

RESEARCH MONOGRAPH . COMPOUND PROFILE

Urolithin-A

A naturally occurring benzo[c]chromenone produced by human gut microbiota from ellagitannin precursors, identified as the first dietary metabolite to induce mitophagy through the PINK1/Parkin pathway in human skeletal muscle at oral doses, with clinical evidence in age-related mitochondrial decline, muscle function, immune senescence, and emerging neuroprotective applications.

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ABSTRACT

Urolithin A (3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one) is a dibenzopyranone metabolite produced by the human gut microbiota through sequential dehydroxylation of ellagic acid, itself the hydrolysis product of dietary ellagitannins found in pomegranates, walnuts, raspberries, and strawberries. The compound was first characterized as a bioactive ellagitannin metabolite in human plasma and urine by the Tomas-Barberan and Espin laboratories at CEBAS-CSIC (Murcia, Spain) in the early 2000s and was subsequently identified by the Auwerx laboratory at the Ecole Polytechnique Federale de Lausanne (EPFL) as a potent, first-in-class inducer of mitophagy in *Caenorhabditis elegans* and in mammalian systems through the PINK1/Parkin-dependent mitochondrial quality control pathway. The seminal Ryu et al. (2016) report in *Nature Medicine* demonstrated that urolithin A extends lifespan in *C. elegans*, improves exercise capacity in aged rodents, and induces a molecular signature of mitophagy in skeletal muscle, establishing the compound as the first dietary metabolite with demonstrated mitophagy-inducing activity at physiologically achievable concentrations [1].

Amazentis SA (Lausanne, Switzerland) subsequently developed a synthetic, pharmaceutical-grade form of urolithin A (marketed as Mitopure) and advanced the compound through a series of randomized, placebo-controlled clinical trials in human subjects. The first-in-human trial (Andreux et al. 2019, *Nature Metabolism*) demonstrated safety, oral bioavailability, and upregulation of mitochondrial gene expression in skeletal muscle of 60 healthy elderly subjects at doses of 250 to 2000 mg administered over 28 days [2]. A subsequent four-month randomized trial in 88 middle-

aged adults (Singh et al. 2022, Cell Reports Medicine) demonstrated significant improvement in muscle strength (approximately 12 percent increase in hamstring muscle strength) and exercise performance at 500 mg and 1000 mg daily doses, with concurrent improvement in plasma biomarkers of mitochondrial health [3]. A parallel randomized trial (Liu et al. 2022, JAMA Network Open) in 66 older adults confirmed improvement in muscle endurance at 1000 mg daily over four months [4]. Most recently, a 2025 randomized trial (Singh et al. 2025, Nature Aging) in 50 healthy middle-aged adults demonstrated that 1000 mg daily urolithin A for four weeks expanded peripheral naive-like CD8⁺ T cell populations, reduced markers of T cell exhaustion, and increased CD8⁺ fatty acid oxidation capacity, establishing the first clinical evidence of urolithin A activity on immune senescence [5]. The molecular pharmacology of urolithin A extends beyond mitophagy induction to include activation of AMP-activated protein kinase (AMPK), inhibition of mechanistic target of rapamycin (mTOR), activation of sirtuin 1 (SIRT1) with consequent deacetylation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha) to promote mitochondrial biogenesis, suppression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) signaling with reduction of proinflammatory cytokines, and inhibition of dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) relevant to Alzheimer's disease pathology. Pharmacokinetics in humans are characterized by intestinal absorption followed by extensive hepatic phase II conjugation (glucuronidation and sulfation), producing circulating glucuronide and sulfate conjugates with peak plasma concentrations at approximately 6 to 8 hours and elimination half-lives of 17 to 24 hours for the predominant

glucuronide species. The compound received Generally Recognized as Safe (GRAS) status from the United States Food and Drug Administration in 2018 (GRN 000791) at dietary intake levels up to 1000 mg per serving. Preclinical safety assessment established a no-observed-adverse-effect level (NOAEL) of 3451 mg/kg/day in male rats and 3826 mg/kg/day in female rats in a 90-day oral toxicity study, with no evidence of genotoxicity or mutagenicity. This monograph reviews the chemistry, endogenous biosynthesis, and chemical synthesis of urolithin A; the multi-target molecular pharmacology encompassing mitophagy, mitochondrial biogenesis, anti-inflammatory, and neuroprotective mechanisms; the human pharmacokinetic profile including interindividual variability driven by gut microbiome metabotype; the clinical evidence base across muscle function, immune health, and emerging neurodegenerative and cardiometabolic indications; sourcing and quality verification for research applications; reconstitution and handling; stack interaction considerations; adverse-event profile; and a structured comparative assessment of five mitochondrial-targeted compounds against urolithin A on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: Urolithin A.

Marketed name: Mitopure (Amazentis SA / Timeline; the synthetic pharmaceutical-grade form used in clinical trials and sold as a dietary ingredient).

Chemical Abstracts Service registry number: 1143-70-0.

International Union of Pure and Applied Chemistry name: 3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one. The compound is also designated 3,8-dihydroxybenzo[c]chromen-6-one under alternative IUPAC nomenclature conventions. Additional synonyms in the literature include 2',7-dihydroxy-3,4-benzocoumarin, 3,8-dihydroxy urolithin, and Uro-A [6].

PubChem CID: 5488186.

Structural class context: Urolithin A is a member of the urolithin family, a group of dibenzo[b,d]pyranone (benzo[c]chromenone) metabolites produced by the sequential removal of hydroxyl groups from ellagic acid by the human colonic microbiota. The urolithin family includes urolithin A (3,8-dihydroxy), urolithin B (3-hydroxy), urolithin C (3,8,9-trihydroxy), urolithin D (3,4,8,9-tetrahydroxy), isourolithin A (3,9-dihydroxy), and several additional minor metabolites [7]. Urolithin A is the terminal or near-terminal metabolite in the dehydroxylation cascade and is the most extensively studied member of the class.

1.2 Structural class and chemistry

Urolithin A is a planar, tricyclic aromatic lactone belonging to the dibenzo[b,d]pyranone structural class (also described as benzo[c]chromenones or 6H-dibenzo[b,d]pyran-6-ones). The core scaffold consists of two fused aromatic rings bridged by a six-membered lactone (pyranone) ring, with hydroxyl substituents at the 3- and 8-positions. The compound does not contain chiral centers and exists as a single constitutional isomer.

Molecular formula: C₁₃H₈O₄.

Exact mass: 228.0423 g/mol (monoisotopic).

Molecular weight: 228.20 g/mol.

Physical properties: Off-white to pale yellow crystalline powder. Soluble in dimethyl sulfoxide (approximately 30 mg/mL), dimethylformamide (approximately 30 mg/mL), and slightly soluble in ethanol. Poorly soluble in water (estimated aqueous solubility less than 0.1 mg/mL at neutral pH). The compound exhibits characteristic fluorescence under ultraviolet excitation, a property that has been exploited for analytical detection in biological matrices. Melting point is approximately 310 to 312 degrees Celsius with decomposition [6, 8].

LogP: Approximately 2.5, indicating moderate lipophilicity consistent with intestinal absorption and tissue distribution.

1.3 Endogenous biosynthesis

Urolithin A is not a primary natural product; it is produced exclusively by microbial metabolism in the human colon. The biosynthetic pathway begins with the dietary intake of ellagitannins (polymeric esters of hexahydroxydiphenic acid and glucose, found at high concentrations in pomegranate arils, walnuts, raspberries, strawberries, and certain oak-aged beverages). Gastric and small intestinal hydrolysis releases ellagic acid from the ellagitannin polymers. In the colon, specific bacterial species perform sequential lactone ring opening and dehydroxylation reactions to convert ellagic acid through a cascade of intermediates (urolithin M5, urolithin M6, urolithin D, urolithin C) to the dihydroxy product urolithin A and the monohydroxy product urolithin B [7, 9].

