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The planetary biology of ascorbate and uric acid and their relationship with the epidemic of obesity and cardiovascular disease

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Summary Humans have relatively low plasma ascorbate levels and high serum uric acid levels compared to most mammals due to the presence of genetic mutations in L-gulonolactone oxidase and uricase, respectively. We review the major hypotheses for why these mutations may have occurred. In particular, we suggest that both mutations may have provided a survival advantage to early primates by helping maintain blood pressure during periods of dietary change and environmental stress. We further propose that these mutations have the inadvertent disadvantage of increasing our risk for hypertension and cardiovascular disease in today's society characterized by Western diet and increasing physical inactivity. Finally, we suggest that a ''planetary biology'' approach in which genetic changes are analyzed in relation to their biological action and historical context may provide the ideal approach towards understanding the biology of the past, present and future.

The reductionist paradigm for human biology has, over the past century, been remarkably successful. It has, in one sense, reached its apotheosis through the complete sequencing of the human genome [1]. This, together with analyses of the transcriptome, the metabolome, and their higher organization has provided a ''parts list'' for a living organism.

As these parts lists have become more complete, it has become increasingly clear that they do not provide anything approaching an understanding of human biology [2]. Additional approaches are needed if that understanding is to emerge and, with it, the opportunity to manipulate the biological parts to manage, treat, and cure human disease.

One approach is to exploit the axiom that a system can be fully understood only if we understand both its structure and its history. This is certainly true for the terrestrial living systems, which are the products of four billion years of random

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variation and natural selection, all constrained by physical and chemical law. Without understanding this history, we are no more likely to understand biology than we are to understand the QWERTY computer keyboard without knowing of the typewriter or the Federal Reserve banking system without knowledge of the Panic of 1896.

This has led us and others to suggest that systems analyses involving a wide field disciplines (including paleontology, paleoecology, evolutionary molecular biology), a process that has been termed planetary biology [3], may provide a better approach to understand how we evolved to our current state. This approach may also provide hypotheses into why humans have certain diseases that appear rare among other species, including obesity, essential hypertension, preeclampsia, cardiovascular disease, alcoholism, and other conditions. Perhaps most importantly, a planetary analysis may provide means for testing evolutionary hypotheses, by reconstructing the ancestral genes and testing how the various mutations may have affected survival given the ecological conditions at that time [4,5]. This approach may also be useful in understanding current genetic polymorphism in human populations that generates differential disease incidence and differential responses to therapies. Furthermore, by providing insight into active evolutionary processes, these analyses may also help predict the needs of our species in response to future changes in climate and ecology.

This paper concerns the differences between humans and other mammal species, with a focus on two key genetic differences. The first is the absence of the final enzyme (L-gulonolactone oxidase) in the pathway of vitamin C (ascorbate) biosynthesis. This enzyme was present in primitive primates, but was lost in the primate lineage leading to monkeys and apes in the Eocene (55-35 MYA). This generated a need for humans, monkeys, and other ''higher'' primates to obtain ascorbate from the diet.

The second is the absence of an active enzyme (urate oxidase, or uricase) involved in the degradation of purines. This arose through mutation of the gene encoding uricase in lineages leading to higher primates in the Miocene (5-23 MYA). This caused urate to be the final enzymatic endproduct in purine metabolism.

As a consequence of these mutations, the plasma concentrations of ascorbate and urate are quite different in humans and most other mammals. Interestingly, this means that two of the three most important water-soluble antioxidants in mammals (the other being glutathione) are different in humans and (for example), rats, an organism widely used in biomedical research to model human biology. We will discuss current hypotheses for why these mutations occurred, and how planetary biological studies might be able to provide a better understanding for the mechanisms leading to these evolutionary changes.

Ascorbate synthesis and its role as an antioxidant

Ascorbate (vitamin C) is synthesized in most mammals by an active five-enzyme process that begins with an activated form of glucose, UDP-glucose. UDP-glucose is present primarily in the liver or kidney, depending on species (Fig. 1). As such, its concentration is highly dependent on intracellular glucose reserves and glycogenolysis. Further, factors that reduce glucose reserves (starvation, low carbohydrate diets) or inhibit glycogenolysis (such as fructose) inhibit ascorbate synthesis [6].

