Weight Loss Mediated Reduction in Xanthine Oxidase Activity and Uric Acid Clearance in Adolescents with Severe Obesity

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Abstract

Background: Increased xanthine oxidase (XO) activity and uric acid levels are known to be associated with obesity and hypertension; however, it is not known if obesity is directly responsible for these associations in youth. This study investigated the effect of weight loss on XO activity, uric acid, and their relationship to blood pressure change in obese youth to provide greater insight on how obesity increases cardiovascular risk.

Methods: This was an ancillary study in which 16 adolescents (mean age 15 ± 2 years) received meal replacement therapy over a period of four weeks. Outcomes measured at baseline and after intervention included weight, blood pressure, XO activity, plasma uric acid, uric acid clearance, and creatinine clearance.

Results: After the meal replacement intervention, participants experienced reductions in body weight (109.2 ± 16 kg vs. 105.2 ± 14 kg, $p < 0.0001$) and BMI (38.7 ± 4 kg vs. 37.4 ± 3 kg, $p < 0.0001$). Plasma XO activity was reduced by 9.8% ($p = 0.016$). Uric acid clearance was decreased by 39% ($p = 0.006$). SBP (systolic blood pressure) and plasma uric acid concentrations were reduced but did not achieve statistical significance ($p = 0.34$ and 0.38 , respectively). DBP (diastolic blood pressure) was unchanged ($p = 0.86$). No significant relationships were found between changes in blood pressure and changes in either XO activity or plasma uric acid levels.

Conclusion: Weight loss led to decreases in uric acid production by lowering XO activity and decreases in uric acid clearance by reducing glomerular filtration (GF) and increasing reabsorption. Changes in XO activity and uric acid levels did not correlate with changes in blood pressure.

Introduction

Obesity rates in the United States among children and adolescents ages 2–19 years old have risen nearly threefold since 1980 to a rate of nearly 17% in 2010.¹ Childhood obesity is associated with increased xanthine oxidase (XO) activity and blood pressure.^{2–4} Specifically, elevated XO activity leads to increased production of uric acid, which has been shown to modulate blood pressure in a two-phase mechanism.⁵ The first phase of blood pressure modulation is uric acid dependent, whereby uric acid induces the renin-angiotensin system (RAS), which leads to acute vasoconstriction and a rise in systemic pressures. The second phase results in chronic hypertension and is uric acid independent and sodium de-

pendent. Therefore, studying the effects of uric acid and XO before the uric acid independent second phase of hyperuricemic hypertension will be critical in determining whether treatment of hyperuricemia is beneficial to treating obesity-mediated hypertension.

Elevated XO activity, uric acid levels, and cytokines are known to correlate with obesity. Studies have demonstrated that cytokines, including interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), regulate XO expression.⁶ Mechanistically, weight loss has also been shown to reduce the elevated cytokine levels and inflammation seen in obese adolescents.⁷ Therefore, changes in the levels of cytokines after weight loss may lead to changes in XO activity and blood pressure. It is still not known whether obesity drives increases in XO and uric

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acid. Studying the effect of weight loss on XO activity, uric acid, and blood pressure will provide greater insight into one potential mechanism of how obesity increases cardiovascular risk. Additionally, investigating this question in a pediatric population allows for the examination of the effect of weight loss on uric acid mediated changes in blood pressure before chronic hypertension has developed.

Since meal replacement therapy has been demonstrated to be an effective short-term weight loss therapy in adolescents,⁸ it was chosen as our model to determine if weight loss mediated changes in uric acid levels are associated with changes in blood pressure. Therefore the primary objective of this study was to determine if modest weight loss in youth with severe obesity would reduce blood pressure via changes in XO activity and plasma uric acid levels. As secondary objectives, we investigated the role of changes in cytokine levels on XO activity. We hypothesized that weight reduction would result in decreased cytokines which in turn would decrease XO activity. Decreased XO activity would lead to decreased uric acid which in turn would result in decreased blood pressure.

Methods

This was an ancillary study within the context of a larger clinical trial in which 16 adolescents (mean age 15 ± 2 years) received meal replacement therapy for 4 weeks. Subjects were recruited from the Pediatric Weight Management Clinic at the University of Minnesota Masonic Children's Hospital. The meal replacement therapy consisted of two frozen entrees (Weight Watchers, Smart Ones®), three SlimFast® shakes, two servings of fruit, and three servings of vegetables per day for a total of approximately 1370 kcals daily. Parental consent and child assent were obtained from participants in this study, and the protocol was approved by the University of Minnesota's institutional review board.