The bacterial species responsible for urolithin production have been identified principally through the work of the Tomas-Barberan laboratory. *Gordonibacter urolithinifaciens* and *Gordonibacter pamelaee* were the first pure-culture isolates demonstrated to convert ellagic acid to urolithin C [10]. Subsequent work identified *Ellagibacter isourolithinifaciens* as a producer of isourolithin A [11]. More recently, *Enterocloster* species have been identified as encoding the dehydroxylase responsible for the final conversion steps to urolithin A in the human gut [12].

A critical feature of urolithin A metabolism is the interindividual variability in production capacity. Population studies have classified individuals into three urolithin metabotypes: metabotype A (producers of urolithin A as the predominant metabolite, approximately 40 to 60 percent of Western populations), metabotype B

(producers of urolithin B and isourolithin A in addition to urolithin A, approximately 25 to 40 percent), and metabotype 0 (non-producers, approximately 10 to 15 percent) [13]. The metabotype is determined by the composition of the colonic microbiota and is relatively stable within individuals over time. The non-producer phenotype provides the primary rationale for supplementation with synthetic urolithin A, bypassing the requirement for microbial conversion.

1.4 Chemical synthesis

Synthetic urolithin A is produced commercially by Amazentis SA and by multiple chemical suppliers for research applications. The published synthetic routes proceed through two principal strategies. The first strategy employs a Hurtley reaction or copper-catalyzed coupling of a 2-halobenzoic acid with a resorcinol derivative, followed by intramolecular cyclization to form the lactone ring [14]. The second strategy uses a Suzuki-Miyaura cross-coupling of a boronic acid-substituted phenol with a halogenated benzoic acid precursor, followed by lactonization under acid or thermal conditions. Both routes are scalable and produce urolithin A at greater than 97 percent purity after recrystallization, typically from ethanol or ethanol-water mixtures. The absence of stereocenters simplifies the synthesis and quality control relative to chiral natural products.

Industrial synthesis for the Mitopure product line is conducted under current Good Manufacturing Practice (cGMP) conditions, with batch release specifications including HPLC purity greater than 98 percent, heavy metals analysis, residual solvent testing, and microbiological quality control.

2. Abstract

Urolithin A is a gut microbiota-derived dibenzo[b,d]pyranone metabolite that has emerged as the first dietary compound clinically demonstrated to induce mitophagy in human skeletal muscle. The compound is produced in the human colon by bacterial dehydroxylation of ellagic acid, itself derived from dietary ellagitannins present in pomegranates, walnuts, and berries. Approximately 40 to 60 percent of the Western population produces urolithin A endogenously upon ellagitannin consumption; 10 to 15 percent are non-producers owing to absence of the requisite microbial species, a finding that has driven development of synthetic urolithin A as a supplement.

The molecular pharmacology of urolithin A centers on induction of mitophagy through the PINK1/Parkin pathway. When mitochondrial membrane potential is dissipated (as occurs in damaged or dysfunctional mitochondria), PTEN-induced kinase 1 (PINK1) accumulates on the outer mitochondrial membrane and recruits the E3 ubiquitin ligase Parkin, which ubiquitinates outer membrane proteins to target the organelle for autophagic degradation. Urolithin A enhances this surveillance pathway, increasing the rate of clearance of damaged mitochondria and thereby improving the overall quality of the mitochondrial pool [1, 15]. Downstream and parallel mechanisms include activation of AMPK, inhibition of mTOR complex 1, activation of SIRT1 with deacetylation of PGC-1alpha to promote mitochondrial biogenesis, and suppression of NF-kappaB-driven proinflammatory cytokine production [16, 17].

The preclinical evidence base is substantial. In *C. elegans*, urolithin A extended lifespan by 45 percent in a mitophagy-dependent manner [1]. In aged mice, oral urolithin A improved running endurance by approximately 42 percent. In rodent models of Alzheimer's disease, long-term treatment improved learning, memory, and olfactory function while reducing amyloid-beta and tau pathology [18, 19]. In models of cardiac injury, urolithin A attenuated doxorubicin-induced cardiotoxicity through PINK1-regulated mitophagy [20].

The clinical evidence base now comprises multiple randomized, placebo-controlled trials. The Andreux et al. (2019) first-in-human study demonstrated safety and upregulation of mitochondrial gene expression in elderly subjects [2]. The

Singh et al. (2022) trial demonstrated significant improvement in muscle strength and exercise performance in middle-aged adults [3]. The Liu et al. (2022) trial confirmed muscle endurance improvements in older adults [4]. The Singh et al. (2025) trial demonstrated expansion of naive-like CD8⁺ T cell populations and improved immune cell metabolic fitness [5]. A large clinical trial examining brain health endpoints with approximately 650 participants is anticipated to report results in 2026.

The compound received FDA GRAS status in 2018 (GRN 000791) at intake levels up to 1000 mg per serving. Safety data across all completed clinical trials have demonstrated tolerability comparable to placebo, with mild and transient gastrointestinal effects (bloating, flatulence) as the only consistently reported adverse events. Preclinical 90-day rat toxicology established a NOAEL of 3451 mg/kg/day in males with no genotoxicity signal [21].

This monograph documents the chemistry, endogenous biosynthesis, chemical synthesis, and physicochemical properties of urolithin A; the multi-target molecular pharmacology; the human pharmacokinetic profile; the clinical evidence base across all studied indications; sourcing and quality verification; reconstitution and handling; stack-interaction considerations; adverse-event and safety data; and a comparative assessment of five mitochondrial-targeted compounds (nicotinamide mononucleotide, coenzyme Q10, pyrroloquinoline quinone, MitoQ, and spermidine) against urolithin A on five competency standards.

3. Discovery and Development History

3.1 Identification of urolithins as ellagitannin metabolites

The urolithin class of dibenzo[b,d]pyranones was first identified in the context of ellagitannin metabolism in ruminant animals in the 1950s and 1960s, but the systematic characterization of urolithins as human gut microbiota metabolites of dietary ellagitannins was accomplished primarily by the laboratory of Francisco Tomas-Barberan and Juan Carlos Espin at the Centro de Edafologia y Biologia Aplicada del Segura (CEBAS-CSIC) in Murcia, Spain, beginning in the early 2000s. The Cerda et al. (2004) report demonstrated that ellagic acid is converted to urolithin A and urolithin B in the human colon and that these metabolites are detectable in human plasma and urine after consumption of pomegranate juice [22]. Subsequent publications from the same group characterized the full urolithin metabolic cascade, established the metabotype classification system (metabotypes A, B, and O), and identified the principal bacterial genera responsible for the biotransformation [7, 9, 13].

3.2 Discovery of mitophagy-inducing activity

The transformative finding for urolithin A biology was the Ryu et al. (2016) report from the laboratory of Johan Auwerx at EPFL, published in Nature Medicine [1]. This study arose from a systematic screen of natural metabolites for compounds that induce mitophagy in *C. elegans* using a fluorescent mitophagy reporter strain. Urolithin A emerged as a potent hit, extending median lifespan by approximately 45 percent in wild-type worms in a manner dependent on the mitophagy genes *pink-1*, *pdr-1* (the *C. elegans* Parkin ortholog), and *dct-1* (the BNIP3L/NIX ortholog). The study further demonstrated that oral urolithin A administration to aged (22-month-old) C57BL/6J mice at 50 mg/kg/day for 8 weeks improved running endurance by approximately 42 percent and increased mitochondrial respiratory capacity in skeletal muscle. Muscle transcriptomic analysis confirmed upregulation of mitophagy, autophagy, and mitochondrial biogenesis gene signatures.

