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Forum

Lipid Profiles and Signals for Long Life

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Historically, fat was considered detrimental to health and lifespan. However, lipidomics, the quantification of all lipid molecules in a biological sample, and genetic studies in model organisms are revealing specific fats that may promote longevity. These emerging findings provide insight into the complex relationship between lipids and longevity.

Lipids and Lifespan

Lipids are essential components of biological membranes, energy sources, and signaling molecules. Lipid signals influence fat synthesis, storage, and catabolism, creating intricate metabolic networks that allow organisms to respond to nutrient availability and energy demands or otherwise adapt to changing environments [1]. Storage lipids (triglycerides) and circulating lipid–protein complexes (lipoprotein particles) have previously been linked to diseases of aging [2], and obesity limits longevity [3]. But can lipids beneficially influence lifespan? Lipid profiles of long-lived humans and model organisms and genetic studies of lipid metabolism suggest that this is the case.

Box 1. Lipid Profiling Challenges and Technologies

Lipids exist in a staggering array of sizes, biophysical properties, and relative abundance. An organism's lipid profile is determined by the combined influences of dietary lipids, *de novo* lipogenesis, and the hundreds of enzymes that modulate the length and desaturation of FA chains and their incorporation into more complex lipid molecules. For example, desaturases convert saturated FA chains to MUFAs (one carbon–carbon double bond) and PUFAs (two or more double bonds) (Figure 1). Lipases liberate FAs from lipid molecules to serve as energy sources or signals or to facilitate transport to other tissues [10]. Current methods utilizing liquid chromatography–mass spectrometry (LC–MS) can detect hundreds of unique lipid species, including novel lipid molecules [13]. Although it is still not possible to detect all types of lipid molecules in a sample, LC–MS-based methods combined with sophisticated software to aid in lipid identification can detect hundreds of distinct lipids. Other methods that degrade lipids into FA chain components can detect differences in FA structure at high resolution, revealing trends in lipid profiles [1,13]. Analysis of metabolites without specifically targeting lipids can also identify some lipid molecules [9].

Characterizing lipids presents unique challenges, but new technologies facilitate quantitative detection of diverse lipids in human samples and model organisms typically used in aging studies (Box 1). The nematode *Caenorhabditis elegans* has emerged as a powerful model for lipid studies due to the detailed characterization of metabolic pathways and ease of genetic manipulation [4,5]. Despite differences in how lipids are stored and synthesized between worms and mammals [4], some lipid profiles associated with longevity may be conserved, and work with *C. elegans* has uncovered molecular mechanisms relating lipids to lifespan that could be explored in mammals.

Lipid Profiles Associated with Longevity

Several lipidomic studies in humans have revealed trends in lipid composition associated with long life. Total lipids extracted from plasma or isolated from erythrocyte membranes of children of long-lived individuals (nonagenarians or centenarians) contain a higher ratio of monounsaturated (MUFA) to polyunsaturated (PUFA) fatty acid (FA) chains relative to matched controls [2]. An increased MUFA:PUFA ratio may influence lifespan by reducing oxidative stress and damage. All lipids can be oxidized by free radicals but PUFAs are the most susceptible to oxidation [2,6]. Because oxidation of FAs propagates further free radical production, high levels of PUFAs could increase oxidative damage on a much larger scale [6].

Several long-lived mutants in *C. elegans*, including mutants with reduced insulin-like signaling and dietary restriction mimics, also exhibited modestly increased MUFA:PUFA ratios when analyzed for general FA chain composition, with the longest-lived mutants having the highest MUFA:PUFA ratios [6]. These worms, and worms lacking a germline (which are also long lived), express higher levels of enzymes that convert saturated FAs to MUFAs (Δ9 desaturases) [3,6] (Figure 1). Additionally, knockdown of Δ5 desaturase, which produces highly unsaturated FAs, appears to promote oxidative stress resistance and lifespan extension in wild-type worms [6]. These findings support a link between PUFA synthesis, oxidative damage, and aging. In mammals, pathways that influence longevity, including insulin and growth factor signaling, regulate Δ9 desaturase expression [3], suggesting that these enzymes may be important targets of other modes of lifespan extension. Future studies are needed to explore the contribution of FA desaturases and their products to

