The Modulation of Lifespan by Perceptual Systems

Scott D. Pletcher

University of Michigan Geriatrics Center, Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan, USA

Could the mere perception of food availability reverse the beneficial effects of diet restriction, which robustly increases lifespan and reduces aging-related disease in many species? We had noted from previous work in *Drosophila* that the effects of dietary restriction were fast acting, completely reversible, and largely independent of the energetic content of the food. Indeed, some characteristic of the diet seemed to be "sensed" by the flies independent of their tendency to eat it. We quickly realized that the *Drosophila* olfactory system, with its well-understood architecture and associated set of genetic tools, was the ideal model to test our hypothesis. Subsequent work resulted in the identification of particular odorants and populations of sensory neurons with potent effects on lifespan, obesity, and metabolism.

Key words: **lifespan; dietary restriction; odorants**

In the nervous system, ancient signaling pathways detect, decode, and relay environmental input to coordinate metabolism and growth. Evolutionary biologists have long studied the ability of environmental cues to stimulate alterations in life history patterns that can range from relatively minor adjustments in the distribution of lifetime reproduction to dramatic changes in the course of development itself. The benefit of such plasticity is clear. Variable environmental conditions challenge individuals to use external information and make calculated decisions, such as whether to wait out the bad times or begin reproduction, to maximize reproductive fitness.

Although sensory input can have dramatic consequences on many aspects of development and behavior,[1,2] remarkably little is known about the mechanisms that link sensory perception with physiological decisions in general, and with aging in particular. The most prominent work on aging has come from the Kenyon laboratory, using the nematode worm, *Caenorhabditis elegans*. Apfeld and Kenyon[3] have shown that neurosensory organs in the worm can regulate lifespan. Specifically, mutations that disrupt the structure and/or function of sensory neurons result in increased longevity, as does laser ablation of the two amphid sheath cells, which support the amphid neurons.[3]

We became interested in sensory perception and its effects on lifespan through the study of dietary restriction (DR). DR is a reduction in nutrient availability that robustly ameliorates aging-related disease in mammals and extends lifespan in many species. This phenomenon was first described over 70 years ago in rats,[4] and it has since been established as the most powerful modulator of aging and aging-related disease in mammals.[5] Over these last four score years researchers have learned a great deal about the impact of DR on nearly all aspects of mouse biology, but very little is known about the underlying molecular mechanisms that are responsible for its effects in any species.

While a postdoctoral fellow in London in the laboratory of Dr. Linda Partridge at

Address for correspondence: Scott D. Pletcher, Ph.D., University of Michigan Geriatrics Center, Department of Molecular and Integrative Physiology, University of Michigan, 109 Zina Pitcher Place, Ann Arbor, MI 48109. Voice: 734-647-9383. spletch@umich.edu

International Symposium on Olfaction and Taste: Ann. N.Y. Acad. Sci. 1170: 693–697 (2009).
doi: 10.1111/j.1749-6632.2009.04926.x

