

Lactate-sensitive PGC-1 routing and pyruvate-linked content signaling as separable control layers in mitochondrial biogenesis

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Keywords: lactate; pyruvate; mitochondrial biogenesis; MCT1; mitochondrial pyruvate carrier; PGC-1alpha; lactylation; skeletal muscle

Abstract

Lactate and pyruvate are frequently treated as interchangeable three-carbon fuels in discussions of mitochondrial biogenesis, yet primary studies indicate that monocarboxylate identity, transport context, redox handling, post-translational signaling, compartment, and PGC-1alpha dependence can separate mitochondrial adaptation into distinct routes. This working paper evaluates a two-route monocarboxylate-control model across skeletal-muscle cells, mouse skeletal muscle, human exercise biopsies, hippocampal lactate-transport experiments, cardiac mitochondrial lactate-import experiments, lactate electron-transport-chain signaling experiments, skeletal-muscle mitochondrial-pyruvate-carrier perturbation, live-cell lactate/pyruvate imaging, and 2026 lactylation-linked PGC-1 studies. PubMed, PubMed Central, NCBI metadata, and publisher records were searched and verified through 27 September 2026. Seventeen primary studies were coded on seven mechanistic axes using a conservative ordinal evidence scheme, and leave-one-study sensitivity checks were performed. Evidence supported a lactate-biased route involving MCT exposure, mitochondrial lactate oxidation, ETC-linked or lactylation signaling, and PGC-1-family nuclear routing, and a pyruvate-biased route in which mitochondrial content, respiratory-chain abundance, or whole-body carbon partitioning can change without proportional PGC-1alpha induction. Ordinal support was highest for test-discrimination value, functional metabolic output, mitochondrial content, and PGC-1-family routing. This synthesis does not estimate pooled effect sizes. Instead, it defines a falsifiable perturbation matrix that separates transport dependence, MPC status, redox buffering, metabolism-independent lactate signaling, lactylation-linked routing, PGC-1alpha dependence, mitochondrial content, and respiratory quality. The model predicts that MCT disruption should preferentially alter lactate-linked routing or mitochondrial lactate oxidation, whereas selected pyruvate-linked content or partitioning outputs should persist under PGC-1alpha attenuation while requiring separate tests of respiratory quality, ATP yield, and stress tolerance.

Keywords: lactate; pyruvate; mitochondrial biogenesis; MCT1; PGC-1alpha; skeletal muscle; lactylation; mitochondrial lactate import

Introduction

Mitochondrial biogenesis in skeletal muscle is commonly organized around PGC-1alpha-centered exercise adaptation. Acute human exercise induces transient PGC-1alpha transcription and mRNA accumulation [6], and low-volume high-intensity training increases mitochondrial enzymes, Tfam, SIRT1, and nuclear PGC-1alpha abundance [7]. However, several primary studies show that this framing is incomplete. Exercise-linked mitochondrial transcriptional events can begin before total PGC-1alpha protein increases [8], and muscle-specific PGC-1alpha loss does not abolish all exercise-induced mitochondrial biogenesis phenotypes [9].

Monocarboxylate metabolism adds a second control layer. Lactate increased MCT1, reactive oxygen species, cytochrome c oxidase, PGC-1alpha expression, and NRF-2 DNA-binding activity in L6 cells [1]. MCT1-mediated lactate handling in skeletal muscle reshaped TCA flux, NAD+/SIRT1 signaling, fiber composition, respiration, mitochondrial proteins, and PGC-1alpha protein [2]. Lactate also increased fatty-acid oxidation, TCA-cycle activity, and mitochondrial respiration in skeletal muscle from obese mice [3]. Two recent ovarian or granulosa-cell studies provide tissue-limited but mechanistically informative comparators linking lactate-sensitive protein or histone lactylation to PGC-1-family nuclear routing, NRF1/2 target expression, mitochondrial mass, and reproductive metabolic output [11,12]. A cardiac isotope-tracing study adds an orthogonal transport comparator: lactate carbon entered TCA metabolites when mitochondrial pyruvate-carrier function was impaired, and mitochondrial lactate oxidation depended on MCT1 [13]. A separate Molecular Cell study complicates a simple oxidation-only explanation by showing that lactate can activate ETC-linked oxidative phosphorylation even when lactate metabolism is bypassed by D-lactate [14].

