

Mitochondrial calcium uptake sets a workload-coupling threshold rather than a universal muscle-performance signal

Andrew Friedl

Precision Mito, Florida, USA

Correspondence: afriedl@precisionmito.com

Abstract

Background: Mitochondrial Ca^{2+} uptake through the MCU complex is often described as a direct bridge from contraction-linked Ca^{2+} signals to mitochondrial energy production. That framing is useful, but it can overstate what follows from biomarker activation alone.

Objective: This working paper evaluates whether skeletal-muscle mitochondrial Ca^{2+} uptake should be interpreted as a universal performance-enhancing signal or as a workload-coupling threshold whose meaning depends on endpoint, timing, and gate state.

Methods: Ten primary records were verified by PMID, DOI, title, model, endpoint class, correspondence evidence where available, and conflict statements where relevant. Evidence was coded by endpoint level, response context, perturbation, direction, and transfer risk. Quantitative pooling was not attempted because the studies differ by organism, age, intervention, muscle context, and outcome scale.

Results: The coded set contained 10 primary records spanning skeletal-muscle molecular endpoints, morphology, function, and clinical-performance-adjacent human outcomes. Five records supported a role for mitochondrial Ca^{2+} uptake in muscle size, fuel selection, exercise capacity, aging biology, or fatigue resistance; three records defined boundary conditions through opposite acute versus fatigue-state behavior, inducible MCUB down-tuning, or human MICU1 disease; and two human exercise records supplied context rather than causal proof. The evidence therefore supports a non-causal, non-pooled threshold interpretation rather than a treatment or supplement claim.

Conclusions: Mitochondrial Ca^{2+} uptake is best treated as a workload-coupling threshold. Too little, mistimed, or poorly gated uptake can impair energy matching, but simple activation is not sufficient evidence for generalized performance benefit.

Keywords: mitochondrial calcium uptake; mitochondrial calcium uniporter; MICU1; MICU3; MCUB; skeletal muscle; exercise adaptation; sarcopenia

1 Introduction

Skeletal muscle faces a recurrent coordination problem: contraction raises ATP demand at the same time that cytosolic Ca²⁺ signals change. The MCU complex provides a plausible coupling mechanism because matrix Ca²⁺ can stimulate pyruvate dehydrogenase and tricarboxylic-acid-cycle dehydrogenases, while regulatory subunits such as MICU proteins, MCUR1, and MCUB shape when and how strongly matrix uptake occurs.

The competing interpretation is narrower. Mitochondrial Ca²⁺ uptake may not be a universal enhancer; it may be a threshold system that fails when uptake is too low, mistimed, or ungated, and may be deliberately down-tuned during specific metabolic states. This distinction matters for exercise, sarcopenia, and nutrition-adjacent claims because molecular activation can be mistaken for function, and acute exercise bioenergetics can be mistaken for durable training adaptation.

The evidence base now includes AAV manipulation of MCU in mouse muscle, germline and muscle-specific M_{cu} deletion, MCUB gain/loss models, MICU3 deletion, older-adult training biopsies, MICU1 human genetic disease, aging-associated MCUR1 and oleuropein experiments, and a 2026 human acute-exercise olive-leaf extract study [1-10]. These studies are well suited for an endpoint-coded synthesis because they contain both supportive and constraining observations.

2 Methods

Primary literature was searched and verified through PubMed, PubMed Central, DOI, publisher pages, and institutional author pages available on 2026-10-07. Records were eligible when they experimentally tested MCU-complex function, mitochondrial Ca²⁺ uptake, MICU regulation, MCUB tuning, muscle contraction or exercise outcomes, aging-associated skeletal-muscle bioenergetics, or human MICU-related disease. Reviews were used only for orientation and were not included in the evidence table. The final synthesis included ten primary records with PMID and DOI identifiers [1-10].

Each study was coded before interpretation by endpoint level: blood proxy, skeletal muscle molecular, morphology, function, or clinical performance. The response context was coded as baseline, acute response, training adaptation, or disease rescue. A transfer-risk field prevented biomarker movement from being interpreted as performance efficacy unless the same study measured a functional endpoint. Source-study involvement, sponsor ties, and patent interests were handled as interpretation boundaries rather than as reasons to discard otherwise relevant primary evidence.