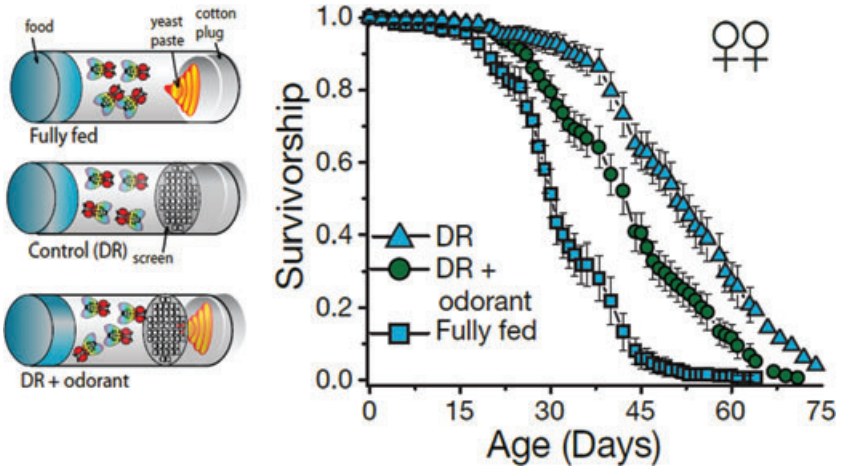

Figure 1. Yeast odorants shorten *Drosophila* lifespan. (Left) Cartoon representation of the protocol used to investigate the impact of yeast odorants on the lifespan of flies exposed to dietary restriction (DR). (Right) Canton-S flies maintained in low-nutrient environments (5% sugar/yeast, labeled DR) exhibited significantly shorter lifespan when presented with odorants from live yeast (blue triangles vs. green circles). Lifespan was further shortened by yeast consumption (blue square). (In color in *Annals* online.)

University College London, I used the fruit fly, *Drosophila melanogaster*, as a tool to investigate genetic mechanisms of DR. During this time our lab executed large-scale biodemography experiments and characterized the phenotypic consequences of diet on *Drosophila* aging, including the timing and dynamics of the effect. These experiments revealed that flies respond extraordinarily quickly to new nutritional environments; age-specific mortality was reduced by up to 95% within 48 h of exposure to nutrient-restricted conditions.[6,7] Such sudden and rapid changes in patterns of aging suggested the hypothesis that sensory perception, alone or in cooperation with other biological processes, may participate in regulating this response. Our attention was drawn to olfaction by whole-genome expression data that compared age-dependent patterns of gene expression between long-lived flies that were diet restricted to fully fed control flies.[8] We found that expression of genes encoding odorant-binding proteins and mediating G protein-coupled receptor signaling was strongly affected by both age and nutrient availability, as were a significant number of genes identified as differentially regulated in smell-impaired flies.[9]

Age- and diet-dependent changes in the expression of odorant-binding proteins led us to ask whether the smell of excess nutrients is sufficient to affect lifespan in *Drosophila*. To test this hypothesis we measured the lifespans of flies in the presence and absence of odorants emitted from live yeast. Yeast odorants were used because yeast availability is a major component of the longevity response to diet in *Drosophila*.[10,11] Our assay for the impact of yeast odorants on fly lifespan involved an odorant-permeable membrane, which prevented flies from accessing one compartment of the vial. Live yeast paste was affixed to a tight cotton plug and placed far behind the screen. Thus, flies could smell and see the yeast but not consume it. Similar constructions where yeast paste was absent and where the dividing screen was absent served as controls (Fig. 1, left). We found that exposure to odorants from live yeast paste was sufficient to reduce lifespan. In long-lived flies that were subjected to DR, we observed decreases in longevity up to 18%. Notably,

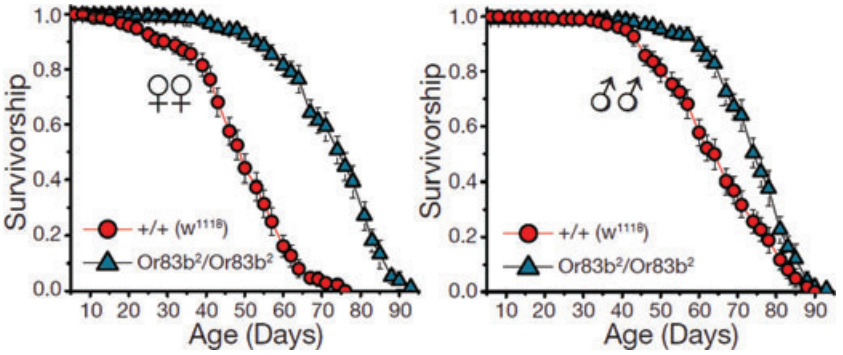

Figure 2. Flies homozygous for a null mutation of the gene Or83b are long lived. The $Or83b^2$ mutation was backcrossed into the w1118 background, and longevity was examined. (In color in *Annals* online.)

lifespan was reduced to a greater extent when flies were allowed to consume the yeast paste, suggesting that olfaction is not the only mechanism through which changes in diet impact aging (Fig. 1, right).

