**Trigonelline is a Novel NAD+ Precursor that Improves Muscle Function during Ageing and is Reduced in Human Sarcopenia**

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**Abstract**

Mitochondrial dysfunction and low nicotinamide adenine dinucleotide (NAD+)levels are hallmarks of skeletal muscle aging and sarcopenia1-3, but it remains unclear whether these defects result from local changes or can be mediated by systemic or dietary cues. Here we reporta functional link between circulating levels of the natural alkaloid trigonelline, which is structurally related to nicotinic acid4, NAD+ levels and muscle health in multiple species. In humans, serum trigonelline levels are reduced with sarcopenia, and correlate positively with muscle strength and mitochondrial oxidative phosphorylation in skeletal muscle. Using naturally-occuring and isotopically-labelled trigonelline, we demonstrate that trigonelline incorporates into the NAD+ pool, and increases NAD+ levels in *C. elegans*, mice and primary myotubes from healthy and sarcopenic humans. Mechanistically, trigonelline does not activate GPR109A but is metabolized via the NAPRT/Preiss-Handler pathway5,6 across models. In *C. elegans*, trigonelline improves mitochondrial respiration and biogenesis, reduces age-related muscle wasting and increases lifespan and mobility through an NAD+-dependent mechanism requiring sirtuin. Dietary trigonelline supplementation in male mice enhances muscle strength and prevents fatigue during aging. Collectively, we identify nutritional supplementation of trigonelline as a novelNAD+-boosting strategy with therapeutic potential for age-associated muscle decline.

**Main**

Sarcopenia is the functional decline of skeletal muscle during aging which impairs mobility and leads to loss of physical independence and disability7. Clinically, sarcopenia is characterized by the pathological decrease of muscle mass, strength and gait speed3,8,9, and arises from myofiber wasting and a combination of molecular and cellular hallmarks of aging that collectively impair contraction10-12. Among these, mitochondrial dysfunction plays a prominent role1,3,13-17, with decreased mitochondrial biogenesis, altered mitochondrial dynamics and proteostasis, and reduced mitochondrial respiration and ATP production being established drivers of muscle aging phenotypes3,18,19. Given that inter-tissue crosstalk controls the availability of metabolic fuels for mitochondrial bioenergetics and influences muscle function and quality of life, research efforts have also characterized systemic contributions to sarcopenia. These include chronic low grade inflammation via pro-inflammatory cytokines, altered metabolic fluxes via reduced circulating levels of anabolic amino acids, and perturbations of glucose, vitamin, and lipid metabolism20-25.

The MEMOSA study has recently identifed mitochondrial dysfunction and declined NAD+ levels as prominent molecular hallmarks of sarcopenia in human cohorts from different geographies3. NAD+ is an essential coenzyme, derived from precursors of the vitamin B3 family, and a key cofactor for cellular and organismal metabolism. Mammals can produce NAD+ from different dietary precursors; these include nicotinamide riboside and nicotinamide mononucleotide (NR and NMN, respectively) converted via the nicotinamide riboside kinase (NRK) pathway, nicotinic acid (NA, also known as niacin) metabolized via the nicotinic acid phosphoribosyltransferase (NAPRT)-dependent Preiss-Handler pathway, tryptophan used in the *de novo* pathway for NAD+ biosynthesis, and nicotinamide (NAM) which generates NAD+ via the NAMPT salvage pathway26. NAD+ levels decline during aging in several metabolic tissues in rodents and humans1,17,27,28, including in skeletal muscle where there is replicated clinical evidence of age-related NAD+ deficiency2,3. However, it is still largely uncharacterized whether alterations in muscle mitochondrial and NAD+ homeostasis are reflected in the circulating metabolome and could be used to define clinical biomarkers and therapeutic interventions to manage later life muscle decline.

To complement our previous analyses of muscle biopsies of human sarcopenia3 and understand if mitochondrial dysfunction and altered NAD+ metabolism could be linked to systemic changes, we investigated serum levels of the kynurenine/B vitamin metabolome in sarcopenic vs healthy control subjects from the MEMOSA cohort (**Supplementary Table 1**). No changes were observed during sarcopenia for the majority of the metabolites analyzed, including the vitamin B3 forms that could act as potential NAD+ precursors. However, sarcopenic patients had lower circulating concentrations of trigonelline, a natural alkaloid found in plants4 and animals, including in humans29,30 (**Fig.1a**). Trigonelline levels were positively correlated with muscle mass assessed via Appendicular Lean Mass index (ALMi) measured by Dual-energy X-ray absorptiometry (DXA), grip strength, and gait speed, all parameters used in the clinical definition of sarcopenia3,9 (**Fig.1b**). Serum levels of trigonelline also associated with NAD+ levels in skeletal muscle (**Extended Data Fig.1a**), which we also previously found to be linked with clinical measures of sarcopenia and muscle health3. Finally, a gene-set enrichment analysis identified a positive association between serum trigonelline levels and several metabolic and signaling pathways in skeletal muscle, with mitochondrial oxidative phosphorylation (Oxphos) showing the strongest association with trigonelline (**Fig.1c**,**d** and **Extended Data Fig.1b**). Analysis of the Bushehr elderly health cohort31 (**Supplementaty Table 2**) demonstrated that serum trigonelline also associates with muscle function in an independent replication study (**Extended Data Fig.1c**). Dietary records indicate that serum trigonelline levels are independent of dietary caffeine and vitamin B3 intake in this cohort, but possibly linked to other dietary factors such as folate and fiber intake (**Extended Data Fig.1c** and **Supplementary Table 3**). In addition, correction for dietary caffeine and vitamin B3 intake did not affect the association between trigonelline and muscle strength (**Supplementary Table 4**). Collectively, our targeted metabolomic profiling of human sarcopenia discovered trigonelline as a new metabolite associated with muscle function, mitochondrial metabolism and NAD+.

Trigonelline is an *N-*methylated form of NA (**Extended Data Fig.1d**) that is synthesized by various plant species4 and is also a metabolite produced by the gut microbiome and endogenous metabolism in humans29,30. Based on this structural proximity to NA and the established link between muscle NAD+ and mitochondria in aging and sarcopenia1,14,15,17, we tested if trigonelline could act as an NAD+ precursor, and directly impact NAD+, mitochondrial and muscle homeostasis. Primary human skeletal muscle myotubes (HSMM) were treated with increasing therapeutic doses of trigonelline in the presence or absence of the nicotinamide phosphoribosyltransferase (NAMPT) inhibitor FK86614,32 to block NAD+ salvage (**Fig.1e,f**), thus mimicking low NAD+ via decreased *NAMPT* expression in human sarcopenic muscle3. Trigonelline increased NAD+ in control cells (EC50=315 µM) (**Fig.1e**), fully rescued NAD+ deficiency in FK866-treated cells (EC50=110 µM) (**Fig.1f**), and also increased NAD+ levels after prolonged treatment (**Extended Data Fig.1e**). When compared to other NAD+ precursors in primary human muscle cells, trigonelline and the salvage precursor NAM induced NAD+ by ~50% while the NRK pathway precursors NR and NMN33 increased NAD+ levels ~2-fold (**Extended Data Fig.1f**). The Preiss-Handler pathway of NAD+ biosynthesis requires the conversion of its substrate NA into nicotinic acid mononucleotide (NAMN) via the rate-limiting enzyme NAPRT6. When we tested trigonelline and other precursors in additional cell lines of muscle, liver and kidney, trigonelline or NA failed to raise NAD+ levels in HepG234 and C2C12 cells, which have low *NAPRT* expression (**Extended Data Fig.1g,h**), while all precursors had similar efficacy in renal proximal tubular epithelial cells (PTECs) (**Extended Data Fig.1h**). To further understand the biological activity of these precursors after *in vivo* absorption, we compared their stability in human serum. Trigonelline is remarkably stable in serum over 72h, while NR and NMN rapidly disappear within hours after conversion to NAM (**Extended Data Fig.1i**). Given its lower serum levels in sarcopenic individuals (**Fig.1a**), we tested trigonelline in sarcopenic muscle cells. Treating primary myotubes from different sarcopenic donors and aged-matched healthy controls35 raised cellular NAD+ (**Fig.1g**)with comparable efficiency between groups (**Extended Data Fig.1j**). Similarly, trigonelline also increased NAD+ levels in primary myotubes derived from aged mice (**Fig.1h**).

