Can heavy isotopes increase lifespan? Studies of relative abundance in various organisms reveal chemical perspectives on aging

Xiyan Li and Michael P. Snyder**

Stable heavy isotopes co-exist with their lighter counterparts in all elements commonly found in biology. These heavy isotopes represent a low natural abundance in isotopic composition but impose great retardation effects in chemical reactions because of kinetic isotopic effects (KIEs). Previous isotope analyses have recorded pervasive enrichment or depletion of heavy isotopes in various organisms, strongly supporting the capability of biological systems to distinguish different isotopes. This capability has recently been found to lead to general decline of heavy isotopes in metabolites during yeast aging. Conversely, supplementing heavy isotopes in growth medium promotes longevity. Whether this observation prevails in other organisms is not known, but it potentially bears promise in promoting human longevity.

Keywords:
aging; kinetic isotopic effects; longevity; stable isotope

Introduction

Understanding the causes of aging in life has emerged as a major research subject in recent years for both scientific and social reasons. On the one hand, aging, manifested as the chronological deterioration in biological functions, is such a complex process that today's aging studies have encompassed virtually all research fields known in life sciences: theories address the molecular, cellular, and organismal aspects from all known angles [1]. This is not surprising, because any incidental impairment of functions, whether for intrinsic or extrinsic reasons, could lead to profound long-term negative impacts on the fitness in individuals. Reduced fitness likely entails lower chances of survival and shorter life expectancy.

On the other hand, understanding and handling aging is an important issue for society world-wide. The global population, especially in the developed countries, has an increasingly longer lifespan expectancy, which has been made possible because of medical and technological advances. The expanding fraction and duration of seniority in the global population may pose a heavy socioeconomic burden on society, as manifested by the predicted spike of neurodegenerative diseases in seniors in the United States, and soaring costs in medicine and healthcare in the later years of life [2, 3]. Understanding aging and promoting healthy aging have thus, become an imminent and imperative mission for the scientific community.

Consistent with the complexity of biological systems, the regulation of aging has been shown to occur at multiple levels, ranging from inherited traits (i.e. genetics), to fostered life styles, to incidental environmental exposure. Understanding all these factors in terms of their relative contribution to aging is obviously a daunting job from a technical point of view. To date, two types of factors that retard aging have been widely recognized: acquired and inherited. Acquired factors include reduced nutritional uptake, termed calorie restriction (CR, diet from carbon sources), or dietary restriction (DR, diet from carbon and/or nitrogen sources) [1]. As the only means identified so far that extends lifespan across experimental organisms, DR is believed to stimulate the organism to maintain an active metabolic hunger state that may benefit its long-term fitness, although the detailed mechanisms involved are not understood.

DOI 10.1002/bies.201600040

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Abbreviations:
KIE, kinetic isotopic effect; **ROS,** reactive oxygen species.

Hypotheses

Inherited aging factors have received much more attention. Through experimental studies of lifespan and senescence, aging has been genetically and functionally linked to a variety of genes that regulates nutrition uptake, metabolism, DNA and protein quality, cell regeneration, intercellular communication, immunity, and cognitive activities [1]. An interesting pattern among these genes is that their lifespan extending effects often result from their absence (or absence of their product) or suppression. A few notable examples include amino acid uptake facilitators, mTORC, histone-modifying enzymes, and nutrient-sensing insulin/IGF-1 pathway [1]. The very presence of these genes is considered to promote aging. This may sound counterintuitive, and raises an intriguing question: how can a gene that, after having been subjected to so many years of functional selection in evolution, becomes detrimental to the survival of an individual? The answer may lay in how we understand aging and interpret the results from aging studies: minimally one can safely contend that these genes underscore the importance of fitness adjustment in aging regulation.

Are there aging factors that are independent of organismal fitness rectification? Some hints can be garnered from a classical aging assay: the yeast replicative lifespan assay. In this assay, individual cells are cultured with infinite supply of nutritional resources on a solid surface. Whereas nutritional effects on fitness are essentially masked, the ability of cells to produce progeny slows and stops after 20–30 cell divisions [4]. Clearly, something has diminished irreversibly over time, even when cells are maintained in nearly "perfect" conditions. These underlying factors may be intrinsic features of the process we call "living," and explain, at least in part, the inevitable reason for aging. And because yeast cells cease to divide, despite ideal conditions, it is likely that such factors bear characteristics that are out of reach of genetic regulation, a feature reminiscent of aging. To find out what they are, scientists have to look at more fundamental aspects of life.

