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Certain metabolic interventions such as caloric restriction, fasting, exercise, and a ketogenic diet extend lifespan and/or health span. However, their benefits are limited and their connections to the underlying mechanisms of aging are not fully clear. Here, these connections are explored in terms of the tricarboxylic acid (TCA) cycle (Krebs cycle, citric acid cycle) to suggest reasons for the loss of effectiveness and ways of overcoming it. Specifically, the metabolic interventions deplete acetate and likely reduce the conversion of oxaloacetate to aspartate, thereby inhibiting the mammalian target of rapamycin (mTOR) and upregulating autophagy. Synthesis of glutathione may provide a high-capacity sink for amine groups, facilitating autophagy, and prevent buildup of alpha-ketoglutarate, supporting stem cell maintenance. Metabolic interventions also prevent the accumulation of succinate, thereby slowing DNA hypermethylation, facilitating the repair of DNA double-strand breaks, reducing inflammatory and hypoxic signaling, and lowering reliance on glycolysis. In part through these mechanisms, metabolic interventions may decelerate aging, extending lifespan. Conversely, with overnutrition or oxidative stress, these processes function in reverse, accelerating aging and impairing longevity. Progressive damage to aconitase, inhibition of succinate dehydrogenase, and downregulation of hypoxia-inducible factor-1α, and phosphoenolpyruvate carboxykinase (PEPCK) emerge as potentially modifiable reasons for the loss of effectiveness of metabolic interventions.

1. Introduction

"It may be well to keep in mind that cell nutrition probably implies a replenishment of electrons…Esoteric processes go on within the cell which draw in electrons, liberated from the breakdown of food substances, and distribute them for the renewal of the component atoms and molecules. All of which is accomplished under nature in the animal organism at present with errata of excess or failure, a per cent. of deficit and damage constantly accumulating through the period known to us as the individual lifetime." —Charles Asbury Stephens, 1920, pp. 205-206.[1]

Lifespan, defined as life expectancy at birth in the longest-lived country in a given year, has increased linearly at the rate of 1 additional year of life for every 4 calendar years since 1840.[2] Health span, however, has not fully kept pace, and advanced age is the main risk factor for numerous chronic, disabling, life-limiting diseases.[3] Thus, understanding and intervening in aging is likely to be the most efficient means of further improving human health.

Such an understanding will surely include a focus on cellular energetics, for energy metabolism and aging are intimately connected. ATP levels decline linearly with age in animal models and in human calf and heart muscle.[4] Bioenergetic failure is seen in certain senescent cells,[5] certain animal models of aging,[6] and such human age-related diseases as Alzheimer's,[7] osteoporosis,[8] sarcopenia,[9] heart failure,[10] glaucoma,[11] and COPD.[12] Cellular functioning depends on intact energy generation.[4,13] Indeed, so reliable is the association that a reasonably accurate estimation of a person's age can be derived almost exclusively from the energetic parameters of their peripheral blood mononuclear cells.[14]

Not surprisingly, then, the most effective means of extending life- and/or health-span—caloric restriction (CR), fasting, a ketogenic diet, and physical exercise—alter how energy is produced and utilized. However, even these "gold standard" interventions are limited, achieving at best an increase in average lifespan of ≈65% in mice,[15] ≈50% in lemurs,[16] and perhaps ≈ 15–20% in rhesus monkeys.[17] Moreover, their mechanistic relationship to key variables in modern theories of aging—DNA hypermethylation, accumulated DNA damage, stem cell exhaustion, oxidative stress, and inflammation—is uncertain. Clarifying the connections between energy metabolism and the mechanisms of aging could help integrate seemingly disparate data, explain the current limitations of metabolic interventions, point to ways of overcoming these limitations, and contribute to the prevention of age-related diseases.

In this review, these connections are explored in the light of the tricarboxylic acid (TCA) cycle specifically. The TCA cycle is

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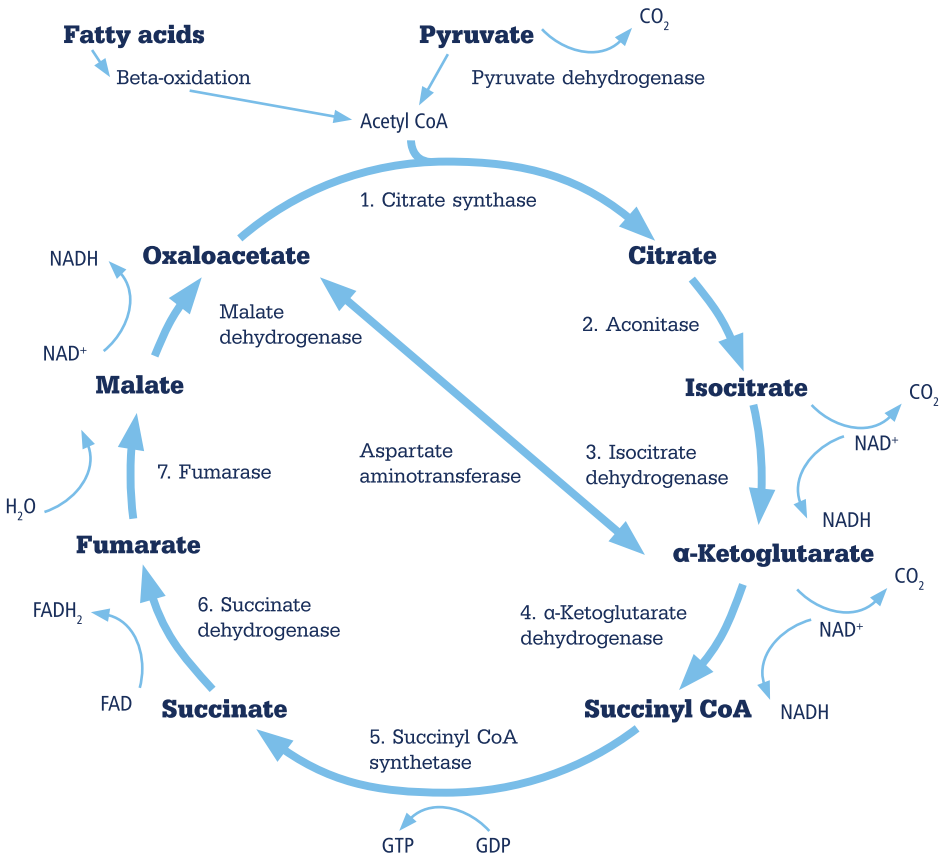

Figure 1. The tricarboxylic acid cycle. CoA = coenzyme A; FAD = flavin adenine dinucleotide; $FADH_2$ = flavin adenine dinucleotide (reduced); GDP = guanosine diphosphate; GTP = guanosine triphosphate; NAD^+ = nicotinamide adenine dinucleotide; NADH = nicotinamide adenine dinucleotide (reduced).

fruitful ground because it is central to energy generation, integrates numerous aspects of cellular physiology, has broad signaling effects within and beyond the cell,[18] and alters its functioning in response to environment, lifestyle, and behavior. Indeed, the TCA cycle is central to so many aspects of cellular homeostasis, it would be surprising if aging, in which homeostasis is broadly disrupted, were not affected by the TCA cycle.

Moreover, preserved TCA cycle functioning characterizes long-lived strains of rats[19] and human centenarians,[20] and the insertion of a gene in mice or roundworms that, among its effects, increases flux through the TCA cycle, confers longevity.[21,22] Conversely, a shift in energy generation away from the TCA cycle to glycolysis may limit lifespan. Overexpression of pyruvate kinase, the last, rate-limiting step of glycolysis, is sufficient to shorten lifespan in *Caenorhabditis elegans*[22] while inhibition of glycolysis confers longevity in some studies of model organisms.[23]

Before discussing these connections, however, let us first review the TCA cycle itself.

2. Tricarboxylic Acid Cycle

2.1. Structure and Function

The classic role of the TCA cycle is energy generation. Through glycolysis in the cytoplasm, carbohydrates are converted to pyruvate. The pyruvate then enters the mitochondrial matrix, where the TCA cycle is found, and is converted to acetyl-Coenzyme A (acetyl-CoA) by the pyruvate dehydrogenase complex (PDH). Similarly, fatty acids are transported into the mitochondria and are converted to acetyl-CoA through β-oxidation. Acetyl-CoA is the canonical input to the TCA cycle.

The TCA cycle is shown in **Figure 1**. Its 8 enzymatic reactions extract high energy electrons in the form of hydride groups (NADH and $FADH_2$) and feed them into the electron transport chain (ETC) for producing ATP. In the process, 2 carbons are lost as CO_2. These carbons are replaced with the 2-carbon acetyl-CoA in the next iteration of the cycle.

As seen in Figure 1, the 8 enzyme complexes of the TCA cycle are citrate synthase, aconitase, isocitrate dehydrogenase (IDH), α-ketoglutarate dehydrogenase (AKGDH), succinyl-Coenzyme A synthetase, succinate dehydrogenase (SDH), fumarase (or fumarate hydratase; FH), and malate dehydrogenase (MDH). As discussed in Sections 3.1.7 and 3.2, aconitase and succinate dehydrogenase seem to be particularly relevant to aging.

The enzymes produce the intermediates citrate, aconitate, isocitrate, α-ketoglutarate (AKG), succinyl-CoA, succinate, fumarate, malate, and oxaloacetate. Particular roles in aging seem to be played by citrate, AKG, succinate, and oxaloacetate.

