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Nutrient control of *Drosophila* longevity

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Abstract

Dietary restriction (DR) extends lifespan of many animals including *Drosophila melanogaster*. Recent work with flies shows longevity is controlled by the ratio of consumed protein relative to carbohydrates. Since reduced insulin/IGF and TOR signaling increase *Drosophila* lifespan, these pathways are candidate mediators of DR. This idea, however, has ambiguous experimental support. The Nutritional Geometric Framework, which dissects the impact of nutrient protein relative to carbohydrates, may provide an approach to resolving the roles for these pathways in DR. Nutrient sensing of protein and carbohydrate may occur in the fat body through signals to hypothalamic-like neurons in the fly brain, and thus control secretion of insulin-like peptides that regulate longevity.

Keywords

dietary restriction; aging; geometric framework; lifespan extension; insulin/IGF signaling; TOR

A long life by eating less

A longstanding problem in the biology of aging is to understand how DR (see glossary) extends life expectancy [1]. In one branch, research has aimed to uncover the cellular mechanisms whereby DR improves survival, for instance through increasing stress resistance, genomic maintenance, or protein homeostasis. On the other hand, these cell-centric mechanisms must be integrated across tissues to assure longevity. The need for such coordination motivates work to understand how nutrients contribute to physiological signaling that affects survival. This question has progressed using *Drosophila* to uncover the nutrient components that modulate longevity assurance, and to analyze the roles of sensory and hormonal systems that systemically integrate longevity control. These data together reveal mechanisms of nutrient sensing that could stimulate new hypotheses to identify the

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metabolic pathways responsible for DR-induced longevity [2] and can suggest endocrine interventions that may emulate the benefits of DR. [3]

The nutrient lever of restricted diet

Restricting food intake without malnutrition was reported to extend lifespan in rats some 80 years ago [4]. Research has since focused on deciphering which aspect of restricted-diet induces longevity, how this dietary feature is sensed and what are the mechanisms by which it regulates aging [5]. Likewise, more than 40 years ago David et al. [6] reported that longevity of laboratory *Drosophila melanogaster* was extended when adults were maintained on diluted yet freely available diets – diets made in agar-based media with different combinations of yeast, sugar or molasses, cornmeal, and other carbohydrates [7, 8]. As with rodents, for *Drosophila* there is keen interest to understand the nutrient effectors of DR, how they are sensed and integrated, and how this affects the molecular mechanisms of survival. The order of these goals is important: before we can understand the cellular or molecular mechanisms of DR, we must first clearly define what nutrients actually modulate lifespan when flies eat less.

Reduced caloric intake *per se* was long thought to be the longevity determinant of DR for all animals, contributing to theories whereby DR modulates aging by reducing metabolic rate and associated costs of oxidative stress [1]. This interpretation was based on mouse studies where food consumption was measured directly [9]. In *Drosophila*, however, the usual DR protocol is to provide adults a diluted but *ad libitum* diet. By reducing all dietary components or by maintaining adults on media with a fixed concentration of sugar with reduced amounts of autolyzed yeast, longevity can be extended 50% or more [7, 10]. But because ingestion rates are not typically measured or controlled, whether adults consumed fewer calories was assumed rather than observed. Techniques to track ingestion eventually revealed that adults partially compensated for dilute diets by eating more [11, 12]. These data however did not determine if flies compensated by consuming more yeast and carbohydrates, or by selectively increasing their intake of one nutrient relative to the other. Overall, *diet dilution* extends lifespan but it remained unknown whether longevity assurance is modulated by a reduction in net calories, protein, carbohydrates, or other nutrient features such as essential fatty acids and micronutrients.

Contribution of specific nutrients

To understand the impact of caloric intake relative to specific nutrients in *Drosophila*, studies have simultaneously manipulated the quantity of carbohydrate (sugar) and protein (provided in hydrolyzed yeast) available from *ad libitum* diets, while directly measuring food intake or net energetics [13–15]. Lee et al. [12] provided adult with diets consisting of sugar and yeast at seven different protein-to-carbohydrate ratios, each at four concentrations. Consumption of each nutrient and lifespan were measured for individually held females. The lifespan of each fly was plotted on the z-axis as a function of its intake of yeast-derived protein (P) and of sugar-derived carbohydrate (C), plotted on the x- and y-axis respectively. The resulting landscape surface reveals isoclines of lifespan where the maximum elevation indicates the P:C ratio of greatest survival (Fig 1). Notably, *Drosophila* lifespan was greatest

along a topological ridge with 1P:16C, and this diet ratio maximizes lifespan at any level of total food intake. DR extends lifespan by reducing the *relative* quantity of dietary yeast, not by modulating the total amount of consumed yeast, sugar or net calories (Box 1).