In a recent effort to look for the intrinsic factors that cause aging, we have discovered a potential candidate [5]. By examining the intracellular small-molecule metabolites in yeast cells undergoing aging, we found that as yeast cells age, the overall heavy isotopic content, such as that of carbon-13, nitrogen-15, and hydrogen-2 (deuterium) declines in the amino acids, an essential group of metabolites that serve as building blocks in protein biosynthesis and precursors in all living organisms (Fig. 1). Moreover, supplementing heavy isotopes through nutritional uptake extends the lifespan of yeast by more than 80% in aging assays, likely via eliciting a DR-like effect [5]. If this observed trend represents a wide-spread phenomenon in the isotopic composition of the metabolome, proteome, and genome in other organisms as well, new perspectives on understanding aging and retarding the end of life may open up. In the rest part of this manuscript we will speculate on why this might be the case.

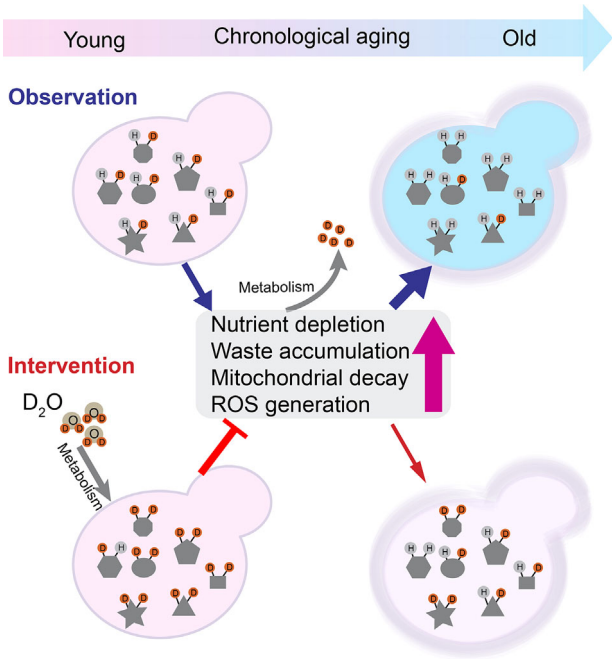

Figure 1. A model summarizes the involvement of natural deuterium decline in yeast aging. Polygons: different metabolites. D, deuterium; H, hydrogen-1; O, oxygen; ROS, reactive oxygen species. For details, see [5].

The kinetic isotopic effect in brief

Carbon, hydrogen, nitrogen, and oxygen, four of the most abundant chemical elements found in life, account for 96% by weight and 99.9% by atomic composition [6]. They all have naturally occurring non-radioactive heavy isotopes, which exist only in low levels (Table 1). Heavy isotopes are usually neglected in biological experiments because they largely resemble their light counterparts in chemical properties due to the identical numbers of protons in the atoms. However, because of their heavier atomic masses, heavy isotopes form more stable bonds. This difference in bond stability is known to shift the kinetics in chemical reactions up to sixfold (e.g. for deuterium) (Table 1), a phenomenon known as the kinetic isotopic effect (KIE). KIE may be subtle in biology in the case of momentary observations, but it may become relevant when cumulative effects are considered. A significant effect of the KIE may thus, occur in aging in the longer term.

Widespread isotope discrimination exists in living organisms

The ability of biological systems to discriminate among isotopes has been widely documented by comparing the isotope abundance in a specific organism

Table 1. A sampling of heavy isotope abundances in the literature

Carbon-13 (^{13}C)	Deuterium (^{2}H)	Nitrogen-15 (^{15}N)	Oxygen-18 (^{18}O)	Sulfur-34 (^{34}S)	Organism (Ref.)
Natural isotopic abundance (%)					
1.07	0.115	0.368	0.205	4.29	
KIE discrimination coefficient[a]					
0.961	0.707	0.966	0.943	0.970	
Isotopic ratio range (δ, ‰)[b]					
−8 (1)	−180 (2)	−13 (1)	−25 (2)	3.0 (1)	Earth atmosphere (1) and water (2) [43–45]
−30 to −20					C_3 plants photosynthesis [7]
−33 to −24					C_3 plant body [46]
−5.7					C_4 plants photosynthesis [7]
−16 to −10					C_4 plant body [46]
			5–25		C_3 plants [8]
				−1.9 to 5.2	Plants [45]
−20 to −16		8–12			Human adults and infants, finger nails [9]
	−38 to −28		−6 to −3		Human infant, urines [10]
−30 to −12		1–8			Beef, chicken, and fries [11]
−24 to −18		13–18			Fish (grouper) [12]
−26 to −24		6–8			Bird (quail) [13]
−16 to −15					Fish (sole larvae) [14]
−38 to −14					Nematodes, Crustaceans, insects, mice [15]
−23.5		4.2–5.8			Bird (quail) and sheep [16]