Ascorbate has numerous biological functions, including important roles in the synthesis of collagen, creatine and catecholamine. However, one of its most important roles is to function as an electron donor, or antioxidant. Antioxidants are thought to play a key role in the protection of species by blocking lipid peroxidation, DNA damage and alkylation, and cell membrane injury [7,8].

An interesting feature of ascorbate is that it is recyclable [9]. Specifically, when ascorbate reacts with an oxidant, it is oxidized to the semidehydroascorbate radical and then dehydroascorbate. While some dehydroascorbate may be catabolized to various end products, dehydroascorbate can also be reduced back to ascorbate by reaction with glutathione or via an enzymatic pathway [6].

Ascorbate and dehydroascorbate enter cells via different transporters, with the ascorbate transporter being specific Na^+ dependent co-transporters (SVCT1 and SCVT2) [10] and the dehydroascorbate transporter using members of the Glut family. Once transported, dehydroascorbate is reduced immediately into ascorbate [11]. The ascorbate transport system allows intracellular ascorbate levels to be in the range of 2—4 mM despite plasma levels that are significantly lower (40—120 μM) [10]. Interestingly, when some cells, such as human neutrophils are activated by a variety of stimuli, intracellular concentration of ascorbate increases even more and can reach 14 mM, while its extracellular level remains unchanged [12].

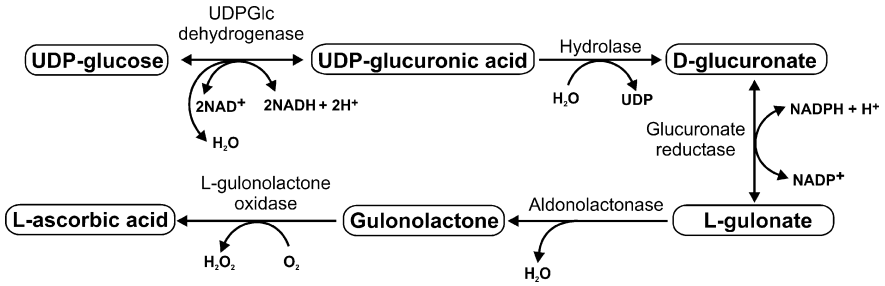

Figure 1 Ascorbate metabolism.

Ascorbate is produced by many fish species, lobe-finned fish (lungfish and the coelacanth), amphibians, and other terrestrial vertebrates. Biosynthesis of ascorbate occurs primarily in the liver (most mammals and some birds) or kidney (amphibians and reptiles, some fish, and some birds) [13]. However, most primates lack the ability to make ascorbate due to a mutation in L-gulonolactone oxidase, which is the final enzyme involved in ascorbate synthesis [14]. The inability to synthesize ascorbate is observed among all primates except the prosimians (with the possible exception of *Tarsius* [15]) suggesting that the mutation occurred during the Eocene between 55 and 35 million years ago [14,16]. A variety of other species also lack the ability to make ascorbate; these include the Indian fruit-eating bat, the guinea pig, the Indian red vented bul—bul bird, and some species of fish [17,18].

One of the consequences of the loss of ascorbate synthesis is the disease scurvy, which was first recognized to be due to a dietary deficiency of fruits by James Lind [19]. It was nearly two centuries later when vitamin C (ascorbate) was identified as the critical nutrient for which a deficiency was responsible for the disease [20]. Today, scurvy is rare, and plasma ascorbate levels are generally in the range of 40—120 μM, which are well above levels associated with this disease (typically <10 μM). These blood levels are two to four times lower than that observed in mammals that synthesize ascorbate [21]. Although the missing L-gulonolactone oxidase in humans might be a reason for somewhat lower concentration of ascorbate in the blood, its concentration in other biological fluids [22] and especially intracellular levels are much higher and in most cases comparable with the mammals that synthesize ascorbic acid [12,13,23,24].

Uric acid and the mutation of uricase

Uric acid is a metabolic product of purine metabolism generated from the breakdown of DNA, RNA and ATP (Fig. 2). The immediate precursor enzyme is xanthine oxidoreductase which converts xanthine to uric acid with the generation of oxidants (superoxide anion or hydrogen peroxide) or NADH. In some species uric acid is then metabolized to allantoin by the enzyme uricase (urate oxidase). Depending on the species, allantoin may be further degraded by allantoinase and allantoincase to generate ammonia.