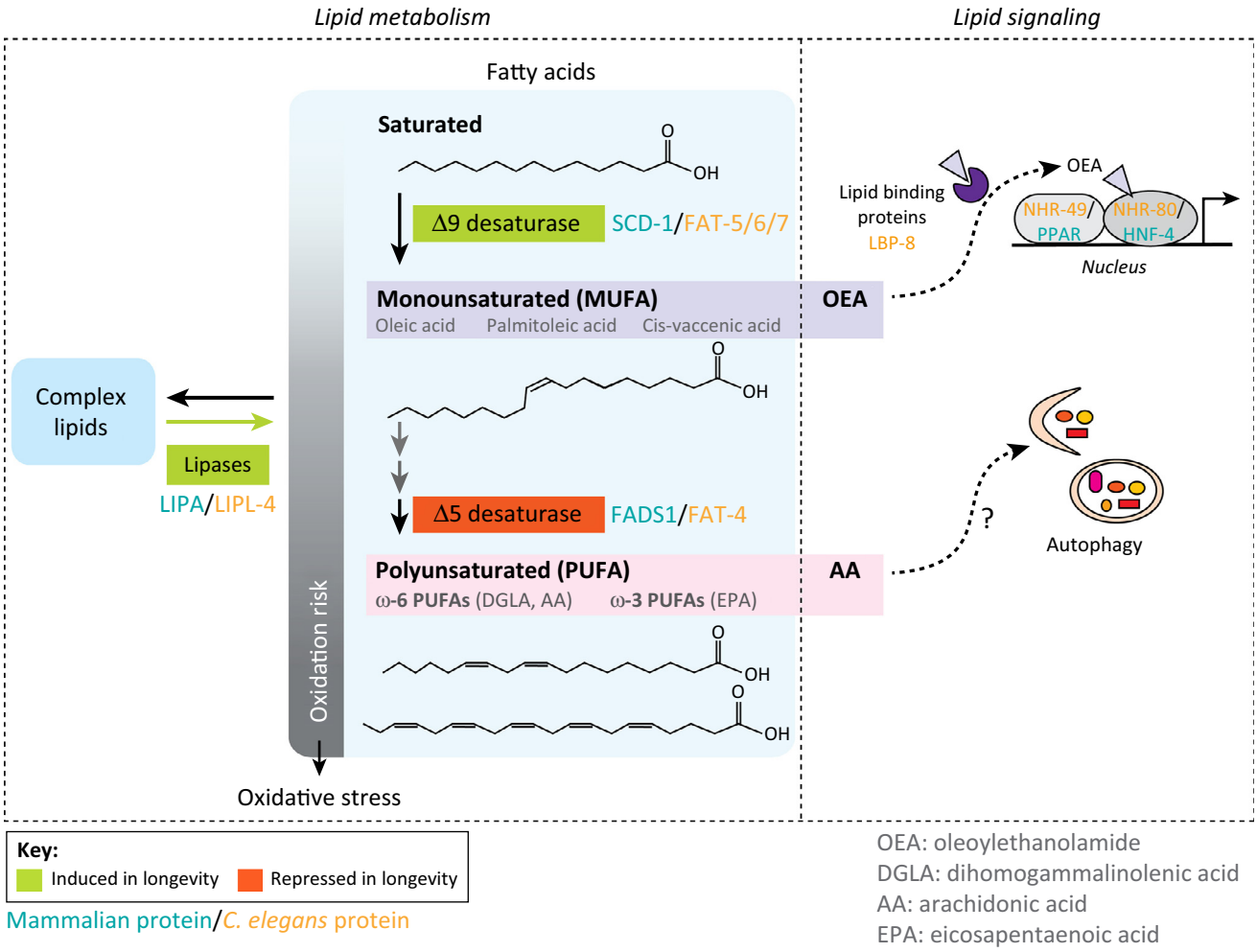

Figure 1. Lipid Metabolic Pathways, Profiles, and Signals Implicated in Longevity. Free fatty acid (FA) chains can be liberated from complex fats by the activities of lipases. Several lipases are elevated under conditions that extend lifespan in *Caenorhabditis elegans*, including LIPL-4. FAs can be saturated (no double bonds), monounsaturated (MUFAs; one carbon–carbon double bond) or polyunsaturated (PUFAs; two or more carbon–carbon double bonds). Increasing desaturation can make FAs more susceptible to oxidation by free radicals. In *C. elegans*, elevation of Δ9 desaturases (FAT-5/6/7), which produce MUFAs, is associated with longevity under several conditions. Conversely, Δ5 desaturase, which can produce highly polyunsaturated FA chains, is reduced in long-lived worms. FA desaturases produce many other MUFAs and PUFAs that are present in biological samples. Exploring how these lipid molecules determine longevity would be of great interest. FAs and other lipids can also act as signaling molecules to influence lifespan. Oleoylethanolamide (OEA), which is elevated in response to increased LIPL-4 expression in worms, activates key metabolic transcriptional regulators to extend lifespan. Lipid-binding proteins mediate OEA signaling and may be important for other lifespan-extending lipid signals within cells or across tissues. ω-6 PUFAs, also elevated by increased LIPL-4 expression, can induce autophagy through unknown mechanisms.

longevity in worms and mammals, especially in the context of long-lived mutants that are known to exhibit numerous other cellular effects.