[a]KIE discrimination coefficient: for simplicity, the KIE coefficient is theoretically estimated as the square root of the reversal of heavy and light isotope mass ratio, and expressed as % of the kinetic constant of light isotope. The experimental KIE coefficient for deuterium-carbon (vs. protonium carbon) bond is ~1/10 to 1/6.
[b]Reported isotopic ratio range is expressed as levels below or above respective international standards.

with its surrounding environment (Table 1). In plants, carbon isotopes are discriminated in photosynthesis, a natural chemical process of carbon fixation, and the entry point of carbon into the whole biosphere [7]. Notably, carbon-13, the heavy isotopes with 1% natural abundance, is discriminated against in typical C_3 plants, which results in its depletion [7]. Conversely, plants enrich oxygen-18 in their leaves, an observation thought to be related to transpiration or water movement in the plant body [8].

Heavy isotope discrimination has been observed in humans as well. Infants assimilate more carbon-13 content and more nitrogen-15 in their fingernails than food sources contain [9], and excrete less deuterium and oxygen-18 in their urines [10]. Similarly, differential utilization of carbon-13 and nitrogen-15 has also been reported for human adults and animals including fish, birds, and cattle, as summarized in Table 1 [9, 11–15]. In addition, food prepared from animal sources tends to have much higher nitrogen-15 content compared with food from plant sources, as observed in infant formulas and whole animals [9, 15]. This suggests that the position in the food chain/web also has an impact on heavy isotope content in different forms of life.

Intriguingly, heavy isotope enrichment seems to occur in many animals on a temporal scale. In one study, both carbon-13 and nitrogen-15 spiked in the fingernails of human infants at early stages (solely breast feeding) but declined with onset of weaning [9]. Conversely, human infants excrete urine that is depleted in both deuterium and oxygen-18, suggesting that human bodies tend to retain heavy isotopes of oxygen and hydrogen [10]. In addition, breast milk feeding has been found to cause a 25–50% boost in both urinal oxygen-18 and deuterium in babies [10]. Similar enrichment was also observed in sole fish larvae, where carbon-13 content increased by 25% in the first 4 weeks of their lives after hatching [14] and in various other animals fed with different diets [15]. It should also be noted that carbon-13 does not appear to be further depleted in animals after its entry into the biosphere through carbon-13 depleting photosynthesis (Table 1), which is probably due to the fact that no extensive metabolic rearrangement of carbon skeletons occurs in metazoan consumers. This observation is also well in line with the general trend of heavy isotope enrichment in various organisms.

Why heavy isotopes are enriched or depleted through biological activities in metazoans still requires more in-depth examination, but the underlying mechanisms, especially in the context of development and aging, could provide important quantitative clues to understand the chemical bases for life. In addition, it should be noted that all aforementioned studies only analyzed the overall isotope composition, yet did not provide more information at the molecular level. It is, thus, not clear whether some processes and enzymes contributed more to the isotopic preference through biological activities.

In a recent study, we have directly measured the heavy isotope content for individual small metabolites by high-resolution mass spectrometry [5]. In yeast cells that have been grown in non-dividing conditions via nutrient

limitation, the overall content of carbon-13, nitrogen-15, deuterium, and sulfur-34 in free amino acids all decline with the progression of chronological aging. This decline is accompanied by diminishing capability of the cells to form colonies in rich media, a measure of vitality. Given the fact that the experiments were carried out in starvation conditions, the decline in heavy isotopes suggests that a constant assimilation of heavy isotopes might be essential to retaining vitality. This notion is supported by other experiments in our study, where supplementation of deuterium or carbon-13 through nutritional uptake, albeit with different efficacy, was confirmed to extend the chronological lifespan of yeast [5]. Consistent with our findings, it has been observed that both carbon-13 and nitrogen-15 are depleted in animal blood and muscle samples after 8-weeks of preservation under incomplete denaturation in vitro, such as in DMSO [16]. This suggests a connection of gradual loss of vitality with the decline of heavy isotope content in the organic matter from living cells.