Uric acid is also recognized as a water-soluble antioxidant and is considered to be one of the most important antioxidants in the plasma [25]. Uric acid can donate an electron to form the urate radical; but unlike ascorbate this radical is not recycled but rather is degraded via several different pathways. Uric acid can react with a variety of substances including hydrogen peroxide, hydroxyl radical, peroxynitrite, and nitric oxide [26—28]. For example, uric acid reacts with hydroxyl radical to form allantoin, with peroxynitrite to form triuret, and with nitric oxide to form 6-aminouracil (Fig. 3). While these reactions might be beneficial under certain conditions as a means for reducing oxidative stress, some of these reactions, such as the uric acid reaction with peroxynitrite, also produces radicals and alkylating species that may be damaging [27,29].

Uric acid has also been proposed to have neurostimulant properties based on its similarity in chemical structure with caffeine [30] and due to epidemiological and experimental studies suggesting it may have a role in increasing reaction time, locomotor activity, and mental performance [31—35].

Uric acid is also important in innate immune function. Specifically, uric acid may aid in the immune recognition of dying cells [36], help activate the inflammasome critical for interleukin-1 beta release [37], and in the immune rejection of tumor cells [38].

Most mammals have functional uricase, and have uric acid levels in the 1—2 mg/dl range (0.06—0.12 mM). In contrast, serum uric acid levels are higher in man and the Great and Lesser Apes due to parallel mutations of the uricase gene that