Measures

The following were measured at baseline and at the end of the 4-week meal replacement therapy: height, weight, waist circumference, blood pressure, fasting insulin, glucose, lipid panel, plasma XO activity, plasma uric acid, uric acid clearance, creatinine clearance, IL-6, and TNF- α . Height and weight were obtained using a standard stadiometer and electronic scale, respectively, with participants wearing light clothes and no shoes. BMI was calculated as weight in kilograms divided by height in meters squared. Uric acid clearance was calculated using serum creatinine levels and urine creatinine levels (1–6 hour collection interval) by using the following equation: (urine uric acid/plasma uric acid) $\times$ (urine volume/collection interval). Creatinine clearance was calculated using serum creatinine levels and urine creatinine levels (1–6 hour collection interval) by using the following equation: (urine creatinine/plasma creatinine) $\times$ (urine volume/collection interval).

Seated blood pressure was obtained after 5 minutes of quiet rest, on the right arm using an automatic sphygmomanometer. Three consecutive blood pressure measurements were taken with a 3-minute rest between. Blood pressure was then averaged. Blood samples were collected after a minimum 8-hour fast. IL-6 and TNF- α were measured using a Luminex multiplex bead array assay (Luminex, Austin, TX). Plasma was stored frozen at -70°C until assayed at the University of Minnesota Cytokine Reference Laboratory (Clinical Laboratory Improvement Amendments licensed) using enzyme-linked immunosorbent assay. The ranges for cytokine concentration for each cytokine are as follows: TNF- α ranged from 1.4–4.0 pg/mL and the sensitivity of the kit was 1.4 pg/mL. IL-6 ranged from 0.7–5.5 pg/mL and the sensitivity of the kit was 0.5 pg/mL).

Plasma XO activity was determined by measuring the XO-specific conversion of lumazine to isoxantholumazine (in plasma, this reaction is inhibited by 96%–99% with allopurinol, a specific XO inhibitor, after accounting for negative control samples). Briefly, reaction mixtures contained 100 μL plasma and 100 μL of 80 μM lumazine in 50 mM potassium phosphate buffer (pH = 7.4) and were incubated at 37°C for 3 hours (this reaction is stable and linear for up to 4 hours) followed by addition of 400 μL methanol (with umbelliferone as internal standard) to stop the reaction. After centrifugation and filtration, the supernatants were saved for high-performance liquid chromatography (HPLC) analysis of isoxantholumazine; 100 μL of the supernatant was injected onto a 250 mm YMC-Pack Phenyl column (Phenomenex, Torrance, CA) and fluorescence was measured on a Waters 474 Scanning Fluorescence Detector (Waters, Milford, MA) set at excitation wavelength = 340 nm and emission wavelength = 400 nm. Lipids, glucose, and insulin assays were also conducted with standard procedures at the Fairview Diagnostic Laboratories, Fairview University Medical Center (Minneapolis, MN), a CDC-certified laboratory.

Statistical Analyses

Paired t-tests were used to compare the changes in outcome variables before and after the meal replacement intervention. Linear regression analysis was used to determine the associations between (1) changes in uric acid vs. changes in blood pressure, (2) uric acid clearance vs. creatinine clearance, and (3) XO activity and blood pressure. Data are presented as mean $\pm$ standard error unless stated otherwise. Statistical significance was set at a p -value ≤ 0.05 .

Results

Baseline and postintervention characteristics are shown in Table 1. After the meal replacement intervention, participants had a decrease in body weight of 3.7% (109 ± 16 kg vs. 105 ± 14 kg, $p < 0.0001$) and BMI of 3.4% (38.7 ± 4 kg vs. 37.4 ± 3 kg, $p < 0.0001$). Systolic blood pressure (SBP) was reduced but did not achieve statistical significance (122 ± 10 mm Hg vs. 119 ± 10 mm Hg, $p = 0.34$);

Table 1. Baseline and Postmeal Replacement Therapy Characteristics

	Baseline (n = 16)	Post (n = 16)	p-value
Gender (% females)	69	N/A	-
Age (years)	14.6 ± 2	N/A	-
Height (cm)	168 ± 8	167 ± 8	
Weight (kg)	109.2 ± 16.0	105.2 ± 14.0	<0.0001*
BMI (kg/m ²)	38.7 ± 4.0	37.4 ± 3.0	<0.0001*
Waist circumference (cm)	110 ± 12	108 ± 12	0.36
Systolic blood pressure (mm Hg)	122 ± 10	119 ± 10	0.34
Systolic blood pressure percentile (%)	73 ± 18	70 ± 23	0.53
Diastolic blood pressure (mm Hg)	69 ± 8	69 ± 10	0.86
Diastolic blood pressure percentile (%)	5 ± 22	64 ± 25	0.39
Heart rate (beats/minute)	76 ± 7	74 ± 10	0.37
Total cholesterol (mg/dl)	169 ± 34	155 ± 33	0.0066*
High-density lipoproteins (mg/dl)	43 ± 12	39 ± 11	0.0009*
Low-density lipoproteins (mg/dl)	103 ± 31	94 ± 28	0.023*
Triglycerides (mg/dl)	119 ± 58	114 ± 56	0.68
Glucose (mg/dl)	84 ± 8	81 ± 7	0.30
Insulin (mU/l)	24 ± 12	23 ± 9	0.60
IL-6 (pg/mL)	2.2 ± 1.2	1.8 ± 0.7	0.03*
TNF- α (pg/mL)	2.6 ± 0.8	2.6 ± 0.6	0.69
Plasma uric acid (mg/dL)	8.5 ± 2.1	8.1 ± 2.2	0.38
Creatinine Cl (mL/minute)	214.5 ± 128.7	162.7 ± 57.7	0.10