3.3 Formation of Amazentis and clinical development

Amazentis SA was founded in Lausanne, Switzerland, by Chris Rinsch in collaboration with Johan Auwerx to translate the urolithin A mitophagy findings into a clinical product. The company developed a proprietary synthetic manufacturing process for pharmaceutical-grade urolithin A (branded Mitopure) and initiated a regulatory and clinical development program.

In 2018, Amazentis obtained FDA GRAS status (GRN 000791) for synthetic urolithin A as a food ingredient at intake levels up to 500 mg per serving in protein shakes, meal replacements, instant oatmeal, protein bars, and yogurt drinks, and up to 1000 mg per serving in Greek yogurts, high-protein yogurts, and milk-based protein shakes [23]. The European Food Safety Authority (EFSA) subsequently approved urolithin A as a novel food ingredient in the European Union.

The clinical trial program has proceeded through multiple phases. The first-in-human Phase 1 study (Andreux et al. 2019) established safety and pharmacodynamic proof of concept [2]. Subsequent Phase 2 studies examined muscle function in middle-aged and elderly adults (Singh et al. 2022; Liu et al. 2022) [3, 4]. The most recent completed trial examined immune function (Singh et al. 2025) [5]. As of the current monograph revision, multiple additional trials are registered on ClinicalTrials.gov, including studies of urolithin A in brain health (NCT07231783, approximately 650 participants), mitochondrial quality in type 2 diabetes (NCT06555706), and immune health in oncology settings (NCT05735886).

In 2022, Nestle Health Science acquired Amazentis, integrating the Mitopure product line into its health and wellness portfolio and expanding commercial distribution.

3.4 Expansion of the research literature

The period from 2016 to 2026 has seen rapid expansion of the urolithin A research literature. PubMed-indexed publications mentioning urolithin A have increased from fewer than 50 per year before 2016 to several hundred per year by 2024. The research scope has extended from the original mitophagy and muscle function focus to encompass neurodegenerative disease (Alzheimer's disease models, Parkinson's disease models), cardiovascular disease (heart failure with preserved ejection fraction, doxorubicin cardiotoxicity), inflammatory bowel disease, osteoarthritis, obesity and metabolic syndrome, cancer biology, and immune aging.

The mechanistic foundation across all these applications is the mitophagy and mitochondrial quality control axis, with additional contributions from the anti-inflammatory and antioxidant activities of the compound.

4. Mechanism of Action

Urolithin A is a multi-target compound whose biological activities converge on mitochondrial quality control and cellular stress response pathways. The principal molecular mechanisms, characterized in cell-based systems, genetic model organisms, and mammalian tissues, are mitophagy induction through the PINK1/Parkin pathway; activation of AMPK; inhibition of mTOR complex 1; activation of SIRT1 and promotion of mitochondrial biogenesis through PGC-1alpha; and suppression of NF-kappaB-mediated inflammatory signaling. These activities operate at concentrations achievable by oral dosing in humans and contribute differentially to the observed effects on muscle function, immune fitness, neuronal health, and inflammatory resolution.

4.1 Mitophagy induction through the PINK1/Parkin pathway

Mitophagy is the selective autophagic degradation of damaged or dysfunctional mitochondria. The canonical pathway is initiated when PTEN-induced kinase 1 (PINK1), a serine/threonine kinase normally imported and degraded in healthy mitochondria, accumulates on the outer mitochondrial membrane of mitochondria with depolarized membrane potential. Stabilized PINK1 phosphorylates ubiquitin and recruits the cytosolic E3 ubiquitin ligase Parkin (encoded by PRKN/PARK2), which ubiquitinates multiple outer membrane proteins. The ubiquitinated mitochondrion is then recognized by autophagy adaptor proteins (p62/SQSTM1, NDP52, OPTN, NBR1), engulfed by a phagophore, and delivered to the lysosome for degradation and recycling of macromolecular components [15].

Urolithin A induces mitophagy by increasing the expression and stabilization of PINK1 on the outer mitochondrial membrane and by promoting Parkin translocation from the cytosol to mitochondria [1, 16]. In *C. elegans*, the mitophagy-inducing activity was demonstrated to be dependent on the *pink-1*, *pdr-1*, and *dct-1* genes; genetic ablation of any of these components abolished the lifespan extension [1]. In mammalian cells, urolithin A treatment at concentrations of 10 to 50 micromolar

induced dose-dependent increases in mitophagy flux as measured by mitochondria-targeted Keima fluorescence, mt-mKeima assays, and colocalization of mitochondrial markers with lysosomal markers [1, 16].

A parallel mitophagy mechanism involving the Ambra1 (activating molecule in Beclin 1-regulated autophagy) pathway has been identified. Urolithin A was reported to attenuate doxorubicin-induced cardiotoxicity by enhancing PINK1-regulated mitophagy through an Ambra1-dependent mechanism, providing evidence for multiple converging pathways through which the compound enhances mitochondrial quality control [20].

4.2 AMPK activation and mTOR inhibition

AMP-activated protein kinase (AMPK) is a master sensor of cellular energy status that is activated when the AMP-to-ATP ratio increases. AMPK activation promotes catabolic pathways (including autophagy and mitophagy) and suppresses anabolic pathways (including mTOR complex 1 signaling). Urolithin A activates AMPK through phosphorylation by upstream kinases, likely liver kinase B1 (LKB1) and calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2), though the proximal target through which urolithin A triggers this cascade has not been definitively identified [16, 17].

The consequent inhibition of mTOR complex 1 releases the block on ULK1 (unc-51-like autophagy activating kinase 1), the initiating kinase of the autophagy cascade. ULK1 activation promotes phagophore nucleation and autophagosome formation, the structural prerequisites for mitophagic engulfment of tagged mitochondria. The AMPK-mTOR-ULK1 axis therefore provides the macroautophagy machinery that is directed to mitochondria by the PINK1/Parkin tagging system.

4.3 SIRT1 activation and mitochondrial biogenesis

Urolithin A activates sirtuin 1 (SIRT1), an NAD-dependent protein deacetylase that regulates multiple longevity-associated pathways [17]. SIRT1 activation leads to deacetylation of PGC-1 α , a master transcriptional coactivator of mitochondrial biogenesis. Deacetylated PGC-1 α translocates to the nucleus and co-activates nuclear respiratory factors 1 and 2 (NRF1, NRF2) and mitochondrial transcription

factor A (TFAM), driving transcription of nuclear-encoded mitochondrial genes and replication of the mitochondrial genome. The net effect is the generation of new, functional mitochondria to replace those removed by mitophagy.

SIRT1 activation also leads to deacetylation of FOXO transcription factors, promoting the expression of antioxidant enzymes (manganese superoxide dismutase, catalase) and autophagy-related genes. The composite effect of urolithin A on the SIRT1-PGC-1 α -FOXO axis is therefore a coordinated program of mitochondrial renewal (removal of damaged mitochondria by mitophagy, generation of new mitochondria by biogenesis, and enhancement of antioxidant defense in the interim).

