Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Urolithin A

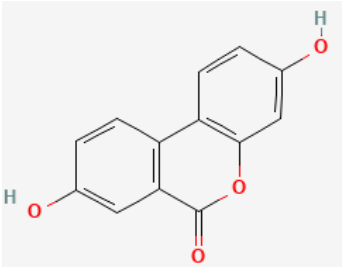
Evidence Summary

Urolithin A may protect against age-related declines in mitochondrial function by promoting autophagy/mitophagy. It has shown good safety but limited benefits in short term studies so far.

Neuroprotective Benefit: Urolithin A may help protect against neuronal loss due to oxidative stress damage and neuroinflammation, but treatment likely needs to begin very early to offer a meaningful benefit in the context of neurodegenerative disease.

Aging and related health concerns: Urolithin A may help protect against age-related diseases by preserving mitochondrial function. Mechanistically it is better suited for prevention than treatment and may need to be started early in life to maximize benefit.

Safety: Urolithin A has been safe in short clinical trials in middle aged and older adults, but long-term safety has not been established.

Availability: OTC	**Dose**: Not established. Oral doses of 500-1,000 mg/day have been associated with effects on mitochondria in pilot clinical trials.	**Chemical formula:** $C_{13}H_8O_4$ **MW**: 228.2g/mol  Source: PubChem
Half-life: ~17-22 hours	**BBB**: Penetrant	
Clinical trials: Urolithin A has been tested in pilot trials in healthy middle-aged to older adults for muscle bioenergetics/function (n=60; n=66; n=88), skin aging (n=178), immunomodulation (n=50), as well as in patients with heart failure (n=10).	**Observational studies**: The urolithin A-producing metabolome is associated with better cardiometabolic health.	

What is it?

Pomegranates contain natural polyphenols including ellagitannins which are hydrolyzed to ellagic acid. Ellagic acid is transformed by the gut microbiota into urolithins. It is found in other foods as well, such as walnuts, and strawberries, but is found in the highest concentration in pomegranates. Studies have looked at urolithin's antioxidant effects, and urolithin A has also been reported to induce autophagy and mitophagy.

Since urolithin A is metabolite of pomegranates, this report will also consider studies using pomegranate extract. However, an important caveat is that individuals metabolize pomegranate differently. Additionally, pomegranate juice contains many other compounds (e.g. other polyphenols). Non-metabolized components of pomegranate have low bioavailability. One study reported that human subjects taking 180ml of pomegranate juice (25mg of egallic acid, 318mg of ellagitannins) had 31.9ng/ml of ellagic acid in plasma one hour post-ingestion, which was eliminated by four hours [1].

Three different urolithin metabotypes (UMs) have been identified: UM-B (subjects that produce isourolithin A and/or urolithin B as well as urolithin A), UM-A (only produce urolithin A), and UM-0 (urolithin non-producers) [2]. In a study of overweight individuals, UM-B individuals had higher cardiovascular risk biomarkers (LDL-c, apoB, oxLDL-c, etc.) than those with UM-A and UM-0, and only those individuals with UM-B showed improvements in those biomarkers when consuming pomegranate

extract for three weeks [3]. It is not clear whether this is due to the UM, per se, or whether it is because UM-B individuals had worse cardiovascular biomarkers at baseline.

Another study found that age was the major determinate of urolithin metabotype (not gender or BMI), where the UM-A metabotype was greatest at younger ages and UM-B increased over time [2] .The authors suggest that interindividual differences in urolithin metabotype could explain the failures of pomegranate extract human trials. Due to this, the plasma levels of urolithins after consumption of pomagranate extract may be variable between individuals.

To get around this variability, some companies are developing formulations of urolithin A that are not subject to variability in human metabolic profiles. A bioavailable form of Urolithin A is currently available from Amazentis. The company has sponsored several pilot clinical trials testing their compound in healthy populations, primarily examining the effects on muscle health and function. One study including 100 healthy adult participants found that only 12% had detectable levels of urolithin A at baseline, and only 40% produced significant amounts of urolithin A following the consumption of pomegranate juice, which was related to differences in the composition of the gut microbiome across participants [4]. Supplementation directly with 500 mg of urolithin A (Mitopure, from Amazentis) resulted in plasma urolithin A levels that were six-fold higher than what was observed following the consumption of pomegranate juice.

Since urolithins undergo phase II metabolism, questions remain regarding the bioavailability and bioactivity of ingested urolithins in target tissues [5]. As a result, some companies and academic groups have been making synthetic analogs or derivatives of urolithin A, which may have more potent drug-like properties [6; 7]. However, these have not been clinically tested to date.

Neuroprotective Benefit: Urolithin A may help protect against neuronal loss due to oxidative stress damage and neuroinflammation, but treatment likely needs to begin very early to offer a meaningful benefit in the context of neurodegenerative disease.

Types of evidence:

- 1 biomarker study on urolithin levels and brain volume
- Numerous preclinical studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

The ability of urolithin A supplementation to prevent cognitive decline has not been directly assessed. The 18 month Green-MED diet trial (NCT03020186) assessed the effect of a Mediterranean diet higher in polyphenols and lower in red/processed meat on age-related brain atrophy in 284 participants (mean age 51 years old) [8]. In this study, higher urinary levels of urolithin A were associated with a lower level of hippocampal volume loss. Since urolithin A is just one of many polyphenols contained in the Green-MED diet, the effect on brain volume cannot be directly tied to urolithin intake, but it suggests that urolithin A may have a protective effect.

***Human research to suggest benefits to patients with dementia*:** None

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Pomegranate extract*:* The benefits of pomegranate extract in animal models of Alzheimer's disease (AD) provided early evidence toward the potential neuroprotective effects of urolithins.

