Uric Acid Levels in Tissues and Plasma of Mice during Aging

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Uric acid (UA) is the end product of purine metabolism in primates. In mammals, the liver is generally considered to be the primary site for the synthesis and degradation of UA. In that process, xanthine oxidase makes UA from xanthine and hypoxanthine which are products of adenine- and guanine-based purine breakdown. Xanthine oxidase is a large enzyme whose active site consists of the metal, molybdenum, bound to sulfur and oxygen. Uricase is a copper-containing oxidase responsible for the hydrolysis of UA to allantoin. In humans, the enzymatic catabolism of adenine- and guanine-based purines proceeds only as far as UA, because uricase mutated in hominoids during primate evolution. Two nonsense mutations at codon positions 33 and 187 and an aberrant splice site were found in the human gene. The loss of uricase in higher primates parallels a similar loss of the ability to synthesize ascorbic acid, leading to the suggestion that UA may partially substitute for ascorbic acid in such species, because UA is known to functions as a strong scavenger of peroxyl radicals, hydroxyl radicals, singlet oxygen, peroxynitrite anion, and peroxynitrite-derived intermediates. In addition to their potency as antioxidants, both UA and ascorbic acid are strong reducing agents (electron donors). In contrast, most mammals including mice possess uricase activity and excrete allantoin and urea as the major nitrogen-containing degradatives of purines. Consequently, the plasma levels of UA encountered in these species are far less than those in humans. In fact, UA content in the sera of rats is only $65\mu M$ and in the plasma of dogs is $20\mu M$, although the UA levels in plasma vary widely among individual humans and, on average, are somewhat higher in males than in females (i.e., $370\mu M$). Because of a lack of uricase activity, humans are likely to develop hyperuricemia and hyperuricosuria as a consequence of ingesting excessive amounts of purine gluten. Hyperuricemia is also a causative agent of hypertension, cardiovascular disease, and gout. Gout is a disease that affects mainly males, but with a clearly increased prevalence in both genders with aging, and is caused by the tissue deposition of UA crystals that elicit an intense self-limiting inflammatory reaction. In gouty patients, the bladder and kidneys have an almost a linear increase in UA stone formation based upon the degree of hyperuricosuria. At levels of UA excretion above 1000 mg/day, about 50% of these patients develop a UA stone. However, no UA stones have been observed in animal species that have uricase activity, as confirmed in lions, tigers, horses, cows, rabbits, dogs, cats, pigs, and rats. Here, we extended that study to mice in which UA levels were quantified in multiple tissues and in plasma as the animals aged. The results verified substantial amounts of UA in all of the 12 sites tested; furthermore, those levels increased in most tissues during aging.

Key words aging; uric acid; uricase; mouse tissue

Materials and Methods

Animals Male mice of the C57BL/6 strain at 3, 6, 12, 24, and 30 months of age were obtained from the animal facility at Tokyo Metropolitan Institute of Gerontology (Tokyo, Japan). All mice were fed a CRF-1 solid diet (Oriental Yeast, Tokyo, Japan). Mice were maintained on a 12-h light/dark cycle in a controlled environment. All experimental procedures using laboratory animals were approved by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Gerontology.

Preparation of Tissues and Plasma Mice were sacrificed, and their blood was collected from the inferior vena cava. Blood was gently mixed with ethylenediaminetetraacetic acid (EDTA) and centrifuged at $880\times g$ for 15 min at 4°C. The resulting supernatants were used as plasma for further analysis. Afterward, mice were perfused systemically with ice-cold phosphate buffered saline through the left ventricle to wash out the remaining blood cells. Tissues of interest were collected and stored at $-80°C$ until use.

Measurement of UA Level UA levels in tissues and plasma were measured by using high-performance liquid chromatography (HPLC) and an electrochemical detector (ECD). Tissues were homogenized in 1 mol of 5.4% metaphosphate (MPA) containing 1 mM EDTA (MPA/EDTA) and centrifuged at 21,000 $\times g$ for 10 min at 4°C. Plasma was mixed separately with equal volumes of 10% MPA/EDTA and centrifuged at 21,000 $\times g$ for 10 min at 4°C. Samples were analyzed by HPLC using an Atlantis dC18 5 μm column (4.6×150 mm; Nihon Waters, Tokyo, Japan). The mobile phase was 50 μl metaphosphate.

Here we quantified the uric acid (UA) levels in 11 tissues and plasma of C57BL/6 male mice to track its turnover during 3 to 30 months of aging. UA levels in the adrenal glands, heart, and spleen increased with aging until 30 months of age. Similarly, UA levels in the liver, kidneys, pancreas, and testes increased until the mice were 24 months old. UA levels also rose in the lungs and skeletal muscles from 3 to 6 months and 6 to 12 months, respectively, and then remained at almost the same levels until 30 months of age. In the skin, UA decreased from 3 to 6 months and then stayed nearly constant until 30 months of age. Moreover, the small intestines and plasma had quite stable UA levels during aging. Thus, our assessment of 11 tissue types from mice showed that the UA levels increased in most tissues during aging.