Taken together, isotopic discrimination exists widely in the biota. The relative magnitude of this discrimination might be elusive, but the trend of change may reveal trace marks for long-term processes such as development and aging. Most of the studies prior to ours have focused on measuring the overall isotopic abundance. However, it is possible that some key processes and key enzymes, such as phosphoenolpyruvate carboxylase for carbon-13 discrimination in plants [7], contribute more significantly to isotopic enrichment or depletion. If this is the case, it is necessary to investigate this issue at the molecular level rather than by simply performing global abundance assays.

Modern analytic instruments are already capable of measuring isotopes in molecules. For example, mass spectrometry has the capability to measure individual adducts from different element isotopes in a particular metabolite (see Fig. 2 for an example). Operating at a mass resolution of 100,000, for example, the mass spectrometer can discern the subtle difference between different isotopes in masses. With this tool in hand, we can measure the isotope content for a particular metabolite in various samples [5]. We should note that technical limitations, such as mass resolution and dynamic range, do limit our scope to detect and distinguish small molecules with a weight of 200 Da or less, as shown for tryptophan in Fig. 2. Technical improvement is certainly needed to expand our scope, but that issue is beyond the remit of this manuscript, and will be addressed elsewhere.

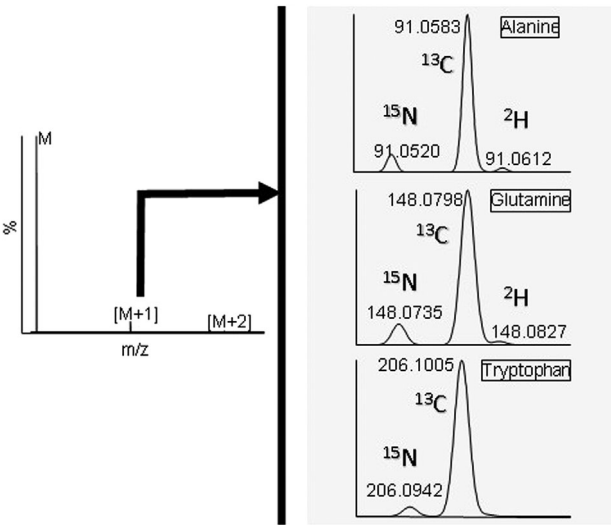

Figure 2. A conceptual demonstration of measuring heavy isotopes in metabolites by mass spectrometry. The separation of mass peaks for three amino acids is simulated in Xcalibur (v2.2, Thermo, Sunnyvale, CA), with a setting of mass resolution at 100,000 in positive mode.

Heavy isotopes profoundly modify biological functions

Despite extensive experimental and theoretical studies on stable isotopes in the chemistry field, there exist only limited – but intriguing – functional studies addressing heavy isotopes in the biological contexts. Most of these studies used deuterium, probably because deuterium could produce the most obvious KIE (Table 1) and is abundantly available as heavy water (D_2O) at a reasonable price.

Water is the most abundant and most used metabolite in living organisms on Earth (Fig. 3), which makes it an appealing vector to introduce heavy isotopes into biological subjects. Shortly after its discovery by Harold Urey, deuterium was used in the form of heavy water to treat various organisms including plants, yeast, and animals [17, 18] and lately fruit flies [19, 20]. Amazingly, feeding experimental organisms with heavy water, despite its pronounced KIE effects, does not seem to have outstanding negative effects on life, and it merely slows down biological processes, such as seed germination, seedling development, fermentation in yeast, motion ability in flatworms, and produces tolerable behavioral changes in mice that are thought to be non-toxic [17]. Heavy water treatment at high dosage (50% or above) does not alter cell size, growth, metabolome structure, or oxygen consumption in yeast [5, 18]. Different species exhibit different levels of tolerance to heavy water uptake, e.g. a level above 20% of heavy water by weight was thought to be toxic in mammals, and only produced negligible growth effects in yeast and algae [17, 18, 21]. In most cases, the adverse effects caused by heavy water treatment can be reversed upon treatment withdrawal. These studies suggest that heavy isotopes, in the case of high dose of deuterium, are well tolerated in biological systems, and thus, do not seem to do serious harm to basic biological activities. Also, as suggested by the fact that heavy water only elicits metabolic slowdown that is akin to