Although these findings imply that reducing PUFAs delays aging, increasing specific PUFAs may also promote lifespan extension. In *C. elegans* and cell lines, supplementation with ω-6 PUFAs activates autophagy, a prolongevity process that promotes survival under nutrient deprivation [7,8]. In worms, increased expression of lipases, which can liberate FAs from complex lipid molecules, promotes high levels of ω-6 PUFAs [8] but also of many other FAs [9]. Both induction of lipases, particularly LIPL-4 (homologous to mammalian LIPA), and increased autophagy are required for longevity in worms lacking a germline [3]. Lipases also support autophagy induction during starvation [8], raising the possibility that lipases and the free FAs they generate are important for longevity in dietary restriction. Additional genetic experiments in nematodes and mammalian cellular and organismal models, combined with lipidomics to identify relevant products of lipid metabolic enzymes such as lipases and desaturases, might shed light on the relative roles of MUFAs and PUFAs in longevity in different contexts. In addition, more work is needed to determine the effects of MUFA

or PUFA supplementation on health span and lifespan. As supplementation experiments have yielded differing results so far [6,8], it will be important to compare the effects of dose and timing of supplementation with various FAs on longevity.

Lipid Signaling in Lifespan Regulation

Lipid signaling can systemically influence cellular functions connected to aging. Steroids are classic lipid signaling molecules that can influence fat metabolism, reproduction, and lifespan [3]. Other lipid signaling pathways may be altered to promote or curtail longevity. For example, certain sphingomyelins are enriched in the plasma of long-lived individuals and their offspring [2]. Sphingomyelins are important components of plasma membranes in the nervous system, but are also precursors for ceramides. These signaling lipids can interfere with insulin signaling [10] and have been implicated in neurodegenerative diseases [2], two disorders characteristic of aging. The accumulation of sphingomyelins may indicate reduced proaging ceramide signaling. The potential signaling roles of other individual lipids altered in long-lived humans [2] remain to be explored.

Studies in *C. elegans* have revealed additional lipid signaling pathways that modulate lifespan. One example involves dafachronic acid, a steroid that promotes longevity in worms lacking a germline [3]. Recently, metabolomic analysis of worms overexpressing LIPL-4/LIPA identified high levels of the lipid oleoylethanolamide (OEA), which can interact with and promote transcriptional activities of the worm functional orthologs of hepatocyte nuclear factor 4 (HNF4) and peroxisome proliferator-activated receptor alpha (PPARα) (Figure 1) [9]. Interestingly, feeding worms OEA is sufficient to activate these transcriptional regulators and extend lifespan. In mammals, HNF4 and PPARs are key regulators of lipid metabolism, inflammation, and cell death [1,10]. In mice, OEA is induced by feeding, regulates satiety, and activates PPARα [1,9]. A large number of lipids have been shown to directly modulate PPAR activity [1], suggesting that PPARs and other nuclear receptors (NRs) may be key targets in prolongevity lipid signaling pathways. Further studies into the effects of PPAR-modulating lipids on lifespan in mammals should uncover new roles for signaling lipids and NRs in aging.

How do signaling lipids reach the appropriate organelle or tissue to influence lifespan? In the case of OEA, a lipid-binding protein, LBP-8, may transport this lipid to the nucleus, facilitating activation of NRs [9] (Figure 1). Recently, chemically modified lipid probes were used to identify proteins that bind common FAs [11]. This method detected many lipid carriers specific to different FAs, including FABP5/e-FABP, a FA-binding protein expressed in epidermal cells that interacts with PPARδ [12]. If candidate signaling lipids could be modified to form lipid probes, this technology could prove instrumental in understanding how lipid transport and signaling impact lifespan.

Resolving the Complex Relationship between Fat and Aging

Emerging lipidomic technologies provide exciting opportunities to understand how lipids influence lifespan. First, lipid profiling could reveal how genetic variants and metabolism are integrated in longevity. Several lipid metabolism genes have longevity-associated variants in humans, including ceramide synthase (CerS) and lipoprotein lipase (LPA) [2], which may influence lipid profiles or lipid signaling to impact longevity. Second, most of the lipid molecules associated with longevity in humans are associated with female longevity only [2]. Males and females inherently express different levels of many lipid metabolic enzymes and have distinct energetic requirements during reproductive years [2]. Intriguingly, most of the lifespan-extending mutations in *C. elegans* that involve altered lipid profiles or signaling also reduce reproductive fitness. Studying sex-specific differences and how lipid metabolism interacts with reproduction will be key to drawing conclusions about the roles lipids play in longevity. Third, the heritability of prolongevity lipid profiles is of great interest. Human studies have revealed that long-lived individuals and their children have similar lipid profiles [2], but whether this inheritance is due to genetic or epigenetic mechanisms remains unknown.