4.4 Anti-inflammatory activity through NF-kappaB suppression

Urolithin A suppresses NF-kappaB signaling in multiple cell types, including macrophages, microglia, intestinal epithelial cells, and chondrocytes [24, 25]. The mechanism involves inhibition of IkappaB kinase (IKK)-mediated phosphorylation and degradation of IkappaB α , resulting in retention of the p50/p65 NF-kappaB heterodimer in the cytosol and reduced transcription of proinflammatory target genes including tumor necrosis factor alpha (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS).

In lipopolysaccharide-stimulated macrophages, urolithin A at 10 to 50 micromolar reduced TNF- α and IL-6 secretion by 40 to 70 percent in a dose-dependent manner [24]. In a dextran sulfate sodium-induced colitis model, oral urolithin A reduced colonic inflammation and preserved gut barrier integrity through both NF-kappaB suppression and enhancement of tight junction protein expression [25]. The anti-inflammatory mechanism is partially dependent on mitophagy (damaged mitochondria release mitochondrial DNA and reactive oxygen species that activate the NLRP3 inflammasome; removal of damaged mitochondria by mitophagy reduces this inflammasome activation), providing a mechanistic bridge between the mitophagy and anti-inflammatory activities.

4.5 Neuroprotective mechanisms

In the central nervous system, urolithin A exerts neuroprotective activity through multiple convergent mechanisms. The compound crosses the blood-brain barrier at pharmacologically relevant concentrations [18]. In Alzheimer's disease models, long-term urolithin A treatment reduced amyloid-beta plaque burden and tau phosphorylation, improved long-term potentiation at hippocampal synapses, and restored lysosomal function (including normalization of cathepsin Z activity) [18, 19]. The restoration of lysosomal function is particularly notable, as lysosomal dysfunction is increasingly recognized as a central feature of Alzheimer's disease pathology.

An additional neuroprotective mechanism involves inhibition of dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), a kinase implicated in tau hyperphosphorylation in Alzheimer's disease and in neuronal dysfunction in Down syndrome [26]. Urolithin A inhibits DYRK1A activity in cell-free and cell-based assays, providing a mechanism for the reduction of tau pathology observed in transgenic mouse models independent of the mitophagy pathway.

In activated microglia, urolithin A suppressed neuroinflammatory cytokine release and reduced markers of microglial activation, consistent with the peripheral anti-inflammatory activity and with a protective role in neuroinflammation-driven neurodegeneration [18].

4.6 Composite pharmacology

The composite pharmacology of urolithin A at oral doses of 500 to 1000 mg produces circulating concentrations of glucuronide conjugates in the low-micromolar range (peak plasma concentrations of urolithin A glucuronide approximately 2 to 15 micromolar depending on dose and individual pharmacokinetics) [2, 3]. The free (unconjugated) urolithin A concentrations in plasma are substantially lower (nanomolar range), but tissue concentrations, particularly in skeletal muscle and potentially in brain following blood-brain barrier penetration, may exceed plasma free concentrations owing to intracellular deconjugation by tissue glucuronidases.

The clinical implication is that the mitophagy, AMPK, and anti-inflammatory effects at oral doses of 500 to 1000 mg are supported by the achieved tissue concentrations. The dose-response relationship in human trials has been shallow, with both

500 mg and 1000 mg producing measurable effects on muscle function and mitochondrial biomarkers, though 1000 mg has been used in the majority of published trials and is the standard research dose.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

Urolithin A is absorbed from the gastrointestinal tract after oral administration. When produced endogenously from dietary ellagitannins, absorption occurs principally in the colon, the site of microbial production. When administered as synthetic urolithin A, the compound is absorbed from both the small intestine and the colon. The oral bioavailability of the parent unconjugated compound is low owing to extensive first-pass metabolism (glucuronidation and sulfation); the circulating species are predominantly phase II conjugates [2].

Time to peak plasma concentration of urolithin A glucuronide after oral administration of 500 to 1000 mg is approximately 6 to 8 hours, reflecting the combined absorption and conjugation kinetics [2, 3]. Maximum plasma concentrations of total urolithin A species (conjugated plus free) after a 1000 mg oral dose range from approximately 2 to 15 micromolar across individuals, with substantial interindividual variability attributable to differences in conjugation enzyme activity, gut transit time, and microbiome composition.

5.2 Distribution

Urolithin A and its conjugates distribute to multiple tissues. The moderate lipophilicity of the parent compound (LogP approximately 2.5) supports tissue penetration. Preclinical studies have demonstrated accumulation in skeletal muscle, liver, kidney, and brain tissue. Blood-brain barrier penetration has been confirmed in rodent studies with detection of urolithin A in brain parenchyma at pharmacologically relevant concentrations following oral administration [18]. Plasma protein binding has not been extensively characterized in published literature but is estimated to be moderate based on the physicochemical properties.

5.3 Metabolism

Urolithin A undergoes extensive phase II metabolism in the intestinal epithelium and liver. The principal metabolic transformations are glucuronidation (catalyzed by UDP-glucuronosyltransferases, principally UGT1A8 and UGT1A10 in the intestine) and sulfation (catalyzed by sulfotransferases, principally SULT1A1 and SULT1A3) [27]. The predominant circulating metabolite is urolithin A-3-O-glucuronide, with urolithin A-8-O-glucuronide and urolithin A sulfate as secondary metabolites. The glucuronide conjugates retain some biological activity in cell-based assays, though at reduced potency relative to the free aglycone. Intracellular deconjugation by beta-glucuronidase in target tissues (skeletal muscle, inflammatory sites, tumor microenvironment) is postulated to regenerate the active aglycone at the site of action, a mechanism analogous to that established for other phenolic glucuronides.

Phase I oxidative metabolism is minimal; urolithin A does not undergo substantial cytochrome P450-mediated biotransformation. This distinguishes the compound pharmacokinetically from many research compounds where CYP polymorphism is a major determinant of interindividual variability. The principal source of pharmacokinetic variability for urolithin A is instead the UGT and SULT conjugation capacity and the gut microbiome metabotype (which determines endogenous production when ellagitannins rather than synthetic urolithin A are the source).

5.4 Elimination

The plasma elimination half-life of urolithin A glucuronide is approximately 17 to 24 hours after oral administration, supporting once-daily dosing [2]. Elimination is predominantly renal, with urolithin A glucuronide and sulfate conjugates excreted in urine. A proportion of the dose undergoes biliary excretion and enterohepatic recirculation, which may contribute to the prolonged elimination half-life.

Total recovery of urolithin A metabolites in urine over 72 hours after a single oral dose accounts for approximately 30 to 40 percent of the administered dose [2]. The remainder is presumably eliminated in feces (unabsorbed compound) and through minor metabolic pathways.

5.5 Routes of administration

The only clinically studied route of administration is oral. Urolithin A is administered as a powder in capsules, as a soft-gel formulation, or as a powder mixed into food or beverages (the Mitopure product line includes powder sachets and soft-gel capsules). Intravenous, intramuscular, and subcutaneous routes have not been studied in humans. Preclinical studies have used oral gavage and intraperitoneal injection in rodents.

5.6 Special populations

Pharmacokinetic studies in special populations (renal impairment, hepatic impairment, pediatric, geriatric) have not been systematically published. The elderly population has been the principal target of clinical trials, and the pharmacokinetic profile in the 60- to 80-year-old age range appears consistent with the younger adult data. Given the predominance of phase II conjugation metabolism and renal elimination, dose adjustment in mild-to-moderate renal impairment is unlikely to be necessary, though data are absent. Pregnancy and lactation data are not available.

6. Clinical Evidence Base

6.1 Preclinical foundation

The preclinical evidence base for urolithin A is extensive and spans multiple disease models and organisms.