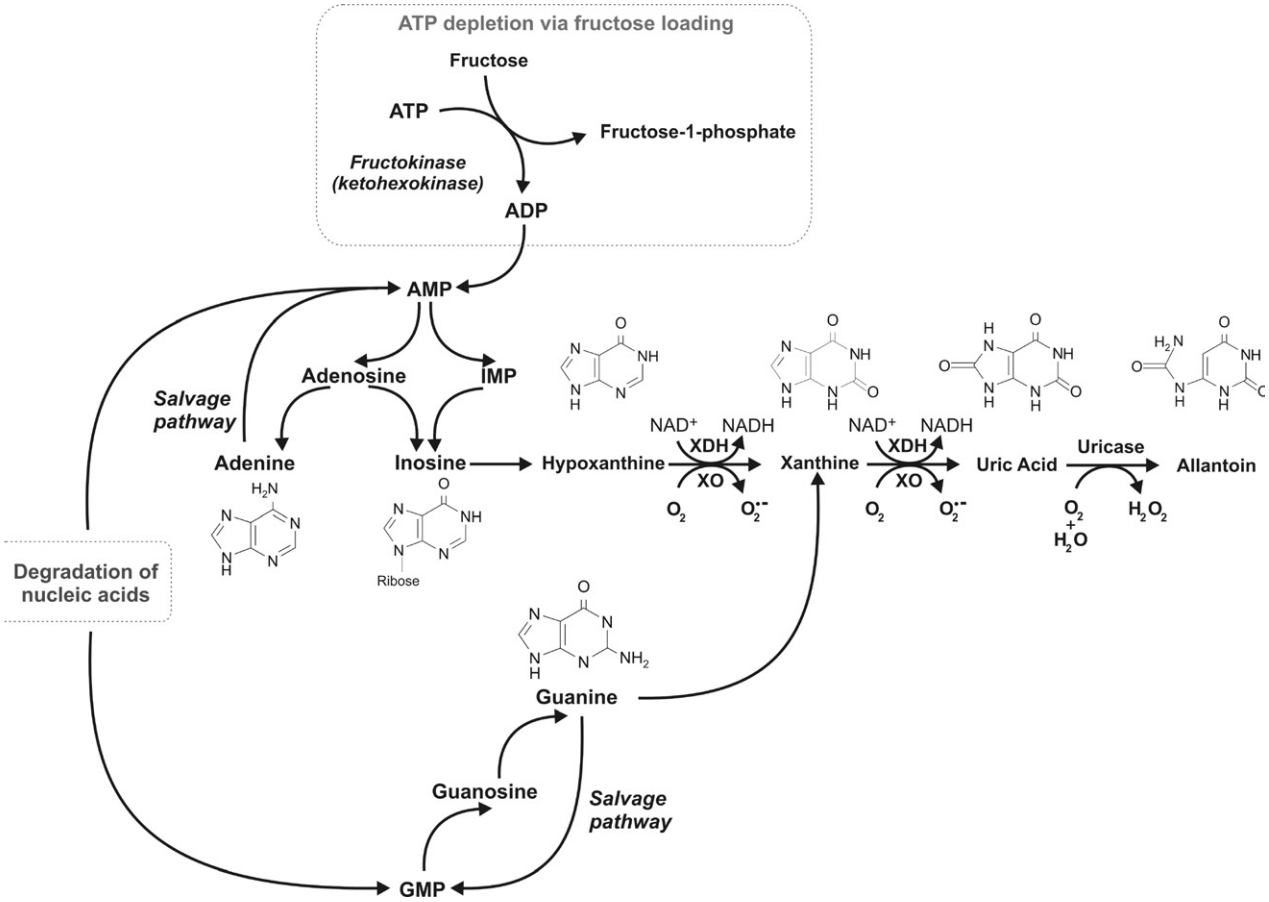

Figure 2 Uric acid metabolism.

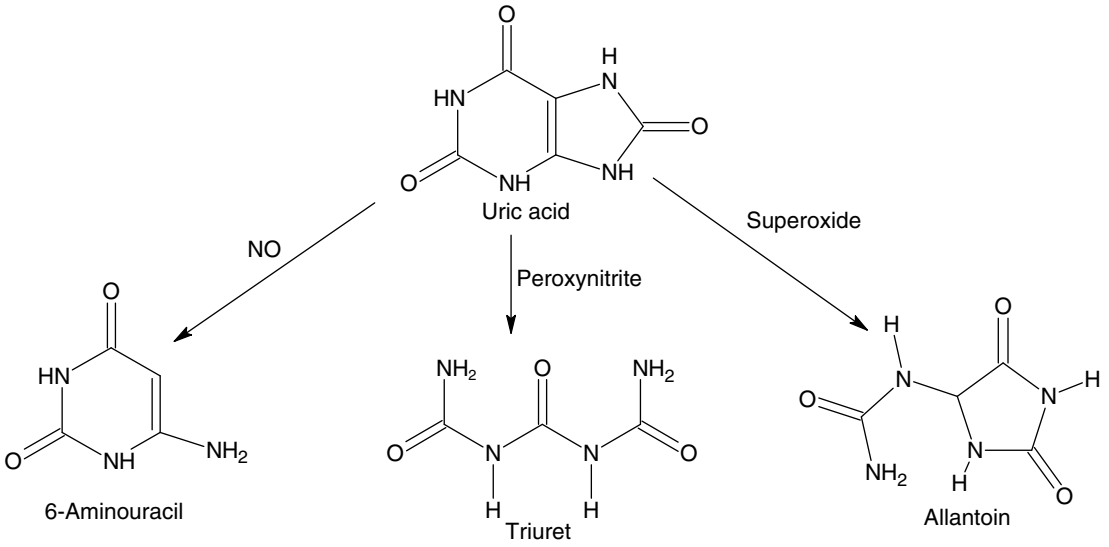

Figure 3 Non enzymatic pathways of urate degradation.

occurred during the mid Miocene [39] (Fig. 4). Uricase activity is also functionally absent or immunologically undetectable in certain New World monkeys (such as the woolly monkey (*Lagothrix*) and the macaque (*Cynomolgus*), consistent with a mutation of the uricase gene [40,41]. Uricase activity is also lower in both Old World and New World monkeys compared to other mammals [42,43].

Recent studies suggest that this may be due to mutations in the promoter regions [44]. Indeed, evidence now suggests that the loss of uricase in humans may have been stepwise, with a progressive loss in activity (due to mutations in the promoter region) followed by complete silencing of the gene [44].

Why did early primates lose their ability to synthesize ascorbate?

Several hypotheses have been proposed to account for the loss of ascorbate synthesis by early primates. Pauling suggested that the loss of ascorbate synthesis resulted as a consequence of the lack of need, as early primates may have had ample access to dietary ascorbate from root tips, seed sprouts, fruits and green leaves [45]. However, Darwinian theory suggests that there was likely a positive selection mechanism that made it advantageous to lack ascorbate synthesis; otherwise, both polymorphisms would have been predicted to survive. In this regard, the enzyme L-gulonolactone oxidase generates not only ascorbate but also hydrogen peroxide (Fig. 1), and therefore is redox neutral, whereas the dietary intake of ascorbate would increase antioxidant activity. Thus, Banhegyi et al have proposed there was a selection advantage to supplying ascorbate stores primarily by diet [6].