An *in silico* computational study was conducted to evaluate the potential of different pomegranate extract constituents and urolithins to cross the BBB [9]. No pomegranate constituents were predicted to cross the BBB; however, urolithin A and B (and their methyl derivative) were all predicted to penetrate the BBB. Urolithin A also reduced Aβ fibrillization *in vitro* and improved survival in a worm model of AD.

Short-term treatment (three weeks) with pomegranate extract in old Alzheimer's mice reduced Aβ42 but did not alter cognition [10]. Longer treatment in young or old Alzheimer's mice decreased amyloid plaques, oxidative stress, inflammation (TNF-α and other cytokines, microgliosis, but not astrogliosis) and increased synaptic density, the growth factor BDNF, autophagy, and improved cognitive performance [11; 12; 13; 14] [15; 16]. It should be noted however, that most of these studies were conducted in one university in Oman, a country with a high production of pomegranates, and may be subject to bias.

Pomegranate juice (500 mg/kg/day) was also found to protect against rotenone-induced neurotoxicity in a rat model of Parkinson's disease [17]. The pomegranate-based intervention began ten days prior to the start of the rotenone injections. The mitigation of motor deficits and partial preservation of

dopaminergic neurons was related to an elevation of urolithin A in the brains of the treated animals, following consumption of pomegranate juice.

Urolithin A: More recent studies have demonstrated neuroprotective benefits in a variety of models testing formulations of purified urolithin A. While many studies come from the same general group of researchers, the protective effects have also been observed by other groups of researchers in various model systems. Several mechanisms have been identified in these studies, which may be more relevant under some conditions than others, but the most consistent mechanism of benefit relates to its role as a mitophagy enhancer.

Mitophagy: An adequate supply of healthy mitochondria is necessary for the maintenance of cellular bioenergetics and redox homeostasis [18]. This requires balance between the clearance of damaged mitochondria, known as mitophagy, and the production of new mitochondria. Mitophagy is a form of autophagy and utilizes aspects of autophagy machinery, and similar to other autophagic processes, is generally promoted by AMPK signaling, and inhibited by mTOR signaling. There are two major pathways of intracellular mitophagy. PINK1/Parkin-dependent mitophagy is triggered in dysfunctional mitochondria that cannot maintain their membrane potential. The presence of the PINK1/Parkin complex leads to the recruitment of LC3, autophagosome engulfment, and digestion by lysosomal enzymes. Hypoxic conditions can trigger a form of PINK1/Parkin-independent mitophagy which involves the proteins BNIP3 and Nix.
The process of mitophagy declines with aging, which can result in the accumulation of damaged mitochondria [18]. These dysfunctional mitochondria have a diminished capacity to meet the energy needs of the cell, and propagate damage through the production of reactive oxygen species (ROS). Disease-associated proteins, such as Aβ and tau, can interact with mitochondrial membranes, and contribute to mitochondrial dysfunction.
Several natural products have been identified that act as mitophagy enhancers, including NAD+ and urolithin A [18]. Screens conducted in cellular models and in *C. elegans* to identify the most potent mitophagy enhancers have consistently found urolithin A to be amongst the best natural mitophagy enhancers. Urolithin A was shown to enhance cellular respiration and promote synaptic function in immortalized mouse primary hippocampal neurons (HT22 cells) expressing mutant tau or mutant APP [19; 20]. An enhancement in the expression of synaptic proteins and a restoration of mitochondrial respiratory capacity to wildtype levels was also observed in iPSC-derived neurons with an ApoE4/E4 genotype [21]. Similarly, urolithin A enhanced PINK1/Parkin-dependent mitophagy in *C. elegans*, and restored memory performance in tau-expressing (BR5270) worms [21]. One study identified proteins

that are part of the core insoluble proteome, which are associated with a variety of aging-related diseases [22]. Many of these proteins are associated with the modulation of lifespan. Treatment with urolithin A was found to reverse dysfunction in this core insoluble proteome in *C. elegans* through the augmentation of mitophagy.

DYRK1A: DYRK1A is a kinase involved in the phosphorylation of tau. It is upregulated in the context of AD and Down syndrome (see DYRK1A Inhibitors report). Epigallocatechin gallate (EGCG) has been known to act as a DYRK1A inhibitor. Urolithin A is a metabolite of the related polyphenol, ellagic acid. Kinase profiling suggests that urolithin A also acts as an inhibitor of DYRK1A [23]. Urolithin A inhibited tau phosphorylation at pT181, pT212, and pT199/202 in a cell-free system. Additionally, urolithin A pretreatment (100 mg/kg/day i.p. for three days) prior to intracerebroventricular injection of okadaic acid, prevented tau phosphorylation at Thr-212 and memory deficits on the Morris water maze.

Neuroinflammation: Mitochondrial dysfunction can contribute to inflammation. The inflammatory profile of immune cells is regulated by their metabolic status, thus changes to mitochondrial function can impact the activity and phenotype of various immune subsets. Additionally, the production of excess ROS by dysfunctional mitochondria can result in oxidative stress damage which triggers inflammatory immune responses. Urolithin A treatment has been found to mitigate the induction of inflammatory processes in a variety of models [24]. This may be related to a reduction in the levels of damaged mitochondria, as well as more direct effects on signaling pathways related to inflammation.

Antioxidant induction: The mitigation of oxidative stress is thought to be a key aspect of urolithin A's neuroprotective activity. This can occur through augmentation of mitochondrial function via the restoration of mitophagy, or through the induction of endogenous Nrf2 antioxidant signaling [6; 25].

AD models: POTENTIAL BENEFIT (Preclinical)
Urolithin A treatment has been found to mitigate amyloid and tau pathology as well as cognitive deficits in various AD mouse models. They appear to be most beneficial when started prior to the onset of significant neuropathology or symptoms. It is currently unclear how well this would translate to human patients, and how early patients would need to start taking it in order to achieve a meaningful benefit.