In *C. elegans*, urolithin A at 50 micromolar extended median lifespan by approximately 45 percent in a mitophagy-dependent manner (effect abolished in *pink-1*, *pdr-1*, and *dct-1* loss-of-function mutants) and improved pharyngeal pumping rate and mobility in aged worms [1].

In aged C57BL/6J mice (22 months), oral urolithin A at 50 mg/kg/day for 8 weeks improved involuntary treadmill running endurance by approximately 42 percent, increased mitochondrial complex I and II respiratory capacity in isolated muscle mitochondria, and upregulated mitophagy and biogenesis gene expression in quadriceps muscle [1].

In rodent Alzheimer's disease models (APP/PS1 transgenic mice and 3xTg-AD mice), long-term oral urolithin A treatment improved spatial learning and memory (Morris water maze), improved olfactory function, reduced amyloid-beta plaque burden by approximately 40 to 50 percent, reduced phosphorylated tau levels, restored long-term potentiation at hippocampal CA1 synapses, and normalized lysosomal cathepsin activity [18, 19].

In doxorubicin-induced cardiotoxicity models, urolithin A attenuated cardiac dysfunction through enhancement of PINK1-mediated mitophagy via the Ambra1 pathway [20].

In colitis models (dextran sulfate sodium-induced), oral urolithin A reduced colonic inflammation scores, preserved epithelial tight junction integrity, and reduced proinflammatory cytokine levels in colonic tissue [25].

6.2 First-in-human study: safety and mitochondrial biomarkers

The Andreux et al. (2019) study, published in *Nature Metabolism*, was the first randomized, placebo-controlled, double-blind clinical trial of synthetic urolithin A in humans [2]. The study enrolled 60 healthy sedentary elderly subjects (aged 61 to 85 years) at the University of Lausanne. Part 1 was a single ascending dose study (250 mg, 500 mg, 1000 mg, 2000 mg) in 36 subjects. Part 2 was a 28-day multiple-dose study at 250 mg, 500 mg, and 1000 mg daily in 24 subjects. Primary endpoints were safety, tolerability, and pharmacokinetics; secondary endpoints included plasma acylcarnitines and skeletal muscle mitochondrial gene expression (assessed by vastus lateralis biopsy at baseline and day 28).

Urolithin A was safe and well tolerated at all doses, with no serious adverse events and no dose-limiting toxicities. Pharmacokinetics confirmed dose-proportional increases in plasma urolithin A glucuronide with a half-life of approximately 17 hours. At the 28-day time point, urolithin A at 500 mg and 1000 mg daily produced significant downregulation of plasma acylcarnitines (C2-carnitine, C4-carnitine, and long-chain acylcarnitines), a biomarker pattern consistent with improved mitochondrial fatty acid oxidation. Skeletal muscle RNA sequencing demonstrated upregulation of mitochondrial gene sets, including genes encoding electron transport chain complex subunits and mitophagy pathway components. This study established the pharmacodynamic proof of concept for urolithin A activity on human mitochondrial biology.

6.3 Muscle strength and exercise performance in middle-aged adults

The Singh et al. (2022) study, published in *Cell Reports Medicine*, was a randomized, double-blind, placebo-controlled trial in 88 overweight, sedentary middle-aged adults (aged 40 to 64 years) randomized to placebo, 500 mg urolithin A daily, or 1000 mg urolithin A daily for 4 months [3]. Primary endpoints included muscle strength (maximal voluntary contraction of the hand and leg muscles) and exercise performance (6-minute walk test, peak oxygen consumption). Secondary endpoints included plasma biomarkers of mitochondrial health.

At 4 months, the 1000 mg group demonstrated a significant improvement in hamstring muscle strength (approximately 12 percent improvement relative to placebo). The 500 mg group showed a trend toward improvement that did not reach

statistical significance for the primary muscle strength endpoint. Both doses produced significant improvements in plasma acylcarnitine biomarkers and in C-reactive protein (a marker of systemic inflammation). Exercise performance measures showed trends toward improvement but did not reach statistical significance in the primary analysis. The study supported the clinical relevance of urolithin A for age-related muscle function decline at oral doses of 500 to 1000 mg daily.

6.4 Muscle endurance in older adults

The Liu et al. (2022) study, published in JAMA Network Open, examined the effect of urolithin A supplementation on muscle endurance and mitochondrial health in 66 older adults (aged 65 to 90 years) randomized to 1000 mg urolithin A daily or placebo for 4 months [4]. The primary endpoint was change in maximal ATP production rate (assessed by phosphorus-31 magnetic resonance spectroscopy in the first dorsal interosseous muscle of the hand). Secondary endpoints included muscle endurance measures and plasma biomarkers.

The study reported improvements in measures of muscle endurance (including increased number of repetitions to fatigue in hand grip exercises) and improvements in plasma acylcarnitine profiles. The phosphorus-31 MRS primary endpoint showed directional improvement that did not reach statistical significance. The study contributed to the evidence base for urolithin A effects on muscle function in the elderly population.

6.5 Immune health and T cell function

The Singh et al. (2025) study, published in Nature Aging, examined the effect of urolithin A on age-related immune decline in 50 healthy middle-aged adults (aged 40 to 65 years) randomized to 1000 mg urolithin A daily or placebo for 4 weeks [5]. The study assessed peripheral blood T cell populations by flow cytometry, T cell metabolic fitness by Seahorse extracellular flux analysis, and systemic inflammatory markers.

At 4 weeks, urolithin A supplementation significantly expanded peripheral naive-like CD8+ T cell populations, reduced the proportion of terminally exhausted CD8+ T cells (PD-1+ TIM-3+ cells), and increased CD8+ T cell fatty acid oxidation ca-

capacity. The results provide the first clinical evidence of urolithin A activity on immune senescence and suggest that mitophagy induction in immune cells may partially reverse the age-related shift from naive to exhausted T cell phenotypes.

6.6 Neurodegenerative disease

Clinical evidence for urolithin A in neurodegenerative disease in humans is limited to registered clinical trials that have not yet reported results. A large randomized trial examining brain health endpoints (NCT07231783, approximately 650 participants) is anticipated to report in 2026. The preclinical evidence in Alzheimer's disease models is strong (see Section 6.1) and provides the mechanistic rationale for the clinical program. The Hou et al. (2024) publication in *Alzheimer's and Dementia* is the most recent and comprehensive preclinical demonstration of urolithin A efficacy in AD models, reporting improvements in cognition, reduction of amyloid-beta and tau pathologies, restoration of mitophagy and lysosomal functions, and anti-neuroinflammatory effects in activated microglia [18].

6.7 Cardiovascular and metabolic disease

Clinical trials examining urolithin A in type 2 diabetes (NCT06555706, assessing mitochondrial quality in skeletal muscle by phosphorus-31 MRS) and in heart failure with preserved ejection fraction (preclinical data from a 2025 bioRxiv preprint demonstrating reversal of cardiac remodeling in a murine HFpEF model) are in progress or in planning [28]. No human efficacy data in cardiovascular or metabolic disease have been published.

6.8 Sports performance

Emerging evidence from 2025 publications suggests potential applications of urolithin A in sports nutrition, bridging the preclinical findings of improved mitochondrial function and exercise capacity to athletic applications [29]. Clinical studies specifically in athletes have not been published, and extrapolation from the sedentary elderly and middle-aged trial populations to trained athletes requires caution.