Box 1

The Nutritional Geometric Framework

The Nutritional Geometric Framework (NGF) is a state-space approach to understand how dietary components affect feeding behavior, food choice, metabolic disease (obesity, metabolic syndrome, diabetes), reproduction, the evolution of trade-offs, physiology, longevity and other traits [68]. In general, the NGF can represent any number and nature of nutrient dimensions although in current analyses for DR, only two food dimensions are modeled. Individuals have a preferred nutrient intake target with a specific ratio of calories derived from protein relative to non-protein (carbohydrates and fats) designated as the P:C ratio, and a total caloric intake that satisfies daily energy balance. Factors affecting the intake target, which can be determined empirically, include age, gender, genotype, activity, health, season, etc. A balanced diet provides nutrients whereby the individual consumes its needed calories while satisfying its target P:C ratio. Diets of any particular P:C ratio can be represented across a caloric range as vectors described in the NGF as ‘nutritional rails’. In practice, available diets can provide nutrients upon a nutritional rail that does not intersect with an individual’s intake target. Feeding behavior in these cases requires ‘rules of compromise’ whereby individuals sacrifice some nutritional needs in order to satisfy those that have high regulatory priority. In this way, individuals will over- or under-consume protein, carbohydrates or total calories to meet its needs for its highest priority nutrient. The NGF uses state-space modeling to estimate, analyze and predict these behaviors and their consequences. Furthermore, phenotypes can be measured and overlaid atop the dietary component graph to provide a multidimensional landscape for how traits are expressed as a function of nutrient composition. In this way the NGF has provided insights for how diet affects longevity during DR.

Yeast, of course, is a complex food consisting of many nutrients besides protein, including essential fatty acids, minerals, vitamins, and cholesterol (which insects cannot synthesize). What feature of reduced dietary yeast relative to carbohydrates modulates lifespan, and in particular is this protein or a specific amino acid? Although caloric intake has dominated views of rodent DR, methionine restriction alone extends lifespan in both mice and rats [16, 17]. In *Drosophila*, flies fed a media with casein as the sole source of protein were long-lived upon diets with intermediate levels of protein although even this best casein-based concentration produced suboptimal longevity relative to diets made with yeast [18]. Likewise, flies were longest lived when fed an intermediate level of methionine in a defined diet with relatively low total amino acids [19]. Interpreting these data is complicated because the only tested low-methionine diet might reflect malnutrition while the only tested high-methionine diet increased total food consumption. Grandison et al. [20], on the other hand, found longevity was reduced when a restricted diet was supplemented with essential

amino acids or with all amino acids, but not when restricted diet was supplemented only with methionine.

Ultimately, to sort out the contribution of nutrient components on aging requires a fully defined diet. Piper et al. [21] thus optimized a holidic diet to mimic the nutrient composition of adult sugar-yeast medium. Fed to adults, this diet supported normal lifespan and egg production. Lifespan was shortened by completely dropping out specific components of the diet, such as cholesterol, all amino acids or the essential amino acids arginine or isoleucine. Although *Drosophila* lifespan was also reduced in a diet that completely lacked methionine, the holidic diet has not yet been used to test whether intermediate levels of methionine restriction extends lifespan. All the same, trials with the holidic diet confirmed that reduced dietary amino acid intake is sufficient to extend longevity. Holidic media now provides a promising tool to dissect the role of specific nutrients in longevity control, and to resolve how flies adjust lifespan in response to the relative concentration of protein and carbohydrate rather than to the absolute quantities of these nutrients.