Trigonelline is N-methylated on its pyridine ring and needs to be demethylated prior to entry into the Preiss-Handler pathway and pyridine N-ribosylated for NAD+ biosynthesis (**Extended Data Fig.1d**). Since there is no trigonelline demethylase identified in plants or in mammals36, we explored potential candidates that could be linked to trigonelline demethylation by correlating serum trigonelline levels with the expression of genes from human sarcopenic muscle RNA-seq filtered for demethylating or methyltransferase activity (**Supplementary Table 5**). *SHMT2*, which is involved in mitochondrial one-carbon metabolism, had the strongest associatonwith serum trigonelline (**Extended Data Fig.1k**), and correlated positively with grip strength and muscle mass in humans (**Extended Data Fig.1l**). While this observation uncovers a potential link between trigonelline and a methyltransferase involved in 1-carbon metabolism that will require further exploration, SHMT2 is unlikely to directly demethylate trigonelline as it is not a N-methyltransferase and is known to cross-talk with NAD+ metabolism indirectly37,38.

To assess whether trigonelline incorporates into the NAD+ molecule, we used an isotopically-labelled form of trigonelline carrying a 13C on the carboxylic acid group, and 3 deuterium (2H) atoms on the methyl group (-CD3) (**Fig.2a**). Administration of isotopically-labelled trigonelline in mice was highly bioavailable with high levels of the parent trigonelline molecule detected in liver, gastrocnemius muscle, kidney, blood and urine 2 hours after oral intake, and were largely cleared following overnight wash-out (**Extended Data Fig.2a**). This was mirrored by increased NAD+ content in liver, muscle, kidney and whole blood, detected with either liquid chromatography coupled high resolution mass spectrometry (LC-HRMS) (**Fig.2b**), or NAD enzymatic assay (**Extended Data Fig.2b**). Based on our labelling strategy, direct incorporation of trigonelline into NAD+ implies the loss of thedeuteratedmethyl group, leading to a 13C-labeled NAD+ structure with a mass of M+1 (**Fig.2a**). Treatment of HSMM cells with labelled trigonelline significantly increased both total cellular NAD+ and [13C]-NAD+ M+1 (**Fig.2c**). *In vivo,* administration of labeled trigonelline also significantly enriched M+1 NAD+ in liver, and whole blood, and to a smaller extent in muscle, with hepatic incorporation being faster likely via a first-pass effect (**Extended Data Fig.2c**). Altogether, these results demonstrate that trigonelline is a *bona fide* NAD+ precursor that directly incorporates in NAD+ in cells and multiple tissues.

Given the similarity of trigonelline with NA and its ability to overcome NAD+ salvage inhibition (**Fig.1e,f**), but also its inefficacy in low *NAPRT*-expressing cells (**Extended Data Fig.1g,h**), we tested whether NAPRT would be required for utilization of trigonelline, and used NR as a reference compound able to generate NAD+ independently of the Preiss-Handler pathway (**Fig.2d**). shRNA-mediated knockdown of *NAPRT* in HSMM cells blocked the generation of NAD+ by trigonelline or NA (**Fig.2e** and **Extended Data Fig.2d,e**). Similarly, the NAPRT inhibitor 2-hydroxynicotinic acid (2-OHNA)39 blocked the conversion of trigonelline to NAD+ in HSMMs, but as expected did not impair the NAD+ boosting effect of NR (**Fig.2f**). Conversely, various levels of cellular NAD+ depletion by blocking NAD+ salvage with FK866 for 24 hours were rescued by trigonelline (**Fig.1f** and **Extended Data Fig.2f**), but not when co-treating with 2-OHNA (**Extended Data Fig.2g**). LC-HRMS analysis also confirmed that 2-OHNA blocks increased NAD+ after trigonelline treatment (**Fig.2g**), without affecting cellular trigonelline levels in the treated groups (**Extended Data Fig.2h**). Of note, endogenous levels of trigonelline increased in cells treated with 2-OHNA in the absence of trigonelline treatment (**Extended Data Fig.2h**), demonstrating that there is an endogenous flux of trigonelline through NAPRT in muscle cells. Following trigonelline treatment, the Preiss-Handler pathway-related metabolites NAAD and NAMN downstream of NAPRT6,33 were increased and blocked by NAPRT inhibition with 2-OHNA (**Fig.2h,i**), whereas NA which is upstream of NAPRT was accumulated (**Fig.2j**), and the salvage pathway metabolite NAM did not change (**Extended Data Fig.2i**). Acute dosing of trigonelline in wild-type (WT) and *Naprt* KO mice40 (**Fig.2k** and **Extended Data Fig.2j**) equally increased trigonelline in tissues (**Extended Data Fig.2k**), distal NAD+ metabolite fluxes in liver and blood (**Extended Data Fig.2l,m**), and tissue NAD+ levels (**Extended Data Fig.2l-n**). However, in *Naprt* KO mice treated with trigonelline, NA accumulated in blood and liver (**Fig.2l**), while NAMN was strongly downregulated (**Fig.2m**), and NAAD induction was blunted in liver (**Fig.2n**), similarly to what is observed in HSMM (**Fig.2h,j**). Upon trigonelline gavage *Nampt* and *Nrk1* expression was downregulated in liver, while *Nrk1* and *Nrk2* were upregulated in muscle in both treatment groups (**Extended Data Fig.2o**). This suggests that trigonelline engages the Preiss-Handler pathway during first pass metabolism, and that secondary compensations through the NR/NMN/NRK pathway also contribute to NAD+ in *Naprt* KOs.Altogether, our *in vitro* and *in vivo* results indicate that trigonelline is metabolized through the Preiss-Handler pathway via NAPRT and promotes NAD+ biosynthesis via both direct and indirect mechanisms.

To compare the functional relevance of modulating NAD+ metabolism via trigonelline and NR through the NAPRT and salvage routes, we measured mitochondrial respiraton and membrane potential in HSMM cells where low NAD+ was modeled with FK866 for 72 hours. Similar to the 24 hour treatment (**Extended Data Fig.2g**), trigonelline boosted NAD+ and this effect was blocked by NAPRT inhibition with 2-OHNA (**Fig.2o**), while no detrimental effect on cell viability was observed (**Extended Data Fig.2p**). Low NAD+ in response to FK866 lowered mitochondrial membrane potential (**Fig.2p**), which was fully restored by trigonelline and NR, while 2-OHNA abolished the effects of trigonelline but not NR (**Fig.2p**). FK866 treatment also significantly decreased maximal mitochondrial respiration (**Fig.2q**), which was also rescued by trigonelline, but blocked when trigonelline was co-treated with 2-OHNA (**Fig.2q**). In contrast, NR was still able to bypass NAPRT inhibition and increase respiration (**Fig.2q**). Finally, respirometry performed with mitochondrial complex inhibitors revealed higher levels of complex II and IV-driven respiration in trigonelline-treated cells, again dependent on NAPRT (**Extended Data Fig.2q**). The clinical use of NA is limited by its effects on skin flushing caused by binding to the G protein-coupled receptor 109A (GPR109A)41. We therefore also tested whether trigonelline may activate this receptor using a GPR109A-overexpressing stable cell line coupled to -arrestin based detection, and validated the assay with NA (EC50=2.5 µM) (**Extended Data Fig.2r**). Trigonelline did not activate GPR109A when tested up to 1 mM, a dose 400-fold higher than the EC50 of NA (**Extended Data Fig.2r**), suggesting that it could be better tolerated than NA. Collectively, these results demonstrate that trigonelline functionally rescues NAD+ deficiency and confirm that trigonelline requires NAPRT and a functional Preiss-Handler pathway for physiological activity on mitochondrial bioenergetics.