Figure 1 shows the analysis process. The workflow began with mechanism selection and current primary-literature verification, then proceeded through eligibility screening, endpoint coding, boundary analysis, sensitivity checks, and synthesis.

3 Results

3.1 Evidence synthesis

The included primary studies supported the existence of a muscle mitochondrial Ca²⁺ workload-coupling system, but not a universal enhancer claim. MCU overexpression or silencing in mouse muscle altered trophic signaling through PGC-1α and IGF1-Akt/PKB pathways [1]. Germline MCU loss removed rapid mitochondrial Ca²⁺ uptake and reduced peak skeletal-muscle performance [2]. Muscle-specific M_{cu} deletion studies then sharpened the endpoint boundary: acute performance could fall, while fatigue-state performance or fatty-acid reliance could improve under some conditions [3,4].

Regulatory-subunit evidence made the threshold model more precise. MCUB induction during fasting reduced mitochondrial

Ca²⁺ influx and favored fatty-acid use; M_{cu}b deletion increased PDH activity and glucose oxidation but worsened adiposity and glucose tolerance [5]. MICU3 loss impaired treadmill exercise capacity and shortened fatigue resistance while reducing mitochondrial Ca²⁺ uptake and contraction-linked NADH matching [6]. Human MICU1 loss-of-function disease showed that gate failure can cause myopathy and altered mitochondrial Ca²⁺ signaling, which constrains claims that more uptake or less gatekeeping is always favorable [8].

Human exercise and intervention records supplied context rather than full causal closure. In older adults, training-associated functional and structural improvement was linked with increased MCU protein and mitochondrial remodeling markers, but the design did not isolate MCU as a sufficient cause [7]. The 2025 Cell Metabolism oleuropein study linked aging-associated MCUR1 decline with impaired uptake and showed MCU-dependent performance effects in models, but sponsor and patent ties require independent replication for translational claims [9]. The 2026 human acute-exercise olive-leaf extract study was more constrained: molecular and PDH-response signals were most evident with moderate exercise, while maximal-intensity and fatigue outcomes were less responsive [10].

3.2 Evidence-coded sensitivity analysis

Three sensitivity checks were applied. The sponsor-linked oleuropein records were treated as relevant but not decisive; the threshold conclusion remained supported by MCU, MCUB, MICU3, and MICU1 studies [1-6,8]. The human-only restriction was exploratory and yielded a cautious interpretation: human records support association, disease relevance, and acute mode-specific bioenergetic modulation, not a chronic training or treatment claim [7,8,10]. Excluding biomarker-only efficacy inference was decision-changing because it converted a potential activation narrative into a threshold model requiring matched functional endpoints.

Figure 2 presents the conceptual synthesis. Solid connectors indicate links supported by at least one included primary study. Dashed connectors mark transfer-limited predictions, especially intervention and disease-boundary claims that require direct replication.

4 Discussion

The evidence supports a threshold interpretation. Mitochondrial Ca²⁺ uptake links contraction signals to enzyme activation, substrate selection, and muscle signaling, but its value depends on the gate state and endpoint being measured. Deficient uptake can impair workload matching, yet down-tuning through MCUB can be adaptive during fasting. Similarly, activation signals can coexist with unresolved questions about chronic benefit, safety, sponsor-independent replication, and clinical transfer.

This interpretation helps separate four claims that are often compressed. First, MCU-complex function is mechanistically important in skeletal muscle. Second, endpoint movement is context-specific: molecular, morphology, function, and clinical-performance endpoints do not carry the same evidentiary weight. Third, rare human disease and mouse genetic deletions show that improper gate function can be harmful. Fourth, nutritional or pharmacologic activation requires human replication with durable training endpoints before performance or sarcopenia claims can be made.

The most useful next experiments would compare matched contraction protocols across MCU, MICU3, MCUR1, and MCUB states; measure PDH activity, substrate oxidation, fatigue, and recovery in the same preparation; and test whether acute bioenergetic response predicts training adaptation. Independent replication of oleuropein or olive-leaf extract effects should use