Received February 26, 2012; accepted May 25, 2012

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The authors declare no conflict of interest.
buffer (pH 2.8), 0.2 g/L of EDTA, 2% methanol at a flow rate of 1.3 mL/min, and electrical signals were recorded by using an ECD (2465, Nihon Waters) with a glassy carbon electrode at +0.6 V.

**Statistical Analysis** Results are expressed as means±S.E.M. The probability of statistical differences between experimental groups was determined by analysis of variance (ANOVA) or Student’s t-test as appropriate. One-way ANOVA followed by Tukey’s Honest Significant Difference (Tukey’s HSD) post-hoc and Student’s t-test were performed using PASW Statistics 18 software (SPSS, Inc., Chicago, IL, U.S.A.). Statistical differences were considered significant at p<0.05.

**RESULTS AND DISCUSSION**

For this analysis of UA levels in tissues and plasma, we first confirmed UA’s chromatographic separation profile. UA was dissolved in 5% MPA/EDTA and analyzed by HPLC. Although the retention times changed depending on column batch, flow speed, and consistency of the mobile phase, the UA peak was detected at 4.2 min of retention time (Fig. 1A). Moreover, no other peaks were detected at this retention time of UA in any tissues or plasma measured in this study. The UA standard curve represented a high correlation coefficient from 10 to 100 μM UA (Fig. 1B).

The source of all tissues and plasma used here to document UA levels during aging was male mice of the C57BL/6 strain obtained from the Tokyo Metropolitan Institute of Gerontology. The mean body weights of the mice, measured to assure normal growth, were 23.5±0.5, 30.5±1.1, 31.8±1.6, 30.1±1.6 and 28.3±0.9 g, respectively, at 3, 6, 12, 24 and 30 months. The mice increased in body weight a significant 105% from 3 months to 6 months and then remained constant from 6 to 30 months with no significant differences among monthly measurements. The quantity of UA rose in the skeletal muscles a significant 104% from 6 to 12 months and was then maintained at a constant point from 12 to 30 months. The small intestines held a consistent level of UA from 3 to 30 months without any significant variation. Unlike the other measurements, the skin had a decrease in UA, which was 26% lower at 6 months than at 3 months of age and then stabilized from 6 to 30 months. Finally, the plasma of these mice routinely contained approximately 50 μM of UA that never varied to a significant extent.

The foregoing evidence that UA levels in adrenal glands, heart, spleen, liver, kidneys, pancreas, and testes of mice increased with aging is the first comprehensive analysis of this type in mice. The increase of UA levels in most tissues of mice over time might relate to an age-related reduction of uricase activity in the liver. In fact, Périchon and Bourre reported that uricase activity decreased significantly in the livers of aged mice.

Wu et al. developed uricase knockout mice and showed that their uricase deficiency caused pronounced hyperuricemia and UA nephropathy so that more than half of these mutant mice died before 4 weeks of age. Thus, a complete lack of uricase in mice evidently led to death, although its absence in humans does not.

UA was analyzed by HPLC and ECD as described in Materials and Methods. (A) Typical chromatograms of UA. (B) UA standard curve from 10 to 100 μM UA.

![Fig. 1. Representative Chromatograms and Standard Curve of UA](image)
Furthermore, Vida et al.\(^7\) described significantly increased xanthine oxidase activity in the liver, cerebral cortex, and plasma from old mice compared with those samples from younger adults. This increase of xanthine oxidase activity with aging is considered to contribute to the age-dependent increase of UA level.

Tissue specific changes of UA levels with aging are thought to arise from differing extents of regulation governing intracellular UA levels by the high-capacity, low-affinity UA glucose transporter (GLUT) 9, the expression of which varies in each tissue.\(^18\text{--}^{23}\) GLUT9 exists as two alternatively spliced variants, GLUT9a and GLUT9b, which have different amino-terminal cytoplasmic tails.\(^20\text{,}^{22}\) In humans and mice, GLUT9b expression is restricted to the liver and kidney, whereas GLUT9a has a broad tissue distribution including the liver, kidney, intestine, heart, skeletal muscle, brain, leukocytes, and chondrocytes.\(^20\text{,}^{21}\) Therefore, GLUT9a and GLUT9b might contribute to the age-dependent increase of UA level, but confirmation is not yet clear.

In conclusion, we demonstrated here that the amount of UA in most tissues of mice, *i.e.*, the adrenal glands, heart, spleen, liver, kidneys, pancreas, and testes, clearly increased with aging. The cause might be an age-associated increase of xanthine oxidase activity and/or decrease of uricase activity in the liver.

**Acknowledgments** This study is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to A.I.). We thank Ms. P. Minick for the excellent English editorial assistance.

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