An alternative hypothesis has been proposed by Challem [46] and Challem and Taylor [47]. These authors have reviewed evidence that a primate retrovirus may have inserted *Alu* elements in the gene for L-gulonolactone oxidase that silenced the gene. While the loss of ascorbate may have been beneficial for the survival of the retrovirus, it may have also led to increased levels of free radicals that could increase the frequency of free radical-induced mutations that could help accelerate the evolution of these early primates [46—48].

What was the advantage to having uricase mutated?

The observation that parallel mutations involving uricase occurred in early hominoid evolution strongly suggests that there must have also been a selection advantage during the Miocene to having higher serum uric acid levels.

The most quoted hypothesis is that originally proposed by Proctor [49] and later Ames [25] who suggested that the uricase mutation may have occurred as a means to replace serum antioxidant activity after the loss of ascorbate synthesis. Indeed, uric acid can help maintain ascorbate levels [50]. Furthermore, the activity of other antioxidant systems, such as superoxide dismutase, are also higher in species lacking ascorbate [51]. Ames has suggested that the reason humans have longer longevity compared to other mammals may relate to the antioxidant benefits provided by the higher uric acid levels [25]. Challenging this hypothesis,

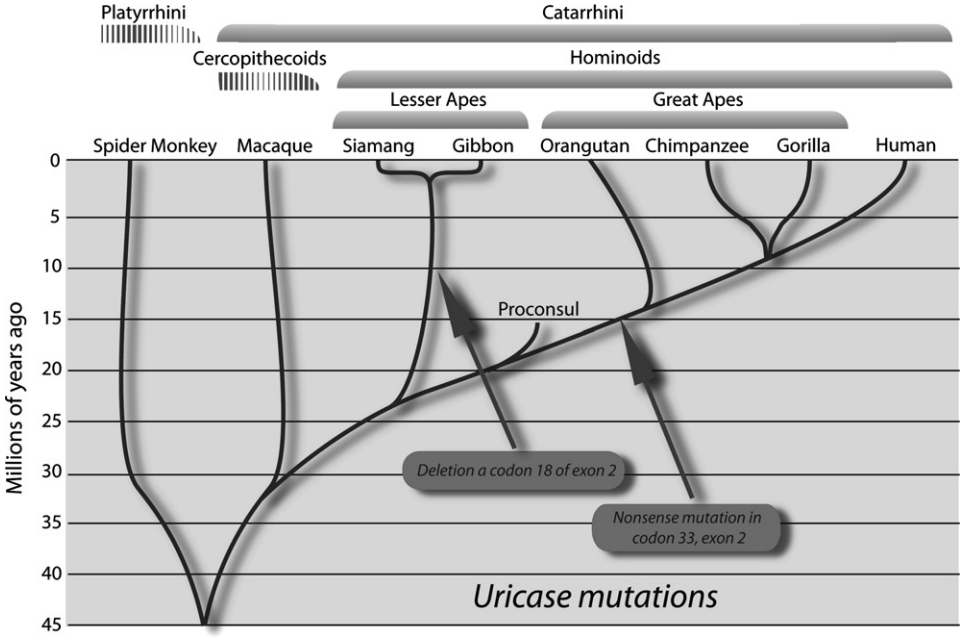

Figure 4 Uricase mutation in early hominoid evolution.

however, is the observation that neither ascorbate or urate levels correlate with maximum life span in vertebrates [52].

Another hypothesis is that the increase in uric acid resulted in better reaction time and higher mental performance that helped accelerate the evolution of man [30]. While some epidemiological studies have supported this, the evidence has been weak at best. Alternative hypotheses could include the possibility that an increase in uric acid might improve innate immune function and the ability to ward off infections or tumors [36–38].