Table 1. Endpoint-coded primary-study evidence matrix

Study	Model and perturbation	Key finding	Interpretive limitation
[1]	AAV-mediated MCU overexpression or silencing in developing and adult mouse skeletal muscle; Gain and loss of MCU-dependent mitochondrial Ca ²⁺ uptake	MCU-dependent mitochondrial Ca ²⁺ uptake altered fiber size and engaged PGC-1α and IGF1-Akt/PKB trophic signaling; overexpression protected against denervation-associated atrophy.	AAV gain/loss biology defines muscle trophism and disease-rescue logic, not a general exercise-performance intervention.
[2]	Germline MCU-null mice and isolated mitochondria; Whole-animal loss of rapid MCU-mediated mitochondrial Ca ²⁺ uptake	MCU-null mice lacked rapid mitochondrial Ca ²⁺ uptake and showed reduced skeletal-muscle peak performance despite viability.	Developmental compensation and germline physiology make this a threshold and compensation study rather than a direct training-adaptation study.
[3]	Embryonic, postnatal, and adult skeletal-muscle Mcu deletion in mice; Skeletal-muscle-specific loss of MCU	Mcu deletion reduced acute exercise performance while improving some fatigue-condition performance and shifting fuel preference toward fatty acid metabolism.	Opposite acute versus fatigue-state outcomes argue against a one-direction performance claim.
[4]	Skeletal-muscle-specific MCU knockout mice and myofibers; Loss of muscle MCU and rescue by PDP2 overexpression	MCU deletion impaired mitochondrial Ca ²⁺ uptake, muscle force, exercise performance, PDH activity, glucose oxidation, and fiber-type balance while increasing fatty-acid reliance.	Metabolic rewiring partially compensated the defect, so mitochondrial Ca ²⁺ uptake acts as a substrate-routing threshold, not a simple more-is-better axis.
[5]	Skeletal-muscle-specific Mcub deletion or MCUB overexpression in mice; Fasting-inducible inhibitory MCUB tuning of mitochondrial Ca ²⁺ influx	MCUB induction reduced mitochondrial Ca ²⁺ influx and favored fatty-acid use; Mcub deletion increased PDH activity, glucose oxidation, adiposity, and glucose intolerance.	The same complex can be physiologically down-tuned during fasting, which blocks a universal activation claim.
[6]	Systemic MICU3 knockout mice with skeletal-muscle contraction assays; Loss of MICU3 threshold-setting protein	MICU3 loss impaired treadmill exercise capacity, shortened time to fatigue in stimulated muscle, reduced mitochondrial Ca ²⁺ uptake, and altered NADH oxidation during contraction.	Systemic deletion and compensatory MICU1.1 changes leave the muscle-specific therapeutic window unresolved.
[7]	Human biopsies from 70-year-old participants after 9 weeks of electrical stimulation or leg press training; Training-associated change in muscle MCU, OPA1, and mitochondrial structure	Improved muscle function and structure after training were associated with increased MCU protein and mitochondrial remodeling markers.	Association in a small human training subgroup does not isolate MCU as a sufficient cause of adaptation.
[8]	Human genetic families plus patient fibroblast functional assays; Biallelic MICU1 loss of function	MICU1 mutations caused proximal myopathy with neurological signs and altered mitochondrial Ca ²⁺ signaling in patient-derived cells.	Human disease evidence establishes harm from gate failure, not benefit from pharmacologic activation.
[9]	Human muscle profiling, patient-derived myotubes, bioactive-molecule screen, and mouse intervention models; Aging-associated MCUR1 decline and oleuropein activation of MCU/MICU1-dependent uptake	MCUR1 downregulation associated with sarcopenia; oleuropein increased mitochondrial Ca ²⁺ uptake, respiration, endurance, and fatigue resistance in models, with MCU-dependence in mice.	Sponsor and patent ties plus preclinical performance endpoints require independent replication before clinical-benefit claims.
[10]	Human double-blind crossover acute exercise study in healthy untrained young adults; Oleuropein-based olive leaf extract before moderate continuous or sprint interval exercise	Olive leaf extract enhanced transcriptomic and bioenergetic response most clearly after moderate-intensity exercise and increased PDH activity with that exercise mode; maximal-intensity and fatigue outcomes were less responsive.	The human signal was acute and mode-specific, with product/in-kind industry involvement, so chronic training benefit and clinical treatment claims remain unproven.

Analysis workflow

Primary evidence was coded before interpretation; boundary records could change the conclusion.