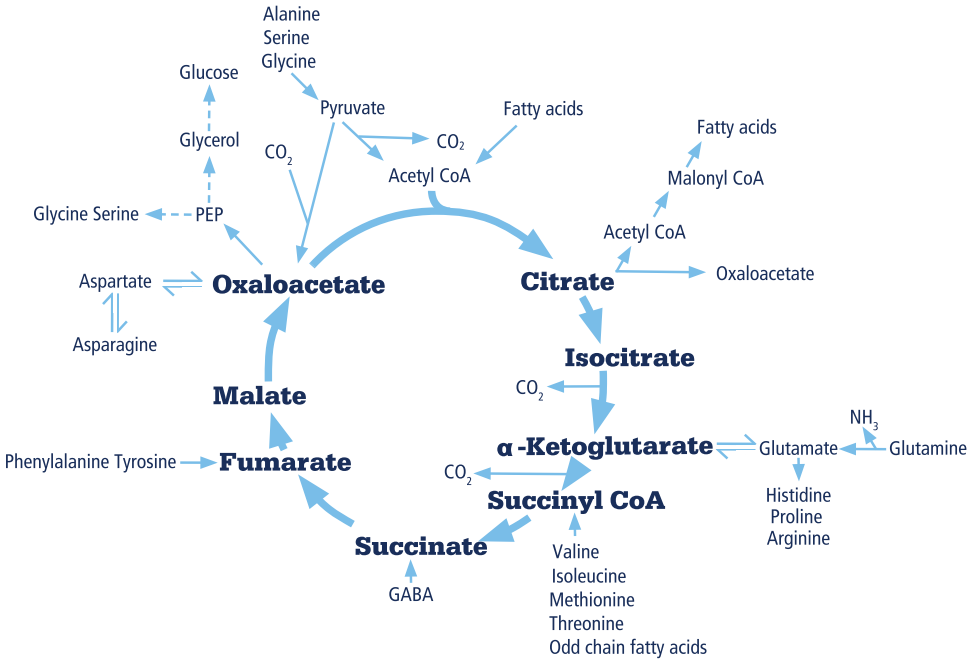

Figure 2. The inputs (anaplerosis) and outputs (cataplerosis) of the TCA cycle allow it to both catabolize glucose, amino acids, and fatty acids for energy and to produce them when needed. PEP = Phosphoenolpyruvate; NH_3 = ammonia.

2.2. Inputs and Outputs

The TCA cycle also produces many of the building blocks for cellular structure and function. In fact, biosynthesis was probably the original role of TCA cycle enzymes, before earth's atmosphere became oxygenated.[18] This removal of intermediates from the TCA cycle is termed cataplerosis (**Figure 2**).

Thus, excess citrate, as occurs when there is a surfeit of acetyl-CoA input to the TCA cycle or if there is damage to the downstream enzymes aconitase and IDH, is exported from the mitochondria to the cytosol, where it is dissociated back into acetyl-CoA and oxaloacetate by ATP-dependent citrate lyase (ACLY). The cytosolic acetyl-CoA is used for lipid synthesis[24] and signaling. The oxaloacetate can be used for producing glucose (gluconeogenesis), glycerol, or the amino acid aspartate.[24] As discussed in Section 3.1, aspartate seems especially consequential for aging.

Similarly, AKG can be used to produce the amino acid glutamate through the enzyme glutamate dehydrogenase (GDH):

$$\text{GDH}: \quad \text{AKG} + \text{NH}_3 \leftrightarrow \text{Glutamate} \tag{1}$$

although in vivo, GDH nearly always functions in the opposite, Glutamate → AKG direction.

Amine groups in amino acids can be transferred by transaminase enzymes, allowing amino acids to interconvert. Thus, alanine aminotransferase (ALT) shifts the amine group in glutamate to pyruvate, producing alanine and returning AKG as a byproduct. The reverse reaction occurs during fasting and exercise, when alanine is converted to pyruvate for use as an energy source:[24]

$$\text{ALT}: \quad \text{AKG} + \text{Alanine} \leftrightarrow \text{Glutamate} + \text{Pyruvate} \tag{2}$$

Similarly, aspartate aminotransferase (AST) catalyzes the reversible reaction:

$$\text{AST}: \quad \text{Glutamate} + \text{Oxaloacetate} \leftrightarrow \text{AKG} + \text{Aspartate} \tag{3}$$

The direction of the reactions depends on the relative concentration of the substrates. A buildup of oxaloacetate, for example, will push the AST reaction in the forward direction, generating aspartate, while a depletion of oxaloacetate will pull it in the reverse direction and consume aspartate.

Because aspartate has a key signaling function in aging (discussed below, in Sections 3.1.2 through 3.1.7), it is likely that the AST reaction in the forward direction accelerates aging and in the reverse direction decelerates it.

Enzymes downstream of cataplerosis include the above-noted ACLY, and phosphoenolpyruvate carboxykinase (PEPCK), which transforms oxaloacetate into phosphoenolpyruvate (PEP), a usually irreversible step in the production of glucose, glycerol, and the amino acids serine, glycine, and cysteine.

$$\text{PEPCK}: \quad \text{Oxaloacetate} + \text{GTP} \leftrightarrow \text{Phosphoenolpyruvate} + \text{CO}_2 + \text{GDP} \tag{4}$$

As intermediates are exported from the TCA cycle for biosynthesis, there must be counterbalancing input.[25] As we have seen, the canonical entrance to the TCA cycle, PDH, provides acetyl-CoA but there are numerous other entrance points that can replenish the TCA cycle.[26] Replenishment of intermediates through these alternative entrances is termed anaplerosis. One of the most important is the direct conversion of pyruvate to oxaloacetate by the pyruvate carboxylase (PC) enzyme (Figure 2):

$$\text{PC}: \quad \text{Pyruvate} + HCO_3^- + \text{ATP} \leftrightarrow \text{Oxaloacetate} + \text{ADP} + P_i \quad (5)$$

Because of its direct production of oxaloacetate, pyruvate carboxylase is favored in the liver when oxaloacetate is needed for gluconeogenesis, for example during fasting,[27] and in the brain, by a ketogenic diet.[28]

TCA cycle intermediates also have signaling functions, helping to coordinate metabolism. For example, succinate can signal by becoming attached to lysine residues on enzymes (succinylation), by inhibiting certain AKG-dependent enzymes, and through the succinate receptor.[29] As discussed in Section 3.2, succinate is relevant to several features of aging, including inflammation, hypoxic signaling, overuse of glycolysis, and increased damage and hypermethylation of DNA.

2.3. Internal Dynamics of and around the TCA Cycle

Interactions between intermediates and enzymes help govern the TCA cycle. This allows the cycle to adapt to the environment. Thus, although substrate will often proceed smoothly around the TCA cycle in the standard clockwise direction, this is not always the case. Enzymes can be blocked, segments of the cycle can be skipped, and a buildup of substrate can cause segments to reverse. Indeed, only 3 steps, the formation of acetyl-CoA from pyruvate, of citrate from acetyl-CoA and oxaloacetate, and of succinyl-CoA from AKG, are regarded as thermodynamically unidirectional.[30]

Moreover, we have seen that through transamination, substrates can jump across the cycle, the direction depending on their relative concentrations. That is, oxaloacetate (in the 11:00 position in Figure 1) can be converted to AKG (in the 3:00 position) and vice versa via the AST reaction.[24]

Thus, the TCA cycle, central to cellular homeostasis, can reconfigure itself in response to the environment, lifestyle, and behavior. Let us now examine how this reconfiguration can affect the rate of aging.

3. Mechanisms Connecting the TCA Cycle to Aging and Longevity

3.1. Autophagy and Its Regulation by Aspartate

3.1.1. Autophagy

In autophagy, damaged molecules (e.g., proteins) and organelles (e.g., mitochondria) are sequestered into autophagosomes, which merge with lysosomes (digestive sacs), allowing them to be broken down within the cell.[31] This allows amino acids to be catabolized for energy and component molecules to be reused, providing for cellular self-renewal.[32,33] The lifespan extension from caloric restriction requires autophagy.[34]

3.1.2. mTOR and Aspartate Signaling

Mammalian target of rapamycin (mTOR) is a key signaling node, integrating information about the amount of energy, glucose, amino acids, hormones, and growth factors, and activating anabolic processes when these are plentiful.[33] Lactate, too, the end-product of excessive glycolysis, activates mTOR.[35] Importantly, mTOR downregulates autophagy by phosphorylating autophagy-related proteins.[36]

Of note, amino acid activation of mTOR occurs whether the amino acids are derived from diet or from the TCA cycle.[32] In particular, the two amino acids directly produced from the TCA cycle—aspartate and glutamate—activate mTOR.[37,38] However, aspartate seems more influential. Inhibiting AST and thereby suppressing aspartate production while allowing glutamate to be produced (through breakdown of glutamine, transamination of alanine by ALT or, rarely, oxidation of AKG by GDH), is sufficient to decrease mTOR activity.[32]

Thus, cytoplasmic depletion of glutamate and, especially, aspartate, raises the level of autophagy. Indeed, replenishing these two amino acids may be a key function of autophagy, as they are central nodes of cellular nitrogen trafficking.[39]