3xTg: Supplementation with urolithin A mitigated declines in cognitive function in the 3xTg AD mouse model. Accumulation of Aβ and tau pathology typically starts around 12 months of age, and females exhibit more profound cognitive deficits in this model [26]. Intermittent (every other week) dietary

supplementation of urolithin A (~5 mg/kg/day) starting at three months of age attenuated Aβ burden in the hippocampus and cortex, and preserved performance on cognitive tasks, including the Morris water maze and cued fear conditioning task, at 12–13-month-olds of age in female 3xTg mice [27]. The protective effect is thought to be related to the enhanced clearance of autophagosomes, as observed in cell culture.

Other studies have found that urolithin A also has some neuroprotective properties when administered after the onset of pathology in this model, starting around 12 months of age. Treatment with urolithin A (200 mg/kg/day) for five months mitigated cognitive declines on the Y-maze and novel object recognition tasks in 3xTg mice [26]. This was accompanied by better preservation of synaptic plasticity (long-term potentiation) and a reduction in the degree of oxidative stress. Additionally, urolithin A treatment was associated with reduced levels of tau pathology, a decrease in the level of activated pro-inflammatory NF-kB, and the normalization of GFAP levels. Benefits were also observed in a model (3xTgAD/Polβ+/−) with more advanced AD pathology, but they were less pronounced, suggesting that urolithin A may be best suited to presymptomatic/early phases and have less efficacy at more advanced stages of disease [26]. The protective effects may stem from a reduction in mitochondrial oxidative stress or increased DNA repair. Treatment of 13-month-old 3xTg mice with urolithin A (200 mg/kg/day) for one month was also found to reduce tau phosphorylation at Thr181, Ser202/Thr205, and Ser262, and showed similar efficacy in males and females in this study [21].

APP/PS1: Neuropathology and behavioral symptoms typically become apparent around six months of age in the APP/PS1 mouse model. Supplementation with urolithin A (200 mg/kg/day) for five months starting at two months of age, which is prior to the onset of pathology, attenuated cognitive declines, such that performance on cognitive tests at seven months of age was still similar to that of wildtype mice [26]. Notably, performance on the Y-maze was maintained in these animals for at least one month after discontinuing treatment with urolithin A. Levels of soluble and insoluble Aβ42 were also reduced in the prefrontal cortex of the treated mice, but the effect on Aβ was only maintained during treatment, as levels increased after cessation of urolithin A. Supplementation with urolithin A also reduced IL-1β-mediated neuroinflammation and mTOR signaling in these mice. Another study found that urolithin A treatment (300 mg/kg/day) starting at four months of age also attenuated Aβ deposition and neuroinflammation, through the inhibition of P65NF-κB, P38MAPK, and BACE1 activity [28]. In cell culture, urolithin A promotes lysosomal acidification and improves lysosomal function, to help restore cellular clearance mechanisms [26]. Impairments to autophagy and mitophagy are thought to occur very early in the disease course, well in advance of the onset of symptoms, such that treatment during the presymptomatic phases are expected to offer the greatest degree of potential benefit. However, similar

to the 3xTg model, benefits have also been observed with the start of treatment during the early symptomatic phase [26]. APP/PS1 mice treated with urolithin A (200 mg/kg/day) for two months starting at six months of age had less accumulation of insoluble Aβ and better cognitive performance on the Morris water maze relative to their untreated counterparts [21]. Urolithin A treatment led to increases in mitophagy-associated proteins, such as PINK1, which is thought to underlie the protective effects.

hAbKI: Humanized homozygous amyloid beta knock-in mice (hAbKI) serve as a model of late-onset sporadic AD. Amyloid protofibrils and cognitive impairments become apparent around 10-14 months of age [29]. A treatment regimen of urolithin A (2.5 mg/kg i.p. 3x/week for four months) starting at three months of age, prior to the onset of symptoms and pathology, led to the upregulation of the mitophagy-associated genes, PINK1 (by 2.63-fold) and Parkin (by 2.3-fold), and an enhancement of mitochondrial respiratory capacity [30]. Urolithin A treatment was also associated with the reduction of oxidative stress and lipid peroxidation markers. Cell culture screens of mitophagy inducers indicated that the combination of urolithin A with EGCG had superior capacity to promote mitophagy [19]. A similar effect was observed in the hAbKI mice, with the combination of urolithin A with EGCG (25 mg/kg i.p.) showing more pronounced benefits relative to urolithin A alone [30].

Cognitive aging models: POTENTIAL BENEFIT (Preclinical)
Impaired mitophagy has been implicated in cognitive aging, and urolithin A has shown protective effects in several mouse models of cognitive aging through the induction of mitophagy and preservation of mitochondrial function. In general, the benefits are most prominent when urolithin A is administered in close proximity to the onset of mitochondrial-damaging processes, as it appears to be better suited to the preservation of tissue function, rather than restoration after damage.

D-galactose-induced: Excess levels of D-galactose can induce an accelerated aging phenotype, largely through the production of oxidative stress and associated cellular damage. Mice treated with urolithin A (150 mg/kg) for two months during the period of D-galactose exposure were protected against cognitive impairment on the Morris water maze, had preserved antioxidant activity, and showed decreased levels of proinflammatory cytokines (TNF-α, IL-6, and IL-1β) in brain tissue [31]. Urolithin A treatment also mitigated the induction of the microRNA, miR-34a, which is associated with regulation of the SIRT1/mTOR signaling pathway. The induction of miR-34a may contribute to autophagy dysfunction. Another study found that methylated urolithin A protected against cognitive impairment by preventing the induction of the NLRP3 inflammasome in this model [32].

Natural aging: Upregulation of miR-34a was also found to impair autophagy in the context of natural aging in mice [31]. Treatment of 12-month-old (middle-aged) mice with urolithin A led to a decrease in levels of miR-34a and mTOR-mediated signaling in the hippocampus.