Data are presented as mean ± standard deviation. All blood measurements performed in fasting state.

*p-value <0.05.

whereas diastolic blood pressure (DBP) was unchanged (69 ± 8 vs. 69 ± 10 mm Hg, $p = 0.86$). IL-6 decreased by 19% (2.2 ± 1.2 pg/mL vs. 1.8 ± 0.7 pg/mL, $p = 0.03$) and TNF- α was unchanged (2.6 ± 0.8 pg/mL vs. 2.6 ± 0.6 pg/mL, $p = 0.69$). Plasma XO activity was significantly reduced by 9.8% (0.045 ± 0.007 pmol/hour vs. 0.040 ± 0.007 pmol/hour, $p = 0.0159$) (Figure 1). Plasma uric acid was reduced but did not achieve statistical significance (8.5 ± 0.5 mg/dL vs. 8.1 ± 0.6 mg/dL, $p = 0.38$) (Table 1). Additionally, creatinine clearance was reduced but did not achieve statistical significance (199 ± 17 mL/minute vs. 160 ± 17 mL/minute, $p = 0.10$) (Table 1); whereas uric acid clearance was significantly decreased by 39% (8.9 ± 1.4 mL/minute vs. 5.4 ± 0.7 mL/minute, $p = 0.006$) (Figure 2). Using regression models, no significant relationships were found between changes in plasma uric acid levels and blood pressure changes (Figure 3).

Discussion

In this study we found that modest weight loss with meal replacement therapy results in reduction of XO activity

among adolescents with severe obesity. Interestingly, this reduction in XO activity was not associated with similar decreases in uric acid, blood pressure, or cytokine levels. There are multiple factors that can regulate XO activity including cytokines⁶ and lipopolysaccharide (LPS).^{9,10} The cytokine IL-6 plays an important regulatory role in the expression of XO, due the presence of repressor proteins that bind the promoter region of the xanthine oxidoreductase gene and which are inactivated by IL-6.^{11–13} Therefore the 19% decrease in plasma IL-6 concentrations observed in the current study may be partially responsible for the lowering in overall XO activity due to an increase in the repression of XO expression, but the correlation between the changes in IL-6 were not significantly associated with the changes in XO activity (data not shown).

Contrary to other studies, we observed weight loss mediated changes only in IL-6 and not in TNF- α concentrations (Table 1). Jung et al. and Ziccardi et al. both reported significant decreases in multiple inflammatory cytokines including IL-6 and TNF- α after weight loss of at least 10% of baseline weight in adults;^{14,15} however, our short-term intervention resulted in only 3.7% reduction in body

Figure 1. Plasma xanthine oxidase activity before and after the meal replacement intervention. Data are means $\pm$ SEM. * p -value = 0.0159 compared to the baseline.

weight. Therefore, TNF- α may not have been as sensitive to weight loss as IL-6, and/or the degree of weight loss might have been insufficient to observe TNF- α changes.

Another possible factor that may regulate plasma XO activity is plasma LPS concentration generated by bacteria. Kurosaki et al. reported that mice exposed to LPS induced XO expression in various organs and tissues; therefore changes in the gut microbiota and LPS exposure from this source may contribute to changes in XO activity in our study.¹⁰ For example, a high-fat diet or high consumption of dietary fats, which are common in subjects with extreme obesity, may also increase LPS levels by altering the gut microbiota to express more gram-negative bacteria.^{16,17} Meal replacement therapy may be partially responsible for changes in XO activity by altering the gut microbiota to

Figure 2. Uric acid clearance before and after the meal replacement intervention. $n = 14$. * p -value = 0.006.