In fact, aspartate is a key signaling hub in metabolism; of the 20 proteinogenic amino acids, in *Escherichia coli* an estimated 27% of all nitrogen flows through aspartate specifically.[40] Aspartate is readily transported from the mitochondria to the cytosol, where mTOR is located, in exchange for glutamate.[41] Aspartate is a precursor for purine and pyrimidine nucleotides, is rate-limiting for the proliferation of cancer cells,[41] and exogenous aspartate is sufficient to restore proliferation to ETC-deficient cells.[42,43] Indeed, in cancer cells, the urea cycle is often suppressed to conserve aspartate for proliferation.[41] Aspartate is also a precursor of asparagine, an amino acid that itself supports anabolic signaling, as it activates mTOR directly and by facilitating the import of other amino acids into the cell.[44]

3.1.3. Aspartate Signaling and Caloric Restriction

Caloric restriction (CR) is the best-validated method for lifespan extension in multiple species.[45] It has several effects on the TCA cycle:

First, because sufficient glucose is not being obtained from diet, it must be created internally, first from glycogen and then from gluconeogenesis. In the liver, flux through pyruvate carboxylase (to convert pyruvate to oxaloacetate) and PEPCK (to convert oxaloacetate into PEP for gluconeogenesis) markedly increase in mice with the duration of fasting.[46] In *C. elegans*, increased activity of PEPCK and another gluconeogenic enzyme, fructose-1,6-bisphosphatase, are each required for the longevity effects of CR.[47]

This consumption of oxaloacetate to produce glucose should drive the AST reaction in the reverse direction, depleting aspartate (Reverse AST: AKG + Aspartate → Glutamate + Oxaloacetate).

Gluconeogenesis increases demand for ATP (PEPCK requires ATP) and for NADH (glyceraldehyde-3-phosphate dehydrogenase, downstream from PEPCK, consumes NADH), which drive increased flux through the TCA cycle.[25] Thus, CR speeds the TCA cycle[26] at the expense of glycolysis,[48] thus extracting as much energy as possible from the available nutrients.

Concurrently, there is a shift towards non-carbohydrate fuel sources. CR increases β-oxidation of fatty acids and the longevity benefits of CR depend in part on this increase.[49]

Second, in CR and, especially, fasting, protein is catabolized for energy, a process that involves autophagy.[50] The amine groups thus liberated are detoxified through the urea cycle. Intrinsic to the urea cycle is the conversion of aspartate to fumarate, further reducing cytosolic aspartate.

Third, CR leads to increased synthesis of glutathione,[51] the body's most prevalent nonenzymatic antioxidant and detoxifying molecule. We will see in Section 3.1.6 how its synthesis can deplete aspartate.

Thus, CR, through utilization of oxaloacetate to form glucose, through increased processing of amine groups through the urea cycle, and through increased production of GSH, can lower levels of aspartate. This should suppress mTOR, facilitating autophagy and therefore longevity.

3.1.4. Aspartate Signaling and Overexpression of Cytoplasmic PEPCK in Skeletal Muscle

That routing oxaloacetate away from aspartate production might be important for longevity is supported by the increased lifespan of roundworms and mice overexpressing PEPCK.[21]

In mice, upregulating cytoplasmic PEPCK (PEPCK-C) in skeletal muscle by a factor of ≈112 produces hyperactivity, a high rate of energy expenditure, and an approximately 2 year increase in lifespan.[21] The mice appear to age more slowly, giving birth to normal litters at the advanced age of 30–35 months.[21]

Similarly, Yuan et al.[22] found that in the roundworm *C. elegans*, overexpression of PEPCK-C in skeletal muscle after reproductive age increased lifespan proportionate to the degree of overexpression. The effect was non-cell autonomous; the entire organism benefitted from PEPCK-C overexpression in skeletal muscle specifically. Of note, the pro-longevity effect depended on a large increase in autophagy beginning in late adulthood.[22]

In the liver and kidney, PEPCK is used for producing glucose, as discussed above. In skeletal muscle, it is used for producing glycerol for forming triacylglycerols, an important energy source for muscles.[52] Moreover, PEPCK speeds oxidation of amino acids into ammonia,[22] which is processed through the urea cycle. Presumably, through the rerouting of oxaloacetate by PEPCK less aspartate is produced, and through the urea cycle more aspartate is consumed. The lower levels of aspartate should deactivate mTOR, accounting for the marked increase in autophagy.

PEPCK-C expression in *C. elegans* naturalistically declines by up to 79% following the reproductive stage, an effect that, from knockdown versus overexpression studies, leads to senescence, loss of motor function, reduced mitochondrial function, vulnerability to the superoxide generator paraquat, and shortened lifespan.[22] Similarly in mice, PEPCK transcription and activity decline with age.[53] The reason for this decline has not been determined but because it is tied to development and reproduction, it is presumed to be a programmed process that optimizes energy generation during the reproductive stage.[22]

3.1.5. Aspartate Signaling and a Ketogenic Diet

A ketogenic diet (≤10% of calories from carbohydrate, 20% from protein, and ≥70% from fats[54]) extends the lifespan and health span of mice.[55] In a ketogenic diet, the supplied nutrients (amino acids and fatty acids) cannot be processed directly by glycolysis but rather must be catabolized initially by the TCA cycle, increasing TCA cycle flux.[56] Moreover, a ketogenic diet requires that glucose be manufactured internally out of oxaloacetate. Thus, as in fasting, CR, and overexpression of PEPCK-C in skeletal muscle, oxaloacetate is repurposed away from aspartate production.

3.1.6. Aspartate, Glutamate, and Dietary Polyphenols

Glutathione (GSH), a tripeptide comprised of glutamate, cysteine, and glycine, is the main nonenzymatic antioxidant in the cytoplasm, present in high concentrations ($1–10 \times 10^{-3}$ M).[57,58] It reduces oxidants directly and through facilitation by glutathione peroxidase. It is also used in detoxification, becoming irreversibly conjugated to electrophilic xenobiotics and to endogenous lipid peroxides and other reactive oxygen and nitrogen species (ROS and RNS) by the glutathione-S-transferases, facilitating their elimination.[59,60]

GSH levels decrease with age,[57] correlate with better physical and mental health in the elderly,[61,62] and are inversely associated with numerous age-related diseases.[63]

Although oxidized glutathione can be recycled to its reduced form, it is also exported by the cell into the bloodstream, catabolized in the kidneys, can bind to proteins and, as noted, its conjugation to electrophiles is irreversible. Therefore, cellular levels of GSH require its de novo synthesis.[64] This synthesis is energy-dependent, consuming 2 molecules of ATP per molecule of GSH.[65]

Overexpression of the gene for the rate-limiting step in GSH synthesis, γ-glutamyl-cysteine ligase (GCL), markedly increases the lifespan of *Drosophila*.[66] Longer-living Chinese hamster ovary cells (used in biotechnology) also show upregulation of glutathione production.[67] Interestingly, however, there is little indication that improved antioxidant capacity is responsible for the lifespan extension. In *Drosophila*, e.g., GCL overexpression does not slow the age-related decline in resistance to oxidative stress,[68] and while GSH-dependent detoxification pathways are upregulated, antioxidant defense is not.[69] Indeed, some gene transcription changes accompanying GCL overexpression overlap with those in CR.[69]

Similarly, *E. coli* genetically engineered to produce high levels of GSH show enhanced resistance to gamma irradiation. Of note, it is the increased capacity to synthesize GSH rather than the measured levels of GSH, that predicts the degree of resistance.[70]

In mice, meanwhile, a remarkable lifespan extension was achieved by Kumar et al.[71] with the administration of N-acetylcysteine (a cysteine precursor) and glycine. Cysteine and glycine are thought to be rate-limiting for the body's production of glutathione. The supplemented mice had a mean lifespan 24% longer than the control group, and the longest-lived supplemented mouse reached an age of 39 months, 40% greater than the longest-lived control mouse.

The purpose of supplementation was to boost GSH levels and reduce oxidative stress, and it achieved both. Moreover, the supplemented group had lower levels of genomic damage, commensurate with the improved antioxidant status.

However, the supplemented group also had higher levels of peroxisome proliferator-activated receptor-α (PPARα), which increases β-oxidation of fatty acids, and sirtuin-3 (SIRT3), which increases NADPH production and activates autophagy, all of which are usually elicited by CR rather than by GSH. Moreover, the supplemented group had higher levels of mitophagy. Mitophagy, the autophagic degradation of damaged mitochondria, slows aging by eliminating them as a source of ROS.[72,73] Because mild oxidative stress is a sign of mitochondrial impairment, it induces mitophagy,[73] while N-acetylcysteine and—of note, GSH—oppose mitophagy.[72,74] Further, the degree of lifespan extension found by Kumar et al. is characteristic of a CR rather than an antioxidant study.

