COMMENTARY

Constant molecular aging rates vs. the exponential acceleration of mortality

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Laëtitia Gorisse, Philippe Gillery, and their coworkers (1) at the University of Reims elegantly document carbamylation as a biochemical change of aging in long-lived proteins that accumulates progressively across the life span. In human skin samples, the main carbamylation product, homocitruline (HCit), shows intriguing linear increases across the life span, with faster accumulation in two shorter lived species. Carbamylation thus joins other markers of protein oxidation and DNA methylation that accumulate at constant rates throughout the adult life span.

A key question is how to connect such invariant aging processes to the exponential rates of accelerating mortality that set life spans. This apparent divergence points to gaps in understanding the relationships of constant rates of molecular aging to the individual health span and the nonlinear increases of morbidity during aging. Although we can readily assess molecular aging, such biomarkers of aging are rarely robust as predictors of individual morbidity and mortality risk in populations. Also noted is the need to test the generality of carbamylation further as a biomarker in species with very slow senescence.

Molecular Aging, Morbidity, and Mortality

Collagen and elastin are very long-lived proteins in skin and blood vessels: Being formed early in life and with very slow turnover, they become progressively modified by biochemical processes that are embedded in our metabolism. Best recognized is glycoxidation, in which our essential fuel glucose chemically attacks the ε-amino group of Lys residues of proteins. Further complex chemistry leads to the formation of advanced glycation end products (AGEs), which accumulate as a linear function of age in connective tissues of humans and rodents, commonly assayed as carboxymethyllysine (CML) and glucosepane, a cross-linker. Now, Gorisse et al. (1) show that the ε-amino group of Lys is also modified by carbamylation from biochemical pathways distinct from glycoxidation that are readily assayed as HCit.

The carbamylation of ε-amino Lys came to light 55 y ago at the Rockefeller Institute, where George Stark of the famed Moore and Stein laboratory showed that isocyanate formed at equilibrium with urea could generate the HCit studied here (2). This equilibrium was the basis for Friederich Wöhler's synthesis of urea in 1828, which was revolutionary as the first artificially synthesized organic compound. Carbamylation soon acquired clinical significance with findings that treatment of patients with the sickle-cell trait with urea to decrease the aggregation of hemoglobin-S also caused cataracts by carbamylation of lens proteins.

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The present analysis of skin collagen shows progressive carbamylation during aging at rates that varied inversely with life span in three species: mouse, bovine, and human. AGE products were also assayed in these samples at specific Lys residues, showing that HCit was several fold more abundant than CML, in human, bovine, and mouse. In old age, HCit and CML together may occupy up to half of the total collagen Lys.

In addition to its accumulation in skin collagen and lens crystallines, carbamylation is detected in arterial atheromas (plaques), and during chronic kidney disease, it is associated with endothelial dysfunctions from elevated blood urea (3). Importantly, there is no evidence that carbamylation promotes cross-linking of collagen and elastin, unlike glycoxidation. Although sagging skin is a mere bane of human aging, declining arterial elasticity has mortal sequelae from increasing blood pressure.

HCit and CML give us two biomarkers of aging that that progressively accumulate in connective tissue collagen through inevitable exposure to ubiquitous circulating metabolites, urea and glucose, respectively. Gorisse et al. (1) show that HCit increased linearly with age in skin samples from 25 healthy individuals, aged 1–98 y, and that levels of HCit were consistently higher than CML after the age of 20 y. However, both

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See companion article on page 1191.

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biomarkers record not only normative exposure, but also pathophysiological excesses that commonly increase during aging: carbamylation from the uremia, cyanate of chronic kidney disease, and glycoxidation from the glycemia of obesity and diabetes. The benchmark studies of AGE by Sell et al. (4, 5) also show progressive accumulations of AGE products in human skin samples, but with slight curvilinear trends. Because clinical diabetics have up to twofold higher AGE, we suggest that the upswing of AGE accumulation at later ages may represent obesity and diabetes, as well as trends for subclinical glucose dysregulation at later ages. Importantly, end-stage kidney disease, which increases skin collagen carbamylation (discussed above), did not significantly alter AGE. We anticipate that larger samples of skin collagen will show some curvilinearity in HCit accumulation at later ages due to the increasing prevalence of kidney dysfunctions.