Figure 3. Linear regression of the changes in uric acid vs. the change in blood pressure. Linear regression analysis was used to determine the association between the change in uric acid clearance vs. systolic blood pressure ($r^2 = 0.12$) and vs. diastolic blood pressure ($r^2 = 0.13$).

decrease LPS production, since the diet included less fat than an average diet (approximately 19% fat in current diet regimen vs. 33% in an average US diet), and meal replacement therapy has been shown in previous studies to lower LPS levels in obese adolescents.¹⁸

Uric acid has been shown to be important in regulating blood pressure in children with hypertension.¹⁹ Plasma uric acid levels depend on its production rate via XO and its elimination rate primarily via the kidneys. We observed that XO activity was reduced after weight loss in the current study (Figure 1). Specifically, 75% of study subjects experienced a reduction in XO activity. Unfortunately, we did not observe a correlation between change in XO activity and amount of weight lost. Both plasma uric acid and blood pressure were unchanged after the intervention (Table 1). We measured creatinine clearance and uric acid clearance before and after weight loss to determine if weight loss would affect plasma uric acid levels via changes in kidney function and the elimination of uric acid. Uric acid clearance decreased by 39% with weight loss (Figure 2) and was mirrored by a reduction in creatinine clearance (Table 1). These decreases are likely due to reversion of renal hemodynamic overload with glomerular hyperfiltration that is seen with obesity. For example, it has been previously shown that weight loss decreases creatinine clearance in obese adults with metabolic syndrome and diabetic nephropathy.^{20,21}

The decrease in uric acid clearance that we observe may be due to changes in the filtration rate of uric acid, given that creatinine clearance—a common surrogate for glomerular filtration rate (GFR)—is related to uric acid clearance ($r^2 = 0.77$) (Figure 4). Or there may be changes in

Figure 4. Creatinine vs. uric acid clearance. Linear regression analysis was used to determine the association between uric acid clearance and creatinine clearance ($r^2=0.77$).

the reabsorption of uric acid, given that the change in uric acid clearance is greater than the change in creatinine clearance uric acid (39% decrease vs. 19% decrease), and uric acid is known to be highly reabsorbed by multiple transporters. The discrepancy between the decreases in creatinine clearance and uric acid clearance indicate that there is an increase in uric acid reabsorption. If uric acid reabsorption was saturated before weight loss, decreasing the filtration rate of uric acid would effectively increase the percentage of uric acid that is reabsorbed, and this would compound the change in GFR to generate a greater change in uric acid clearance. Therefore, future studies should continue to measure uric acid clearance as changes in creatinine clearance underestimate the changes in uric acid clearance due to the reabsorption of uric acid.

Overall, the decreased uric acid production and decreased elimination resulted in no net change in plasma uric acid levels, which would potentially explain the lack of change in blood pressure after weight loss. Having no net change in plasma uric acid was surprising, as participants in the current study had very high levels of plasma uric acid (mean of 8.5 mg/dL at baseline) that would be considered hyperuricemic even in adults (cutoff values of hyperuricemia are 5.8 mg/dL in women and 6.8 mg/dL in men). Hyperuricemia persisted even after weight loss, which would indicate that the degree of weight reduction was insufficient to lower cardiovascular risk by affecting either plasma uric acid or blood pressure. This high level of uric acid in our subjects may lead to eventual damage of the kidneys via RAS activation and sodium-dependent hypertension in the future.

Early intervention and hypertreatment of hyperuricemia before sodium-dependent hypertension is established may be a future therapeutic target in adolescents with severe obesity, as treatment of hyperuricemia in adults has been shown to not be as effective of a measure to treat hyper-

tension compared to children (3.3 mm Hg decrease in SBP vs. 6.9 mm Hg decrease in SBP).^{19,22}

Limitations of this study were lack of a control group and the modest degree of weight reduction (3.7%) experienced by participants, since it is unknown if greater degrees of weight loss via this method would lead to significant changes in plasma uric acid and blood pressure. Blood pressure at baseline was also slightly elevated, but most were not classified as hypertensive (≥ 95 th percentile), and this may have been a contributing factor to why we did not observe a significant change in blood pressure with weight loss. Future studies should employ interventions associated with greater weight loss in a population with higher levels of blood pressure and compare LPS levels with XO activity to determine if decreased LPS could be responsible for changes in XO activity due to weight loss.

In conclusion, results of this study demonstrate that modest weight loss reduces XO activity in adolescents with severe obesity. Despite this finding, there was no net change in uric acid levels and subsequently no meaningful change in blood pressure. Taken together, these results highlight the fact that the relationship between XO, uric acid, and blood pressure in obesity is complex and remains to be fully elucidated.

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Author Disclosure Statement

AK serves on pediatric obesity advisory committees for Novo Nordisk Pharmaceuticals and Takeda Pharmaceuticals but does not accept personal or professional income for his services. The other authors have no relevant disclosures.

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