Lower NAD+ levels during aging are linked to mitochondrial dysfunction, muscle decline and reduced fitness and lifespan in lower organisms, such as nematodes14,42-45. Given that NAD+-enhancing interventions can improve these phenotypes14,42-45, we next tested the impact of trigonelline during aging in *C. elegans* by treating N2 wild-type worms starting from Day 1 of adulthood (**Fig.3a**). Trigonelline significantly extended lifespan, with comparable effects to equimolar levels of NR (**Fig.3b** and **Extended Data Fig.3a**). Akin to that observed in human and mouse cells and tissues, trigonelline raised NAD+ levels in aged nematodes (**Fig.3c**), and its NAD+-boosting effects were comparable to NA and NAM, and partially lower than NR and NMN (**Extended Data Fig.3b**). Trigonelline increased mitochondrial content assessed via the mtDNA/nDNA ratio (**Fig.3d**), activated the expression of genes linked to mitochondrial respiration and proteostasis (**Fig.3e**), similar to NR (**Extended Data Fig.3c** and14,43), and increased mitochondrial respiration (**Fig.3f** and **Extended Data Fig.3d**). We next evaluated muscle structure and mobility in aging nematodes. Trigonelline supplementation improved myofiber integrity during aging (**Fig.3g** and **Extended Data Fig.3e**), and this was mirrored by reduced worm paralysis (**Fig.3h**), and increased spontaneous mobility (**Fig.3i**). Interestingly, worms treated with trigonelline later in adulthood showed only a mild lifespan extension (**Extended Data Fig.3f**), but maintained better mobility than control animals during aging (**Extended Data Fig.3g**), indicating benefits of trigonelline on healthspan even when treating for a shorter duration and later in life. Importantly, the effects of trigonelline on NAD+ and mitochondrial content were lost with knockdown of the *Naprt* worm ortholog *nprt-1* (**Fig.3j,k**), in line with what was observed in HSMM. Knock-down of *nprt-1* or *sir-2.1* (**Extended Data Fig.3j**), the predominant sirtuin in N2 worms (**Extended Data Fig.3k**), both blunted the lifespan-extending effects and mobility benefits of trigonelline (**Fig**.**3l-n**), in line with similar effects observed with NA (**Extended Data Fig.3h,i**) and other NAD+ boosters14,42-45. As expected, trigonelline increased NAD+ levels after *sir-2.1* knockdown since sirtuins act as NAD+ sensors downstream of NAD+ biosynthesisand *sir-2.1* knockdown did not change baseline NAD+ levels (**Extended Data Fig.3l**). Together, these results validate the physiological requirement of the Preiss-Handler pathway for longevity, mitochondrial and functional benefits of trigonelline in an *in vivo* model, and demonstrate that the observed benefits on aging and health-span require the NAD+-dependent sirtuin deacetylase.

To translate the functional impact of trigonelline on muscle health from nematodes to mammals, we finally supplemented aged mice with dietary trigonelline. A 5-day treatment increased the expression and activity of mitochondrial Oxphos complex I and complex II in skeletal muscle (**Fig.4a-d** and **Extended Data Fig.4c**), without influencing mitochondrial abundance measured with mt/nDNA and citrate synthase (**Extended Data Fig.4a,b**). A 12-week dietary trigonelline supplementation increased plasma, liver and muscle levels of trigonelline in aged mice (**Fig.4e,f**), with no signs of toxicity (**Extended Data Fig.4d,e**). Body composition was not impacted by the treatment, with no changes in lean and fat distribution in trigonelline-treated mice (**Fig.4g** and **Extended Data Fig.4e**), no change of liver and muscle mass (**Fig.4h** and **Extended Data Fig.4h,i**), and no effects on TA muscle histology assessed via myofiber size, capillary area, glycogen accumulation, or fibrosis in TA and in diaphragm muscle (**Extended Data Fig.4j-n**). We next measured NAD+ in different tissues, where chronic trigonelline exposure significantly increased NAD+ in liver and kidney (**Extended Data Fig.4o**). Elevation of muscle NAD+ (see **Fig.2b; Extended Data Fig.2b,4o**) and Oxphos proteins (**Fig.4b,d; Extended Data Fig.4p**) were flattened in chronic *vs* acute exposure, likely because of long-term physiological compensation similar to those observed in clinical studies in muscle with dietary administration of other precursors46,47.

The effects of trigonelline on *in vivo* muscle function were assessed by recording spontaneous activity, and measuring grip strength and *in situ* contraction of tibialis anterior (TA) muscle via electrical stimulation of the sciatic nerve to overcome any effects on volition. Chronic supplementation of trigonelline significantly increased grip strength of forelimb muscles in aged mice (**Fig.4i**), and normalized age-related decline of fine spontaneous activity which mobilizes the hindlimb and frontlimb muscles (**Extended Data Fig.4q**). Maximum tetanic force of TA muscles was not impacted in aged trigonelline-supplemented mice (**Extended Data Fig.4r**), likely because tetanic force relies on muscle mass and neuromuscular coupling, both of which were not affected by trigonelline. However, when TA muscles were repeatedly stimulated *in situ* to assess muscle fatigue from high-intensity contractions, the age-related decline of force was attenuated in aged mice treated with trigonelline (**Fig.4j**). In this setting that mimics a physiological situation where the performance of healthy young mice decreases by 10% during the fatigue protocol, muscle fatiguability was tripled in aged mice, but trigonelline prevented approximately 50% of this age-related decline. Together, these functional data indicate that chronic trigonelline administration mitigates muscle decline during aging by stimulating mitochondria and increasing muscle performance and and resistance to fatigue during high-intensity contraction.

Although there is a common trajectory of age-related muscle decline in all individuals, the rate and extent of loss of muscle mass and strength varies between older individuals and the prominent factors that drive sarcopenia and disability *vs* healthy aging are poorly understood for therapeutic intervention. Our previous profiling of muscle biopsies from sarcopenic patients *vs* age-matched healthy controls revealed that mitochondrial dysfunction and reduced NAD+, two well-established molecular hallmarks of organismal aging11, are prominent features of pathological progression and sarcopenia3. In this study, we expanded our initial analysis to a targeted serum metabolomic profiling of sarcopenia, and discovered that the NA-related alkaloid trigonelline is a circulating metabolite correlating with muscle strength, and gait speed in humans, and declines during sarcopenia. Trigonelline is a well-described plant metabolite where its synthesis from NA via methylation confers protective and adaptive functions4,48, without an established reutilization as a Preiss-Handler substrate49,50. Consequently, trigonelline is particularly abundant in plant-derived food products such as coffee, beans and fenugreek seeds, which have been reported to modulate endogenous trigonelline levels in humans4,51,52. Although high coffee consumption has been reported to associate with a lower prevalence of sarcopenia in some populations53,our correlation analyses did not detect an association between circulating trigonelline and dietary caffeine intake levels in a population from the middle-east, possibly because coffee consumption is low in this population. Serum trigonelline levels were also not correlated to dietary vitamin B3 intake, suggesting that methylation of dietary vitamin B3 does not directly control endogenous trigonelline. It is also unlikely that intake of coffee and niacin directly drives the link between trigonelline and sarcopenia as association of serum trigonelline with muscle function was not markedly influenced by correction of dietary caffeine and vitamin B3 intake. Interestingly, food may still contribute to trigonelline metabolism as the dietary intake of folate and fibers was linked to circulating trigonelline levels. In addition, trigonelline can be produced by the microbiome, and was proposed as a biomarker of metabolic health or physical fitness since urinary trigonelline levels decrease during obesity and increase in professional athletes29,30. It is therefore likely that the association of trigonelline and sarcopenia has multifactorial contributions via a complex cross-talk between different food groups and endogenous metabolism.

Our work also describes for the first time trigonelline as a novel NAD+ precursor, and demonstrates the benefits of its therapeutic dietary supplementation for mitochondrial function, muscle health and mobility during aging. NAD+-boosting by trigonelline is conserved across multiple model organisms and primary human muscle cells from healthy and sarcopenic donors. Trigonelline treatment in aged mice increased different domains of muscle performance that mobilize both leg and arm muscles by improving both muscle fatigue and grip strength, all of which are important for healthy physical aging. Fatigue and grip strength are also well established measures of sarcopenia and physical frailty54,55 and are commonly used to assess the pathological impairment of quality of life in older people in clinical practice. However, sarcopenia is a multifactorial disease where both muscle mass and function decline beyond pathological thresholds7,8. Since trigonelline improved grip strength and fatigue independent of changes of muscle mass and maximal tetanic strength, we conclude that trigonelline cannot reverse all causes of sarcopenia and will have to be combined with other nutrients that support muscle mass like protein, omega 3 fatty acids or vitamin D for the nutritional managemement of sarcopenia. At the cellular level, the benefits of trigonelline on muscle performance did not arise from structural adaptations in the skeletal muscle architecture such as myofiber cross-sectional area, vascularization and fibrosis, but were linked to functional improvements of mitochondrial respiratory complex I and II activity. Using inhibitors and genetic loss of function *in vitro* and *in vivo,* we show that trigonelline is metabolized via the Preiss-Handler pathway and requires NAPRT to boost NAD+, stimulate mitochondrial metabolism, and increase mobility and lifespan during aging. Our work with tracers and metabolomics also indicates that trigonelline is demethylated prior to entering the flux of NAD+ biosynthesis, in line with early biochemical evidence that an enzymatic activity from liver may demethylate trigonelline in mammals36. However, there is no known demethylase that catalyzes trigonelline demethylation. Although the correlation we discovered between *SHMT2* expression and serum trigonelline, muscle mass and muscle strength suggests that trigonelline could cross-talk with the SAM-dependent methyltransferase activity of SHMT2 and folate/1-carbon metabolism, future studies will be required to characterize the mechanisms of trigonelline demethylation and the identity of the trigonelline demethylase.