7. Reported Research Dose Ranges

7.1 Human clinical doses

All completed human clinical trials have used oral synthetic urolithin A (Mitopure) at doses of 250 mg to 2000 mg daily. The dose range evaluated across trials:

Single ascending dose (safety): 250 mg, 500 mg, 1000 mg, 2000 mg (Andreux et al. 2019) [2].

Multiple daily dosing (28 days to 4 months): 250 mg, 500 mg, 1000 mg daily [2, 3, 4, 5].

The standard research and commercial dose is 500 to 1000 mg daily. The 1000 mg dose has produced the most consistent efficacy signals across trials and is the dose used in the majority of published studies. The 500 mg dose has produced directional improvements in most endpoints but with smaller effect sizes. Doses above 1000 mg have not been studied in multiple-dose trials.

7.2 Preclinical doses

C. elegans: 50 micromolar in culture media [1].

Mice (oral gavage): 25 to 50 mg/kg/day, corresponding to a human-equivalent dose of approximately 200 to 400 mg/day by allometric scaling [1, 18].

Rats (toxicology): up to 3451 to 3826 mg/kg/day (NOAEL in 90-day study) [21].

7.3 Dietary intake from food sources

Endogenous urolithin A production from dietary ellagitannins varies enormously across individuals (metabotype-dependent) and diets. A typical serving of pomegranate juice (240 mL) provides approximately 100 to 300 mg of ellagitannins (as ellagic acid equivalents), of which a metabotype A individual might convert approximately 5 to 15 percent to urolithin A. The resulting plasma urolithin A concentra-

tions from dietary sources are substantially lower than those achieved by supplementation with 500 to 1000 mg of synthetic urolithin A, providing the pharmacokinetic rationale for supplementation.

8. Sourcing and Quality Verification

Pharmaceutical-grade synthetic urolithin A (Mitopure, Amazentis SA / Timeline) is the highest quality source for research and clinical applications. This material is manufactured under cGMP conditions, is the form used in all published clinical trials, and carries FDA GRAS status. The product is available commercially in capsule and powder sachet formats at standardized doses.

Research-grade urolithin A is supplied by multiple chemical and biochemical suppliers (Sigma-Aldrich [catalog SML1791, greater than 97 percent purity by HPLC], Cayman Chemical [catalog 22607], Santa Cruz Biotechnology, Selleckchem, StressMarq, MedChemExpress, and others) at purities typically greater than 97 percent by HPLC. Supply is in milligram to gram quantities. Pricing varies by supplier and quantity; typical research-grade pricing is in the range of \$30 to \$100 per 50 mg at the time of monograph preparation.

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity, ideally greater than 97 percent
- Mass spectrometry confirmation of molecular weight (229.0501 for the protonated molecular ion $[M+H]^+$ or 227.0344 for the deprotonated molecular ion $[M-H]^-$)
- Proton and carbon-13 nuclear magnetic resonance spectroscopy for structural confirmation
- Karl Fischer or loss-on-drying analysis for residual water content
- Residual solvent analysis (gas chromatography) for common synthesis solvents
- Heavy metals analysis (inductively coupled plasma mass spectrometry) for arsenic, cadmium, lead, and mercury

Third-party analytical laboratories provide independent confirmation of vendor certificates of analysis. The urolithin A research supply market is well established; however, the compound's emergence as a consumer supplement has expanded the

number of suppliers, and investigators should exercise caution with suppliers that do not provide detailed certificates of analysis or that offer the compound at prices substantially below established research suppliers.

9. Reconstitution and Handling

Urolithin A is a crystalline solid with limited aqueous solubility. For research applications, stock solutions are typically prepared in dimethyl sulfoxide (DMSO) at concentrations of 10 to 30 mg/mL (approximately 44 to 132 millimolar), yielding clear solutions. The compound is also soluble in dimethylformamide (DMF) at similar concentrations. For cell culture experiments, DMSO stock solutions are diluted into culture media to achieve final DMSO concentrations of 0.1 percent or less; investigators should verify that the DMSO vehicle concentration does not produce confounding effects in their specific assay systems.

For aqueous formulations (oral administration in animal studies), urolithin A can be suspended in 0.5 percent carboxymethylcellulose or in corn oil by sonication. The low aqueous solubility necessitates suspension rather than solution for oral gavage at the doses used in preclinical studies (25 to 50 mg/kg).

Storage of the solid compound should be at minus 20 degrees Celsius, protected from light and moisture, under which conditions the compound is stable for at least 2 years. DMSO stock solutions should be stored at minus 20 degrees Celsius in single-use aliquots to avoid repeated freeze-thaw cycles; stability under these conditions is at least 6 months. Working solutions in aqueous media should be prepared fresh on the day of use. The compound is sensitive to photodegradation under prolonged ultraviolet exposure; amber glass or foil-wrapped containers are recommended for storage and handling.

For cell-based assays, typical working concentrations are 5 to 100 micromolar, with 25 to 50 micromolar being the most commonly reported effective concentrations for mitophagy induction in published studies [1, 16].

10. Stack Interaction Considerations

Urolithin A has a favorable drug interaction profile relative to many research compounds owing to the absence of cytochrome P450-mediated metabolism. However, investigators conducting research with urolithin A in combination regimens should consider the following interactions:

Mitophagy and autophagy modulators: Concurrent administration with other mitophagy or autophagy inducers (rapamycin, metformin, spermidine, resveratrol) may produce additive or synergistic effects on mitophagy flux. The combination of urolithin A with rapamycin has been studied in preclinical models with evidence of synergy; however, excessive autophagy induction can be detrimental, and dose titration of combinations should be empirically optimized.

NAD⁺ precursors: Concurrent administration with nicotinamide mononucleotide (NMN), nicotinamide riboside (NR), or niacin is pharmacologically rational (urolithin A promotes mitophagy and mitochondrial biogenesis; NAD⁺ precursors support the sirtuin and PARP enzymes that regulate mitochondrial function). No pharmacokinetic interaction is expected. This combination is widely used in consumer longevity supplement stacks and has not produced safety signals, though formal clinical study of the combination has not been published.

Coenzyme Q10 and MitoQ: Concurrent administration with coenzyme Q10 or its mitochondria-targeted derivative MitoQ is pharmacologically complementary (CoQ10/MitoQ support electron transport chain function; urolithin A promotes quality control of the organelles housing the electron transport chain). No pharmacokinetic interaction is expected.

Anti-inflammatory agents: Concurrent use with non-steroidal anti-inflammatory drugs, corticosteroids, or biologic anti-inflammatory agents may produce additive anti-inflammatory effects. No antagonistic interactions have been characterized.

Ellagitannin-rich foods: Concurrent consumption of pomegranate juice, walnuts, or berries with synthetic urolithin A would add endogenously produced urolithin A to the supplemented dose in metabotype A individuals. The incremental contribution from dietary sources is modest relative to the supplemented dose but should be considered in research protocols that require precise dose control.

UGT substrates and inhibitors: Because urolithin A is metabolized primarily by glucuronidation, concurrent administration with UGT inhibitors (probenecid, valproic acid, certain flavonoids) could theoretically increase circulating free urolithin A concentrations. Conversely, UGT inducers could reduce exposure. These interactions have not been formally studied.

Anticoagulants: In vitro studies have reported weak antiplatelet activity of urolithin A at high concentrations. The clinical relevance is uncertain, but investigators should exercise caution with concurrent anticoagulant or antiplatelet therapy until clinical interaction data are available.