All of these suggest that—in *E. coli*, *Drosophila*, and mice—it is the process of synthesizing GSH, rather than the amount of GSH or the cell's redox status, that confers longevity.[66,70]

Consideration of the TCA cycle can shed light on this. Supplementing glycine and cysteine to increase GSH requires that the cell itself produce the third constituent, glutamate. This can be accomplished in four ways:

(1) The transamination of AKG, depleting aspartate (Reverse AST: AKG + Aspartate → Glutamate + Oxaloacetate). This is the same reverse-AST reaction as encountered in CR (Section 3.1.3), but its purpose here is the resulting glutamate rather than oxaloacetate. In this reaction, aspartate is consumed to produce glutamate.
(2) Glutaminolysis, the breakdown of glutamine (Glutamine → Glutamate + NH_3). The NH_3 (ammonia) is then detoxified through the urea cycle, depleting aspartate.
(3) The oxidation of AKG into glutamate (GDH: AKG + NH_3 + NAD(P)H → NAD(P)$^+$ + Glutamate). This requires that GDH function in reverse, which it tends not to do in vivo,[75] but to the extent that it does, it likely promotes autophagy by consuming reducing equivalents (NADH and NADPH).[75]
(4) The transamination of AKG, depleting alanine (Reverse ALT: AKG + Alanine → Glutamate + Pyruvate). Alanine is an exception among amino acids in that it is a byproduct of muscle breakdown, connotes a negative energy balance, and may activate AMP-activated protein kinase (AMPK).[76] Thus, producing glutamate through ALT, lowering alanine, might increase mTOR signaling. However, given equal availability of substrate, the AST pathway appears to be 13-fold more active than ALT in producing glutamate (Table 3 in Ellinger et al.[77]).

Thus, producing glutamate depletes aspartate. Moreover, once the glutamate is created, it is sequestered in glutathione and thus there is also less free glutamate. The net effect is likely to deactivate mTOR and stimulate autophagy.

Similarly, glycine is produced endogenously from oxaloacetate. Cysteine production, too, through the transsulfuration pathway, consumes glycine and therefore oxaloacetate. Moreover, the transsulfuration pathway produces as a byproduct hydrogen sulfide (H_2S), a readily diffusible gas that, as an antioxidant and through post-translational modification of proteins and the K_{ATP} potassium channel, mediates many of the benefits of CR.[78]

The production of glutathione, then, likely has metabolic benefits separate from the fact that glutathione is an antioxidant. The internal production of each of its constituents routes oxaloacetate away from the production of aspartate.

GSH synthesis is upregulated by polyphenols,[79] alpha lipoic acid (ALA),[80] and resveratrol,[81] via the Nrf2/ARE transcription factor. ALA may also support GSH synthesis by facilitating cellular uptake of cystine and its reduction to cysteine.[82]

3.1.7. Aspartate Signaling and Oxidative Stress

Among TCA cycle enzymes, aconitase is particularly vulnerable to inactivation by ROS because they cause iron to dissociate from the iron-sulfur clusters at its core.[83–85] Under oxidative stress, aconitase is essentially 100% blocked.[86,87]

This has four effects. First, it leads to an increase in citrate in the mitochondria because the citrate is not being processed into aconitate. This is likely adaptive as the citrate can chelate the iron liberated from the damaged aconitase, preventing production of the hydroxyl radical.[88,89]

Second, the increased citrate ultimately spills over to the cytosol,[90] where it is broken down into acetyl-CoA and oxaloacetate. A portion of the latter is used by cytosolic AST to produce aspartate,[91] especially in high-glucose conditions when gluconeogenesis is not active. This is considered a low-capacity pathway, but cell proliferation depends on the export of citrate from the mitochondria.[92]

Third, in the mitochondria, to bypass the blocked aconitase and continue respiration, transamination is used to produce AKG from glutamate and, simultaneously, consume oxaloacetate: (Forward AST: Oxaloacetate + Glutamate → Aspartate + AKG). This allows two-thirds of the TCA cycle, from AKG to oxaloacetate, to function in its canonical, clockwise direction. As a result, however, under oxidative stress, the AST reaction proceeds in the opposite direction as it does under CR, and instead of consuming aspartate, the reaction produces it.

A fourth effect is possible as well. The inhibition of TCA cycle flux by oxidative stress should cause an increase in cytosolic pyruvate, a portion of which may be converted to malate by the malic enzyme (ME), consuming NADPH. The high cytoplasmic ratio of NAD$^+$/NADH would then drive MDH to convert malate to oxaloacetate, supporting further cytosolic aspartate synthesis.[91]

Thus, oxidative stress likely increases the production of aspartate. The aspartate is readily, and essentially irreversibly, transported to the cytosol in exchange for glutamate. Because aspartate facilitates mTOR more strongly than does glutamate,[32] the net effect is to activate mTOR. Thus, prolonged oxidative stress alters TCA cycle functioning in a way that inhibits autophagy.[32]

Oxidative stress has other pro-aging effects as well, including increases in succinate and acetate. These will be discussed below in Sections 3.2.3 and 3.4, respectively.

Of note, the inactivation of aconitase is the most common finding of the TCA cycle in aged animals.[93–95]

3.2. Succinate, Alpha-Ketoglutarate, and Genetic and Epigenetic Maintenance

3.2.1. Succinate and DNA Methylation

Cellular identity—distinguishing, for example, a hepatocyte in the liver from a fibroblast in the skin—depends on stable

epigenetic marks that determine which genes are transcribed. In particular, the attachment of methyl groups to the DNA of a gene's promoter region tends to repress transcription. Changes in DNA methylation patterns over time correspond so closely to biological age that they constitute an aging clock.[96] In particular, the promoter regions of certain genes, found in areas with a high concentration of cytosine-phosphate-guanosine sequences ("CpG islands"), become increasingly methylated.[96] Moreover, there is early in vivo evidence for rejuvenation by demethylating agents.[97]

Thus, aging may be due in part to a loss of epigenetic information.[98] Why epigenetic maintenance progressively fails is not yet known, but DNA damage, and particularly difficult-to-repair double-strand breaks (DSBs), may account for the increasing hypermethylation. DNA and histone methyltransferases (including DNMT1 and DNMT3B), histone acetyltransferases, and the SIRT1 deacetylase are recruited to the sites of oxidative DNA damage and DSBs.[99,100] This is likely to prevent transcription, providing unimpeded access to the DNA for repair.[101] However, the additional methylation does not always reverse.[99] In the short run this may be an adaptive strategy against cancer, by decreasing the transcription of genes that were damaged and perhaps not fully repaired.[100] Over decades, however, it means that certain genes become progressively switched off.

Moreover, AKG is an obligatory cofactor for certain dioxygenase enzymes,[18] including the ten-eleven translocation (TET) enzymes that demethylate DNA, and the Jumanji enzymes that demethylate histones. TET2 in particular may remove the methylation marks that were placed on DNA during repair.[102] Intrinsic to this enzymatic demethylation is the conversion of AKG to succinate.[18]

Like all AKG-dependent dioxygenase enzymes, the histone- and DNA-demethylases require oxygen, iron, and a reducing environment to keep the iron in its ferrous (Fe^{2+}) state.[18] Thus, excessive ROS will inhibit, and vitamin C will ordinarily potentiate, demethylation.[18] In addition, because succinate, and indirectly fumarate, are products, they inhibit these reactions.[18] That is, excess succinate and fumarate may exacerbate the age-related hypermethylation of CpG islands.

Note, too, that DNA demethylation (and histone acetylation) are energy-consuming.[103] Epigenetic maintenance is among the processes that depend on bioenergetic competence.

We will discuss shortly the conditions under which succinate and fumarate build up in the cell. First, however, let us consider another effect of increased succinate.

3.2.2. Succinate and DNA Stability and Repair

Epigenetics aside, DNA damage, and particularly DSBs, is suspected of contributing directly to aging by triggering apoptosis, stem cell depletion, cellular senescence, and inflammation, and by causing transcription errors.[104]

DNA DSBs are repaired primarily by the homologous recombination and the nonhomologous end-joining pathways, the former of which depends on the Jumanji histone demethylases KDM4A and B.[105] Thus, accumulation of fumarate and succinate to very high levels, as occurs when the genes encoding FH and/or SDH are mutated, causes accumulation of DNA DSBs.[105]

Moreover, decreased functioning of SDH causes a buildup of succinate and succinyl-CoA. The succinyl-CoA provides succinyl groups that attach to certain lysine residues on histones. This likely enhances gene transcription by opening the associated chromatin[106] but if not reversed by SIRT7 it also prevents the compaction of chromatin needed for DNA repair. As a result, loss of SDH function causes an accumulation of DNA damage, even in the absence of any external genotoxic agent.[106]

This refers to the special case of loss-of-function mutations of FH and SDH but there are also more prosaic circumstances in which succinate builds up: hypoxia and oxidative stress.