Streptozotocin-induced: A high glucose environment, such as occurs in the context of diabetes, promotes a state of persistent oxidative stress characterized by inflammation and mitochondrial dysfunction. Urolithin A treatment (2.5 mg/kg/day i.p.) for eight weeks following the induction of streptozotocin-induced diabetes preserved performance on the Y-maze cognitive test in male mice, and mitigated the induction of Aβ and p-tau pathology [33]. Additionally, urolithin A protected against mitochondrial dysfunction by preventing mitochondrial calcium influx and the related induction of mitochondrial ROS. Urolithin A also protected against NLRP3-mediated neuroinflammation and reactive gliosis in this model [34].

Olanzapine-induced: The antipsychotic olanzapine is associated with accelerated cognitive decline, and was found to impair mitophagy by blocking mitophagosome-lysosome fusion. In *C. elegans*, treatment with urolithin A attenuated olanzapine-induced deficits in learning and memory [35]. The protective effect may be related to the preservation of mitochondrial function due to the partial amelioration of mitophagic flux, as observed in cell culture.

Sleep deprivation: Pretreatment with urolithin A (2.5 mg/kg or 10 mg/kg i.p.) for seven days prior to a 24-hour sleep deprivation event mitigated subsequent cognitive deficits and neuroinflammation in young (3 months old) and middle-aged (12 months old) mice [36]. Urolithin A also protected against sleep deprivation-induced mitochondrial dysfunction.

Amyotrophic lateral sclerosis model: POTENTIAL BENEFIT (Preclinical)
In $SOD1^{G93A}$ mice exposed to copper (0.13 PPM) starting at six weeks of age, treatment with urolithin A (50 mg/kg/day) starting at 13 weeks of age helped preserve motor neurons and mitigated motor dysfunction and neuroinflammation [37]. The copper exposed mice experienced mitochondrial dysfunction and an acceleration of motor decline. The preservation of motor neuron number and function with urolithin A was accompanied by the activation of PINK/Parkin-dependent and independent mitophagy.

Traumatic brain injury (TBI) model: POTENTIAL BENEFIT (Preclinical)
Treatment with urolithin A (2.5 mg/kg i.p.) starting immediately after the induction of TBI via a controlled cortical impact attenuated BBB leakage and neurological deficits in mice [38]. Urolithin A treatment led to a partial preservation of tight junction proteins and a reduction in brain edema. It was also associated with the induction of autophagy via regulation of the PI3K/Akt/mTOR pathway.

APOE4: None

Aging and related health concerns: Urolithin A may help protect against age-related diseases by preserving mitochondrial function. Mechanistically it is better suited for prevention than treatment and may need to be started early in life to maximize benefit.

Types of evidence:

- Two systematic reviews of studies testing urolithin A
- 3 RCTs testing urolithin A for mitochondrial function in muscle
- 3 related RCTs testing topical urolithin A for skin aging
- 1 RCT testing urolithin A's effects on the immune system
- 1 clinical trial testing urolithin A in patients with heart failure
- Four meta-analyses for pomegranate in humans
- 2 RCTs for pomegranate extract in prostate cancer
- Several observational studies assessing urolithin A levels and cardiometabolic profiles
- Numerous preclinical studies

Lifespan: POTENTIAL BENEFIT IN ANIMAL MODELS
The accumulation of dysfunctional mitochondria with aging is thought to contribute to age-related tissue damage, such that interventions to restore the balance of healthy mitochondria may rejuvenate tissues, leading to the prolongation of healthspan and/or lifespan [39]. The restoration of mitophagy and mitochondrial dynamics with urolithin A has been associated with lifespan extension in some model systems. These effects were generally observed with treatment starting early and lasting through the duration of the lifespan, so it is unclear whether that would translate to humans initiating supplementation with urolithin A in middle to late life. However, pilot studies in middle-aged to older adults suggest that urolithin A may offer modest benefits to healthspan, such as improvements to mitochondrial health, and possibly tissue function, even at later stages of life.

A systematic review of five studies including 250 participants ranging from 36 to 88 years old testing urolithin A (10 to 1000 mg/day) from one to four months assessed the geroprotective potential of urolithin A in humans [40]. While improvements in mitochondrial activity, autophagy, inflammation, and muscle strength were observed, most of the effects were modest, and they concluded that the available evidence was insufficient to indicate whether urolithin A has meaningful geroprotective activity in humans. The ability to detect an effect may have been limited by small samples sizes and the relatively short durations of treatment, relative to the durations associated with benefit in preclinical models.
A scoping review of 15 articles assessing the impact of urolithin A on the prevention of age-related pathologies indicated that urolithin A has primarily been tested in models related to neurodegenerative disease, musculoskeletal-related disease, cardiovascular disease, skin health, and cancer [41]. Most of the benefits observed to date have been in animal models.

Pomegranate supplementation to fruit fly food increased male and female lifespan by 18% and 8%, respectively [42].

Urolithin A increased the lifespan of worms by 45%, though no effects were seen with ellagic acid. It increased worm activity (e.g. pharyngeal pumping) and improved muscle fiber organization. Life extension was dependent on mitochondrial function and mitophagy. Urolithin A decreased mitochondrial content in young worms while maintaining the maximal respiratory capacity and increased mitochondrial number in old worms. In two mammalian cell lines (muscle and intestine), Urolithin A also induced autophagy. Six-week treatment in old mice increased running endurance by 42% while treatment in young mice increased running capacity by 65%. Lean muscle mass did not increase, suggesting that these benefits were due to muscle efficiency [43].

An intermittent urolithin A regimen, in which the chow was supplemented with 25 mg/kg of urolithin A (resulting in an estimated consumption of 5 mg/kg/day) every other week starting at three months of age increased survival in wildtype (C57Bl/6N) male mice [27]. The median lifespan at 80th percentile mortality of the mice was extended 18.75% from the start of urolithin A supplementation.