11. Adverse Events and Safety

11.1 Preclinical toxicology

A comprehensive toxicological assessment of synthetic urolithin A was conducted in support of the FDA GRAS application and published by Heilman et al. (2017) [21]. Key findings:

- 28-day oral toxicity study in Sprague-Dawley rats: no adverse effects at the highest dose tested
- 90-day oral toxicity study in Sprague-Dawley rats: NOAEL of 3451 mg/kg/day in males and 3826 mg/kg/day in females (the highest doses tested), with no target organ toxicity
- Bacterial reverse mutation assay (Ames test): negative for mutagenicity
- In vitro mammalian chromosomal aberration assay: negative for clastogenicity
- In vivo micronucleus assay: negative for genotoxicity
- Reproductive and developmental toxicity: not formally assessed in published form

The safety margins are substantial. The NOAEL of 3451 mg/kg/day in male rats translates to a human equivalent dose of approximately 557 mg/kg/day by allometric scaling, which for a 70 kg adult corresponds to approximately 39,000 mg/day, approximately 40-fold above the highest clinical dose studied (1000 mg/day).

11.2 Common adverse events in human trials

Across all published clinical trials, urolithin A at doses of 250 to 2000 mg was well tolerated, with adverse-event profiles comparable to placebo [2, 3, 4, 5]. The most commonly reported adverse events (generally mild and transient):

- Gastrointestinal discomfort: bloating, flatulence, mild nausea (approximately 5 to 10 percent of subjects at 1000 mg, not statistically different from placebo in most trials)
- Headache (approximately 3 to 5 percent, comparable to placebo)

- Muscle soreness (approximately 2 to 3 percent, context-dependent in trials involving exercise testing)

No dose-limiting toxicities were observed at any studied dose. No serious adverse events were attributed to urolithin A in any published trial.

11.3 Laboratory safety parameters

Liver function tests (AST, ALT, alkaline phosphatase, bilirubin), renal function tests (creatinine, blood urea nitrogen), complete blood counts, and cardiac biomarkers were monitored in clinical trials and remained within normal limits throughout dosing periods at all studied doses [2, 3].

11.4 Pregnancy and lactation

No human data are available. The compound has not been assessed for reproductive or developmental toxicity in published preclinical studies. Use during pregnancy and lactation is not recommended owing to absence of data.

11.5 Long-term safety

Published human safety data extend to 4 months of continuous daily dosing at 1000 mg. Long-term safety beyond 4 months has not been established in controlled clinical trials. Consumer use of Mitopure has been ongoing since approximately 2020, and no post-market safety signals have emerged in published form. However, the absence of long-term controlled data should be noted.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five mitochondrial-targeted compounds against urolithin A, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The five compounds were selected as the principal mitochondrial function agents with substantive clinical or preclinical pharmacology, with priority on those that address overlapping mechanisms or indications with urolithin A.

12.1 Nicotinamide mononucleotide (NMN)

Mechanism: NAD⁺ precursor. NMN is converted to NAD⁺ by nicotinamide mononucleotide adenylyltransferase (NMNAT) enzymes, replenishing cellular NAD⁺ pools that decline with age. Elevated NAD⁺ supports sirtuin-mediated deacetylation (SIRT1 through SIRT7), PARP-mediated DNA repair, and CD38-mediated calcium signaling. The mechanism is complementary to but distinct from urolithin A: NMN supports NAD⁺-dependent enzymatic functions, while urolithin A directly induces mitophagy and mitochondrial quality control [30].

Development trajectory: NMN has been studied in multiple human clinical trials. The Yi et al. (2023) meta-analysis of randomized controlled trials reported modest improvements in physical performance measures in older adults [31]. The compound is widely available as a dietary supplement and has not been developed as a pharmaceutical. Regulatory status varies by jurisdiction; in 2022, the FDA ruled that NMN could not be marketed as a dietary supplement in the United States owing to its prior investigation as a new drug (a decision contested by supplement manufacturers).

Novelty: Low to moderate. NAD⁺ precursor supplementation is a well-established concept; NMN is one of several NAD⁺ precursors (alongside nicotinamide riboside and niacin) with published clinical data.

Effect size: Modest. Meta-analytic evidence suggests small improvements in physical performance; effect sizes in individual trials have been inconsistent, with some trials reporting no significant benefit on primary endpoints.

Promising potential: Moderate. The compound is widely used and researched but has not produced breakthrough clinical results. Regulatory uncertainty in the United States limits commercial development.

Side-effect profile: Favorable. NMN is generally well tolerated at doses of 250 to 1200 mg daily. Gastrointestinal discomfort is the most commonly reported adverse event. No serious safety concerns have been identified.

Overall validation: Moderate. Substantial preclinical evidence; growing but inconsistent clinical evidence; widely available.

Comparison to urolithin A: NMN and urolithin A address different aspects of mitochondrial health. NMN replenishes the NAD⁺ cofactor required for sirtuin and PARP function; urolithin A promotes the physical removal and replacement of damaged mitochondria. The mechanisms are complementary and the combination is pharmacologically rational. Urolithin A has a more consistent clinical evidence base for muscle function than NMN (significant muscle strength improvement in Singh et al. 2022, confirmed muscle endurance effects in Liu et al. 2022) and a clearer regulatory path (FDA GRAS status obtained). NMN has a larger consumer market and a longer history of human use as a supplement.

12.2 Coenzyme Q10 (ubiquinone/ubiquinol)

Mechanism: Endogenous lipid-soluble electron carrier in the mitochondrial electron transport chain. CoQ10 shuttles electrons from complexes I and II to complex III. Supplementation is intended to support electron transport chain efficiency and to provide antioxidant protection against mitochondrial reactive oxygen species. The mechanism is fundamentally different from urolithin A: CoQ10 supports the function of existing mitochondria, while urolithin A promotes the turnover and renewal of the mitochondrial pool [32].

Development trajectory: CoQ10 has been studied in hundreds of clinical trials over approximately 40 years. The most substantive clinical evidence is in heart failure (the Q-SYMBIO trial demonstrated reduced cardiovascular mortality in chronic

heart failure patients supplemented with 300 mg CoQ10 daily) and in statin-associated myopathy [33]. CoQ10 is one of the most widely consumed dietary supplements globally.

Novelty: Low. CoQ10 has been available as a supplement since the 1970s and has been extensively studied.

Effect size: Variable by indication. The Q-SYMBIO heart failure trial reported clinically meaningful mortality reduction. Effects on general mitochondrial health in healthy populations are modest and inconsistent.

Promising potential: Moderate. The compound has an established role in specific clinical contexts (heart failure, statin myopathy) but has not demonstrated transformative effects in general aging or mitochondrial decline.

Side-effect profile: Excellent. CoQ10 is extremely well tolerated at doses of 100 to 600 mg daily, with decades of safety data.

Overall validation: High for specific cardiovascular indications; moderate for general mitochondrial support.

Comparison to urolithin A: CoQ10 and urolithin A are mechanistically orthogonal. CoQ10 optimizes the function of the electron transport chain within existing mitochondria; urolithin A removes damaged mitochondria and promotes generation of new ones. The combination addresses both the quality control (urolithin A) and functional optimization (CoQ10) of the mitochondrial pool. CoQ10 has a much larger evidence base and longer clinical history; urolithin A has a more novel mechanism and more targeted activity on the fundamental process of mitochondrial quality control.

12.3 Pyrroloquinoline quinone (PQQ)

Mechanism: Redox cofactor that stimulates mitochondrial biogenesis through activation of PGC-1alpha and CREB signaling. PQQ also functions as a potent antioxidant (cycling between oxidized and reduced forms with a catalytic capacity approximately 100-fold greater than ascorbic acid on a molar basis). The biogenesis-stimulating activity of PQQ is complementary to the mitophagy-inducing activity of urolithin A: PQQ promotes the creation of new mitochondria, while urolithin A promotes the removal of damaged ones [34].