3.2.3. Succinate, Hypoxic Signaling, and Oxidative Stress

Aging tissues are prone to localized hypoxia due to loss of capillaries, changes in hemodynamics, and a buildup of connective tissue that impairs oxygen diffusion.[107] As a result, flux through the ETC slows, causing NADH to accumulate. The TCA cycle reconfigures itself to contend with this.[108] Specifically:

(1) Pyruvate enters the TCA cycle via conversion to oxaloacetate by pyruvate carboxylase (Figure 2). This bypasses PDH, which would otherwise generate NADH. The latter half of the TCA cycle functions in reverse to metabolize NADH to NAD^+. That is, the oxaloacetate is converted, successively, to malate, fumarate, and succinate.
(2) The NAD^+ allows AKGDH to produce succinyl-CoA, whose conversion into succinate generates ATP or GTP without need for oxygen or the ETC.[30] A portion of the AKG is then converted by MDH and IDH2 to 2-hydroxyglutarate, thus bypassing NADH production by AKGDH.
(3) SDH is both a component of the TCA cycle and Complex II of the ETC. In hypoxia (pO_2 < 8–10 mm Hg) ETC Complex IV, in which hydride groups are joined to molecular oxygen, is compromised.[109] The resulting backup of electrons slows SDH, causing succinate to build up.[10]

As a result of all these, fumarate, 2-hydroxyglutarate and, especially, succinate, whose concentration can be 11-fold higher, accumulate, signaling hypoxia in the cell and throughout the body.[108]

Meanwhile, under oxidative stress, the activity of AKGDH is decreased by c. 40%,[86,87] causing AKG levels to rise.[110] This is likely adaptive in that AKG is an antioxidant, becoming nonenzymatically oxidized to succinate:[111]

$$\mathrm{AKG} + \mathrm{H_2O_2} \rightarrow \mathrm{Succinate} + \mathrm{CO_2} + \mathrm{H_2O} \quad (6)$$

In invertebrates, inhibition of AKGDH can also route isocitrate, by means of isocitrate lyase, through the glyoxylate pathway, producing more succinate.[112,113]

Thus, under oxidative stress, as in hypoxia, succinate levels rise.[110]

We have seen that succinate (and particularly the succinate/AKG ratio) inhibits AKG-dependent dioxygenases. Among them are the enzymes that degrade hypoxia-inducible factor-1α (HIF-1α). Thus, when oxygen levels fall, HIF-1α levels rise. Fundamentally, HIF-1α facilitates adaptation to hypoxic conditions.

It inhibits the TCA cycle and reroutes glucose through (anaerobic) glycolysis.[114]

The adaptation to hypoxia, however, comes at a cost. Succinate, acting through its receptor, increases production of the proinflammatory cytokine IL-1β.[29] Also, via its role in Complex II of the ETC, succinate causes reverse electron transport back to Complex I, where the excess electrons form ROS.[18]

Succinate, and impairment of SDH, implement several features of aging, including inflammation, hypoxic signaling, increased glycolysis, and DNA damage and hypermethylation. Higher succinate levels are associated with obesity[29] and are mechanistically linked to such age-related conditions as osteoporosis,[115] osteoarthritis,[116] atherosclerosis,[117] hypertension,[118] and cancer.[29] In people, serum levels of succinate rise with age.[119]

From one perspective, then, a rise in succinate, as an adaptation to hypoxia, is a biochemical example of allostasis, a distortion from optimal homeostasis for the purpose of meeting an environmental pressure.[120]

However, hypoxia in the right amount is also hormetic—a moderate challenge that strengthens the organism. Hypoxia impairs ATP production, causing the AMP:ATP ratio to rise, which activates AMPK, suppresses mTOR, and increases autophagy.[121] Mitophagy in particular is increased by HIF-1α, to reduce oxygen consumption and production of ROS by damaged, inefficient mitochondria.[73,122] Via the Nrf2 transcription factor, HIF-1α also increases glutathione synthesis.[123] These may explain the anti-aging benefits of hyperbaric oxygen, which induces a temporary, reactive state somewhat resembling hypoxia.[124]

Nonetheless, upregulation of glycolysis and reduced TCA cycle flux were key components of the aging process discussed above for *C. elegans*.[22] To more fully understand this double-edged sword of succinate, let us consider how it interacts with aspartate signaling, mTOR, and autophagy.

3.3. Connections between Aspartate and Succinate Signaling: Age-Related Dysmetabolism

Decreased autophagy via aspartate is not fully separable from inflammation, DNA hypermethylation, and inhibited DNA repair from succinate.

That is, mTOR directly inhibits SDH,[125] causing succinate to build up. Moreover, the export of aspartate from the mitochondria to the cytosol is indirectly coupled to the import of malate through the malate-aspartate shuttle, increasing cytoplasmic NADH. The malate then back-propagates, via reverse TCA cycle flux, to form, successively, fumarate and succinate.[29]

Also, we have seen that under oxidative stress aconitase is blocked because of its iron-sulfur core. SDH also has an iron-sulfur core, and although it is not permanently damaged by oxidative stress like aconitase, it is reversibly inhibited.[94] Moreover, a portion of oxaloacetate is oxidized to form malonate, also potentially inhibiting SDH.[86] Thus, both oxidative stress and overnutrition cause not only increased aspartate but also increased succinate, and likely thereby inflammation, (pseudo-) hypoxic signaling, and DNA hypermethylation and damage.

Is the reverse, then, also true? Does succinate activate mTOR? As a rule, quite the opposite occurs: Via HIF-1α, succinate provides negative feedback that reduces further aspartate production. In particular, the transamination we saw when aconitase is blocked by oxidative stress (Forward AST: Glutamate + Oxaloacetate → AKG + Aspartate) is inhibited by HIF-1α.[126] This seems logical, as hypoxia is surely an anti-proliferative stimulus. Thus, an increase in aspartate causes an increase in succinate, which reduces aspartate production, restoring equilibrium.

However, this homeostatic mechanism depends on HIF-1α, whose content and signaling decline with age.[123] Without this "off switch," mTOR, over-proliferation (and risk of cancer), decreased autophagy, inflammation, DNA hypermethylation, and impeded DNA repair can all remain active simultaneously. We might term this persistent state "age-related dysmetabolism."

This may help explain the health benefits of titrated hypoxia: Although dangerous, hypoxia might temporarily restore the negative feedback on mTOR.

3.4. Acetyl-Coenzyme A Signaling: Autophagy, Histone Acetylation

Pyruvate scavenges ROS with acetate as a byproduct:[127]

$$\text{Pyruvate} + H_2O_2 \rightarrow \text{Acetate} + CO_2 + H_2O \quad (7)$$

In vivo, 5–15% of a cell's acetate production is through this reaction[128] and thus, oxidative stress can increase the level of acetate. Overnutrition can have similar effects, as excess citrate from the TCA cycle is exported to the cytosol where, via ACLY it is parsed back into acetyl-CoA and oxaloacetate. Alternatively, when the supply of pyruvate exceeds the processing capacity of the TCA cycle, as would occur in overnutrition, over-reliance on glycolysis, and/or deficiencies in NAD^+ and/or CoA (synthesized from vitamin B5), the excess pyruvate is transformed into acetate by PDH and, promiscuously, by AKGDH.[128]

The acetate is then converted into acetyl-CoA by acetyl-CoA synthase. Much of the acetyl-CoA is converted into lipids, but some is used to acetylate lysine residues on enzymes and histones.[129] In particular, acetyl-CoA activates the EP300 acetyltransferase, which then acetylates (activates) mTOR, inhibiting autophagy.[130] Moreover, cytoplasmic acetyl-CoA is thought to be used in acetylating histone H4, downregulating autophagy.[131] Of note, acetyl-CoA slows the functioning of SDH as well,[10] thus activating both aspects of dysmetabolism.

Conversely, during CR, acetyl-CoA is directed to the mitochondria for energy production, depleting its levels in the cytosol and nucleus,[132] triggering autophagy.[133,134]

Histones are also acetylated, affecting gene transcription. However, the dynamics of histone acetylation are likely complex, and depend on which histone is being acetylated by which acetyltransferase.[135]

3.5. Alpha-Ketoglutarate and Stem Cell Maintenance and Exhaustion

Hypoxic signaling does not contribute to aging in all cells. In particular, adult stem cell niches are hypoxic.

Maintenance of the adult stem cell pool requires that the majority of stem cells be in a quiescent state.[136] Thus, because adult

stem cell niches are hypoxic; HIF-1α is upregulated, the TCA cycle is suppressed,[137] and there are high levels of fumarate, 2-hydroxyglutarate, and succinate, and a high succinate/AKG ratio. These preserve stemness by inhibiting the TET DNA demethylases, thus keeping differentiation-associated genes in an inhibited, hypermethylated state.[108,137] Conversely, increases in AKG promote demethylation, differentiation, and exiting of the stem cell pool.[137]

Thus, if AKG is converted to succinyl-CoA and then succinate there is the risk of interfering with DNA repair and causing inflammatory signaling. However, when AKGDH is inhibited, reducing further flow through the TCA, then AKG builds up, potentially inhibiting stem cell maintenance. In fact, AKGDH, both a source and a target of ROS, is inhibited by age, and by oxidative stress.[87]

We have seen, however, an alternative path for AKG: It can receive the amino group from aspartate to become glutamate. Thus, synthesizing glutamate lowers the levels of both aspartate and of AKG, supporting autophagy on the one hand, and stem cell maintenance, DNA repair, and decreased inflammatory signaling on the other. Glutathione, used by the body as an antioxidant and detoxifying agent, likely also has utility as a high-capacity glutamate sink.

3.6. TCA Cycle Flux and Its Effects on Aging

The consumption of oxaloacetate to form glucose or glycerol, the consumption of glutamate to form glutathione, and the consumption of aspartate in the urea cycle all require increased flux through the TCA cycle. Conversely, oxidative stress, by reversibly inhibiting AKGDH, slows the TCA cycle.[87] Increased flux might in itself slow aging, for simply depleting ATP through physical exercise, or mitochondrial uncoupling,[138,139] which increase demand for NADH and therefore the activity of the TCA cycle, is sufficient to extend lifespan.

How, then, could a high TCA cycle flux slow aging? There are several possibilities.

The simplest is that in high flux, oxaloacetate joins with acetyl-CoA to form citrate, depleting acetyl-CoA and directing oxaloacetate away from the production of aspartate. These would reduce the activation of mTOR and the suppression of SDH.