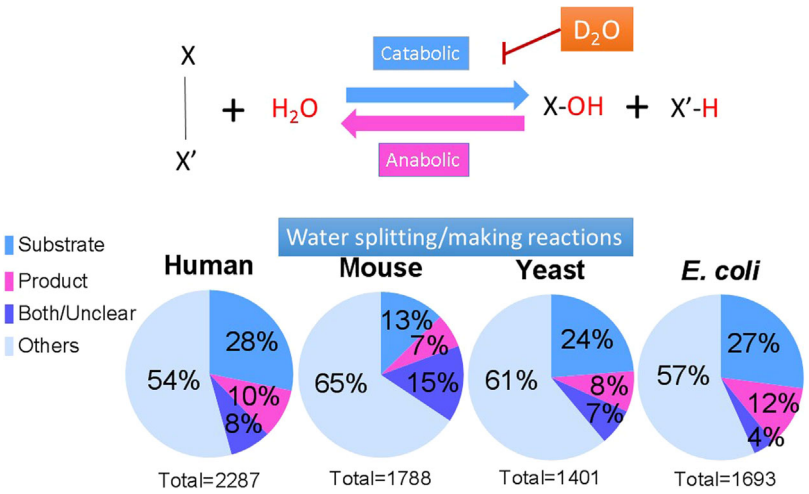

Figure 3. Water prevails in biochemical reactions. Upper panel shows water involvement in catabolism and anabolism. Potential impact of heavy water (D_2O) on the chemical equilibrium is also indicated. Lower panels summarize the percentage of total biochemical reactions that use water as substrate, product, or both, respectively in four model organisms. Data were retrieved from BioCyc website [47]. The light blue and magenta colors denote catabolic and anabolic reactions, respectively, as shown in the upper panel.

low temperature preservation [18, 20, 22], the largely reversible effects caused by heavy water treatment may also find applications in hibernation-like lifespan extension, as would be needed for interstellar travel.

At the cellular and molecular levels, heavy isotopes also produce profound effects. In plants, photosynthetic discrimination against carbon-13 in carbon dioxide (CO_2) is less evident in C_4 plants (mostly grasses) than in C_3 plants (95% of all plants such as trees), which is thought to result from recycling usage of CO_2 due to anatomic separation of photosynthetic reactions in C_4 plants [7]. Furthermore, PEP carboxylase, the major CO_2 fixing enzyme in C_4 plants, exhibited much less discrimination against carbon-13 than Rubisco, the other major CO_2 fixing enzyme in C_3 plants (PEP, 0.2%; Rubisco, 3%) [7]. The general retarding effects of KIE may explain why photosynthesis in C_4 plants is more efficient than in C_3 plants.

Another known discrimination against carbon-13 happens in fatty acid metabolism in both prokaryotic and eukaryotic microbes [23, 24]. The carbon-13 content at the carboxyl group of cellular fatty acids in acyl-CoA form is depleted at the transacylation step leading toward free fatty acids, whereas, most carbon-13 content is diverted to desaturation to produce unsaturated fatty acids in acyl-CoA form. This discrimination likely stems from the enzymatic action of thioesterases, a type of water-splitting hydrolases. Because thioesterases presumably do not distinguish between saturated and unsaturated acyl-CoA, there must be an exit route for the otherwise dynamically accumulating carbon-13 in acyl-CoA. A candidate exit route might be beta-oxidation, a mitochondrial/peroxisomal process that starts from acyl-CoA dehydrogenation but does not involve the carboxyl C-1 position whatsoever. The end product acetyl-CoA can readily act as a substrate for other processes such as the Krebs cycle. In this case, crossing spatial barriers such as the mitochondrial membranes by acylcarnitine translocation system should not affect the net metabolic outcome. A systemic mechanism for carbon-13 fractionation may be depicted, where carbon-13 in fatty acids is preferentially routed for dehydrogenation and in turn subject to catabolic breakdown through beta-oxidation. If this phenomenon also exists in higher organisms, manipulation of carbon-13 content through fatty acids uptake may impact beta-oxidation in mitochondria and peroxisome, both of which activities are linked to longevity and aging symptoms [25–27].