Our results add trigonelline to the list of established dietary NAD+ boosters. These molecules, including NR, NA, NAM and NMN, have shown preclinical potential for ameliorating aging and chronic diseases in model organismsand have been investigated in recent human clinical trials focused on healthy aging46, obesity56, mitochondrial disease57, neurodegeneration58 or insulin sensitivity47, with different outcomes. Our work highlights that the NAD+-boosting capabilities of different precursors vary across tissues and experimental models according to the relative activity of different branches of NAD+ biosynthesis. Trigonelline contributes to the NAD+ pool via a more indirect route than the ribosylated precursors NR and NMN, but our comparative studies demonstrate that trigonelline has similar cellular and physiological benefits to NA and NR in cells or nematodes, most likely owing to its higher stability than ribosylated precursors. NA has lipid lowering properties33 and provides benefits in mitochondrial myopathies with impaired NAD+ salvage57, but has limited tolerability as it induces skin flushing through dermal Langerhans cells due to GPR109A-dependent vasodilation41,59. Our results indicate that unlike NA, trigonelline does not activate GPR109A at physiological and therapeutic levels, and likely provides a more favorable therapeutic profile. While trigonelline primarily engages the Preiss-Handler pathway across models, *in vivo* supplementation also elevates NAD+ metabolites from other biosynthetic routes, supporting the inter-organ cross-talk between NAD+ pathways already observed in humans and rodents with NR supplementation46,60. In particular, trigonelline increases NR and NMN in liver or serum both in WT and *Naprt* KO mice, and these secondary NAD+ fluxes can contribute to the NAD+ pool *in vivo* in the absence of *Naprt*. This highlights the complexity of systemic NAD+ metabolism modulation and the need to study the interplay between NAD+ fluxes and to run future comparative studies with different precursors in specific models and in humans. The heterogeneity of NAD+ biosynthesis and metabolism also suggests that some physiological specificity may exist between different precursors across organs, supporting additional translational applications of trigonelline. Owing to its high bioavailability and serum stability, trigonelline can reach the brain and impact cognitive performance in Alzheimer’s disease mice61, therefore holding potential for brain benefits in addition to muscle health. In addition, trigonelline can protect from metabolic dysfunction and improve glucose tolerance both in mice and humans62,63.

In summary, clinical profiling discovered an association between trigonelline and muscle health in humans, and our preclinical experiments demonstrated that trigonelline is a novel NAD+ precursor that optimizes mitochondrial function to improve muscle strength and prevent fatigue during aging. Trigonelline is therefore a novel nutritional geroprotector with therapeutic potential to manage sarcopenia and other age-related pathologies.

**Author contributions**

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**Competing interests**

M.M., E.M., S.C., M.P.G., L.C., M.V, C.C, V.S., and J.N.F. are or were employees of Nestlé Research, which is part of the Société des Produits Nestlé S.A. (SPN). L.G.K. was an employee of Nestlé Health Sciences. K.M.G. has received reimbursement for speaking at conferences sponsored by companies selling nutritional products, and is part of an academic consortium that has received research funding from BenevolentAI Bio Ltd., Nestlé Research and Danone. The remaining authors declare no competing interests.

**Methods**

*Human studies*

Detailed description of the entire Multi-Ethnic Molecular determinants of Sarcopenia (MEMOSA) study is available in *Migliavacca et al.3*. 20 Chinese male participants aged 65–79 years with a diagnosis of sarcopenia and 20 control participants of the same age/ethnic group were recruited in Singapore under a study approved by the National Healthcare Group Domain-Specific Research Board (NHG DSRB) under reference number 2014/01304, and each participant gave written informed consent. The measurement of the following analytes in overnight fasting venous blood sample was performed by Bevital (https://bevital.no/), using LC-MS/MS: Pyridoxal 5'-phosphate, Pyridoxal, 4-Pyridoxic acid, Pyridoxine, Thiamine, Thiamine monophosphate, Riboflavin, Flavin mononucleotide, Cystathionine, Neopterin, Tryptophan, Kynurenine, Kynurenic acid, Anthranilic acid, 3-Hydroxykynurenine, Xanthurenic acid, 3-Hydroxyanthranilic acid, Picolinic acid, Quinolinic acid, Nicotinic acid, Nicotinamide, N1-methylnicotinamide, Cotinine, Trans-3'-hydroxycotinine, Trigonelline. Primary myotubes were derived from muscle biopsies from participants in the Hertfordshire Sarcopenia Study Extension (HSSe) with approval from the Hertfordshire Research Ethics Committee64. Each participant gave written informed consent. Bushehr nutritional epidemiology study in older people: 186 older men aged 60 years and above participating in the second stage of the Bushehr elderly health (BEH) program were randomly selected from the population-based prospective cohort study conducted in Bushehr, a southern province of Iran31. Grip strength was measured using a digital dynamometer with 3 repeats of each hand, and retaining the highest average handgrip strength for the strongest hand. Appendicular lean mass index (ALMi) was measured using dual energy X-ray absorptiometry (DXA, Discovery WI, Hologic, Bedford, Virginia, USA), with AMLi calculated for each participant as the sum of upper and lower limb lean mass expressed in kilogram divided the height square expressed in meter. Dietary intake assessment was performed by a 24-hour dietary recall of all food and beverages consumed, captured performed by expert nutritionists31. Standard reference tables were used to convert household portions to grams and quantified using the nutritionist IV package, modified for Iranian foods to obtain daily energy, nutrient values and servings of foods consumed. For mixed dishes, food groups and nutrients were calculated according to their ingredients. An overnight fasting venous blood sample was collected, and serum was stored at -80°C to analyze serum micronutrients by mass spectrometry as reported in Panahi et al65. ​The study was approved by the Research Ethics Committee of the Endocrinology & Metabolism Research, Tehran University of Medical Sciences, under reference TUMS.EMRI.REC.1394.0036, and each participant gave written informed consent. Local sample analyses were approved by the cantonal ethics commission for human research (CER-VD) in Vaud, Switzerland under reference 490/14.

*mRNA association and pathway analysis*

Transcriptomics data are available at Gene Expression Omnibus under the subseries GSE111016. All statistical analyses of transcriptomics data were performed using R version 3.3.3 and relevant Bioconductor packages (*e.g.,* edgeR 3.16.5). Briefly, after excluding one sample with abnormally low percentage of uniquely mapped reads and after removing genes with a mean expression lower than 20 reads, data were normalized by the trimmed mean of M-values method as implemented in the edgeR67 function calcNormFactors. Normalized skeletal muscle mRNA expression data were associated with the serum level of trigonelline using Spearman rank correlation and the full dataset of genes expressed in skeletal muscle was used for pathway enrichment. Pathway enrichment analysis was performed using MSigDB v5.2 collections H (hallmark gene sets) from Molecular Signatures Database (MSigDB) and the mean-rank gene set enrichment test68 to assess whether an annotated set of genes was enriched in genes associated with the serum levels of trigonelline. For **Supplementary Table 5**, the list of genes with mRNA expression correlating with serum trigonelline was filtered for genes annotated with demethylase or methyltransferase activity.