Another factor may be improved glucose handling. Slowing of the TCA cycle can cause excess glucose to be routed through secondary pathways—the sorbitol/polyol, hexosamine, diacylglycerol/protein kinase C, and advanced glycation end product pathways.[140] Each of these has pathogenic products implicated in aging. Overreliance on glycolysis itself accelerates aging[47] and one of its rate-limiting enzymes, pyruvate kinase, downregulates PEPCK.[22] Conversely, increased β-oxidation, bypassing glycolysis, confers longevity,[49] and taking supplemental glucosamine, a mild glycolysis inhibitor, prospectively predicts lower all-cause mortality.[141] When glycolysis predominates, the excess pyruvate is converted to lactate, which activates mTOR,[35] suppressing autophagy.

The TCA cycle may also help promote genomic stability. The enzyme topoisomerase II helps ensure proper replication of DNA by maintaining its 3D structure. All TCA intermediates except succinyl-CoA, but especially citrate and oxaloacetate, stimulate topoisomerase II activity, perhaps because, naturalistically, dividing cells are shifting to a more TCA cycle-dependent energy production.[142]

Disrupted homeostasis may also be a factor. Overreliance on glycolysis leads to increased pyruvate and acetyl-CoA that are not broken down by the TCA cycle. The excess acetyl-CoA is processed for lipogenesis, while the excess pyruvate can be processed into glucose.[48] These may contribute to metabolic syndrome.

Moreover, the TCA cycle has a central role in antioxidant defense. Pyruvate, AKG, and oxaloacetate have direct antioxidant properties by scavenging H_2O_2.[26] Citrate, malate, and oxaloacetate are likely indirectly antioxidant by chelating iron,[143] and pyruvate[35] and fumarate[144] induce the expression of antioxidant enzymes by activating the Nrf2 transcription factor.

Further, TCA cycle intermediates contribute to the production of NADPH, a fundamental antioxidant used by the cell directly to regenerate the endogenous antioxidants glutathione, thioredoxin, and cytoplasmic Coenzyme Q10, and indirectly to regenerate vitamins C and E.[145] In particular, GDH and, more so, IDH2, are key sources of NADPH.

Similarly, certain isoforms of malic enzyme (ME), converting malate into pyruvate, produce NADPH (e.g., ME1: malate + NADP $\rightarrow$ pyruvate + NADPH).[23] Slowing of the TCA cycle, by decreasing malate, impairs this reaction, shifting the cytoplasm to a more oxidized, proinflammatory state.[146]

Moreover, through the ETC, NADH from the TCA cycle creates a proton gradient across the inner mitochondrial membrane. This gradient drives the production of ATP but also, through the enzyme nicotinamide nucleotide transhydrogenase, powers a reaction generating the antioxidant NADPH in the mitochondrion:[147]

$$\mathrm{NADH} + \mathrm{NADP^+} \rightarrow \mathrm{NAD^+} + \mathrm{NADPH} \tag{8}$$

In these ways, then, TCA cycle flux may slow the rate of aging.

3.6.1. Longevity Depends on Increased Demand

It seems likely that the benefits obtain only when the TCA cycle is accelerated because of increased demand for its products—NADH, $FADH_2$, and/or cataplerotic output. Artificially increasing TCA cycle velocity by pushing in substrate would simply pass more hydride groups to the ETC than are needed for ATP synthesis, causing an increase in ROS.[148] Similarly, AKGDH in high-NADH conditions generates superoxide and H_2O_2 (oxygen becomes the electron acceptor when NAD^+ levels are low),[87] as do excesses of other TCA cycle intermediates.[149]

Rather, the foundation for speeding the TCA cycle is to increase demand for its downstream products through metabolic interventions such as caloric restriction, fasting, physical exercise, and/or use of a mild uncoupling agent. Indeed, when demand for NADH exceeds TCA cycle capacity, there is an increase in the NAD^+/NADH ratio, supporting the antiaging effects of the SIRT deacetylase enzymes.

The pathways discussed in this paper are summarized in **Figures 3** and **4**.

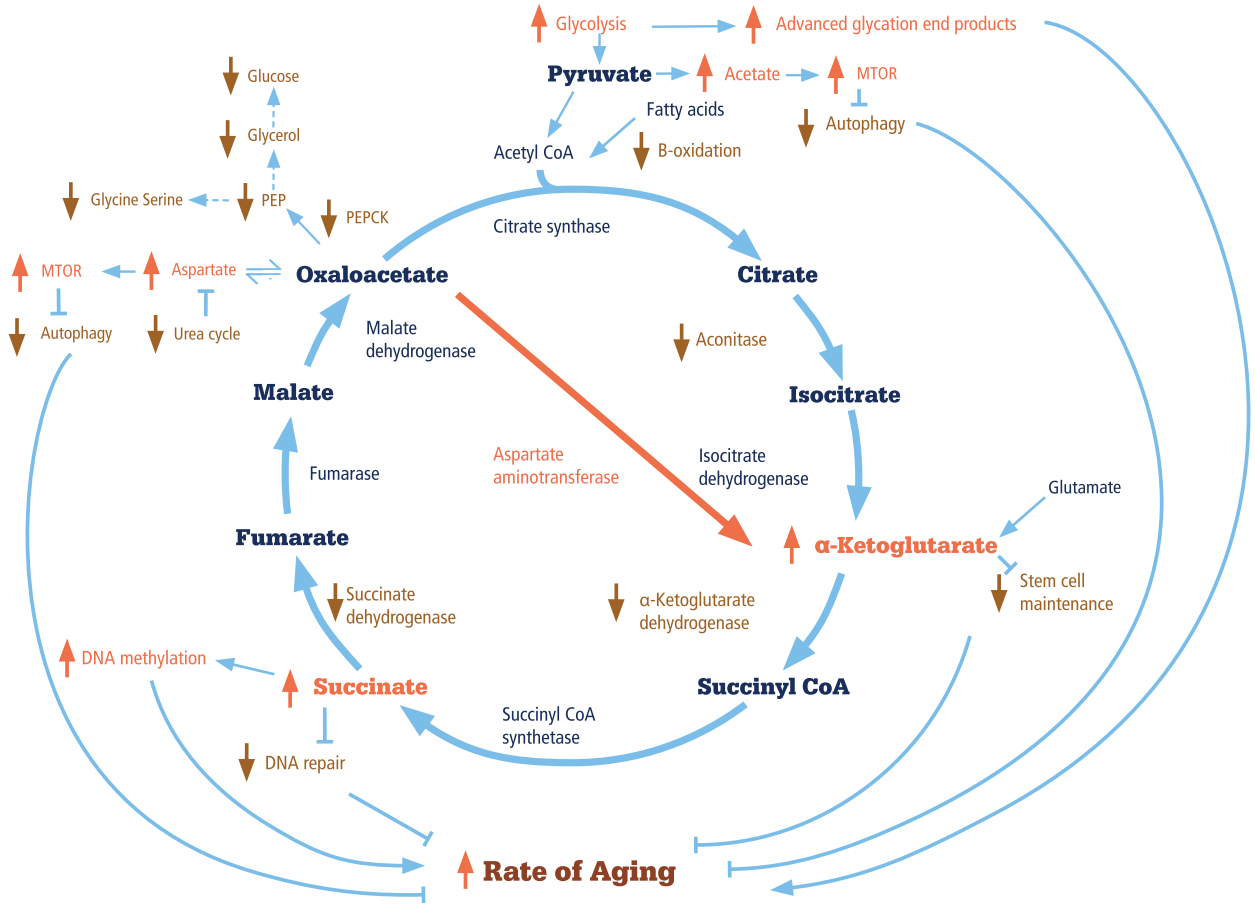

Figure 3. Metabolic processes that accelerate aging. Proceeding clockwise: 11:00 position: With excessive caloric intake and a sedentary lifestyle, oxaloacetate is not as needed to produce glucose, glycerol, or amino acids, and is preferentially routed to the production of aspartate. The aspartate activates mTOR, suppresses autophagy, and increases the rate of aging. 12:00 position: Excessive caloric intake, especially from carbohydrates, decreases β-oxidation and upregulates glycolysis, with advanced glycation end products as a byproduct, contributing to cellular damage and aging. Excess pyruvate and citrate lead to increased levels of acetate in the cytosol, which speeds aging by upregulating mTOR and suppressing autophagy. 2:00 position: The blockade of aconitase by oxidative stress causes the aspartate aminotransferase reaction (in the center of the cycle) to proceed in the forward direction, producing alpha-ketoglutarate from oxaloacetate, with aspartate (at 11:00) as a byproduct. The aspartate inhibits autophagy, accelerating aging. 3:00 position: Under oxidative stress, alpha-ketoglutarate is produced by aspartate aminotransferase from oxaloacetate. Moreover, the processing of alpha-ketoglutarate into succinyl-CoA is impeded by oxidative stress. The resulting buildup of alpha-ketoglutarate interferes with maintenance of the adult stem cell pool, facilitating aging. 7:00 position: Under oxidative stress, and because of a buildup of aspartate and activation of mTOR, the processing of succinate by succinate dehydrogenase is slowed. The increased level of succinate inhibits repair of DNA double-strand breaks and favors hypermethylation of DNA, both of which contribute to aging.

4. Implications and Limitations

4.1. Implications

Consideration of the TCA cycle integrates disparate mechanisms of aging and helps explain the effect of metabolic interventions. It also provides a novel mechanism for the longevity benefits of dietary polyphenols—by increasing GSH synthesis, they upregulate a high-capacity sink for glutamate and, indirectly, aspartate.