DNA methylation also merits our consideration. This recently discovered biomarker of aging also accumulates linearly with age and through processes apparently distinct from protein glycoxidation and carbamylation. Assayed in white blood cells of hundreds of individuals in independent studies, global DNA methylation shows surprisingly linear increases across the life span (6, 7). Moreover, the rate was faster for men than for women, in accord with sex differences in life expectancy (6). Brain and other tissues also show linear increases of DNA methylation (7). Moreover, during treatment of type 2 diabetes, skeletal muscle DNA methylation responded to exercise and to blood glucose variations (8). Intriguingly, the methylation of genes implicated in diabetes also showed correlations with glycated hemoglobin (HbA1c), a short-term marker for blood glucose exposure.

How then can we relate these biomarkers of aging to life expectancy? We see a major gap between the linear changes accumulated in long-lived macromolecules and the exponential increases of morbidity and mortality. First, we must consider sample source and numbers. The skin samples typically have <100 specimens, whereas for DNA methylation, samples are much larger and, in at least in one instance, population-based (7). Looming orders of magnitude larger are the national population data for morbidity and mortality. Critically, these small skin samples underrepresent the diversity of aging populations for preclinical and clinical grade dysfunctions, which tend to elevate blood glucose and urea in association with dysregulation of glucose-insulin and kidney functions, respectively. In a large case-cohort study of diabetics, plasma levels of AGE and its soluble receptor (sRAGE) predicted worsening kidney function and all-cause mortality, with hazard ratios of 1.1–1.2 (9). Importantly, the hazard ratio increases were linear across tertiles of AGE and sRAGE.

Exponentially accelerating mortality remains one of the strongest generalizations of human aging. As described by the Gompertz model, the exponential increase of mortality with age prevails in all human populations living in all circumstances, and has for several centuries (10–12). Underlying the acceleration of mortality are accumulating diseases and dysfunction, collectively known as morbidities, which represent still obscure processes. The major 20th century improvements of mortality across the life span from improved medicine, public health, and nutrition have not altered the exponential force of mortality at late ages. In fact, later age mortality rates are accelerating faster, because the basal mortality level ("minimum mortality") from which the Gompertz exponential arises has been lowered, thereby delaying, but not reducing senescence at later ages (11–13). The mortality rate plateau that was once thought to be reached by the age of 90 y has been remapped to ages of 110–114 y (14), and thus does not pertain to the experience of the vast majority of aging humans (12, 13).

We may approach the protein and DNA biomarkers of aging from another perspective to clarify their relationship to what we call the "morbidity process," or the processes underlying the onset and intensity levels of health dysfunctions. Before mortality accelerations are well defined after the age of 40 y, the average individual already shows preclinical changes in many physiological parameters that may, or may not be, on pathways to disease and disability. There is little evidence that these preclinical changes proceed in an exponential process. As one example, systolic blood pressure increases linearly from young ages (15, 16), causing the majority to develop hypertension during their life span. There is thus a major gap between the linear biomarkers of aging and the current wall of mortality from exponentially accelerating mortality that sets the life expectancy and the observed maximum life span (12, 13). Biomarkers of aging should link not only to the age pattern of the health changes in understandable fashion but also to the diversity of human aging. Mortality rates show that men "age" faster than women, African-Americans age faster than US whites, and people of lower educational and economic status age faster than people of higher status. Further biomarkers of aging may give needed insights for the biological causes and the timing of this diversity.

Questions for the Future

Looking ahead, we anticipate valuable further findings on carbamylation for sex and species differences and for experimental manipulations of life span, using these reliable and convenient assays for HCit in skin samples. For example, how do age slopes compare in species of naked mole-rats or primates with different life spans? Levels of protein carbonylation (chemically distinct from carbamylation and glycoxidation) were surprisingly high in naked mole-rats, despite their 30-y life span (17). Species differences in carbamylation could also covary with levels of blood urea, which Gorisse et al. (1) show to be higher in mice than humans, with correspondingly greater increases of HCit during the short mouse life span. Could the absence of hypertension in aging mice of most genotypes be due to their greater arterial carbamylation, which might compete with the AGE products associated with human arterial stiffening? Lastly, it will be interesting to know if genetic manipulations of life span in mice also alter the rate of carbamylation.

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