*Cell cultures and reagents*

Human primary myoblasts from male donors (Lonza, CC-2580) were seeded in 96 well plates at a density of 12’000 cells per well in skeletal muscle growth medium (SKM-M, AMSbio). After one day, the differentiation was induced by a medium change for 4 days Dulbecco’s Modified Eagle’s Medium (DMEM/F12 life technologies) supplemented with 2% horse serum. Primary human myoblasts from male donors from the HSSe cohort (n= 3 sarcopenic and 3 control) were seeded in 96 well plates coated with matrigel at a density of 15’000 cells per well in myoblast proliferation medium (DMEM, 20% FBS, 10% HS, 1% P/S, 1% CEE). After 48 hrs, differentiation was induced by changing to differentiation media (DMEM, 2% HS, 1% P/S), this was termed differentiation day 0. HepG2 cell line (ATCC, Manassas, VA, USA), were maintained in exponential growth phase in DMEM supplemented with 10% fetal bovine serum, at 37 °C in a humidified atmosphere of 5% CO2. The C2C12 myoblasts were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) high glucose (Gibco, USA) containing 10% fetal bovine serum, and were differentiated into myotubes in DMEM high glucose containing 2% horse serum when the cell proliferation density of 80–90% in a 5% CO2 incubator at 37°C. Primary myotubes from aged male C57BL/6JRj mice (24 months from Janvier Labs, France) were generated from freshly sorted muscle stem cells isolated by flow cytometry as previously described69. Briefly, hindlimb muscles from were rapidly collected, minced, and digested with 2.5 U/ml Dispase II (Sigma), 0.2% Collagenase B (Sigma) and 5 mM MgCl2 at 37°C. The preparation was then filtered sequentially through 100 micron and 30 micron filters and cells were incubated at 4°C for 30 min with antibodies against CD45 (Invitrogen, #MCD4528, 1/25), CD31 (Invitrogen, #RM5228, 1/25), CD11b (Invitrogen, #RM2828, 1/25), CD34 (BD Biosciences, #560238, 1/60), Ly-6A/E (BD Biosciences, #561021, 1/150) and α7-integrin (R&D Systems, #FAB3518N, 1/30). MuSCs identified as CD31-/CD11b-/CD45-/Sca1-/CD34+/Integrin α7+ were isolated with a Beckman Coulter Astrios Cell sorter. FACS-isolated muscle stem cells were plated onto gelatin-coated 384-well plates at a density of 600 cells per well in DMEM supplemented with 20% heat-inactivated FBS (Thermo Fisher Scientific, #16140063), 10% horse serum (Thermo Fisher Scientific, #26050088), 2.5 ng/ml bFGF (Thermo Fisher Scientific, #PMG0035), 1% Penicillin-Streptomycin (Thermo Fisher Scientific, #15070063) and 1% L+-Glutamine (Thermo Fisher Scientific, #25030149). Media was refreshed the next day and cells were grown for 4 days until confluency. Cells were then differentiated for 2 days to myotubes in differentiation medium (DMEM, Thermo Fisher Scientific, #11995065) supplemented with 2% horse serum (Thermo Fisher Scientific, #26050088), and 1% Penicillin-Streptomycin (Thermo Fisher Scientific, #15070063). Treatments were performed on day 4 of differentiation for 24 hours. IM-PTEC cells isolated from Immorto mice70 were a gift from Dr. Alessandra Tammaro (Amsterdam UMC, the Netherlands). Cells were maintained in DMEM/F12 medium (Gibco) with 10% fetal bovine serum (Gibco), 5 ug/mL insulin and transferrin, 5 ng/ml sodium selenite (Gibco), 40 pg/ml triiodo-thyrionine (Sigma-Aldrich), 36 ng/mL hydrocortisone (Sigma-Aldrich), and 20 ng/mL epidermal growth factor (Sigma-Aldrich) with L-glutamine and antibiotics (both from Gibco) and mouse IFN-y (10 ng/ml; Prospec) at 33°C in 5% (v/v) CO2. Before being seeded for experiments, cells were grown at restrictive conditions (37°C 5% (v/v) CO2, in the absence of IFN-γ) for 7 days, to downregulate SV40 large T antigen activity.

Cells were treated with trigonelline iodide (Molport #MolPort-003-944-936) after internal quality control by mass spectrometry showing >99.7% purity, NR chloride (NR, Chromadex), nicotinic acid (NA, sigma #N0761), nicotinamide (NAM, sigma #72340), b-nicotinamide mononucleotide (NMN, sigma #N3501), 2-hydroxy-nicotinic acid (2-OHNA, sigma #251054), and FK866 hydrochloride (FK866, sigma #F8557) for the desired time. Unless otherwise stated, trigonelline treatment was performed at 1 mM. 2-OHNA and FK866 were used at 1 mM and 100 nM, respectively in all experiments, unless differently indicated. NAD+ was assessed using Biovision NAD+/NADH Quantitation Colorimetric Kit #k337-100. Hoechst Stains (Invitrogen; ref: H33342) and JC-10 (Enzo, ref: Enz-52305) were used in mitochondrial function assessment. Isotopically-labeled trigonelline iodide ([13C,2H3]-Trigonelline M+4) was labeled with one 13C on the carbonyl group and three 2H on the nitrogen of the pyridine ring through custom-synthesis and used *in vitro* and *in vivo* at the mentioned concentrations. Briefly, [carbonyl-13C]-nicotinic acid (0.1 g, 0.8 mmol) was stirred in anhydrous EtOH (5 mL), followed by addition of 200 L (0.46 g; 3 mmol) of 2H3-methyl iodide at 23C. The mixture was stirred under reflux at 45-48C in an oil bath for 2 days. Oil bath was removed and the reaction mixture was allowed to cool down to room temperature. Ethanol was evaporated on a rotary evaporator. Yellow powder residue was first washed with EtOH (2 x 1 mL) and then with diethyl ether (2 mL). The product was additionally dried under vacuum at 23C to give yellow solid (0.159 g; 74%). MS: 142.14 [M-I]+.

*NAD+ measurement.*

Quantification of NAD+ in cells was performed with a colorimetric assay (Biovision). Total NAD+ was normalized to the total number of cells. Quantification of NAD+ in tissue samples was performed using an enzymatic method adapted from Dall et al72 with normalization to tissue weight. Analysis of the NAD+ metabolome in cells or *in vivo* samples was performed by HRLC-MS as described below.

*Cellular assays*

*Cell death assays.* Kinetic experiments of apoptosis were performed with the IncuCyte ZOOM instrument (Essen Bioscience, Ann Arbor, MI, USA). The cells were incubated with IncuCyte Annexin-V Green (4642), according to supplier’s instructions. Four images per well were collected at the indicated time using a 10X objective and bandwidth filters (Ex: 440/80nm; Em: 504/44nm).

*Mitochondrial function.* For mitochondrial potential assessment, cells were seeded into 96-well Sensoplate (Greiner; ref: 655090) at a density of 12’000 cells per well in skeletal muscle growth medium (SKM-M, AMSbio). After one day, the differentiation was induced by a medium change for 4 days, mitochondrial potential was performed at desired time and treatment. Briefly, cells were incubated 30 minutes in Krebs buffer with Hoechst Stains and JC-10. Images were acquired using the ImageXpress (Molecular Device) using 10x objective. The following filters were used: JC-10, FITC Filter Cube/ TRITC Filter Cube; Hoechst, DAPI Filter CubeThe total intensity of both FITC and TRITC fluorescence is recorded for each cell and is used to calculate a cellular fluorescent ratio: Ratio per cell = log2 (∑pixel intensityTRITC/∑pixel intensityFITC). Once segmentation was completed, results were analyzed using KNIME software.

The bioenergetic profiles of the cells were analyzed by an XF96 extracellular flux analyzer (Seahorse Biosciences, North Billerica, MA, USA) as described. Briefly, the cells were seeded in XF96 Cell Culture Microplates and oxygen consumption rate was measured into Krebs buffer (NaCl 135 mM, KCl 3.6 mM, NaH2PO4 0.5 mM, MgSO4 0.5 mM, Hepes 10 mM, NaHCO3 5 mM) supplemented with 10 mM glucose, 10 mM pyruvate, and 2 mM glutamin. For substrate-driven OCR measurement in permeabilized HSMM myotubes, assay was performed into Agilent Seahorse XF Plasma Membrane Permeabilizer (PMP), following manufacturer’s instructions. Briefly, the cells were seeded in XF96 Cell Culture Microplates and oxygen consumption rate was measured into Mitochondrial assay solution (MAS buffer; Mannitol 220 mM, Sucrose 70 mM, KH2PO4 10 mM, MgCl2 5 mM, HEPES 2 mM, EGTA 1 mM, BSA 0.2% (w/v)) supplemented with ADP.