Sarcopenia: POTENTIAL BENEFIT
Mitochondrial capacity is a key determinant of the performance capacity of skeletal muscle [44]. The accumulation of dysfunctional mitochondria impairs the bioenergetic capacity of the muscle, which in turn limits exercise capacity. Improvements to muscle efficiency and performance observed with

urolithin A supplementation in animal models provided rationale to test the capacity of urolithin A to improve muscle performance in humans. To date, urolithin A has been tested in three small pilot trials in relatively healthy middle-aged to older adults. All of the trials used the Mitopure formulation of urolithin A from Amazentis.

The first in human Phase 1 trial (NCT02655393), conducted by Amazentis, tested urolithin A in single ascending doses from 250 to 2,000 mg, and in multiple ascending doses of 250, 500, or 1000 mg/day for 28 days in healthy elderly participants (n=60) [45]. The study was powered for the outcome of safety, but benefits to mitochondrial health were observed based on exploratory gene expression analyses in muscle biopsies at doses of 500 and 1,000 mg/day. There were increases in the expression of genes related to mitophagy, mitochondrial biogenesis, and fatty acid oxidation. These included many of the same genes that were found to be downregulated in the skeletal muscle of pre-frail elderly adults. Additionally, increases in the plasma levels of short chain acylcarnitines were also observed with urolithin A supplementation (500 or 1,000 mg/day), suggestive of enhanced efficiency of fatty acid oxidation.

The randomized, placebo-controlled Phase 2 ATLAS trial (NCT03464500) tested the effect of urolithin A (500 or 1,000 mg/day) supplementation for four months on exercise tolerance in untrained overweight middle-aged adults (n=88) [46]. The trial did not meet its primary outcome of power output on the cycle ergometer from baseline to day 120, as there were no significant differences between the urolithin A and placebo groups on this measure. There were significant improvements on some of the secondary outcomes also related to muscle strength and exercise performance. There were improvements on muscle strength based isokinetic Biodex dynamometer strength testing for both doses. Most notably, the level of improvement on the six-minute walk test in the 1,000 mg dose group (+33.43 m) is in the range of what is considered clinically important. Urolithic acid reduced markers of inflammation including CRP, IFN-γ, IL-1β, and TNF-α. Gene expression analysis indicated that urolithin A treatment led to an enrichment of genes related to glycogen metabolism, mitochondria, and Parkin-mediated activity in the muscle tissue. These changes in gene expression, along with an effect on acylcarnitine levels, were only observed at the 500 mg dose, despite potential evidence of an effect on muscle performance at the higher dose. It is unclear why biochemical and expression changes were not observed at the 1,000 mg dose in this study.

In the double-blind, placebo controlled randomized ENERGIZE trial (NCT03283462) older adults (n=66) aged 65 to 90 years old were treated with urolithin A at a dose of 1,000 mg/day for four months [47].

The trial did not meet its primary endpoint of the change from baseline in the six-minute walk distance and change from baseline to four months in maximal ATP production in the hand skeletal muscle. Participants in both the urolithin A (+60.8± 67.2 m) and placebo (+42.5± 73.3 m) groups improved the distance traveled on the six-minute walk test. There were no differences in maximal ATP production in the hand (first dorsal interosseus) (0.07 ±0.23 mM/s vs 0.06±0.20 mM/s) or leg (tibialis anterior) (−0.03 ±10 mM/s vs 0.03 ±0.10 mM/s) muscles. The lack of detectable benefit with urolithin A may have been related to the relatively high baseline mitochondrial function in the study population. This study used a cutoff for maximal ATP production of 1.0 mM/s, which is higher than what is typically used (0.7 mM/s). Urolithin A treatment was associated with reductions in levels of acylcarnitines, primarily long-chain, as well as reductions in levels of the systemic inflammatory marker CRP.

Overall, the studies suggest that urolithin A has the capacity to impact mitochondrial-related expression and possibly activity in the skeletal muscle of humans, but the potential functional implications are limited to date. In comparison to preclinical animal studies, the duration of treatment was relatively short, and the participants were more advanced in age, which may have limited the potential for benefit.

Cardiovascular: POTENTIAL BENEFIT (Preclinical)
Metabolome types relating to urolithin production have been associated with cardiovascular risk profiles, with metabolomes resulting in urolithin A production typically associated with lower risk for cardiometabolic diseases. Since the different metabolomes are associated with differences in gut microbiota composition, the cardioprotective effects cannot be clearly tied to the production of urolithin A per se. However, preclinical studies suggest that urolithin A itself may have properties beneficial for cardiovascular health.

Pomegranate juice: A meta-analysis of eight RCTs [48] reported that consumption of pomegranate juice (doses between 50 and 300ml/day) reduced systolic blood pressure (-5 mmHg) and diastolic blood pressure (-2 mmHg) regardless of the length of the study (> or < 12 weeks) or dose. Systolic reductions were significant, while diastolic reductions were slightly non-significant at high and low doses. Another meta-analysis of 12 RCTs reported that pomegranate juice did not change LDL-c or HDL-c but did lower triglycerides [49] . Two caveats to the studies mentioned above are that pomegranate juice contains many other compounds and individuals metabolize ellagic acid differently and the studies may not be relevant to urolithin A, per se. These mixed results may also be reflected in two meta-analyses showing no benefit with pomegranate juice on glucose management or CRP levels [50; 51].

Pomegranate Extract: In multiple mouse models of atherosclerosis, pomegranate extract reduced atherosclerotic plaque size up to 44% and the proportion of occlusive coronary artery plaques. It also reduced levels of oxidative stress, ox-LDL-c, MCP-1 (which recruits macrophages in to plaques), lipid accumulation, macrophage infiltration, and fibrosis in the myocardium and increased macrophage cholesterol efflux [52; 53; 54].