More evidence about the biological effects of deuterium comes from animal studies. In isolated rat liver mitochondria, depleting deuterium in natural water by distillation to one third of the original abundance has been shown to boost succinate-dependent H_2O_2 generation by 67% in maximum velocity and enhance the substrate affinity by 57% in Michaelis constant Km [28]. Since the monitored reaction is an indicator of overall respiration function in mitochondria, an intracellular hub for energy and material metabolism, one would expect that deuterium in natural water can greatly impact the overall metabolism in any eukaryotes. This notion is also supported by our recent observation in yeast, where heavy water treatment remarkably suppresses the endogenous generation of reactive oxygen species (ROS) by mitochondria [5, 28]. Another line of evidence comes from the protective effects of metabolite deuteration. A recent study has found that deuterated polyunsaturated fatty acids, even supplied in a minor fraction, protected mammalian cells from various damage associated with oxidative stress, such as lipid peroxidation and mitochondrial uncoupling [29]. Deuteration of lipids, a major group of metabolites that are mainly metabolized in mitochondria, is thought to stabilize the aerobic metabolism and prevent uncoupling of respiration from ADP phosphorylation, a major source of endogenous ROS generation and chemical damages [29–31]. The chemical basis for these benefits originates exclusively from KIE.

Perplexingly, contradictory reports have emerged from deuterium depletion studies that may suggest heavy isotopes are detrimental. Tumor regression was reported to result from applying deuterium-depleted water in dogs and humans, and cancer cell growth in vitro was inhibited by deuterium depletion as well [32–34]. The benefits of deuterium depletion in cancer treatment are linked to suppression of oncogenic genes and the promotion of apoptosis, and exhibited strong differences in efficacy between sexes [33, 34].

However, the apparent contradictions with regard to the beneficial claims of deuterium in biology may be reconcilable. First, because cancer cells still use the same regulatory mechanisms of cell cycle control as normal cells, clinical demands for growth

inhibition in cancer may not yield beneficial outcomes in the organismal survival of healthy individuals. Second, before a clear association between deuterium and gene mutation is established, the only plausible explanation for deuterium effects, whether good or bad, is generally attributable to deuterium's capacity for modifying metabolism, or through KIE. These studies all derived from the original observation that deuterium depletion retards cell cycle progression [32], which may also be interpreted as the necessity of deuterium for maintaining optimal growth in normal cells. In a situation similar to that where starvation causes muscle wasting, depletion of water-bound deuterium may elicit material recycling by triggering apoptosis in vitro and in vivo [33, 35]. As a result, deuterium-depleting and augmenting experiments may be united in these regard. What remains to be reconciled is whether metabolic slowdown by deuterium-feeding is consistent with inhibition of cell cycle progression by deuterium-depleting. This might be possible, because it is known that metabolism cycle proceeds independently with cell division cycle in yeast [36].

Heavy isotope content changes in aging

Because KIE is an intrinsic property that applies to all biochemical reactions, the affected biological systems cannot avoid it, but can merely accommodate it, which might leave cumulative chemical traces as an individual life progresses. One such trace has been captured by numerous isotope abundance analyses in various organisms, as noted in Table 1. For example, stable heavy isotope abundance is notably different between living and non-living matter, infant organisms and adults, different body parts, different dietary sources (animal vs. plant diet), and recently, between different stages of the lifespan [5, 9–15]. These studies all suggest that the change of heavy isotope content results from biological activities along the temporal dimension.

Among all the factors that may affect the in vivo abundance of heavy isotopes, the intrinsic time-dependence represents one of the most intriguing features for its implications in aging, a natural consequence of most lives. Ideally, a temporal trend of heavy isotope change at the global and molecular levels might reveal the status of an individual in terms of the aging clock. In addition, intervention with heavy isotope supplements may bear the hope of promoting longevity. In this regard, mere analysis of overall atomic abundance may not provide enough information to understand the underlying biological processes. More robust tools, such as mass spectrometry and nuclear magnetic resonance, may help to provide insights into biological players of relevance.

As the first study of its kind, we have found all three common heavy isotopes in amino acids declined in yeast undergoing chronological aging [5]. This decline can be effectively retarded by the supplementation of heavy isotopes, which, consistent with our hypothesis, also extends lifespan. Although only a small group of metabolites were covered, our study has provided molecular evidence that the temporal change in heavy isotope content is real and relevant in aging. Whether similar isotopic declines occur in other organisms, such as mammals, is still pending. Nevertheless, it would be of interest to compare the heavy isotope decline in long-lived model animals, such as naked mole rat, with that in other rodents of much shorter lifespan.