*NAPRT knockdown in cells.* To knockdown [*NAPRT*](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/calcium) in myotubes, 8,000 cells per well were seeded. Then Skeletal Muscle Cell Growth Medium (AmsBio, #SKM-M medium) was added and cells were incubated overnight. Subsequently, cells were infected with [adenovirus](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/adenoviridae) *NAPRT* shRNA (Sirion Biotech, Germany) at 200 MOI. Adenoviral infection with scrambled shRNA (Sirion Biotech, Germany) was used as a control, and cells were incubated for 48 h before initiating differentiation of myoblasts into myotubes. To check knockdown efficiency, cells were lysed in TriPure RNA reagent. Total RNA was transcribed to cDNA using the QuantiTect Reverse Transcription Kit (QIAGEN). Expression of *NAPRT* was analyzed using the LightCycler480 system (Roche) and LightCycler 480 SYBR Green I Master reagent (Roche). See **Supplementary Table 6** for the primer list.

*GPCR agonist assay.* The GPR109A agonist assay was performed utilizing the PathHunter beta‐arrestin enzyme fragment complementation (EFC) technology. GPCR Internalization Assays provide a quantitative measurement of arrestin‐ mediated GPCR internalization (Eurofins Discovery Services, CA, US). Compounds were tested in duplicates in agonist mode with GPR109A Biosensor Assay. Data were normalized to the maximal and minimal response observed in the presence of control ligand and vehicle.

*Precursors stability.* The stability of the different precursors was assessed in human serum (sigma #H4522) at 37°C at the indicated time points. A liquid-liquid extraction was adopted from *Giner et al*.71 to assess the level of precursors or intermediates in solution at the indicated incubation times (see below for full description).

*Animal studies*

Studies and procedures in WT mice were approved by the Nestlé Ethical committee (ASP-19-03-EXT), the Office vétérinaire cantonal Vaudois (VD2770 and VD3484), and the Animal Ethics Committee at The University of Melbourne (1914961.2). *Naprt* KO animal studies were approved by the Animal Experiment Committee at the University of Toyama (approval A2022MED-19), and were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals at the University of Toyama, which are based on international policies.

For all *in vivo* studies, trigonelline was orally administrated to animals in a dose equimolar to 300 mg/kg of trigonelline anhydrous. For the *in vivo* tracers experiments, 12-week-old C57BL/6JRj male mice were given a dose of labeled [13C,2H3]-Trigonelline M+4 iodide by gavage, and after 2 hours and 24h, blood, urine, muscle and liver were sampled. For the trigonelline supplementation in aged mice, 20-month-old C57BL/6J male mice from The Jackson Laboratory (Maine, USA) were fed a Standard AIN93M Rodent Diet (Specialty Feeds, Australia) supplemented for 5 consecutive days or during 12 weeks with trigonelline monohydrate (Laurus Labs Limited, India) after internal quality control by mass spectrometry showing >99.9% purity. Tissues were harvested at the end of the treatments. In the 12 weeks supplementation study, phenotypic characterization included body composition, using a whole-body composition analyser (LF50, Bruker), grip strength was assessed one week before killing using a grip strength meter. The force applied to the bar at the moment the grasp was released was recorded as the peak tension. At the end of the study, TA muscle function was assessed and at the completion of all functional assessments, a blood sample was obtained via cardiac puncture for subsequent analysis, and the heart was surgically excised and weighed. The skeletal muscles (TA, extensor digitorum longus, soleus, plantaris, gastrocnemius, quadriceps, diaphragm strips) and organs (liver, kidneys) were then surgically excised, weighed and frozen for biochemical and immunohistochemical analysis. Animals were killed as a consequence of the cardiac excision. Plasma parameters were measured using Vetscan Equine Profile Plus. An enzymatic method adapted from Dall et al72 was used to measure NAD+ content in mice. OXPHOS complexes expression was measured in isolated mitochondria by reducing Western blot analysis using OxPhos Rodent WB Antibody cocktail (Invitrogen #45-8099) and Anti-α-Tubulin antibody (Sigma #T6074). Equal protein load (20 µg/well) and consistent gel transfer was verified by Revert total protein stain (Licor Biosciences). Blots were imaged using a Licor IR imager, and quantified with Image Studio Lite (V. 5.0, Li cor Bioscience). Contractile function of TA muscles *in situ* was performed on the 20 month old mice and a reference group of young C57BL/6JRj male mice (12 weeks), as described previously73,74. In brief, maximum isometric tetanic force was determined from the plateau of a complete frequency-force relationship. Assessment of contraction-induced fatigue was determined by maximally stimulating muscles for an isometric contraction once every 4 s for 4 min. Recovery force was assessed after 5 min and 10 min rest after the initial stimulation period. Data were normalized to baseline force. For the *Naprt* KO study, animals were kept under a controlled temperature and humidity (25°C, 50%) with standard light condition (a 12:12 h light–dark cycle) with free access to water and standard chow diet (CLEA Japan Inc., Japan). *Naprt* KO mice of mixed genders were obtained by crossing heterogenic C57BL/6N *Naprt* KO mice described previously40 and treated with custom-synthesized trigonelline iodide at 8 weeks of age. Two hours later, tissues were collected and immediately frozen in liquid nitrogen and kept in -80°C.

*Histological analyses*

TA muscles were excised, blotted, and weighed on an analytical balance and then embedded in optimal cutting temperature compound, and frozen in isopentane cooled in liquid nitrogen for later immunohistochemical and histological analyses. Sections were stained with or reacted for: hematoxylin and eosin for assessment of general muscle architecture; CD31 to visualize capillaries; Van Gieson’s to identify collagen/fibrosis; succinate dehydrogenase activity as a general marker of mitochondrial (oxidative) activity; and Periodic acid Schiff for glycogen content75. Muscle fibre cross-sectional area was estimated from immunolabeling of laminin, myosin heavy-chain I, and myosin heavy-chain IIa in the muscle cross-sections. The signal derived from the antibody against laminin was used to select single muscle fibers and antibodies against myosin heavy-chains I and IIa, were used to differentiate between fiber types. Digital images of stained sections (four images per muscle section) were obtained using an upright microscope (20X objective) with camera (Axio Imager D1, Carl Zeiss, Wrek Göttingen, Germany), controlled by AxioVision AC software (AxioVision AC Rel. 4.7.1, Carl Zeiss Imaging Solutions, Wrek, Göttingen, Germany). Images were quantified using AxioVision 4.7.1 software for average fiber CSA, as described previously76.

*RNA and Western blot analyses in cells and rodent tissues*

For qPCR from tissues from the *Naprt* KO study, RNA extraction was performed by using TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA). ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan) was used to synthesize cDNA. Real-time PCR was performed by using THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan) on Thermal Cycler Dice Real Time System II (Takara Bio, Shiga, Japan). mRNA was quantified by Delta-Delta Ct method against Rpl13a as reference gene. See **Supplementary Table 6** for the primer list.