Urolithin*:* To date, the cardioprotective effects of urolithin A have only been observed in preclinical models. A pilot randomized, double-blind, placebo-controlled crossover trial conducted in 10 patients with heart failure with reduced ejection fraction (HFrEF) did not find evidence of cardioprotection on echocardiographic or biochemical measures [55]. The study involved two 4-week intervention periods testing urolithin A (500 mg BID) or placebo separated by a two-week washout period. There were no significant effects on echocardiographic measures (LVEF, LVEDD, LVESV, and TAPSE), or plasma levels of pro-BNP, glucose, and CRP. With the exception of an increase in HDL-c (+6.46 ± 2.33 mg/dL), there were no significant effects on blood lipid measures (LDL-c, triglycerides, total cholesterol, and VLDL-C). However, the intervention period may have been too short to detect a difference. The population may have also been too advanced, as preclinical studies across indications generally find that the benefits of urolithin A are most prominent during early stages of cellular dysfunction, prior to the onset of major tissue damage/pathology.

In a rat model of aortic injury on a high cholesterol diet, 12 week treatment with urolithin A decreased LDL-c, decreased angiotensin II levels, increased Nrf2 activity, and improved aortic wall morphology [56].
In a rat model of streptozotocin induced type 1 diabetes, 3-week treatment with urolithin A and B improved cardiac function (maximal rate of ventricular pressure, reduced contraction time, cardiomyocyte contractility, etc.) [57]. In an ischemia/reperfusion model, urolithin A reduced cell death and infarct size, improved cardiac function, and reduced levels of ROS. These effects were mediated through the PI3K/Akt pathway [58].
Urolithin A was able to inhibit the TGFβ-induced cardiac fibroblast to myofibroblast transformation in rats, which was accompanied by a reduction in fibrosis in the left anterior descending coronary artery ligation model [59]. The protective effect may have been related to the induction of Nrf2 and its associated antioxidant signaling molecules by urolithin A.
Urolithin A (50 mg/kg) also protected against cardiac remodeling, fibrosis, and diastolic dysfunction in a model of high-fat diet-induced metabolic cardiomyopathy [60]. In cell culture, urolithin A protected against palmitic acid-induced cardiomyocyte hypertrophy through the enhancement of mitophagic flux.

In the atherosclerotic ApoE(-/-) mice fed a high fat and cholesterol diet, urolithin A (50 mg/kg) supplementation reduced lesion load, and the macrophage content of the atherosclerotic plaques [61]. Urolithin A attenuated endothelial inflammation in TNF-α-stimulated human umbilical vein endothelial cells (HUVECs) through the production of nitric oxide (NO) [61]. Impacts on NO synthesis have been observed in other studies as well.
In vitro studies suggest that both urolithin A and urolithin B increase nitrite/nitrate levels and eNOS levels, but had no effect individually [62]. In human artery endothelial cells exposed to ox-LDL-c, urolithin A improved cell survival, increased the expression of NO and eNOS, and decreased the expression of inflammatory proteins [63].

Immune function: POTENTIAL BENEFIT
A variety of cell culture studies indicate the immunomodulatory potential of urolithin A [24]. These effects are largely mediated by the impact of urolithin A on mitochondrial function and ROS production. The phenotype of immune cells is heavily influenced by their metabolic state, such that agents which modulate mitochondrial function have the capacity to also regulate the inflammatory status/polarization of immune cells. As a microbiota-derived ligand of human aryl hydrocarbon receptors, urolithin A has the capacity to impact cell signaling and function of a variety of human cell types, particularly immune cells [24]. These receptors are involved in the regulation of responses to environmental stimuli. Oxidative species act as signaling molecules and impact the functionality of several immune cell subsets, most notably neutrophils. Urolithin A can also modulate the inflammatory status of innate immune subsets through the regulation of NF-kB activation. The activation of autophagy pathways may also regulate the polarization of macrophages, particularly under inflammatory conditions. For example, *in vitro* studies suggest that urolithin A prevented the activation of macrophages which required autophagy [64]. A cell culture study assessing the impact of urolithins on the modulation of LPS-induced inflammatory responses in a variety of human immune cell types found that urolithin A was the most potent in inhibiting pro-inflammatory responses, via the inhibition of TNF-α and induction of IL-10 [65]. Urolithin A was also found to have the capacity to induce ERK1/2 phosphorylation, which is typically associated with pro-inflammatory signaling, suggesting that the immunomodulatory effects of urolithin A may be context dependent.

Urolithin A has also been shown to influence the phenotype of adaptive immune subsets in a manner that may be beneficial for cancer immunosurveillance. Urolithin A supplementation (50 mg/kg/day) for four weeks reduced tumor growth of engrafted MC-38 colon carcinoma cells in wildtype mice, but not in immunocompromised mice, suggesting that it is immune cell dependent [66]. The effect appears to

stem from the remodeling of the T-cell compartment toward a state refractory to tumor cell development, rather than the augmentation of tumor infiltrating lymphocytes [66]. Urolithin A may promote the formation of naïve T cells through induction of FOXO1 and downstream gene expression in CD8+ T cells. Since tumors vary in terms of their immunogenicity, the impact of urolithin A may be dependent on the tumor type as well as the timing of administration. Another study found that urolithin A was able to enhance the effector function of primary CD8+ cytotoxic T lymphocytes and human chimeric antigen receptor (CAR) T cells via ERK1/2 signaling [67]. Engagement of this pathway was found to promote autophagic flux and enhance the respiratory capacity of the T cells. The metabolic remodeling of the T cells influences their cytotoxicity activity.

The pilot randomized, double-blind, placebo-controlled MitoIMMUNE trial (NCT05735886) sought to characterize the effect of urolithin A on the human immune system to assess its potential use as part of cancer immunotherapy [68]. The study included 50 middle aged to older adults (age 45-70) treated with urolithin A (1,000 mg/day) or placebo for 28 days. The primary endpoints were the changes in peripheral CD3+ cells, and alterations in mitochondrial activity in immune cell subsets in the peripheral blood. Urolithin A led to immune remodeling, including an expansion of circulating NK cells and a reduction in the inflammatory signature of monocytes. There was a shift toward more naïve CD8 T cells, coupled with an increase in mitochondrial mass in these cells. This pilot suggests that urolithin A has immunomodulatory properties in humans, though it has not yet been established if or how these changes will impact risk for various age-related conditions.