An alternative hypothesis: the loss of ascorbate and rise in uric acid had a role in maintaining blood pressure during periods of environmental stress

Ascorbate and blood pressure

The inability to synthesize ascorbate would have forced early primates to ingest a vitamin C-rich diet to maintain their ascorbate stores. The gorilla, who lacks the ability to synthesize Vitamin C, eats about 4.5 g of ascorbate per day [53]. Pauling has calculated, based on synthetic rates in other mammals, that the average 70 kg human would have to ingest 1.8–4.1 g/d ascorbate to achieve similar blood levels [45].

What would happen if suddenly a change in climate occurred that resulted in less available dietary ascorbate? Serum ascorbate levels would fall, resulting in less protection from oxidative stress. Furthermore, low concentrations of ascorbate, particularly in the presence of catalytic metals such as copper or iron, can become pro-oxidative [54], and examples have been found where ascorbate increases rather than decreases intracellular oxidative stress [55].

In turn, oxidative stress has been shown to raise blood pressure [56]. For example, the depletion of glutathione in rats rapidly results in a rise in blood pressure [57]. Numerous animal models have shown a key role for oxidative stress (present in the circulation, blood vessels, and kidney) in mediating hypertension [56]. Ascorbate administration also lowers blood pressure in many hypertensive animal models [58–60].

Epidemiologic studies have confirmed a strong inverse relationship between serum ascorbate levels and blood pressure [61–63]. Plasma levels of ascorbate are lower in hypertensive subjects (mean 40 μmol/L) compared to normotensive controls (mean 57 μmol/L) [64]. Plasma ascorbate levels are also lower in African–Americans who have higher rates of hypertension compared to whites [65] and in other hypertensive conditions, such as preeclampsia [66].

Vitamin C supplementation in patients with hypertension have also reported a reduction in blood pressure in some studies [67–69] but was either negative or less effective in other studies [70,71]. One potential explanation is that the effect of vitamin C on blood pressure appears to be more effective in the studies using younger subjects as opposed to studies that enrolled elderly subjects with longstanding hypertension, possibly because in later stages of hypertension the kidney is the primary driving force [72]. In one recent study of young subjects (age 30–59), a strong effect was observed with ascorbate levels and blood pressure after either taking a vitamin C deplete diet or following supplementation [68].

Given these considerations, it is tempting to speculate that the mutation in ascorbate synthesis occurred during a time of high vitamin C intake and was partially advantageous since dietary intake did not require the generation of oxidants that occurs with its synthesis. However, if a climatologic change resulted in famine or starvation, those primates that could still make vitamin C would decrease their synthesis (see above) with potential advantage of stimulating oxidative stress and raising blood pressure; but those who could not synthesize ascorbate at all might develop more severe oxidative stress and higher blood pressures that might have provided a superior survival advantage for that time.

Uric acid and blood pressure

We have recently proposed a hypothesis for how a mutation in uricase might provide a survival advantage by raising blood pressure [73]. Specifically, there is evidence that during the early Miocene there was a marked increase in ape species [74]. However, by the mid Miocene there was global cooling ('the Miocene Disruption') associated with the extinction of numerous species, likely including many species of apes. During this period large areas of rain forests dried out, leaving savannahs and grasslands, and forcing early hominoids to develop knuckle walking and change their diet. The Paleolithic diet was low in sodium [75], and hence survival would be optimized by those species that could maintain blood pressure and salt sensitivity.

Interestingly, while uric acid can function as an antioxidant, it can also function as a pro-oxidant on a variety of cell types and in vivo [27,29]. Indeed,

inhibiting uricase in rats results in a rise in blood pressure associated with systemic reduction in endothelial nitric oxide and a stimulation of the renin angiotensin system [76,77]. Over time uric acid causes microvascular disease in the kidneys due to direct effects of uric acid on vascular smooth muscle cells and endothelial cells [78–81]. Once these changes occur the kidney preferentially holds onto salt (development of salt-sensitivity) [73].

Evidence that uric acid is involved in blood pressure in humans is equally compelling. There are now extensive studies showing that a high uric acid independently predicts the development of hypertension [82–84]; likewise, an elevated uric acid is common in early hypertension and was present in almost 90% of hypertensive adolescents in one study [85]. Furthermore, recent clinical trials have found that lowering uric acid lowers blood pressure in both adolescents and adults with hypertension (Feig and Johnson, unpublished) [86,87].