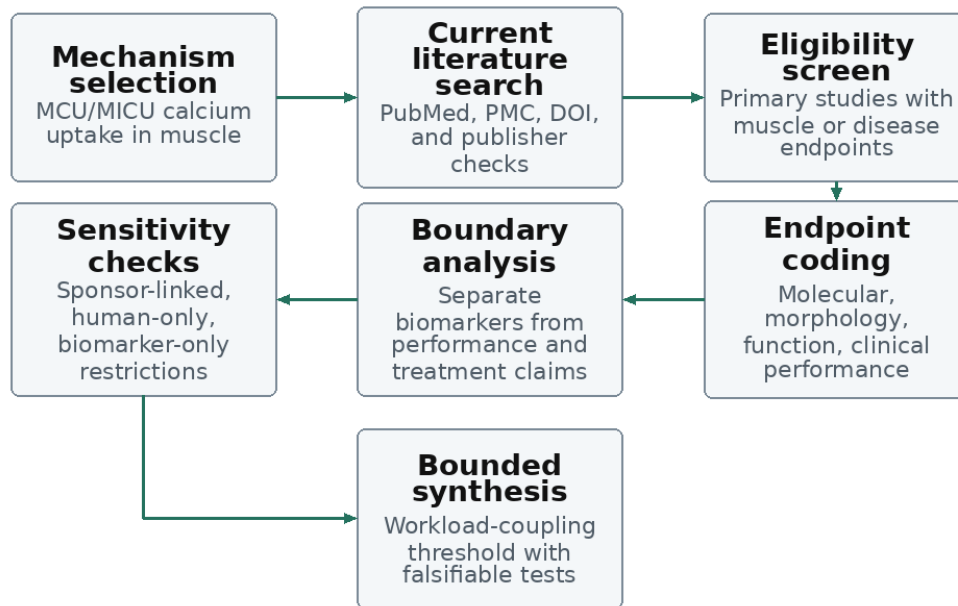


Figure 1. Analysis process. The workflow began with MCU-complex mechanism selection and current primary-literature verification, then proceeded through eligibility screening, endpoint-level evidence coding, boundary analysis, sensitivity checks, and synthesis. Solid connectors indicate executed analysis steps.

Workload-coupling threshold model

Solid links reflect included primary evidence; dashed links identify testable and transfer-limited predictions.

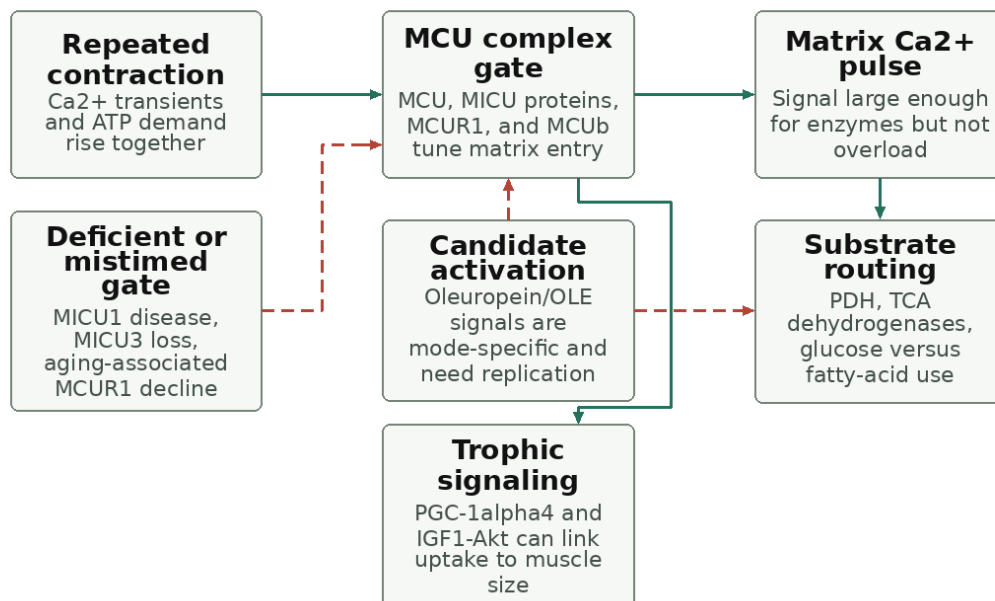


Figure 2. Conceptual workload-coupling threshold model. Solid connectors indicate evidence-supported links from included primary studies. Dashed red connectors identify transfer-limited predictions, including intervention and disease-boundary claims requiring direct replication.

preregistered outcomes, product chemistry disclosure, and exercise-mode stratification.