Neuropathy: POTENTIAL BENEFIT (Preclinical)
A 24-day treatment in a rat model of tibial and sural nerve transection with pomegranate extract attenuated neuropathic pain, reduced levels of TNF-α, and increased levels of glutathione and nitrite [69].

Prostate Cancer: POTENTIAL MINOR BENEFIT/UNCLEAR
Pomegranate has been tested in clinical studies for prostate cancer. In a trial of 183 subjects after primary therapy for prostate cancer, pomegranate extract (dose not specified) did not increase PSA doubling time. However, in a subset of patients with a manganese superoxide dismutase (*MnSOD*) AA genotype (which could indicate a low antioxidant status), PSA doubling time increasedby12 months in the pomegranate group compared to an increase of only 1.8 months in the placebo group. However, the number of *MnSOD* AA patients was small [70]. Pomegranate extract (1,000 mg/day) for 12 months did not significantly affect PSA doubling time or biopsy kinetics in an RCT including 30 men with localized

prostate cancer undergoing active surveillance [71]. Reductions were observed in levels of oxidative stress markers (8-OHdG) and androgen receptor expression in prostate tumor tissue with pomegranate extract.

Cell culture studies suggest that urolithin A may have some direct anti-cancer effects, at least in some tumor types. Urolithin A has been shown to inhibit the proliferation of androgen-dependent (LNCaP) and independent (DU-145) prostate cancer cell lines [7]. Part of the mechanism may be related to the inhibition of androgen receptor signaling. A synthetic analog of urolithin A, ASR-600, was found to downregulate androgen receptor expression and PSA in prostate cancer cell lines and inhibit tumor growth of 22RV1 cells in a xenograft mouse model [7].
Urolithin A exhibited cytotoxicity in colorectal adenocarcinoma cell lines (SW480, SW620, HCT 116, and HT-29) [5]. Urolithin A was able to induce cell death in oral squamous cell carcinoma cell lines through the inhibition of Akt/mTOR signaling and subsequent induction of autophagic signaling [72].

Skin aging: POTENTIAL BENEFIT
A series of controlled clinical trials (NCT05300984; NCT05473832; NCT05300542) were sponsored by Amazentis, testing the impact of topical formulations of urolithin A on skin aging [73]. One study assessed skin barrier function and aging pathways in 48 postmenopausal women, while a second study examined the effect on wrinkles in 108 middle-aged men and women following eight weeks of urolithin A (0.5% or 1%). A UVB-mediated photo damage study in 22 participants assessed the impact on skin redness when applied for 24 hours following UVB exposure. Impacts to biological pathways related to aging were assessed from skin biopsies. The 1% urolithin cream formulation reduced wrinkle appearance and improved skin hydration after eight weeks, relative to placebo, in the first two studies. Transcriptomic analysis from skin biopsies indicated an upregulation of genes related to collagen fibril organization, including fibrillar type I and III collagen, while reducing genes related to (inflammatory) chemokine responses. Acute exposure of cultured human skin cells to urolithin led to downregulation of genes associated with collagen degradation, including matrix metalloproteinases (MMPs), while increasing genes associated with mitophagy (PINK1/Parkin) and autophagy (MAP1LC3 and ULK1). In the photodamage study, 1% urolithin A reduced skin redness by 13%. No significant effects on any of these measures were observed with placebo or the lower (0.5%) dose of topical urolithin A.

Metabolic disease/Obesity: POTENTIAL BENEFIT (Preclinical)
Urolithin metabolome types have been associated with markers of cardiovascular and metabolic health. The metabolome A subtype, in which individuals produce moderate to high concentrations of urolithin A has been associated with a lower propensity for metabolic syndrome [3]. The differences in metabolome are related to the composition of the gut microbiome, suggesting that a metabolically healthy microbiome includes a high proportion of taxa which metabolize ellagic acid into urolithin A. In a study of 560 Spanish adolescents from the SI! Program for Secondary Schools trial, urolithin A levels were found to be inversely associated with diastolic blood pressure [74]. Additionally, higher levels of phenolic metabolites, including urolithin A, were associated with reduced odds of presenting with features of metabolic syndrome, such as abdominal obesity. An analysis of metabolites following pomegranate extract consumption from overweight participants with mild dyslipidemia from the POMEcardio trial found that pomegranate extract reduced bile acids and the coprostanol/cholesterol ratio only in participants that could produce urolithin A [75]. It is unclear whether the prospective cardioprotective effects stem from the production of urolithin A, the modulation of the gut microbiota, or a combination.
In the DIRECT-PLUS testing the impact of the polyphenol-rich green-Mediterranean diet on weight loss in 294 participants, elevated urine levels of urolithin A were associated with a greater reduction in visceral adipose tissue [76]. Although, the effect on fat loss cannot be directly attributed to urolithin in this trial, similar effects have been observed in preclinical studies. Rats treated with urolithin A (2.5 mg/kg i.p.) for four weeks following ten weeks of a high-fat diet had lower levels of visceral fat and reduced serum cholesterol levels relative to those fed a high-fat diet without urolithins [77]. The protective effect may be related to a reduction in oxidative stress and preservation of hepatic lipid metabolism. Urolithin A administration protected against insulin resistance in mice fed a high-fat diet, likely through the promotion of mitochondrial biogenesis and attenuation of lipid accumulation in the liver [78]. It also protected against the infiltration of inflammatory macrophages in adipose tissue. Additionally, some rodent studies suggest that urolithin A may promote brown fat activation [79], though it is unclear whether that effect would be translatable to humans.