In lieu of the observed heavy isotope decline with age, the next question is: where do they go? Two possibilities exist: increased excretion or decreased retention. We favor the latter because so far there is no chemical evidence indicating that heavy isotopes can be actively enriched in a well-defined biochemical process. In contrast, decreased retention may originate from deficient assimilation through anabolism and internal partitioning into inactive stock molecules. Depending on how heavy isotopes are introduced, in general they should retard catabolic reactions but produce much milder effects on the anabolic reactions (see Fig. 3 for an example of deuterium and hydrogen). Therefore, it is more likely that the observed heavy isotope decline derives from internal partitioning, a notion that is supported by observation that the heavy isotope content declines in the same metabolites found in both the cytosol and media during yeast aging, as demonstrated for glutamine [5].

Promoting longevity and health by heavy isotope supplementation

Before our observation of heavy isotope decline during organismal aging, deuterium-bearing heavy water has been shown to promote longevity or improve certain health aspects in several organisms, including fruit flies, rodents, and humans [19, 20, 29, 37, 38]. In fruit flies, transient exposure to heavy water at juvenile stages extends lifespan, and the exposure does not affect the health and reproduction [19]. However, a dosage of 50% heavy water shortens the lifespan [20], and the relative lifespan shortening by heavy water was ameliorated by temperature elevation from 10 to 30°C, suggesting a protective effect of heavy water on fruit fly survival in hot conditions where accelerated metabolic rate normally reduces longevity. Improved thermoresistance was indeed observed at the protein, cell, and organism levels in fruit flies upon heavy water treatment [22]. Similarly, a driving factor in temperature-compensated effects by heavy water was observed to alter the phase relation in circadian oscillation [39]. The heavy water effect is increasingly more pronounced with rising temperature. However, the mechanism is still unknown. The similarity in the biological responses between heavy water and low temperature also correlate well with the general observation that fruit flies and worms have longer lifespan, and retarded brain degeneration when maintained at low temperature [40, 41].

Several functional studies have shown that deuterated polyunsaturated fatty acids, even supplied in a minor fraction (20–50%), can protect yeast and mammalian cells from ROS damage to mitochondria [29, 37]. In whole animals, 25% heavy water was able to normalize high blood pressure induced by high salt diet in rats, possibly through suppressing hypertension-related elevation in calcium uptake [38].

These effects would surely extend lifespan.

In yeast, we also showed that heavy water extends chronological lifespan in a dosage-dependent manner [5]. This pro-longevity effect could be essentially abrogated by mild dietary restriction or mitochondrion removal. Heavy water also suppresses the endogenous ROS generation, which could ameliorate the background chemical damages from ROS and lead to long-term improvement in fitness and survival rate. All these protective effects indicate that heavy water functions as a metabolism modifier to promote longevity, a feature that could be amenable to implementation in the context of other well-known anti-aging interventions [1].

One remarkable feature of heavy water in biology has emerged from several earlier observations [17], in which heavy water at high dosage (50–90%) was found to suppress seed germination, retard seedling germination, disrupt flatworm activities, and even stimulate hyperactivity in mice fed with one volume equivalent of their total body fluid daily. However, heavy water was found to produce only reversible or non-accumulative effects, in other words, no long-term toxicity. If these observations apply to long-term processes, then heavy water seems merely to elicit a hibernation-like dormancy, without producing long-lasting adverse effects. These reversible effects would make the application of heavy water even more appealing in retarding aging and preserving vitality in human activities involving survival over extraordinary time periods, such as interstellar travel.

Although heavy water dominates in our current understanding and appreciation of heavy isotopes in biology, because of the prevalence of water in biochemistry (Fig. 3), other deuterium-bearing metabolites and other elements may also prove useful. Deuterated lipids have shown great healthcare promise in protecting cells from ROS damage [29, 31, 37]. Deuterated drugs are also superior to their non-deuterated peers in safety, efficacy and tolerability, because deuteration alters their metabolic and pharmaceutical profiles in a favorable way [42]. The more abundant and prevalent an element is in biology, the more effects its heavy isotope likely produces (such as hydrogen in water, see Fig. 3). In fact, augmentation of overall heavy isotope uptake through diet has been speculated to provide health benefits over a wide spectrum [6]. It would be fascinating to see whether isotopes of other elements have similar effects: oxygen-18, carbon-13, and nitrogen-15 are especially relevant for their respective elemental prevalence in organic matter.

