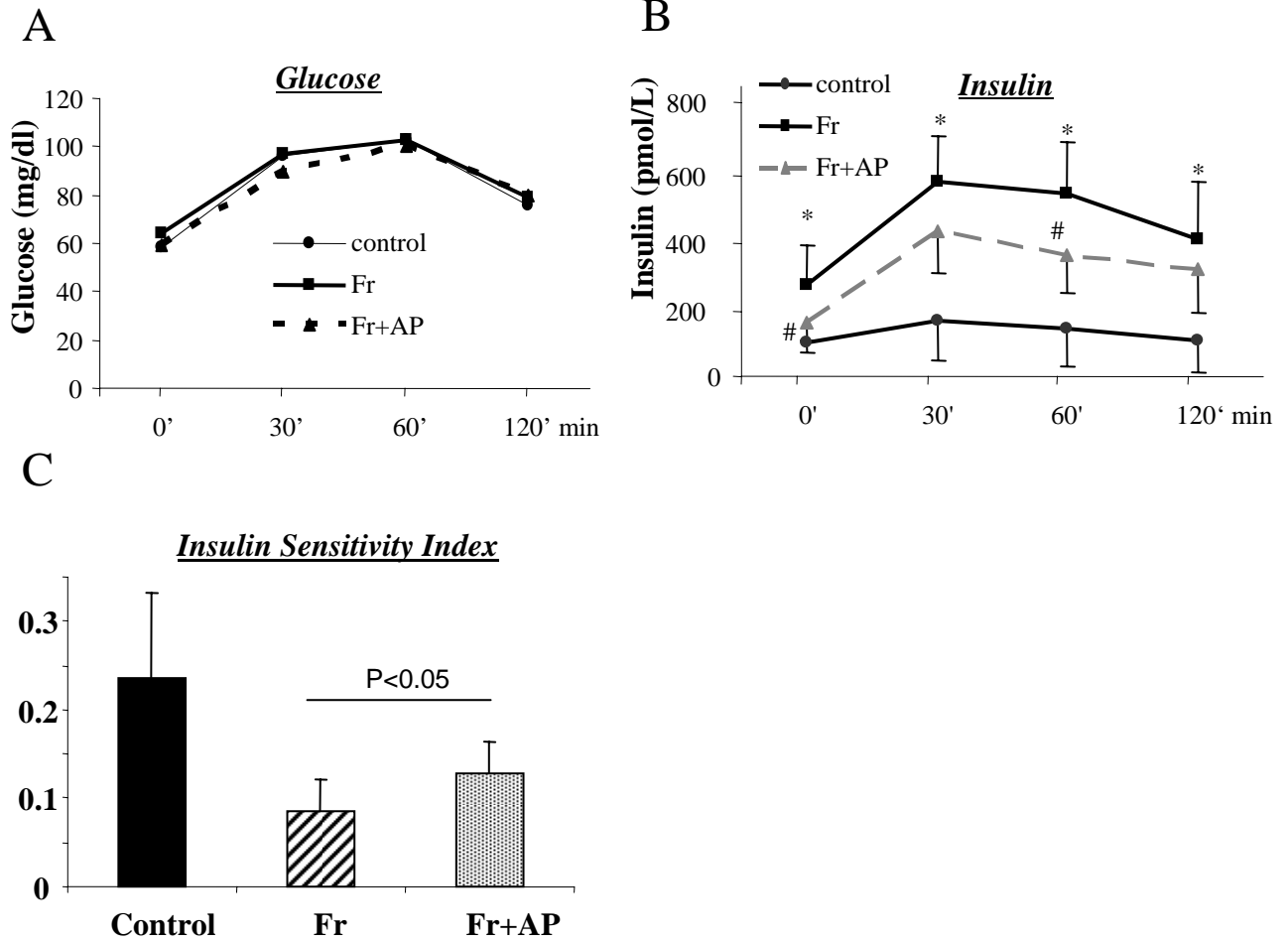
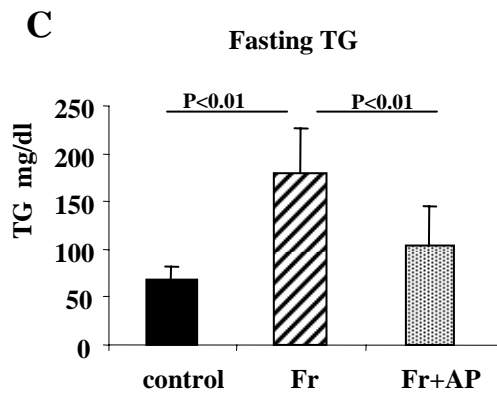
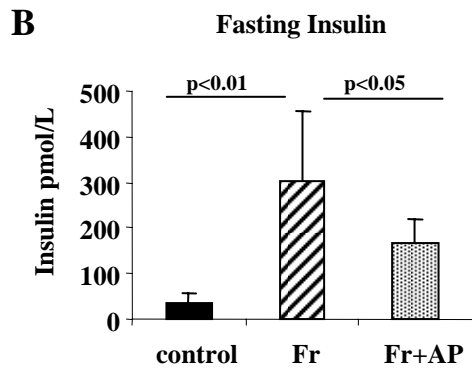
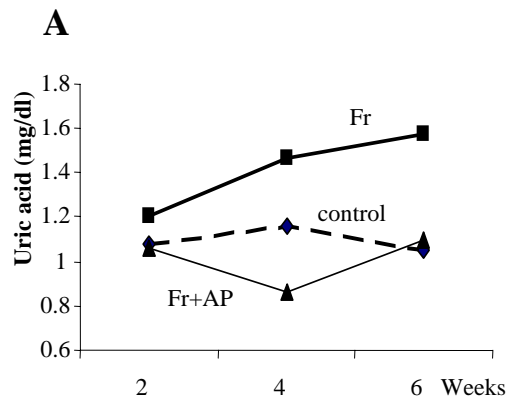


Figure 3



**Figure 4 Uric Acid Dose-dependently Inhibits Acetylcholine-Induced Vasodilation in Rat Aortic Artery Segments**