Olfaction modulates life span

Besides responding to ingested nutrients, flies can modulate lifespan through their perception of nutrient cues. The longevity gained by flies maintained on a dilute sugar-yeast diet is blunted if adults are simultaneously held in vials with yeast they can smell but not ingest [22]. This control of lifespan requires odorant-binding protein Or83b, which is expressed in sensory neurons where it forms dimeric complexes with Or-type odorant receptors [23]. Flies carrying null mutations of *Or83b* have olfactory defects, altered adult metabolism, enhanced stress resistance, and extended lifespan [22]. Nonvolatile protein itself should not account for this olfaction, and the effector appears to be carbon dioxide, which could be a reliable cue for growing yeast [24]. Olfactory neurons that express the gaseous phase CO₂ receptor *Gr63a* are required for the emissions of live yeast to reduce lifespan of dietary restricted adults. Furthermore, *Gr63a* mutant flies exhibit increased longevity when maintained on normal diet. *Gr63a* co-functions with the CO₂ sensitive *Gr21a* receptor in ab1C sensillum of antennae [25]. This sensillum does not express *Or83b* and thus should not use Or83b as a co-receptor, suggesting that CO₂ perception only partly accounts for the sensory control of DR. Other gaseous molecules such as nitric oxide and carbon monoxide [26] might contribute to the longevity regulatory response mediated by *Or83b* expressing neurons. On the other hand, *Or83b* occurs in ab1A, ab1B and ab1D sensilla, which are adjacent to ab1C [25], and grouped olfactory neurons, including those with CO₂ receptors, can interact through non-synaptic, lateral inhibition [27]. CO₂ sensing in ab1C could potentially integrate signaling through interactions with *Or83b* containing neurons. Although, the circuitry and processing of these sensory inputs remains to be fully understood, these findings together indicate that the control of aging by nutrients in *Drosophila* can be non-autonomous and even independent of ingestion.

Neuroendocrine controls of longevity in response to nutrition

A key asset of *Drosophila* is the ability to study how lifespan is affected in mutant adults maintained on a range of diets where gene-by-diet analysis can identify factors required for

DR to increase lifespan [28, 29]. Gene-by-diet analysis is a specific application of gene-by-environment analysis, which characterizes the phenotypic plasticity of a quantitative trait by plotting trait values for each genotype as a function of an environmental variable; these plots are called reaction norms [30]. When diet is the variable, reaction norms of longevity increase to a maximum at some relatively restricted diet and then decrease at even more restricted diets presumably due to malnutrition (fig 2A). In studies with enough diets to accurately estimate reaction norms, mutants of genes required for DR will show less responsive, flatter plots [21]. Testing mutants with this approach has the potential to uncover which sensory and endocrine systems control aging in response to diet.

The Insulin/IGF signaling (IIS) pathway

Insulin/IGF signaling (IIS) is an attractive candidate for endocrine control of DR-induced longevity assurance because many nutrient factors regulate its output [31]. *Drosophila* has a single insulin/IGF-like tyrosine kinase receptor (INR), eight insulin-like peptide loci (*dilps 1-8*), and a single forkhead transcription factor FOXO, encoded by *dfoxo* [32] [33, 34]. As in mammals, ligand activated INR interacts with the insulin receptor substrate-like homolog, encoded by *chico*, to activate the serine/threonine-specific protein kinase AKT which in turn phosphorylates FOXO and represses its activity [35]. Mutants of this IIS system show elevated total body triglycerides and carbohydrates, reduced growth, limited reproduction and extended lifespan [36–38]. Insulin/IGF signaling is strongly reduced by fasting [39] and these facts together suggest that DR might foster longevity by repressing IIS. It is thus surprising that gene-by-diet analyses to date have failed to, or at best only weakly supported this hypothesis.

Lessons from *dfoxo* mutants

The role for IIS in the control of DR-mediated longevity has been directly explored with mutants of *dfoxo*. While *dfoxo* null adults are somewhat short lived, they show the same magnitude of increased longevity upon DR as seen in wild type flies [40, 41] (Fig 2B, C). These data, however, may overlook a real requirement for dFOXO in DR that actually occurs in an untested region of nutrient space. Piper *et al.* [42] argued that two-dimensional reaction norms, such as in Figure 2, are only a slice of the nutritional response landscape (Fig 1B). A mutant of *dfoxo* could change the nutritional response landscape of longevity relative to protein and carbohydrate, yet a particular diet dilution series might only sample the mutant and wild type in a plane where the response surfaces are parallel (Fig 1C). To robustly test if mutants affect the longevity response to diet, future studies are needed to assess differences among genotypes across comprehensive nutritional landscapes.