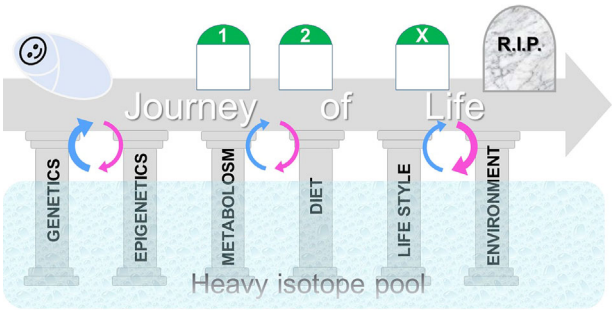

Figure 4. A speculative scheme accounting for the effects of isotopes in aging. The arrows are used in a similar sense as in Fig. 3, to indicate metabolic routes of heavy isotopes.

Despite their potential benefits over the long term, heavy isotopes are generally associated with growth and development retardation, as described earlier in this manuscript. How these adverse effects modify the long-term aging outcome remains to be evaluated, and more mechanistic insights are needed. But innovative approaches, such as deuteration of polyunsaturated fatty acids and transient exposure to heavy water, have already provided promising means to overcome the potential caveats and to maximize the beneficial outcome from heavy isotope supplementation.

Prospects and speculations

Based on our knowledge to date, we speculate that heavy isotope effects in biology represent a new perspective to understanding biological functions and aging, as conceptually illustrated in Fig. 4. This perspective opens a new avenue of inquiry into metabolism as a chemical basis for all biological functions. Moreover, because metabolism is composed of highly connective chemical reactions, heavy isotopes may flow through metabolic flux to proteins and nucleic acids, or simply partition into a subset of metabolites, all of which could be subject to isotopic analysis. Before thorough examination of the isotope composition in well-defined situations provides definitive answers, we can make several speculations:

1) We expect to see an aging-related decline of heavy isotopes in macromolecules such as the proteome and genome. Because the cellular metabolites serve as the building blocks for all macromolecules through anabolic reactions – such as amino acids for proteins and nucleic acids – the loss of heavy isotope content in the cellular metabolites will likely be carried over to proteins and nucleic acids as well.
2) We anticipate an aging-related enrichment of heavy isotopes in a subset of the metabolome, or Heavy Isotope Sink (HIS) metabolites, during aging. These HIS metabolites should be reasonably abundant and metabolically dormant, hence, tolerating potentially acute adverse effects from enriched heavy isotopes due to KIE. Candidates for such HIS metabolites include structural and storage lipids, polysaccharides and proteins as well as excreted waste metabolites.
3) With proper technical innovations, measurement of heavy isotope content may be used as a benchmark predictor to assess the chronological position of any biological subject in its lifespan. More generally, the heavy isotope content may represent a universal consumption marker for all biological activities that drive growth, development, and inevitably aging.
4) Supplements of heavy isotopes through the most relevant metabolic processes will help extend the

lifespan in most, if not all, organisms. Of course, mechanistic insights are needed to reveal the key processes and enzymes that possess the highest discrimination power against heavy isotopes. We anticipate that different pathways and enzymes will exert different and specific effects among different species, especially because environmental adaptation has shaped the metabolism of various organisms according to their natural habitats. Targeted supplementation of heavy isotope-containing metabolites would be a better way to boost efficacy while minimizing adverse consequences.

Conclusions and outlook

Retention of heavy isotopes through metabolism has been pervasively observed in various forms of life. The underlying mechanisms, presumably through KIEs, may be a chemical basis of aging. Recent studies have started to reveal general features and consequences of heavy isotopes in modifying the metabolism during organismal aging, and its application in promoting longevity in simple and complex organisms. How this perspective extends to larger organisms such as mammals remains to be examined. Such investigations will likely have profound implications for understanding aging and promoting longevity by providing new strategies in objective aging measurement and pro-longevity supplements.

Acknowledgments

This work was supported by NIH grant (5R01GM06248012) and California Institute for Regenerative Medicine grant (RB4-06087) to MPS. The authors would like to thank Dr. Peichuan Zhang for critical reading and comments during manuscript preparation.

The authors declare no conflict of interest.

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