See Spot age: a lifelong dog metabonomic study

In 1987, scientists at Nestlé Purina embarked on a landmark study of 48 Labrador retrievers to determine the impact of caloric restriction on canine health and life span. The 14-year Purina Life Span Study found that dogs fed 25% fewer calories than matched littermates during their lifetimes lived 2 years longer and showed reduced signs of aging (J. Am. Vet. Med. Assoc. 2002, 220, 1315–1320). Because the scientists carefully preserved biological samples from the dogs at various stages of their lives, future researchers could reexamine the samples at a later date and exploit new technological innovations. In this issue of JPR (pp 1846–1854), Jeremy K. Nicholson and colleagues at Imperial College London collaborated with scientists at Nestlé Research Centers in Switzerland and Missouri to analyze the metabolic effects of aging and dietary restriction in dogs.

Nicholson and co-workers conducted a metabonomic investigation by performing $^1$H-NMR analyses on urine samples from dogs in the study. Metabonomics is the investigation of the multivariate metabolic response of an organism to a stressor or intervention, in this case aging or dietary restriction. “Our study was entirely retrospective,” says Nicholson. “It would have been impossible to analyze the samples in real time because the analytical technology has changed dramatically over the past 15–20 years. One of the great things about metabonomics is that if samples have been well preserved, you can go back in time and revisit great experiments that were conducted for other reasons.” According to Nicholson, retrospective studies such as these are impossible for transcriptomics and very difficult for proteomics, because RNA and proteins are not as stable as metabolites over long periods of time.

$^1$H-NMR analysis of urine samples provided metabolic signatures of several hundred urinary metabolites for each dog at each time point. Because the spectral data were complex, a series of multivariate statistical analyses was applied to detect global metabolic differences between groups of dogs. The researchers detected a rapid shift in urinary metabolic profiles in dogs <1 year of age, a stabilization between 1 and 2 years, a second metabolic shift between 5 and 9 years, and a third shift after 10 years of age. The metabolic profiles of the dietary-restriction (DR) dogs followed the same global trajectory as the control-feeding (CF) dogs, but differences in the detailed biochemical profiles were detected.

Analysis of $^1$H-NMR spectra identified several urinary metabolites that varied with age. The concentration of creatinine, a breakdown product of the energy-storage molecule creatine phosphate, increased until 5–9 years of age and then decreased. Because creatinine is produced by skeletal muscle, changes in urinary creatinine concentration likely reflect age-related changes in lean-muscle mass. Increased excretion of mixed glycoproteins, probably due to growth processes and changes in renal function, was observed for young dogs. In addition, the concentrations of hippurate and 3-hydroxyphenylpro- pionic acid (HPPA) were increased in 1.5-year-old dogs. Urinary excretion of hippurate and 3-HPPA is modulated by microbes in the colon; therefore, fluctuations in these metabolites might indicate age-related changes in gut microbiota.

Although aging exerted a greater effect on the global metabolic profile than dietary restriction did, significant metabolic differences were observed between CF and DR dogs. DR dogs had reduced levels of metabolites associated with energy metabolism; this finding indicates a decreased expenditure of energy. A reduced metabolic rate may lead to attenuated oxidative damage, possibly explaining the delayed aging observed in DR dogs. Furthermore, DR dogs had increased urinary concentrations of aromatic metabolites such as hippurate and 4-HPPA. This finding suggests that, like aging, dietary restriction modifies the composition or functional activities of gut microbiota.

According to Nicholson, changes in gut microflora with aging and dietary restriction warrant further study. Microbes in the colon are thought to affect the bioavailability of calories, fiber processing, and the processing of fat in the liver, as well as the possible production of toxins that damage the liver and central nervous system. Nicholson says, “Very little is known about the role of gut microflora in longevity and quality-of-life responses to diet restriction in animals and man. All we know is that the activity of gut microbes is probably very important in a wide range of diseases with systemic metabolic components.”

Nicholson stresses the importance of the collaboration between his lab and Nestlé Purina scientists. “In many ways, this is an amazing study because of its boldness and scale—it could not be done in a university department, because of the huge resources and costs required for a 14-year, 48-dog study,” he says. “Furthermore, these studies cannot be done on humans under such controlled conditions, so information from dog studies may be of great value to understanding human metabolic variations.” In this way, the dog can truly be considered man’s best friend.

—Laura Cassiday