Dissecting the role of *dilps*

Data for other manipulations within the insulin/IGF signaling system show mixed outcomes. *Drosophila* heterozygotes of the IRS1-4 homolog encoded by *chico* live longer than wild type but among diets that produce DR they show the same magnitude of longevity extension as wild type (Fig 2F) [43]. The *chico* heterozygote shifts its DR reaction norm to the right, perhaps reflecting a change in its perception of total nutrients but not in how it functionally responds to nutrients. In contrast, genetically ablating the insulin-producing cells (IPC)

located in median neurosecretory cells (MNC), which eliminates insulin secretion as well as any other neuropeptide produced in these neurons (for instance, drosulfakinin [44]), increases longevity and flattens the DR reaction norm relative to wild type controls over a four-fold range of dietary yeast (Fig 2D,E) [45]. On the other hand, individually knocking out *dilp2*, *dilp3* or *dilp5* loci by homologous recombination does not change the longevity diet reaction norm relative to wild type [34]. Interpretation here is complicated because compensatory expression occurs among the various dilps: the *dilp2* null mutant shows elevated *dilp3* and *dilp5* mRNA, while the *dilp5* knockout elevates *dilp3* mRNA. Such compensation does not occur in a combined *dilp2-3, 5* genetic knockout. This genotype is long-lived and its longevity is independent of diet, although in these trials the longevity of the wild type control itself is largely independent of diet. Overall, as with *dfoxo*, it shall be important to evaluate the effect of MSN ablation and *dilp* knockouts using the nutritional response landscape and to do so in regions where wild type flies shows a strong response to changes in the ratio of protein to carbohydrate.

The target of rapamycin (dTOR) pathway

Independent of the dFOXO transcription factor, nutrients might modulate aging via IIS through its effects on target of rapamycin (TOR) signal transduction. The TOR kinase participates in a complex that modulates cellular responses to amino acids by regulating translation initiation, ribosomes, autophagy and endocytosis [46]. When amino acids are abundant, TOR inactivates the translation repressor 4eBP (*4eBP* itself is transcriptionally regulated by dFOXO) and activates kinase S6K, which regulates translation initiation and elongation through its phosphorylation of PDCD4 and eIF4A [47]. While TOR activity is directly regulated by extracellular and intracellular amino acid flux, it is also controlled by systemic nutrient sensing, when AKT is phosphorylated through insulin/PI3K [48, 49]. Phosphorylated AKT represses tuberous sclerosis complex protein (Tsc2 in mammals, Tsc1/2 in *Drosophila*), which in turn de-represses the ras-like small GTPase Rheb [50]. Rheb activates and licenses TOR complex 1 (TORC1) to promote cellular activities in response to local amino acids. In principle, TOR may thus act as both an autonomous and a systemic mediator of DR.

Since *Drosophila* null mutants of *4eBP* are viable (unlike null mutants of *dTOR* [51, 52]), they have been used as a proxy in gene-by-diet analyses to assess the role of TOR in DR [29, 53]. When tested on five diets where yeast was diluted from 16% to 0%, *4eBP* mutants responded to DR as robustly as wild type control (Fig 2G), suggesting that 4eBP and perhaps TOR are not required for DR to extend lifespan. On the other hand, using the same genotypes Zid *et al.* [54] concluded that loss of *4eBP* meaningfully affected the longevity reaction norms in trials with a limited range of protein-restricted diets that lacked essential sterols and lipids. These conflicting conclusions might occur because each study only investigated one slice of the protein-carbohydrate topography (Fig 1). As with *dfoxo* and *dilps*, to robustly test if a gene affects longevity in response to DR may require estimates of longevity reaction norms measured across the P:C response landscape. Thus, while many data suggest that TOR functions in the control of aging [49], and that TOR provides both systemic and local cellular responses to amino acids, we cannot yet resolve if this nutrient nexus is required for DR to extend longevity.

Fat body as endocrine integrator of diet and aging

DILP2, DILP3 and DILP5 in adults are primarily produced in insulin producing cells (IPC) among the MNC. In contrast, DILP6 in adults is produced largely in the adult fat body, although it is also expressed in non-MNC of the adult brain and from glial cells within larvae [55, 56]. Adult fat body has liver and adipose-like functions of metabolism, lipid storage, innate immunity, detoxification and protein synthesis [57–59]. *dilp6* mRNA in fat body is induced by dFOXO – a condition that is associated with increased longevity [60] – and lifespan is extended when transgenic *dilp6* is expressed in fat body [55]. As well, *dilp6* mRNA of fat body is increased in fasted adults [55]. Notably, elevated fat body *dilp6* suppresses the secretion of DILP2 into hemolymph from the MNC, which may explain why elevated *dilp6* increases in lifespan. How *dilp6* of fat body modulates DILP2 secretion in the brain is unknown. *Drosophila* fat body also produces a leptin-like homolog encoded by *unpaired-2* (*upd2*) [61]. Fat body *upd2* is elevated by high sugar and high fat diets. *upd2* from fat body activates JAK/STAT signaling in GABAergic neurons (GN) of the brain, which reduces accumulation of DILP within MNC, presumably by secreting these peptides. Overall, *upd2* appears to function as an endocrine signal for a state of abundant nutrition.