5 Limitations

This working paper is an endpoint-coded mechanistic synthesis, not a systematic review or meta-analysis. The included studies span cells, mice, older-adult biopsies, rare human disease, and an acute human nutrition-exercise protocol. Sample sizes, assays, and endpoints differed materially. Sponsor and patent interests are present in the oleuropein literature and are treated as validation priorities rather than proof of invalidity. No clinical treatment, supplement, or exercise prescription follows from this evidence.

6 Conclusions

Mitochondrial Ca²⁺ uptake in skeletal muscle sets a workload-coupling threshold: adequate and correctly gated uptake can support energy matching and muscle signaling, but activation alone is not evidence of generalized performance improvement, chronic training adaptation, or clinical benefit.

Declarations

Data and code availability. The evidence table, machine-readable data, analysis code, figures, and QA record accompany this repository deposit.

Ethics statement. This literature synthesis used no identifiable patient data and required no new human-participant or animal research.

Funding. No external funding was reported for this working paper.

Competing interests. The author reports no competing interests for this working paper.

Author contributions. Andrew Friedl conceived the project, directed the analysis, reviewed the evidence package, and approved the manuscript for repository review.

AI assistance. AI tools assisted literature synthesis, drafting, data visualization, and formatting. Gemini provided an independent critique. Andrew Friedl remains solely responsible for the content and is the sole author.

References

- [1] Mammucari C, Gherardi G, Zamparo I, et al. The mitochondrial calcium uniporter controls skeletal muscle trophism in vivo. *Cell Reports*. 2015;10(8):1269-1279. PMID: 25732818. doi:10.1016/j.celrep.2015.01.056.
- [2] Pan X, Liu J, Nguyen T, et al. The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nature Cell Biology*. 2013;15(12):1464-1472. PMID: 24212091. doi:10.1038/ncb2868.
- [3] Kwong JQ, Huo J, Bround MJ, et al. The mitochondrial calcium uniporter underlies metabolic fuel preference in skeletal muscle. *JCI Insight*. 2018;3(22):e121689. PMID: 30429366. doi:10.1172/jci.insight.121689.
- [4] Gherardi G, Nogara L, Ciciliot S, et al. Loss of mitochondrial calcium uniporter rewires skeletal muscle metabolism and substrate preference. *Cell Death and Differentiation*. 2019;26:362-381. PMID: 30232375. doi:10.1038/s41418-018-0191-7.
- [5] Huo J, Prasad V, Grimes KM, et al. MCUB is an inducible regulator of calcium-dependent mitochondrial metabolism and substrate utilization in muscle. *Cell Reports*. 2023;42(11):113465. PMID: 37976157. doi:10.1016/j.celrep.2023.113465.
- [6] Roman B, Mastoor Y, Zhang Y, et al. Loss of mitochondrial Ca²⁺ uptake protein 3 impairs skeletal muscle calcium handling and exercise capacity. *Journal of Physiology*. 2024;602(1):113-128. PMID: 38018177. doi:10.1113/JP284894.
- [7] Zampieri S, Mammucari C, Romanello V, et al. Physical exercise in aging human skeletal muscle increases mitochondrial calcium uniporter expression levels and affects mitochondria dynamics. *Physiological Reports*. 2016;4(24):e13005. PMID: 28039397. doi:10.14814/phy2.13005.
- [8] Logan CV, Szabadkai G, Sharpe JA, et al. Loss-of-function mutations in MICU1 cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling. *Nature Genetics*. 2014;46(2):188-193. PMID: 24336167. doi:10.1038/ng.2851.
- [9] Gherardi G, Weiser A, Bermont F, et al. Mitochondrial calcium uptake declines during aging and is directly activated by oleuropein to boost energy metabolism and skeletal muscle performance. *Cell Metabolism*. 2025;37(2):477-495.e11. PMID: 39603237. doi:10.1016/j.cmet.2024.10.021.
- [10] Lanfranchi C, Moreno-Asso A, Horstman AMH, et al. Oleuropein-based olive leaf extract enhances muscle mitochondrial bioenergetics response to moderate - but not maximal - intensity exercise in humans. *Journal of Physiology*. 2026;604(10):3802-3824. PMID: 41979147. doi:10.1113/JP290316.