Pyruvate-focused evidence points in a different direction. Pyruvate increased mitochondrial protein expression, basal respiration, and maximal oxidative capacity despite PGC-1alpha knockdown or PGC-1alpha-null primary myoblasts [4]. In C2C12 myotubes, pyruvate increased respiratory-chain and substrate-use proteins while suppressing PGC1alpha expression [5]. Skeletal-muscle mitochondrial pyruvate-carrier deletion diverted pyruvate into circulating lactate, increased Cori cycling, increased fatty-acid oxidation and energy expenditure, and preserved muscle mass and strength [16]. These studies indicate that mitochondrial content, respiratory-chain abundance, substrate preference, systemic carbon routing, and coordinated respiratory quality should not be collapsed into one unqualified biogenesis endpoint.

The objective was to construct a transparent evidence map and a publication-ready mechanistic summary for testing whether lactate and pyruvate are best modeled as a shared fuel pair or as entry points into separable mitochondrial-adaptation routes. The central hypothesis is that monocarboxylate flux gates at least two partially separable outputs: lactate-biased transport, redox, mitochondrial import, and lactylation routing through PGC-1-family coordination, and pyruvate-biased substrate sensing that can raise selected mitochondrial-content outputs without requiring proportional PGC-1alpha induction.

Methods

Search and screening

Primary literature was searched through PubMed, PubMed Central, NCBI metadata, and publisher records through 27 September 2026 using combinations of lactate, pyruvate, MCT1, monocarboxylate transporter, PGC-1alpha, mitochondrial biogenesis, TCA flux, skeletal muscle, exercise, redox, lactylation, mitochondrial lactate import, mitochondrial pyruvate carrier, and respiration. Representative queries included lactate MCT1 mitochondrial biogenesis skeletal muscle, pyruvate PGC-1alpha independent mitochondrial biogenesis, lactate mitochondrial import MCT1 respiration, lactylation PGC1alpha mitochondrial biogenesis, lactate electron transport chain metabolism independent, mitochondrial pyruvate carrier skeletal muscle lactate Cori cycling, and live-cell lactate pyruvate mitochondrial ATP imaging. Included records had to report primary experimental results, an identifiable model system, a perturbation relevant to monocarboxylate routing or exercise-linked mitochondrial adaptation, and a verifiable bibliographic record. Reviews were used only for orientation and were not entered into the evidence-coded table.

Screening prioritized studies that could discriminate at least one of seven mechanistic boundaries: lactate versus pyruvate, MCT transport dependence, mitochondrial pyruvate-carrier status, metabolism-dependent versus metabolism-independent lactate signaling, redox or post-translational signaling, PGC-1alpha dependence, and mitochondrial content versus respiratory quality. Prior Mito Map weekly publication bundles were screened for relevant methods and limitations. A prior monocarboxylate-routing bundle was relevant because it exposed the same mechanistic fault line and a useful ordinal-coding approach. Its underlying source claims were reverified from primary literature rather than copied forward, and three useful comparator/falsifier studies were added only after PubMed, PubMed Central, or publisher verification [14,16,17]. A withdrawn article was excluded after a PubMed withdrawal notice was identified. Figure 1 summarizes the analysis process from concept scoping and literature search through screening, evidence coding, analysis, sensitivity checks, and synthesis.