Although a role for *upd2* and *dilp6* in DR has yet to be specifically determined, their responses to nutrients suggest how they might work together as dietary sensors to control lifespan. *Drosophila* lifespan is maximized by diets with low protein relative to carbohydrates. We propose that reduced dietary P:C simultaneously increases *dilp6*, which will suppress DILP2 secretion [55], and *upd2*, which favors IIS by releasing accumulated DILP from the IPC [61]. DILP secretion from the IPC may thus respond to the ratio dietary P:C by measuring fat body signals derived from DILP6 relative to those from UPD2. A hypothalamic-like feature of the *Drosophila* brain may thus test a threshold between the countervailing nutrient signals from fat body to control longevity in terms of the ratio of nutrient components, and thus respond to relative nutrient quality rather than to absolute quantity.

Feeding and aging modulated by a *Drosophila* hypothalamic-like center

Hypothalamic-like functions may exist within the adult fly brain where neurons express short neuropeptide F (sNPF), a functional homolog of mammalian hypothalamic neuropeptide Y (NPY) [40, 62]. sNPF secreted from dorsal lateral peptidergic neurons (DLPs) induces *dilp2* and *dilp5* in IPCs via sNPF receptors (sNPF_{R1}) that signal through extracellular signal-related kinases (ERK) [62, 63, 64]. *sNPF* mutants have reduced expression of *dilps*, and they are correspondingly long-lived and have increased circulating levels of glucose [62]. Expression of sNPF positively regulates food consumption [40, 62], as does NPY of the mammalian hypothalamus [65]. In a further role analogous to the hypothalamus, sNPFergic neurons acting upon sNPF_{R1} in medial neurons modulates expression of *Drosophila* *minibrain* (*mnb*) [66]. *mnb* is homologous to mammalian Dual specificity tyrosine-phosphorylation-regulated kinase 1a (Dyrk1a) [67]. Dyrk1a is regulated by NPY within cells of mammalian hypothalamus through PKA-CREB signaling, which is also used by *Drosophila* sNPF to modulate *mnb* [66]. *Drosophila* and mammals thus share hypothalamic-like control of feeding mediated by sNPF/NPY acting through *mnb*/Dyrk1a.

Finally, *Drosophila dilp6* expression from fat body represses neuronal expression of *sNPF* [55]. We predict this should reduce DILP2 expression and secretion. Collectively, these observations suggest that sNPF and sNPFR1 neurons provide *Drosophila* with hypothalamic-like functions through which fat body nutrient sensing may mediate DILP expression and aging.

Concluding remarks and future perspectives

A model starts to emerge whereby *Drosophila* hypothalamic-like control of insulin-like peptides coordinates physiology and aging in response to diet. *Drosophila* appear to modulate lifespan upon DR by responding to reduced proportion of nutrient amino acids relative to carbohydrates, and not in response to the absolute quantify of either nutrient or total calories. While olfactory sensing of dietary yeast is sufficient to *inhibit* longevity assurance induced by DR, the primary *induction* of longevity assurance signaling and cellular mechanisms should require responses to ingested nutrients. The fat body of *Drosophila* appears to be a nutrient sensor that produces hormonal signals to modulate a network of neuropeptides within the brain that control insulin-like peptides. Fat body DILP6 and Upd2 are produced respectively in response to elevated dietary protein or carbohydrates and fat. These hormones may work together to signal the state of dietary P:C ratio to neurons with hypothalamic-like functions to regulate DILP2, DILP3 and DILP5 induction and secretion from insulin producing neuronal cells. Notably, the IPC itself is not thought to have cell-autonomous nutrient sensing capacity. Yet, while it is soundly demonstrated that aging can be slowed when DILP secretion is reduced, or when IIS is systemically inhibited, it remains unclear whether the longevity effects of DR are mediated through this signaling pathway. In part this ambiguity may arise because our standard gene-by-diet approach to analyze when mutants are required for DR explores too narrow a slice of the nutrient response landscape. Alternatively, the translation of nutrient sensing into longevity control may involve systemic hormonal signaling independent of IIS, or autonomous nutrient sensing through TOR, although current evidence based on gene-by-diet interaction analysis again does not yet prove this pathway is required for DR. Given the range of potential systemic and even cell-autonomous nutrient sensors, and the dimensionality of the nutrient space that must be tested with mutants and demographic outcomes, *Drosophila* shall continue to be a powerful model to discover how restricted diet without malnutrition slows aging.