*Metabolomics analyses in cells and rodent tissues*

Metabolomics analyses were conducted on cells and rodent tissues using a liquid-liquid extraction method adapted from Giner et al. (71). The NAD+ metabolome was assessed in various samples, including cells, whole blood, urine, liver, and muscle. For cell extraction, cells were scraped from plates and extracted in a cold mixture of methanol:water:chloroform (5:3:5 (v/v)). Tissue extraction involved homogenization with metal beads in pre-cooled racks (-80°C) using a tissue mixer (Qiagen TissueLyser II). Biofluids, such as whole blood and urine, were directly extracted in cold methanol:water:chloroform (5:3:5 (v/v)). Isotopically labeled internal standards, including fully labeled 13C yeast extract and nicotinamide-D4, were included for data normalization. Additionally, isotopically labeled acyl-carnitines (NSK-B, CIL) were added for normalization in cell metabolomics. After centrifugation, the samples yielded an upper phase containing polar metabolites, a lower phase containing apolar metabolites, and a protein layer in between. The upper phase was dried and dissolved in 60% (v/v) acetonitrile:water for analysis. Protein layers from cells, liver, and muscle samples were quantified using a bicinchoninic acid (BCA) assay (ThermoFisher Scientific) for later normalization of metabolite concentrations. Liquid chromatography high-resolution mass spectrometry was performed using hydrophilic interaction chromatography (HILIC) analytical columns, such as HILICON iHILIC®-Fusion(P) or ZIC-pHILIC columns. The separation was achieved using a linear solvent gradient with solvent A (H2O with 10 mM ammonium acetate and 0.04% (v/v) ammonium hydroxide, pH ~9.3) and solvent B (acetonitrile). The eluting NAD+ metabolites were analyzed using an Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific) with a heated electrospray ionization (H-ESI) source. Data processing and instrument control were performed using Xcalibur v4.1.31.9 software (Thermo Scientific). Trigonelline in tissues was quantified using the same liquid-liquid extraction method, while body fluids (plasma and urine) were extracted using ACN:MeOH:H2O (-20°C) as described by Li et al. (79). A labeled amino acid mix (NSK-A1, CIL) served as an internal standard. Leucine-D4 was used as an internal standard for normalization. Trigonelline concentrations were determined using calibration curves ranging from 10 µM to 1000 µM. For tracer experiments, the same methods were used, and the relative abundance and enrichment of isotopologues of NAD metabolites were calculated by dividing the area of each isotopologue by the sum area of all isotopologues. In the *Naprt* KO rodent study, metabolite extraction and NAD+ metabolomics were performed as previously described (80). Tissues were ground in a 50% methanol-50% water mixture using a multi-beads shocker (Yasui Kikai, Japan). The metabolites were analyzed using an Agilent 6460 Triple Quad mass spectrometer coupled with an Agilent 1290 HPLC system. Trigonelline and other NAD+ metabolites were detected using specific transitions, and data analysis was conducted using MassHunter Workstation-Quantitative Analysis software (Agilent Technologies).

*C. elegans methodologies and treatment*

Wild type hermaphrodites Bristol worms (N2) and GFP-tagged myosin worms81 were treated with Trigonelline chloride (Sigma, #T5509) and NR chloride from Day 1 of adulthood. Nematodes were cultured at 20°C on nematode growth medium (NGM) agar plates seeded with *E. coli* strain OP50. Trigonelline and the other precurors were added to the NGM medium at a final concentration of 1mM just before pouring the plates, unless otherwise stated. An enzymatic method adapted from Dall et al72 was used to measure NAD+ content in worms. 50-100 worms were collected per biological replicate, and NAD+ levels were normalized on the protein content. Bacterial feeding RNAi experiments were carried out as described82. RNAi clones (from GeneService) used were *sir-2.1* (R11A8.4), *nprt-1* (Y54G2A.17).

*Lifespan.* Worm lifespan tests were performed using about 90-100 animals per condition and scored manually every other day, as previously described83. Treatments and experimental measurements were started at Day 1 of wild type N2 worm adulthood, in a regimen of chronic exposure till experiments termination. Statistical significances were calculated by using the log-rank (Mantel-Cox) method.

*Mobility assay. C. elegans* spontaneous mobility test was performed using the Movement Tracker software84. The experiments were repeated at least twice. For paralysis scoring, 45 to 60 worms per condition were manually scored for mobility after poking. Worms that were unable to respond to any repeated stimulation were scored as dead. Results are representative of data obtained from at least two independent experiments. Trigonelline treatment and experimental measurements were similar to what described above.

*RNA analyses.* A total of approximately 6,000 worms per condition, divided into 6 biological replicates, was recovered in M9 buffer from NGM plates at Day 2 of adulthood and lysed in the TriPure RNA reagent. Total RNA was transcribed to cDNA using the QuantiTect Reverse Transcription Kit (QIAGEN). Expression of selected genes was analyzed using the LightCycler480 system (Roche) and LightCycler 480 SYBR Green I Master reagent (Roche). For worms two housekeeping genes were used to normalize the expression data, actin (*act-1*) and peroxisomal membrane protein 3 (*pmp-3*). See **Supplementary Table 6** for the primer list.

*Muscle integrity imaging.* Imaging of muscle structure was performed on RW1596 *myo-3 (st386)* worm strain55. Trigonelline treatment started at Day 1 of worm adulthood, in a regimen of chronic exposure until termination at Day 11. Confocal images were acquired with Leica SP8 inverse STED 3X (Leica Microsystems) under non-saturating exposure conditions. Worms were immobilized with 7.5 mM solution of tetramisole hydrochloride (Sigma-Aldrich) in M9 and mounted on 2% agarose pads on glass slides. Myofibers integrity scoring was performed with the method recently described by *Dhondt et al*.85 on a total of 6 worms group and 9-31 muscle cells per group.

*Oxygen-consumption assays.* Oxygen consumption was measured in N2 worms treated from embryo to L4 with trigonelline using the Seahorse XF96 equipment (Seahorse Bioscience) as previously described86. Respiration rates were normalized to the number of worms in each individual well and calculated as averaged values of 5 repeated measurements.

*Mitochondrial DNA quantification.* One worm was lysed in 2 L lysis buffer (50mM KCl, 10mM Tris pH8.3, 2.5mM MgCl2, 0.45 % NP-40, 0.45% Tween 20, 0.01 gelatin, 100 mg/ml proteinase K (added prior to use) and heated at 65°C for 60 min followed by inactivation heating 90°C for 15 min. Worm lysate was diluted 50 times with DEPC water for SYBRgreen qPCR reaction using LightCycler® 480 SYBR® Green I Master kit (Roche) following kit instructions. Relative values for *nd-1* and *act-3* (**Supplementary Table 6**) were compared within each sample to generate a ratio representing the relative level of mitochondrial DNA per nuclear genome. Experiments were performed on at least ten independent biological samples.

**Data availability**

All data generated or analysed during this study are included in this published article. Source data for uncropped gels and for all the individual p values presented herein are provided with the paper. The transcriptomic datasets analyzed in this study are available at Gene Expression Omnibus under the subseries GSE111016.

**Statistics**

All statistical analysis was carried out using GraphPad Prism 9 or R. The statistical methods used for each analysis are mentioned in the figure legends. Statistical methods for transcriptomic analyses of muscle biopsies were previously reported3. Data distributions were plotted as box-plots representing the 25th percentile, the median, and 75th percentile.Associations between two continuous variables were determined using Spearman rank correlations. For statistical comparisons of two conditions, the Student’s two-tailed unpaired t-test was used. For comparisons of more than two groups, the data were analysed with one-way analysis of variance (ANOVA) followed by Šídák's multiple comparisons tests post hoc test or two-way ANOVA followed by Tukey’s multiple comparisons post hoc test, unless differently specified in the figure legends. P values of <0.05 (\*), <0.01 (\*\*), <0.001 (\*\*\*) and <0.0001 (\*\*\*\*) were considered statistically significant. Normality of each readout was assessed based on historical values of the lab for this readout. All data represent the mean±standard error of the mean (s.e.m.). *n.s.* indicates results that were not statistically significant.

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**Figure legends**

**Figure 1. Serum trigonelline is reduced in human sarcopenia and associates with mitochondrial and NAD+ metabolism in skeletal muscle**. **a**, Serum levels of trigonelline in healthy controls (n=20) and sarcopenic subjects (n=20) from the MEMOSA Singapore Sarcopenia Study (SSS) (unpaired two-tailed Student’s t-test). **b**, Association of serum trigonelline levels with appendicular lean mass (ALM) index, grip strength and gait speed; Pearson correlation coefficient and its p-value were calculated on n = 40 serum samples from SSS. **c**, SSS muscle RNAseq association with serum trigonelline levels. Gene set enrichment ordered according to the significance of enrichment with only the top 10 gene sets being reported, FDR<10-20 were trimmed at FDR=10-20 (n=39 muscle samples). **d**, Enrichment plot for the hallmark oxidative phosphorylation gene set from panel (**c**). **e,f**, Relative NAD+ levels in human primary myotubes (HSMM) following treatment with increasing concentrations of trigonelline, in the presence or absence of FK866 (one-way ANOVA, mean ± s.e.m, n=6 biological replicates per group). **g**, Relative NAD+ levels in human primary myotubes from healthy controls and sarcopenic patients from the HSSe cohort treated *ex vivo* with or without trigonelline (unpaired two-tailed Student’s t-test, mean ± s.e.m, n=3 biological replicates per group). **h**, Relative NAD+ levels in primary myotubes from aged mice (22 months) treated *ex vivo* with trigonelline (unpaired two-tailed Student’s t-test, mean ± s.e.m, n=8 and n=9 biological replicates per group). P: <0.05 (\*); <0.01 (\*\*); <0.001 (\*\*\*); <0.0001 (\*\*\*\*); n.s., non-significant. For all the individual p values, see the Fig.1 Source Data file.