Finally, to what extent does altered lipid composition or lipid signaling support longevity under various modes of lifespan extension? Studies in *C. elegans* have uncovered exciting links between lipids and lifespan in many genetic and environmental interventions, which could be further explored in organisms that better model human fat metabolism and storage. In particular, mice lacking FABPs [12] would be useful in testing the contribution of lipid signaling to longevity. If general prolongevity lipid molecules or trends were identified, would it be possible to extend lifespan by modulating the diets of wild-type organisms, including humans?

Although fat is historically associated with poor health and obesity burdens health care worldwide, specific lipid profiles and signals may delay aging. The continued use of lipidomics and genetic studies of lipid synthesis and signaling pathways in model organisms will undoubtedly reveal many new roles for lipids in the regulation of longevity and deepen our understanding of the complex relationship between fat and aging.

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Forum

Copernicus Revisited: Overturning Ptolemy's View of the GPER Universe

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Whether aldosterone activates the G-protein-coupled estrogen receptor (GPER) has been questioned, recently, in the name of Copernicus. However, for G-protein-coupled receptors (GPCRs) multiple hormone activators are common. Further, studies in mineralocorticoid receptor (MR)-deficient systems, with pharmacological GPER-selective antagonists or regulation of GPER expression, consistently show that some aldosterone effects can be GPER mediated.

'Seek Simplicity and Distrust It' – Alfred North Whitehead

For almost 1500 years, our understanding of the structure of the solar system was based on the work of Claudius Ptolemy, who lived in Rome around 100 AD. His model assumed that Earth was not only the center of our solar system but also the centre of the Universe. Ptolemy's view of the solar system adequately explained the motions of the heavenly bodies based on the evidence in hand. That view changed, in part based on the work of Nicholas Copernicus in the 15th century. Perhaps the most important contribution of Copernicus in the course of his monumental career as a mathematician, physicist, and astronomer was his success in switching scientists' perspective from an Earth-based focus of the Universe to one where Earth's position was much more analogous to that of the other known planets of our solar system.

In some respects the discussion over the exclusivity of estrogen as the sole physiological agonist for GPERs recapitulates the controversies that occurred on a much more celestial level more than 500 years ago when we finally came to understand that Earth was just another planet in a solar-centered system. In this Forum article, we examine an analogous possibility: that in many ways the GPER is just another GPCR.

GPER (also known as GPR30) started life as an orphan GPCR. However, work done in the early 2000s, much from the laboratory of Ed Filardo at Brown [1] and Eric Prossnitz at the University of New Mexico [2], established GPR30 as the receptor mediating at least some of the rapid responses of estrogen. Notably, the perspective of many of the early authors in the GPR30/GPER field was that estrogen was at the center of the GPER universe and was the solitary physiological hormone rotating around it.

However, if one considers the GPER as just another GPCR, the premise that the GPER may have multiple agonist hormones would be neither unexpected nor unusual. From the outset[i] GPCRs interacting with multiple physiological hormones were appreciated to be common (although not universal). After all, the beta-adrenoceptor, the prototype non-visual GPCR, was initially appreciated to have affinities (albeit varying) for two endogenous hormones, epinephrine and norepinephrine, and for countless pharmacological agonists and antagonists. Further, we now appreciate that GPCRs demonstrate not only greater diversity in ligand interactions than previously appreciated, but also a range of conformational states that vary with the ligand occupying the receptor. Different ligands interacting with the same GPCR promote differences in receptor–effector profiles as well as ligand-dependent receptor oligomerization or interaction with one or another G protein, receptor activity modifying protein (RAMP), or receptor component protein (RCP) [3]. Thus, conceptually, shifting from an estrogen-specific perspective of the GPER universe to one that includes aldosterone in its orbit should not be as iconoclastic as the shift from an Earth-centric to a heliocentric planetary system.

Does Aldosterone Mediate Rapid Effects via the GPER?

Four years after our initial demonstration that aldosterone can mediate rapid effects via the GPER [4], numerous lines of evidence supported the generalizability of our findings; namely, that at least some of aldosterone's MR-independent effects are mediated via the GPER, as noted below. However, at the outset it is important to emphasize that the GPER may not be the only non-MR-dependent mechanism by which aldosterone mediates its rapid effects and to not rule out the possibility that there are other hormones acting via the GPER waiting to be discovered.