In addition to raising blood pressure, recent studies support uric acid as having a role in insulin resistance and obesity [88,89]. Indeed, fructose, which rapidly raises uric acid, induces metabolic syndrome in animals and this can be ameliorated by lowering uric acid [88]. The mechanism by which uric acid mediates features of the metabolic syndrome is likely due to the ability of uric acid to block some of insulin's actions by reducing endothelial nitric oxide as well as due to direct effects of uric acid on the adipocyte [76,90].

Thus, the uricase mutation may have conferred a survival advantage by helping to raise blood pressure, stimulate salt-sensitivity, and induce insulin resistance and mild obesity, and thereby help promote survival during a period of famine or stress.

How could a mutation that was a survival advantage in the Miocene now be playing a role in the cardiovascular epidemic? The consequence of the uricase mutation is that humans not only have higher uric acid levels than most other mammals but they also can not regulate levels as effectively [91,92]. Interestingly, because the current Western diet is high in meats and fructose, both which generate uric acid, humans today have higher uric acid levels (range 4–10 mg/dl) compared to primates that lack uricase (where uric acid levels are typically in the 3–4 mg/dl range) [92]. Our preliminary studies in Yanomamo Indians living in their original habitat and with their primitive diet found serum uric acid levels in the 2–4 mg/dl range, suggesting that primitive humans had lower uric acid levels than today (Oliver and Johnson, preliminary data). Thus, in today's society we are ingesting significantly more sweeteners (containing fructose) and meats (containing purines) such that those who obtain the highest uric acid levels develop high blood pressure (hypertension), insulin resistance and obesity, and possibly diabetes and cardiovascular disease.

Planetary biology and the approach to the uricase/ascorbate hypotheses

How can planetary biology help determine which hypothesis is correct? By pinpointing the date of the mutations and cross checking with the paleoecological and anthropological record, we can evaluate the effects of environment with the genetic changes.

Emerging tools in paleogenetics provide an experimental tool to test hypotheses that emerge from this correlative science [93]. In a paleogenetics experiment, the structures of ancestral genes are inferred from the sequences of their descendents; the ancestral genes from extinct organisms are then resurrected in the laboratory, where their behavior is studied. This allows us to evaluate their function under cell culture and in animal models using inferred ancestral environmental conditions.

Finally, paleogenetic evaluation of changes in up- and down-stream genes may allow additional testing of the hypothesis. For example, mice in which uricase has been knocked out do not survive due to a rapid rise in uric acid that leads to massive uricosuria, intrarenal crystal deposition, and acute renal failure [94]. Thus, there were likely changes in other genes that regulate uric acid production or excretion to prevent this complication in the primates that lost uricase. One possible change could have been a contemporaneous reduction in the rate of synthesis of uric acid.

Consistent with this possibility, humans have a 100-fold lower activity of xanthine oxidase than rodents [95]. This appears to be the result of a loss of transcription and core promoter activity for the gene encoding that enzyme [96]. In some New World primates, the level of serum uric acid is also not elevated due to enhanced mechanisms of urinary excretion [40]. Thus, identifying the temporal sequence for changes in xanthine oxidase and in renal urate transport may provide clues to the sequence by which the changes in gene expression occurred as it relates to the paleoecological and paleontological record.

Likewise, the mutation of ascorbate synthesis did not change dramatically the intracellular levels of ascorbate, even as it evidently lowered the serum levels of ascorbate. This suggests that changes in the management of ascorbate also occurred in

more ancient primate lineages contemporary with the loss of ascorbate biosynthesis. An obvious system where compensatory changes might have been effective is in the ascorbate transporters.

These considerations suggest a historical paradigm for future research that should complement in this century the reductionist paradigm of the last. It is partly correlative science; interconnecting the evolutionary histories of related genes should provide hypotheses to interpret of the evolutionary biology of the biomolecular system. Paleogenetic resurrections will provide experimental tests of those hypothese. Animal model studies will provide orthogonal tests, completing the connection between the biomolecules, natural history, and physiology.

Disclaimers

Dr. Johnson is listed as an inventor on several patent applications related to lowering of uric acid in cardiovascular disease and also is author on a book on fructose that will be published in 2008 by Rodale press. Dr. Benner and Dr. Gaucher are listed as inventors on several patent applications to apply evolutionary analysis to understand protein evolution.

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