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Glossary

Dietary restriction (DR)

Consumption of less food (without malnutrition) resulting in increased life expectancy. Historically attributed to the effect of consuming fewer calories (“caloric restriction”), many data now

	demonstrate that DR is stimulated by restricting specific nutrients rather than by limiting total energy intake
Drosophila Insulin Like Peptides (DILP)	Drosophila has eight recognized loci encoding peptides with sequence and putative processing homologous to mammalian insulin. DILPs signal through the insulin-like receptor and variously regulate growth, metabolism, aging, reproduction and other phenotypes
Drosophila Forkhead box type O transcription factor (dFOXO)	the homolog of mammalian FOXO1, FOXO3a and FOXO4
Holidic diet	Nutritional media fed to Drosophila composed entirely of chemically specified ingredients
Insulin/IGF signaling (IIS)	Drosophila has a single (recognized) insulin-like receptor that regulates traits commonly attributed to both insulin and IGF signaling of mammals
Life expectancy	From a given age, the duration of time until half the current cohort dies. Life expectancy from the onset of adulthood (eclosion in the case of Drosophila) is informally referred to as lifespan or longevity
Longevity reaction norm	Life expectancy plotted as a function of diet for a specific genotype. Reaction norms feature in gene-by-environment analysis, a tool of evolutionary genetics used to study phenotypic plasticity
Medial neurosecretory cells (MNC)	Neurosecretory cells in the medial region of the insect brain that contains the insulin-producing cells (IPC), among other neurons
Nutritional rail	a line (vector) representing a give food (or diet) mixture in nutrient space
Nutritional response landscape	A three-dimensional topographic form of longevity reaction norm in the context of the Geometric Framework where life expectancy, or any other response variable, is a function of two dietary variables; calories consumed as protein and as non-protein (carbohydrates and fat). Fitted isoclines on the topographic surface on the z-plane connect values of life expectancy observed among the experimental cohorts
Drosophila Target of Rapamycin (dTOR)	The homolog of mTOR, a kinase that responds to cellular import of amino acid to regulate various processes including translation and autophagy

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Box 2**Outstanding Questions**

- If insulin/IGF signaling is not the nutrient-sensing pathway that mediates the effect of DR upon longevity, what is? Might insulin signaling modulate the impact of DR upon lifespan through its effects on TOR rather than through its control of FOXO? Independent of IIS, how can we robustly determine if TOR signaling modulates the beneficial effects of DR, given that *Drosophila* TOR null-mutants are not viable?
- How do insulin/IGF, TOR, GABA and sNPF pathways interact to maintain stable states of pro-reproduction (with associated aging) relative to longevity assurance (with reproductive quiescence)?
- How do mutants of candidate DR-mediating pathways affect the nutritional response landscape of longevity relative to their wild type controls?
- Are total or specific amino acids sufficient to regulate longevity in response to DR? How do such ingested regulatory nutrients interact with regulatory signals that are derived from distant cues such carbon dioxide?

Highlights

Drosophila is a physiologic, genetic model to determine how dietary restriction extends lifespan

Reduced insulin/IGF extends fly lifespan but may not mediate dietary restriction aging

New tools to study dietary restriction: Nutritional Geometric Framework, holidic diet

Dietary restriction may extend lifespan by limiting amino acids alone or relative to carbohydrates