**Figure 2. Trigonelline is an NAD+ precursor and activates mitochondrial function via the Preiss-Handler pathway**. **a**, Experimental design of isotope-labeled trigonelline experiment & incorporation route into NAD+. **b**, NAD+ levels measured by LC-HRMS in liver, gastrocnemius muscle and whole blood 2 hours (2 h) or overnight (o/n) after labelled trigonelline oral gavage in young mice (One-way ANOVA, n=4-5 biological mice per group). **c**, NAD+ levels measured by LC-HRMS in HSMM myotubes (left panel) and relative isotopic enrichment of NAD+ (right panel) after 24 h incubation with 1 mM labelled trigonelline (unpaired two-tailed Student’s t-test, n=3 biological replicates per group, control values correspond to natural abundance of heavy isotopes in NAD+). **d**, Representation of the Preiss-Handler and salvage pathways of NAD+ production. **e**, Relative NAD+ levels in HSMM cells 48 h after adenoviral infection with a scrambled or *NAPRT* shRNA (unpaired two-tailed Student’s t-test, n=6 biological replicates per group). **f**, Relative NAD+ levels in HSMM myotubes after 24h trigonelline or NR treatment, in the presence or absence of 2-OHNA (One-way ANOVA, n=12 biological replicates per group). **g-j**, NAD+ metabolites measured by LC-HRMS in HSMM myotubes after 24 h incubation with trigonelline, in the presence or absence of 2-OHNA and FK866 or their combination (One-way ANOVA, n=3 biological replicates per group). **k**, qPCR mRNA expression of *Naprt* in liver of wildtype (WT) and *Naprt* knockout (KO) mice (One-way ANOVA, n=4-6 animals per group). **l-n**, LC-HRMS measurement of NA(**l**) and NAMN (**m**) levels in blood and liver, and of NAAD (**n**) levels in liver at 2 hours atfer trigonelline gavage in WT or *Naprt* KO mice (One-way ANOVA, n=4-6 animals per group). **o**, Relative NAD+ levels in HSMM myotubes following 72 h trigonelline or NR treatment, in the presence of FK866 and with or without co-treatment with 2-OHNA (one-way ANOVA, n=10 biological replicates per group). **p**, Mitochondrial membrane potential (ΔΨm) measured via JC-1 staining in HSMM myotubes following the same treatments as in (**o**) (one-way ANOVA, n=16 biological replicates per group). **q**, Maximum oxygen consumption rate (OCR) in HSMM myotubes following the same treatments as in (**o**) following stimulation with 3 M FCCP (one-way ANOVA, n=9-10 biological replicates per group).All data are expressed as mean ± s.e.m with p-value <0.05 (\*); <0.01 (\*\*); <0.001 (\*\*\*); <0.0001 (\*\*\*\*); n.s., non significant. For all the individual p values, see the Fig.2 Source Data file; AU, abitrary units.

**Figure 3. Trigonelline supplementation enhances lifespan and ameliorates age-related muscle decline and mitochondrial dysfunction in *C. elegans*. a,** Experimental design of compound treatments at 1 mM started from Day 1 of adulthood (D1) in N2 wild-type worms. **b**, Lifespan in trigonelline-treated worms (log-rank test, n=90 worms per group). **c**, Relative NAD+ levels in aged worms (*D8=Day 8*; unpaired two-tailed Student’s t-test, n=6 biological replicates per group). **d**, mt/nDNA in aged worms (*D8;* unpaired two-tailed Student’s t-test, n=12 worms per group). **e**, mRNA expression of mitochondrial genes in worms treated with trigonelline (unpaired two-tailed Student’s t-test, n=6 biological replicates per group). **f**, Basal and maximal oxygen consumption rate in L4 worms treated from embryo stage (unpaired two-tailed Student’s t-test, n=36 animals per group). **g**, Confocal images (left) and quantitave integrity scoring (right) of GFP-labeled muscle fibers in adult (*D1*) and aged (*D11=Day 11*) RAW1596 (*myo-3p::GFP*) worms (One-way ANOVA, n=6 worms and 9-31 sarcomeres per group). Scale bar, 10 µm. **h**, Percentage of paralyzed aged worms (*D11*; n=3 independent experiments, unpaired two-tailed Student’s t-test). **i**, Spontaneous mobility of worms at different ages (unpaired two-tailed Student’s t-test, n=33-49 worms per group). **j**, Relative NAD+ levels in D1 adult worms treated from embryo stage and fed with control (*empty vector; e.v.*) or *nrpt-1* RNAi (One-way ANOVA, n=6-14 biological replicates per group). **k**, mt/nDNA in worms treated as in (**j**) (One-way ANOVA, n=10-12 animals per group). **l,m**, Lifespan of control (*e.v.*), *nrpt-1* RNAi (l) and *sir-2.1* RNAi (m) worms (log-rank test, n=100 animals per group). **n**, Spontaneous mobility of control (*e.v.*), *nrpt-1* RNAi and *sir-2.1* RNAi worms at Day 6 (unpaired two-tailed Student’s t-test, n=63-123 individual worms per group). All data are expressed as mean ± s.e.m with p-value : <0.05 (\*); <0.01 (\*\*); <0.001 (\*\*\*); <0.0001 (\*\*\*\*). n.s., non-significant. For all the individual p values and animal numbers per group, see the Fig.3 Source Data file; AU, abitrary units.

**Figure 4. Trigonelline supplementation enhances mitochondrial activity and muscle function in aged mice. a**,Mitochondrial complex I activity in gastrocnemius muscle of aged mice (20 months) following a 5 day dietary supplementation of trigonelline (unpaired two-tailed Student’s t-test, n=7-8 biological replicates per group). **b**,Mitochondrial complex I (NDUFB8) protein levels in the same samples as in (**a**) (unpaired two-tailed Student’s t-test, n=6 biological replicates per group). **c**,Succinate dehydrogenase (SDH) activity in quadriceps muscle of the same groups as in (**a**) (unpaired two-tailed Student’s t-test, n=7 biological replicates per group, **d**, Mitochondrial complex II (SDHB) protein levels in the same samples as in (**a**) (unpaired two-tailed Student’s t-test, n=6 biological replicates per group). **e**,Plasma trigonelline levels measured in aged mice (22-24 months) after 12 weeks of trigonelline supplementation (unpaired two-tailed Student’s t-test, n=13 and 15 biological replicates per group). **f**, LC-HRMS measurement of trigonelline levels in gastrocnemius muscle and liver of the same mice groups as in (**e**) (unpaired two-tailed Student’s t-test,Gastrocnemius: n=5 and 6,Liver: n=13 and 16 biological replicates per group); *<LOQ*, below level of quantification. **g**,Lean mass normalized to body weight of aged mice after 12 weeks of treatment as in (**e**) (unpaired two-tailed Student’s t-test, n=13 and 15 biological replicates per group). **h**,TA muscle mass of aged mice after 12 weeks of treatment as in (**e**) (unpaired two-tailed Student’s t-test, n=13 and 15 biological replicates per group). **i**,Grip strength of aged mice after 12 weeks of treatment as in (**e**) (unpaired two-tailed Student’s t-test, n=13 and 15 biological replicates per group). **j**,*In situ* muscle contractility normalized to initial force after supramaximal stimulation of TA muscle in young controls and aged mice after 12 weeks of treatment as in (**e**) (two-way ANOVA followed by uncorrected Fisher LSD tests; n=11-14 biological replicates replicates per group). All data are expressed as mean ± s.e.m with p-value <0.05 (\*); <0.01 (\*\*); <0.001 (\*\*\*); <0.0001 (\*\*\*\*); n.s., non-significant. For all the individual p values, see the Fig.4 Source Data file.