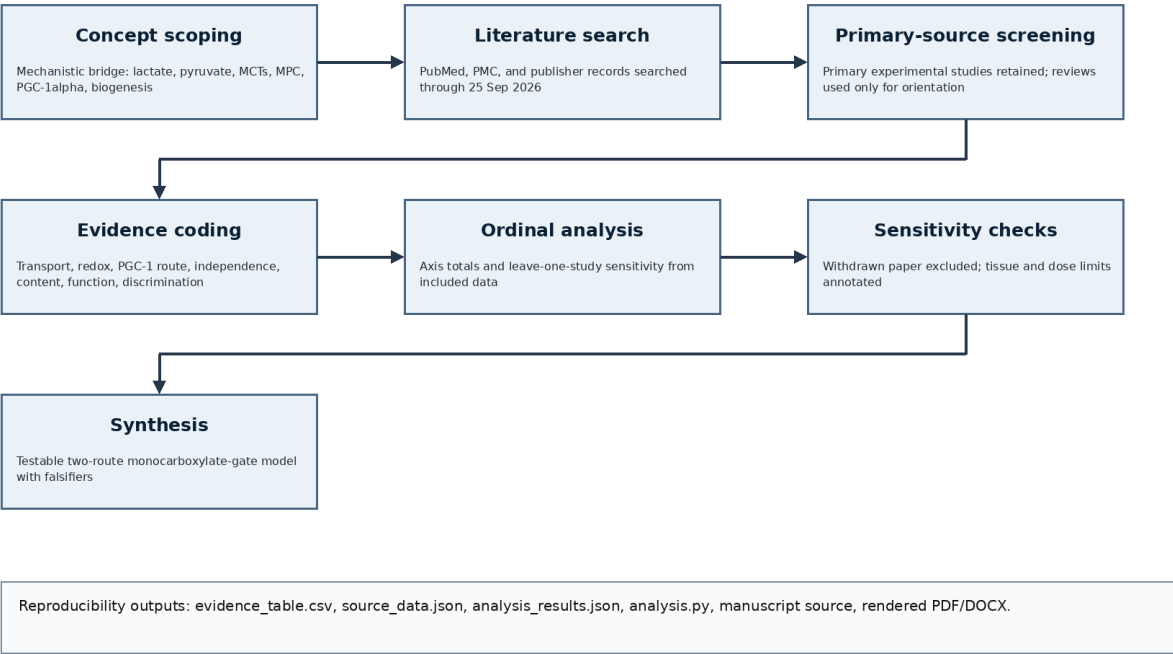


Figure 1. Analysis-process workflow from concept scoping and literature search through source screening, evidence coding, ordinal analysis, sensitivity checks, and synthesis.

Evidence coding

Each included study was coded across seven axes: lactate transport, redox signal, PGC-1-family route, PGC-1alpha-independent branch, mitochondrial content, functional metabolic output, and test-discrimination value. Codes used a conservative ordinal scale: 2 indicated direct or strong evidence in that study, 1 indicated partial or indirect evidence, 0 indicated not assessed or not central, and -1 indicated evidence against the axis as framed. Certainty notes distinguished direct evidence in the tested system from lower-confidence transfer across tissues. Codes and certainty notes were entered in `analysis.py` and exported to `source_data.json`, `evidence_table.csv`, and `analysis_results.json`. The bundle also records rendering metadata in `paper_meta.json` and final artifact hashes in `checksums.sha256` so that manuscript source, analysis outputs, native tables, figures, DOCX, PDF, and QA records can be matched after remediation.

Evidence hierarchy and decision rules

Evidence was interpreted in three ordered tiers: direct skeletal-muscle or human-exercise evidence, mechanistically direct evidence in another tissue, and cross-system comparator evidence. A route was not treated as transferable to skeletal muscle merely because it was mechanistically plausible elsewhere. Support for either proposed route required convergence across more than one experimental system plus at least one discriminating perturbation, such as transporter loss, PGC-1alpha loss, MPC perturbation, redox buffering, or a metabolism-independent lactate control. Negative codes were retained rather than averaged away, and a zero denoted absence of a direct test rather than evidence of no effect. These rules were specified before interpreting the aggregate axis totals so that abundant but indirect findings could not override a direct falsifier.

Each interpretive statement was also assigned one of three claim boundaries during synthesis: observed within the tested system, transferred as a bounded comparator, or proposed as a hypothesis. Only the first category was treated as direct support for that system. Cross-tissue findings could motivate an experiment but could not establish a skeletal-muscle mechanism. Evidence rows retained the model, perturbation, observed result, interpretation limit, and certainty statement together so that a favorable result could not be separated from its dose, tissue, or design limitation. This claim-boundary audit was applied before drafting and repeated during citation QA.

This was a structured qualitative reanalysis rather than a formal meta-analysis. Numerical effect sizes were not extracted from figures, and cross-study pooling was not attempted because the included evidence spans different tissues, species, perturbation durations, doses, and endpoints. Ordinal totals were used only to identify where evidence is concentrated and where discriminant experiments are most valuable.

Reproducible analysis and sensitivity checks

The analysis script writes the evidence table, machine-readable source data, axis totals, leave-one-study sensitivity outputs, and all figures. The leave-one-study check recalculates total ordinal support after removing each study. This check tests whether the conceptual model depends on a single paper. Additional manual checks assessed whether each claim was tied to a cited primary source, whether hypotheses were separated from observed evidence, whether tissue-specific findings were not generalized beyond their experimental context, and whether citation mismatches remained after DOI, PubMed, PubMed Central, or publisher verification.