Development trajectory: PQQ has been studied in small clinical trials examining cognitive function, sleep quality, and fatigue. The evidence base is substantially smaller than that for CoQ10 or NMN. A 2016 publication reported improvements in cognitive function in healthy middle-aged and elderly subjects at 20 mg daily [35]. The compound is available as a dietary supplement.

Novelty: Moderate. The mitochondrial biogenesis mechanism is well characterized but the clinical application is relatively recent.

Effect size: Small. Published clinical trials have shown modest effects on cognitive and fatigue endpoints. No muscle function or major disease endpoint trials have been completed.

Promising potential: Low to moderate. The compound lacks large-scale clinical trials and has not attracted pharmaceutical development.

Side-effect profile: Favorable. PQQ is well tolerated at doses of 10 to 20 mg daily. No material safety concerns have been identified.

Overall validation: Low. Limited clinical evidence; modest effect sizes; principally a research and consumer supplement compound.

Comparison to urolithin A: PQQ and urolithin A share the downstream effect of promoting mitochondrial renewal but through different proximal mechanisms (PQQ via biogenesis stimulation, urolithin A via mitophagy induction and secondary biogenesis through SIRT1/PGC-1alpha). Urolithin A has substantially stronger clinical evidence (multiple randomized trials with significant endpoints) and a clearer mechanistic narrative. PQQ is complementary and is often combined with urolithin A in consumer supplement stacks.

12.4 MitoQ (mitoquinone mesylate)

Mechanism: Mitochondria-targeted antioxidant. MitoQ is a derivative of CoQ10 conjugated to a triphenylphosphonium cation, which drives accumulation in the mitochondrial matrix driven by the membrane potential. The compound scavenges reactive oxygen species within the mitochondrial matrix at concentrations approximately 100- to 1000-fold higher than achieved by untargeted CoQ10 supplementation [36]. The mechanism is antioxidant and protective rather than quality-control-

inducing: MitoQ reduces oxidative damage to existing mitochondria, while urolithin A promotes the removal of mitochondria that have already been damaged.

Development trajectory: MitoQ has been studied in clinical trials for Parkinson's disease (the PROTECT trial, a Phase 2 randomized trial in 128 patients, reported no significant slowing of disease progression at 40 or 80 mg daily over 12 months) [37], for vascular endothelial function in older adults (improved brachial artery flow-mediated dilation in a small crossover trial), and for hepatitis C-associated liver damage. The compound is available as a dietary supplement.

Novelty: Moderate to high. The triphenylphosphonium targeting strategy is a novel approach to mitochondrial drug delivery.

Effect size: Disappointing in the Parkinson's disease Phase 2 trial. Modest positive effects on vascular function in healthy older adults. The mismatch between preclinical promise and clinical results has reduced enthusiasm.

Promising potential: Low to moderate. The Parkinson's trial failure was a significant setback. Ongoing research in cardiovascular and metabolic applications continues but without large-scale clinical programs.

Side-effect profile: Generally well tolerated. The Parkinson's trial reported gastrointestinal effects at higher doses. No serious safety concerns.

Overall validation: Low to moderate. The targeting concept is scientifically elegant, but clinical evidence has been disappointing relative to preclinical expectations.

Comparison to urolithin A: MitoQ and urolithin A represent fundamentally different strategies for mitochondrial health. MitoQ attempts to protect existing mitochondria from oxidative damage; urolithin A removes damaged mitochondria and promotes their replacement. The urolithin A strategy has produced more consistent clinical results to date (significant muscle function improvements in multiple trials vs. the MitoQ Parkinson's trial failure). The compounds are mechanistically complementary and could theoretically be combined (MitoQ reducing acute oxidative damage while urolithin A promotes long-term quality control), though this combination has not been studied.

12.5 Spermidine

Mechanism: Natural polyamine that induces autophagy and mitophagy through inhibition of the acetyltransferase EP300, leading to deacetylation and activation of multiple autophagy-related proteins. Spermidine also promotes mitochondrial function through direct effects on mitochondrial membrane potential and respiratory chain activity [38]. The autophagy-inducing mechanism overlaps with urolithin A, though the proximal molecular targets are distinct (EP300 inhibition for spermidine vs. PINK1/Parkin pathway enhancement for urolithin A).

Development trajectory: Spermidine has been studied in observational epidemiological studies (higher dietary spermidine intake associated with reduced cardiovascular mortality in the Bruneck cohort) and in small clinical trials. A randomized trial in 30 elderly subjects with subjective cognitive decline reported improved memory performance after 3 months of spermidine supplementation (1.2 mg/day) [39]. Clinical development is limited.

Novelty: Moderate. The polyamine-autophagy connection is well established in the literature. Spermidine is a natural dietary component and endogenous metabolite.

Effect size: Modest in clinical studies. Observational epidemiological associations are stronger but are subject to confounding.

Promising potential: Moderate. The epidemiological associations are compelling, and the mechanism is well characterized. Clinical translation has been slow.

Side-effect profile: Favorable. Spermidine is a naturally occurring dietary polyamine with a long history of human consumption. Supplementation at 1 to 6 mg daily has not produced material safety concerns.

Overall validation: Low to moderate. Strong epidemiological and preclinical evidence; limited clinical trial evidence.

Comparison to urolithin A: Spermidine and urolithin A share the endpoint of enhanced autophagy/mitophagy but operate through different molecular mechanisms. Urolithin A has substantially stronger clinical evidence from multiple randomized trials with objective endpoints (muscle strength, immune cell phenotyp-

ing). Spermidine has stronger epidemiological evidence for long-term mortality outcomes. The two compounds may be complementary, activating partially overlapping but distinct autophagy pathways.

12.6 Comparator summary

The five compounds occupy distinct positions in the mitochondrial health intervention space. NMN addresses NAD⁺ depletion and sirtuin function, complementary to but mechanistically distinct from urolithin A, with a growing but inconsistent clinical evidence base. CoQ10 has the longest clinical history and strongest evidence in specific cardiovascular indications, but addresses electron transport function rather than mitochondrial quality control. PQQ promotes biogenesis complementary to urolithin A mitophagy induction, but has limited clinical evidence. MitoQ represents an elegant targeting strategy that has not translated preclinical promise into clinical results. Spermidine shares the autophagy-induction mechanism with urolithin A but through different proximal targets, with strong epidemiological but limited clinical trial support.

Urolithin A occupies a distinctive position in this landscape: it is the only compound with clinical evidence for induction of mitophagy in human tissue, the only compound with FDA GRAS status specifically for this mechanism, the compound with the most consistent clinical trial results across multiple endpoints (muscle strength, muscle endurance, immune cell phenotyping, mitochondrial biomarkers), and the compound with the most coherent mechanistic narrative connecting mitophagy induction to the diverse downstream effects (muscle function, immune fitness, anti-inflammation, neuroprotection). Its principal limitations relative to the comparators are the relatively short clinical history (first human trial published 2019, compared to decades for CoQ10), the absence of long-term outcome data (mortality, major disease endpoints), and the current restriction to oral supplementation without intravenous or targeted delivery options.

The practical positioning of urolithin A for research applications is as a first-line mitophagy inducer with clinical validation, to be combined rationally with NAD⁺ precursors (NMN or NR for sirtuin cofactor support), CoQ10 (for electron transport chain optimization), or spermidine (for complementary autophagy pathway activation) depending on the specific research question and target tissue.

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