Osteoarthritis: POTENTIAL BENEFIT (Preclinical)
Urolithin A was found to enhance mitophagic flux in cultured human chondrocytes from both healthy individuals and patients with osteoarthritis, which was accompanied by dose-dependent increases in mitochondrial respiration [80]. Protective effects on joints were also observed *in vivo* in the meniscal destabilization mouse model of osteoarthritis. Mice treated with a urolithin A supplemented diet (250

mg/kg) for eight weeks post-surgery exhibited an attenuation of pain responses, synovitis, and histological damage in the knee. These studies were sponsored by Amazentis.

Age-related macular degeneration (AMD): POTENTIAL BENEFIT (Preclinical)
Defective mitophagy has been implicated in the progression of AMD, suggesting that mitophagy enhancing therapies might help prevent or delay disease progression [81].
Urolithin A was tested in sodium iodate-induced models of acute retinal degeneration [82]. Sodium iodate is an oxidizing agent that induces retinal cell death and vision loss. Autophagic flux and PINK1/Parkin-dependent mitophagy were preserved in mice treated with urolithin A (2.3 mg/kg i.p.) starting three days prior to sodium iodate [82]. Urolithin A also protected against sodium iodate-induced lysosomal membrane permeabilization and cell death in human-derived ARPE-19 cells. These studies suggest that urolithin A may help protect retinal cells during early stages to preserve mitochondrial function, but it is unclear if it would offer meaningful benefit after significant retinal cell damage/loss has already occurred.

Safety: Urolithin A has been safe in short clinical trials in middle aged and older adults, but long-term safety has not been established.

Types of evidence:

- 1 systematic review of human trials testing urolithin A
- 6 RCTs testing oral urolithin A
- 3 related RCTs testing topical urolithin A
- Multiple meta-analyses for pomegranate

Urolithin A has shown good safety to date, however, all of the trials conducted so far have been small and of relatively short duration [40]. Additionally, the trials have generally been conducted in relatively healthy populations, so it is unclear whether there are any disease-related interactions. Adverse events were generally mild, and there were no clear patterns. There were no drug-related serious adverse events or significant effects on laboratory parameters, biochemical tests, liver or kidney function tests, hematological parameters, or abnormalities on ECG [45; 46]. In a series of studies testing a topical formulation of urolithin A, there were a few cases of mild skin irritation [73].

Urolithin does not have genotoxic effects based on preclinical toxicology studies. The No Observed Adverse Effect Level (NOAEL) was determined to be 3,451 mg/kg bodyweight/day in male rats and 3,826 mg/kg bw/day in female rats, or the human-equivalent dose of approximately 557 mg/kg bw/day in males and 617 mg/kg bw/day in females [45; 83].

Amazentis submitted an application to the FDA that urolithin A should be considered Generally Recognized as Safe (GRAS). Upon review of the application materials, the FDA provided a letter of no objections to this claim in 2018 (GRAS Notice No. GRN 000791).

In clinical studies, pomegranate extract is safe. High doses (3000mg/day over 28 days) increased diarrhea. However, large-scale, long-term studies have not been conducted [84].

Drug interactions:
Information on drug interactions with urolithin A has not been established [85]. However, pomegranate juice interacts with cytochrome P450 and thus may interact with other drugs [84].

Sources and dosing:
Urolithin A can be obtained from the diet through the consumption of foods containing ellagitannins such as pomegranates, walnuts, almonds, and some berries [85]. However, urolithin A can only be obtained from the metabolism of these foods in individuals who have bacteria in their gut microbiome capable of producing urolithin A.

Urolithin A is also available in the form of OTC supplements from a variety of suppliers. However, a study testing the reliability of label claims for urolithin A containing supplements found that relative to the stated concentration, the level of urolithin A in the tested products ranged from -15.5% to +28.6% [86]. The most well validated form of urolithin A is the Mitopure® formulation from Amazentis, as this is the form used in the majority of the clinical trials to date. In clinical trials, impacts to mitochondrial health were observed at doses of 500 to 1,000 mg/day, though it has not yet been shown to have clinical benefit for any disease indication, and is not approved for any indication.

Most studies with pomegranate juice have used 250-500ml/day (or 500-1000mg/day of pomegranate extract).

Research underway:

According to Clinicaltrials.gov, there are currently 10 active clinical trials testing formulations of urolithin A.

These include trials testing the effects of urolithin A on glucose metabolism in adults >55 years old (NCT06274749); the effect of urolithin A on blood flow on middle-aged adults with obesity (NCT05921266); a trial sponsored by Amazentis testing the bioavailability of three Mitopure formulations (NCT06362018); a trial sponsored by Amazentis testing Mitopure on mitochondrial quality in frail older adults (NCT06556706); a feasibility trial testing urolithin A in patients with COPD undergoing pulmonary rehabilitation (NCT06324214); a trial sponsored by Amazentis testing a Mitopure skin cream (NCT06619457); a trial testing the impact of urolithin A with protein supplementation during single leg immobilization (NCT05814705); a trial testing urolithin A in men with prostate cancer undergoing radical prostatectomy (NCT06022822); a biomarker trial sponsored by Amazentis to detect urolithin A in dried blood spots (NCT04985630).

Additionally, there are academic groups and companies that are developing analogs or derivatives of urolithin A for potential clinical use in cancer and/or as mitophagy enhancers [6; 7]. To date, none of these has gone through clinical testing.

Search terms:

Pubmed, Google: urolithin, ellagic, pomegranate +

- Alzheimer's, cognition, neurodegeneration, cardiovascular, metabolism, aging, autophagy, orthostatic, neuropathy, lifespan, apoE, cancer, clinical trials, meta-analysis, safety

Websites visited for: Urolithin A

- Clinicaltrials.gov (pomegranate and urolithin a)
- Examine.com (punicalagins; urolithin a)
- DrugAge
- PubChem
- DrugBank.ca
- Consumerlabs.com

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