Moreover, oxidative stress has traditionally been understood in terms of damage to the cell's enzymes and structural components[150] or as distorting redox-sensitive signaling.[151] Consideration of the TCA cycle extends this to include DNA hypermethylation and inhibited repair, and suppression of autophagy.

The analysis also helps explain why metabolic interventions are not more effective and how this can be remediated. The blockade of aconitase, inhibition of SDH, and downregulation of PEPCK and HIF-1α increasingly compromise metabolism and may establish a vicious cycle of accelerated aging. Thus, protecting iron-sulfur clusters, maintaining PEPCK transcription and signaling, and preserving the capacity to upregulate HIF-1α, may have large effects in sustaining the benefits of metabolic interventions. These are important future research directions.

Protection and repair of iron-sulfur clusters have received some study[152] and factors governing the transcription, translation, and protein stability of HIF-1 have been identified.[153–155]

The decline in PEPCK expression with age presumably reflects, as does its suppression in embryo, methylation of its gene promoter region.[156] However, cAMP increases PEPCK

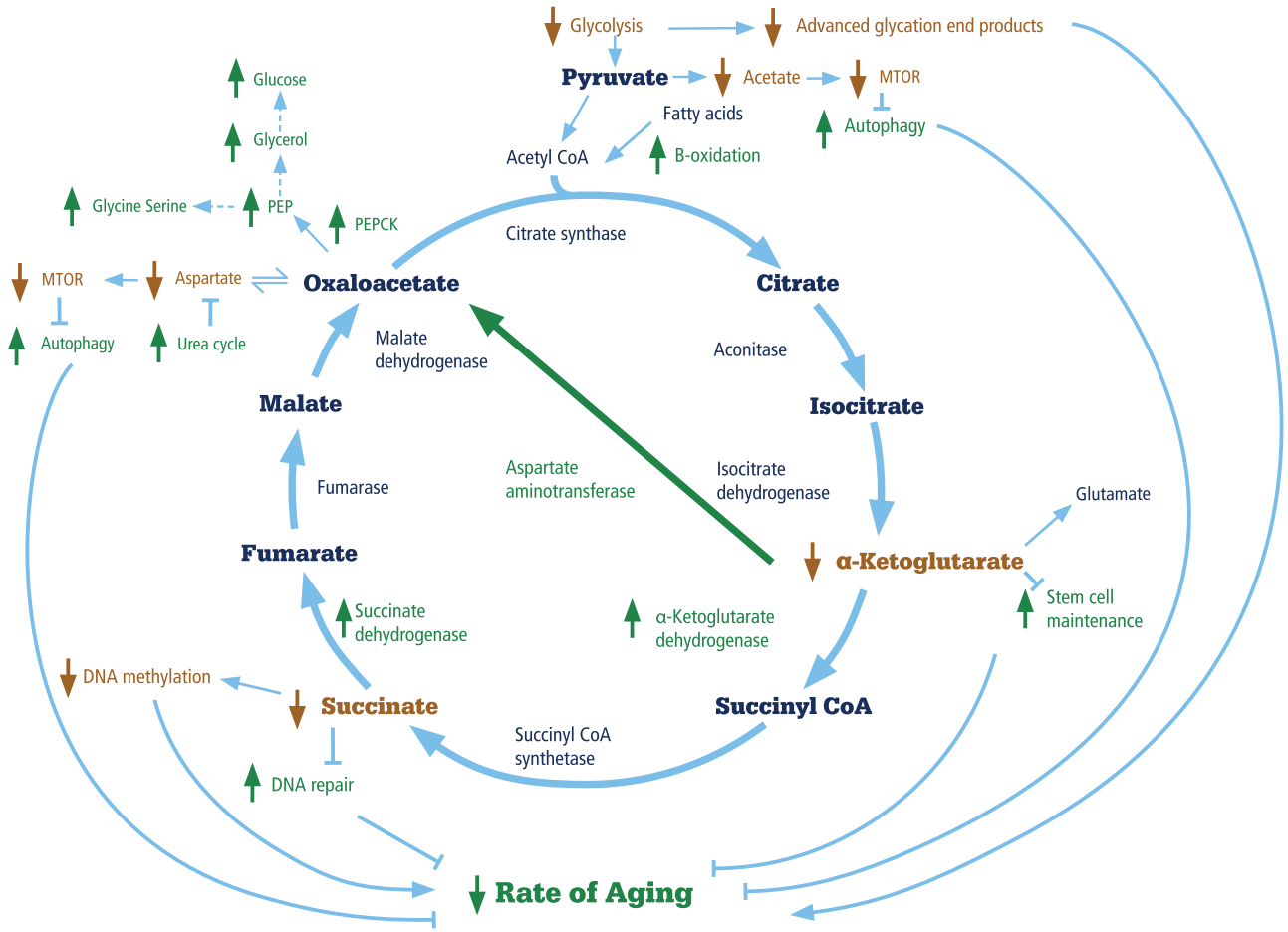

Figure 4. Metabolic processes that decelerate aging. Proceeding clockwise: 11:00 position: During caloric restriction, fasting, physical exercise, and/or a ketogenic diet, PEPCK routes oxaloacetate to the production of glucose, glycerol, and amino acids, and away from the production of aspartate. The decrease in aspartate inhibits mTOR, activates autophagy, and slows the rate of aging. 12:00 position: Decreased caloric intake, particularly from carbohydrates, favors β-oxidation and downregulates glycolysis, resulting in fewer advanced glycation end products and cellular damage. Acetate is consumed by the TCA cycle, thus inhibiting mTOR and increasing autophagy, slowing the rate of aging. 3:00 position: Alpha-ketoglutarate is processed into glutamate by the reverse aspartate aminotransferase reaction (in the center of the circle), which provides oxaloacetate for producing glucose, glycerol, and amino acids. This reverse aspartate aminotransferase reaction consumes aspartate, facilitating autophagy and decelerating aging. It also draws down levels of alpha-ketoglutarate, thus slowing aging by supporting stem cell maintenance. With a diet high in polyphenols, this same reaction is used to produce glutamate for synthesizing glutathione, and has the same effects on aspartate, alpha-ketoglutarate, and aging. 7:00 position: Without oxidative stress or the buildup of aspartate, the processing of succinate into fumarate is unimpeded. The lower level of succinate facilitates repair of DNA double-strand breaks and demethylation of DNA, slowing aging. Not shown: Succinate provides negative feedback on aspartate synthesis via HIF-1α, preventing succinate- and aspartate-mediated promotion of aging from remaining active simultaneously.

transcription within 3 hours despite high levels of methylation and reduces the methylation after 2 days.[157] For PEPCK, targeted demethylation seems achievable, likely via the CREB transcription factor acting on part of the PEPCK promoter region. Oral administration of cAMP to mice increases the level of PEPCK, activates SIRT1, SIRT3, and Nrf2,[158] increases β-oxidation[159] and TCA cycle flux,[160] and modestly extends lifespan.[158]

In the meantime, certain lifestyle changes may have modest benefit. Alpha lipoic acid (ALA) may protect iron-sulfur clusters via antioxidant effects and by increasing the expression of frataxin, a chaperone for aconitase.[88,161,162]

PEPCK-C requires manganese and is potentiated by magnesium,[163] commending a diet sufficient in these. Several dietary constituents, including resveratrol, soy isoflavones, a cyanidin from blueberries, gingerol from ginger, and oleic acid from nuts, increase cAMP signaling in skeletal muscle[164] and thus potentially PEPCK. In contrast, long-term, very high-dose biotin suppresses the expression of PEPCK[165] and inhibits SIRT1.[166,167]

Physical exercise helps to maintain PEPCK-C levels, as does CR.[22] PEPCK-C in skeletal muscle supports glyceroneogenesis for energy, suggesting endurance exercise. However, skeletal muscle PEPCK (cytoplasmic and mitochondrial) also supports the synthesis of serine and other amino acids,[168] proteinogenesis, and muscle regeneration,[169,170] implicating strength training as well.[171] Mitochondrial PEPCK in muscle is upregulated by β-adrenergic signaling,[168] which perhaps should be factored in when considering beta-blockers therapeutically.

Physical exercise induces hypoxia in the mouse intestine, liver, and kidney[172] and raises serum HIF-1α level in people.[173] It

would be interesting to determine whether some of the health benefits of exercise are mediated by HIF-1α. Exercise may help preserve the capacity for HIF-1α signaling, as transcription of a gene likely prevents its methylation. Vitamin C seems to be a general demethylating agent, while vitamin D[174] and combinations of polyphenols[175] inhibit de novo methylation by DNMT3a and 3b.

In the cerebral cortex, neural firing leads to functional hypoxia in regions farthest from capillaries and penetrating arterioles.[109] The hypoxia is thought to underlie release of VEGF in support of the increased synaptic strength underlying learning and memory. It would be interesting to determine whether neural activity also has hypoxia-mediated benefit for the entire body.

GSH synthesis is upregulated by polyphenols,[79] ALA,[80] and resveratrol,[81] via the Nrf2/ARE transcription factor. This may support autophagy via the sequestration of amine groups.