Figure 1. Nutritional response landscapes of median lifespan

A) Topographic representation of *Drosophila melanogaster* median lifespan response landscapes as functions of consumed protein and carbohydrates. Modified from Lee et al. [12]. Isoclines represent life expectancy fit to a spline surface estimated from median lifespan from 28 female cohorts maintained on different diet combinations of protein and carbohydrate. A diet that yields consumption of 1P:16C produces the maximum lifespan (red dash line). Transects of isocaloric intake (black dashed lines): lifespan increases along an isocaloric transect as the ratio of consumed protein to carbohydrate decreases; caloric intake itself can vary without affecting lifespan. Hypothetical response landscapes for a wild type (**B**) and a mutant (**C**) that represent different total elevation and topography. Modified from Piper et al. [31]. The differences among the topographic shape of these landscapes represent an underlying mechanistic interaction between the mutated gene and longevity in response to diet; *the gene participates in how DR modulates lifespan*. Arrows represent potential vectors of diets in a hypothetical experiment to determine how lifespan responds to DR when diet is manipulated by dilution (here with carbohydrates fixed at 1000 μ g and protein diluted from about 1500 μ g to 400 μ g.) **D)** Observed median lifespan that would be obtained in the gene-by-diet analysis with the diet dilution vectors of figures **B** and **C**. Although the mutant (green) is longer-lived than wild type (blue) on all tested diets, its reaction norm to dietary protein has the same response (slope) as wild type controls. Such data would inadvertently suggest that the mutated gene *does not* contribute to the mechanism by which DR modulates lifespan.

Figure 2. Observations for genotype-by-diet interaction to assess the role of insulin/IGF signaling and TOR as mechanisms of DR induced longevity

Diet is manipulated by dilution of yeast alone (Y%), or by sugar and yeast together (SY%).

A) Schema for reaction norm of life expectancy across a single factor diet dilution series. In the range of DR, lifespan increases to its maximum as diet is diluted. Further dilution progressively reduces lifespan, presumably due to malnutrition. **B–G)** Reaction norms of wild type and mutant flies to evaluate gene-by-diet interaction as evidence that a gene contributes to the mechanism of DR: compare reaction norm slopes to the right of the diet that maximizes lifespan. **B)** *foxo* null mutant (*foxo²¹/foxo²⁴*) relative to wild type controls, redrawn from Min *et al.* [40]. **C)** *foxo* null mutant (*foxo²¹/foxo^{25c}*) relative to heterozygotes (+/*foxo²¹*), plotted from data of Giannanku *et al.* [41]. **D, E)** Genetic ablation initiated during development of insulin producing cells (IPC-KO) using a binary UAS/Gal4 to drive apoptosis in cells that induce the *dilp2* promoter relative to intact controls (wild type with only Gal4 or UAS). **D** are females, **E** are males. Drawn from data of Broughton *et al.* [45] excluding the 1% yeast diet where survival was 20–24 days for all genotypes. **F)** Mutant Drosophila insulin receptor substrate (IRS1-4) homolog *chico* (*chico¹/chico¹*), redrawn from Clancy *et al.* [43]. **G)** Null mutants (males) of the Drosophila 4e binding protein (*4eBP*) relative to wild type; redrawn from [29].

Figure 3. Insulin producing cells and regulators of adult *Drosophila* gut and nervous system

Two clusters of insulin producing cells (IPC) among the medial neurosecretory cells (MNC) (inset 3A) insulin producing cells (**illustrated in red**) secrete *dilp2*, *dilp3* and *dilp5* [39]. DILP peptides are secreted within the brain, into the corpora allata (inset 3B yellow marked tissue [69]), to the proventriculus and crop, and to the aorta from which DILP is released into circulation [70]. DILP7 is produced in neuromeres (not illustrated here) of the ventral nerve cord that extend to the hindgut and rectal papillae [70]. Malpighian tubules (**illustrated in blue**) are osmoregulatory tissues with kidney-like functions (Inset 3C); these express *dilp5* thought to act in a paracrine fashion [71]. Fat bodies (Inset 3D) are distributed in the abdomen and within the head capsule (**illustrated in yellow**); this tissue produces *dilp6* as well as the leptin-like Upd2 [55, 61]. Upd2 is secreted into circulating hemolymph and acts upon GABA secretory neurons (GN, **illustrated in blue**) within the brain that induce DILP secretion from the MNC. Insulin production in the MNC is also modulated by input from dorsal lateral peptidergic neurons (DLP, **illustrated in brown**) neurons that produce sNPF as well as the stress responsive neuropeptide corazonin [64]. DILP6 is an insulin-signaling agonist within fat body, but it systemically reduces IIS activity by repressing DILP2 secretion from the MNC [55]. While DILP6 of fat body represses sNPF expression in the brain, it remains unknown whether DILP6 acts as an endocrine to directly affect the MNC or acts through intermediary adipokines or metabolites. This figure was developed from the detailed review of Nassel *et al.* [31].