Yeast odor reduced lifespan only when flies were diet restricted. Longevity was not affected by yeast odor when flies were well fed. These data strongly suggest that yeast odor is not toxic in the traditional sense and that non-nutrient-related odorants are not the primary cause of the effect. They also suggest that there is some degree of overlap in the diet and odorant longevity pathways and that nutrient-related odors can limit the longevity extension imparted by DR. The beneficial effects of DR are due, therefore, in part to reduced perception of nutrient availability.

The idea that the common odorants are sufficient to limit fly lifespan led to the prediction that if the flies were isolated from these odorants, their normal lifespan would be extended. To test this prediction we turned to a gene that was described by the Vosshall laboratory[12] to be required for normal function of the majority of fly olfactory receptors, Or83b. Unlike conventional odorant receptor genes, which are expressed in a restricted subset of odorant receptor neurons, Or83b is expressed in most olfactory neurons, both in the adult and in the larvae.[12] The function of Or83b has been analyzed in *Drosophila* by mutational analysis[12] and by RNA interference.[13] As suggested by its presence in most odorant receptor neurons, reduction in Or83b activity severely reduced odor-evoked physiological and behavioral responses to a wide range of odorants.[12,13] Regardless of the precise molecular nature of fly olfactory receptors, these data support a model in which Or83b acts in concert with conventional olfactory receptors to stimulate response to different odorants.[14]

On the basis of the general requirement of Or83b for normal olfaction, we reasoned that loss of function in olfactory signaling in Or83b mutant flies may be sufficient to increase longevity. Indeed, Or83b mutant flies were strikingly long lived. In low-nutrient and high-nutrient conditions, female Or83b mutant flies exhibited a roughly 50% increase in median lifespan. Males were also long lived, but the magnitude of extension was often smaller (Fig. 2). Finally, while Or83b mutant flies have normal size and metabolic rate, they are resistant to starvation and hyperoxia, and females exhibit increased triglyceride storage. Sensory perception, therefore, appears to be a potent modulator of a range of physiological characteristics.

There are at least two reasonable explanations for the long-lived phenotype of Or83b mutant flies. Or83b mutant animals are broadly anosmic, and it is possible that global loss of chemosensory function and a

general lack of olfaction are required for extended longevity. Alternatively, it may be that the longevity phenotype observed in Or83b[2] mutant animals results from an inability to detect a small number of specific odorants, as identified and processed by specific odorant receptors. Using *C. elegans*, Alcedo and Kenyon[15] performed laser ablation of individual cells to investigate the effects of specific sensory neurons on worm lifespan. They found that removal of specific gustatory and olfactory neurons could increase, decrease, or have no effect on lifespan.[15] Thus, some sensory neurons produce signals that antagonize long life, while others promote it.[16] If the situation is analogous in flies, then we might expect some of the conventional odorant receptors to promote longevity and others to limit it. We are currently addressing this question using single-gene knockout and overexpression. Although it is early in the game, we have identified candidate populations of sensory neurons that initiate longevity programs and effector pathways in target tissues that enact them. These are the initial steps toward our long-term goal: to elucidate the network that couples sensory perception to longevity—from signaling inputs and their associated neurocircuits that detect and decode sensory information; to endocrine cells, hormone identity, and transport that relay that information to target tissues; to transcriptional complexes and gene targets that ensure cell, tissue, and organism survival.

From worms to flies to mammals, signaling networks coordinate chemosensory input to physiological outputs. In worms and flies, these outputs influence lifespan and disease-relevant phenotypes, such as fat deposition and stress resistance. In mammals, olfactory signals mediate developmental and behavioral changes in response to territorial cues, dominance relationships, and mating receptivity. Might some of these inputs also be capable of modulating aging and associated age-related disease? By isolating important regulatory mechanisms that link environment to lifespan in invertebrates, we hope to illuminate how sensory cues influence neuroendocrine function, lipid metabolism, and aging. As with much of the work in short-lived model systems, many of the relevant genes and circuits have evolutionarily conserved counterparts. Therefore, what is learned may be applicable to understanding human aging, as well as disease states in diabetes, obesity, and dementia.

Conflicts of Interest

The author declares no conflicts of interest.

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