The consumption of aspartate by the reverse-AST reaction seems to play a key role in slowing aging. Thus, modest supplementation of its cofactor, vitamin B6, might increase benefit. However, blockade of aconitase with oxidative stress and age propels the transamination reaction in the forward direction, producing aspartate, and supplementation might increase this as well. Also, supplementation to extremely high levels can have unpredictable effects due to the compensatory downregulation of the enzyme.[176]

AKG is consumed when alanine is catabolized for energy, as occurs during exercise (ALT: AKG + Alanine → Glutamate + Pyruvate). Vitamin B6 is a cofactor, and a deficiency in B6 can cause an increase in AKG,[146] perhaps with ramifications for stem cell maintenance.

During CR, the demand for pantothenic acid increases to support formation of fatty acyl-CoA molecules, the form in which fat stores are metabolized into energy via β-oxidation.[132]

Thus, current knowledge suggests combining CR, fasting, exercise, and dietary polyphenols with modest additional resveratrol, vitamins B5 and B6, and R-ALA. Glucosamine may benefit by inhibiting glycolysis. The combination of supplemental glycine and N-acetylcysteine[71] is intriguing and likely works through both antioxidant and CR-mimetic mechanisms.

4.2. Background Diet

Although the discussion of DR and intermittent fasting was neutral with respect to the underlying type of diet, some information is available on how diet type and restriction might interact.

In rhesus monkeys consuming a Western, processed-food diet plus vitamin and mineral supplementation, a long-term 30% reduction in caloric intake lowered the risk of age-related disease and death by approximately two-thirds.[177] Similarly, in a 2 year randomized, controlled study of 220 people, an average 12% caloric restriction led to improved cardiometabolic markers, age-related physiological changes, and DNA methylation age by the Dunedin (although not the GrimAge) clock.[178] A dose-response relationship was shown, with successively greater benefits in DNA methylation age in those achieving ≥10% CR <20% and ≥20% CR compared with <10%.[178] In this study, there were no specific requirements for the type of diet. These suggest that, over a broad range, diet quality is not crucial for the benefits of CR, at least given multivitamin/multimineral supplementation.

This could support a ketogenic diet that confers satiety and weight loss. A ketogenic diet also increases β-oxidation, suppresses glycolysis, and likely reduces aspartate production in favor of gluconeogenesis, all of which should slow aging.

Still, epidemiological studies[179,180] and controlled interventions[181,182] show large decreases in cardiovascular-, cancer-, and all-cause-mortality from a largely plant-based diet. In fact, the polyphenols found in such a diet may be themselves a favorable metabolic intervention. As discussed above, polyphenols can upregulate the synthesis of GSH, most likely facilitating autophagy in the process (Section 3.1.6), and certain polyphenols might raise cytoplasmic PEPCK levels in skeletal muscle via a cAMP-mediated pathway (Section 4.1). Polyphenols may also raise TCA cycle flux through their direct and indirect uncoupling effects and by facilitating PDH activity, and they may promote a shift from glycolysis to β-oxidation.[183,184] Alternatively, by inhibiting ATP synthase, polyphenols may promote autophagy by reducing mTOR activity.[183,184]

Moreover, antioxidants found primarily in plant foods have been shown in clinical trials to prevent DNA DSBs from CT scans,[185,186] and in vitro from other genotoxic agents.[187] The most effective antioxidants for this seem to be N-acetylcysteine (found in onions and spinach) and vitamin C, with some benefit also from vitamin E and β-carotene.[188] DSBs may be important in aging in themselves and by causing DNA hypermethylation. Further, certain polyphenols inhibit DNMT3a and 3b methyltransferases and therefore de novo DNA methylation.[189]

In the Women's Health Initiative database, the GrimAge methylation clock shows rather strong negative correlation with plasma carotenoids (r = -.26), an objective measure of vegetable intake.[190] Similarly, a controlled, 2-year intervention trial of 219 healthy, nonsmoking women ages 50–69 found that adoption of a Mediterranean diet led to slowing of the GrimAge clock.[191] As the GrimAge measure did not improve with even ≥20% CR,[178] CR and polyphenols may possibly make separate contributions to longevity. CR and vitamin C favor demethylation and polyphenols inhibit new methylation, perhaps providing some basis for unique contributions. Taken together, these data may support unprocessed and largely (but not necessarily exclusively) plant-based nutrition, such as the Mediterranean or DASH diets, as a foundation for CR and periodic fasting.

Still, each approach has tradeoffs. A ketogenic diet may improve insulin sensitivity and glycemic control at the expense of cardiovascular risk.[54] A vegan diet may reduce all-cause mortality at the cost of lower bone mineral density and increased fracture risk, perhaps due to limited calcium and vitamin B12 intake.[192] CR, too, may reduce bone mineral density.[193] Intermittent fasting may improve metabolic health and reduce the development of diabetes and cardiovascular disease but be ill-advised for people at risk of hypoglycemia or an eating disorder.[194] Indeed, CR, fasting, and physical exercise are themselves hormetic challenges whose benefits depend on an optimal level that must be adjusted for age, frailty, and health status.

4.3. Limitations

Although the TCA cycle brings together several important processes in aging, it is ultimately a subset of a more complex system. For example, the NAD^+-dependent sirtuins, which deacetylate histones and enzymes, are known to play a large role in aging.[195]

Similarly, the PEPCK mouse shows markedly increased physical activity. This causes an increase in the AMP:ATP ratio, activating AMPK, which inhibits mTOR and is obligatory for the lifespan extension.[22] Moreover, PEPCK mice have low blood levels of insulin,[21] which is characteristic of longevity.[196] Sirtuins, AMPK, demethylating enzymes, and the TCA cycle all interact to affect the rate of aging. For example, the reverse-AST reaction, which consumes aspartate and promotes autophagy, produces oxaloacetate. When citrate synthase is suppressed, the oxaloacetate will be reduced to malate by MDH, in the process oxidizing NADH to NAD^+. NAD^+ is an essential cofactor for the SIRT enzymes. Conversely, SIRT6 may promote longevity by upregulating gluconeogenesis, implying PEPCK as part of the mechanism.[197]

Nonetheless, PEPCK is not everywhere beneficial: When cellular glucose levels are high, the PEPCK enzyme is acetylated by p300. This causes PEPCK to function in reverse, converting PEP into oxaloacetate, presumably for entry into the TCA cycle.[149] This might drive the AST reaction in the forward direction, producing aspartate. Conversely, PEPCK is returned to its deacetylated, gluconeogenic form by SIRT1, which is upregulated by CR.[149]

Blockade of aconitase by oxidative damage may promote aging by increasing aspartate synthesis. However, this likely depends on the degree of blockade. In *C. elegans*, RNA interference knockdown of aconitase by 65% or of IDH1 by 80% causes an ≈20% rise in mitochondrial ROS without significant effect on lifespan.[198] This may be because the accumulation of citrate, a stress signal, triggers the mitochondrial unfolded protein response (UPR^{mt}), a nuclear transcription program that supports mitochondrial proteostasis, repair, and biogenesis.[198] The UPR^{mt} confers longevity in many[199] but not all[200] contexts. On the other hand, 90% knockdown of aconitase shortens lifespan of *Drosophila*, 100% knockout is embryonic lethal, and mutation of the gene for mitochondrial aconitase confers reduced longevity in people.[201] Naturalistically, impairment of aconitase by ROS is likely to be a hormetic stimulus, whose effects depend on the extent of impairment.

Although age-related hypermethylation of DNA at certain gene promoter regions has been comparatively well-studied, the epigenetic methylome is more complex. The DNA of gene bodies can also be methylated, facilitating transcription,[202] while methylation of lysine residues on histones can either support (H3K4) or repress (H3K27) transcription.[203] There is also age-related hypomethylation of repetitive DNA elements, leading to the activation of endogenous retroviral sequences and cancer risk.[204] Methylation of RNA is also widespread, and poorly understood.[204] Thus, hypermethylation of certain DNA promoter regions is likely only a first approximation of how transcription becomes dysregulated with aging. Nonetheless, the high predictive ability of DNA methylation clocks and the rejuvenating effects of demethylating agents suggest that hypermethylation indeed has a major influence on aging.

Consideration of the TCA cycle highlights mechanisms that CR, fasting, physical exercise, and intake of polyphenols share in slowing aging. However, there is also evidence that these interventions activate unique pathways, allowing for additive or synergistic benefit.[205] Research into the unique pathways will likely also be fruitful.

Finally, the model presented here is a tool whose value will depend on its future ability to guide the amelioration of aging and its diseases. The extent of this utility remains to be determined.

Nonetheless, the centrality of the TCA cycle to physiology, and of energetics to aging, implies that the model indeed has relevance. Moreover, the particular susceptibility of aconitase and succinate dehydrogenase to suppression by oxidants, the nonenzymatic production of acetate by oxidants, and the widespread participation of oxidative stress in the diseases of aging, suggest that age-related dysmetabolism and its downstream consequences are not uncommon, and are likely to be useful as therapeutic targets.

5. Conclusions

In response to environment (e.g., oxidative stress) and behavior (e.g., caloric restriction, fasting, a ketogenic diet, physical exercise) the TCA cycle adjusts several processes that likely control the rate of aging, including autophagy, DNA methylation, repair of DNA double-strand breaks, stem cell maintenance, inflammation, and overreliance on glycolysis. Consideration of the specific underlying mechanisms suggests ways of preserving and shaping its effects, potentially slowing aging to a greater extent than has heretofore been possible.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

J.M.B. is responsible for all aspects of this paper.

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