Results

Evidence map

The included evidence formed two complementary clusters. Lactate-focused studies supported transport-sensitive or signaling-linked mitochondrial adaptation. In L6 cells, lactate increased ROS production, MCT1 expression, COX expression, PGC-1alpha expression, and NRF-2 DNA-binding activity [1]. In skeletal-muscle MCT1 deletion, altered lactate handling was linked to TCA flux redistribution, oxidative-fiber changes, respiration, mitochondrial proteins, NAD⁺, SIRT1 activity, and PGC-1alpha protein [2]. Lactate administration in high-fat-diet or obese mice increased fatty-acid oxidation, TCA-cycle activity, and mitochondrial respiration [3]. In mouse hippocampus, lactate injection and high-intensity exercise increased PGC-1alpha and mtDNA copy number, while UK5099 attenuated the response [10]. A 2026 ovarian-cell study identified HSP90alpha lactylation as a mechanism promoting PGC1alpha and LRPGC1 nuclear translocation, NRF1/2 target expression, mitochondrial mass expansion, and estrogen biosynthesis [11]. A separate 2026 granulosa-cell study linked FSH-driven lactate accumulation to P300/CBP-dependent H4K5 lactylation, HDAC4 expression, PGC-1alpha deacetylation, NRF1/2 target transcription, mitochondrial biogenesis, follicular growth, and estradiol production [12]. A cardiac isotope-tracing study did not test biogenesis directly, but it strengthened the transport and redox boundary by showing MCT1-dependent mitochondrial lactate oxidation when pyruvate-carrier function was impaired [13]. The lactate-ETC study added a separate boundary: acute lactate signaling can increase oxidative phosphorylation without requiring lactate catabolism, so mitochondrial ATP or respiration shifts cannot automatically be interpreted as biogenesis or lactate-carbon oxidation [14]. Redox sensitivity remained relevant because vitamin C reduced training-induced endurance and mitochondrial-biogenesis markers in humans and rats, although that trial did not isolate lactate as the upstream signal [15].

Pyruvate-focused studies supported a content and partitioning branch that can separate from canonical PGC-1alpha induction. In C2C12 and primary myoblasts, pyruvate increased mitochondrial proteins, basal respiration, and maximal oxidative capacity, and the response persisted with PGC-1alpha knockdown or in PGC-1alpha-null primary myoblasts [4]. In C2C12 myotubes, pyruvate

increased cytochrome c oxidase subunit 1, cytochrome c, CD36, and HAD while suppressing PGC1alpha expression and altering substrate-use markers [5]. In skeletal-muscle MPC deletion, impaired mitochondrial pyruvate uptake increased circulating lactate, Cori cycling, fatty-acid oxidation, energy expenditure, and insulin sensitivity while retaining muscle mass and strength [16]. These findings argue that mitochondrial content, respiratory-chain abundance, carbon partitioning, and substrate quality should be measured as separable endpoints.

Exercise studies supported the relevance of PGC-1-family routing but also bounded it. Human knee-extensor exercise induced marked transient PGC-1alpha transcription and mRNA changes [6], and practical low-volume high-intensity training increased mitochondrial enzyme activities, COX proteins, Tfam, SIRT1, exercise capacity, and nuclear PGC-1alpha abundance [7]. In rat skeletal muscle, NRF binding and mitochondrial mRNAs increased before total PGC-1alpha protein rose [8]. In muscle-specific PGC-1alpha knockout mice, voluntary wheel running retained exercise capacity and exercise-induced mitochondrial biogenesis [9]. Redox buffering with vitamin C reduced training adaptation and mitochondrial-biogenesis markers in humans and rats, supporting redox sensitivity without assigning lactate as the upstream signal [15].

Live-cell imaging supplied a negative-control style constraint. In hepatocytes and skeletal-muscle cells exposed to lactate overload, genetically encoded indicators showed lactate and pyruvate dynamics in cytoplasm and mitochondrial matrix; in skeletal-muscle cells, mitochondrial ATP remained unchanged despite increased lactate and pyruvate [17]. This result does not refute lactate oxidation in other settings, but it prevents a simple inference that mitochondrial lactate or pyruvate entry is sufficient for ATP production, respiratory quality, or biogenesis.

Table 1. Evidence-coded primary literature matrix.

Study	System	Perturbation	Observed evidence	Interpretive limit
Hashimoto 2007 [1]	L6 skeletal-muscle cells	10-20 mM lactate	Lactate increased ROS, MCT1, COX, PGC-1a expression, and NRF-2 DNA-binding activity.	Cell model with high lactate exposure; not a direct exercise or clinical test.
Zhang 2024 [2]	Skeletal-muscle MCT1 deletion in mice	Muscle-specific MCT1 loss	MCT1 deletion changed TCA substrate flux, increased oxidative fibers, respiration, mito. proteins, NAD+, SIRT1, and PGC-1a protein.	Genetic loss of lactate transport can produce compensatory adaptations, so directionality differs from acute lactate addition.
Park 2024 [3]	High-fat-diet and obese mouse skeletal muscle	Lactate with or without exercise context	Lactate increased fatty-acid oxidation, TCA-cycle activity, mito. respiration, and UCP3 under high-fat conditions.	Supraphysiological dosing and diet-induced obesity limit direct transfer to normal muscle adaptation.
Wilson 2007 [4]	C2C12 and primary myoblasts	72 h sodium pyruvate	Pyruvate increased mito. protein expression, basal respiration, and maximal oxidative capacity despite PGC-1a knockdown or PGC-1a-null background.	Myoblast setting and high pyruvate exposure require dose and differentiation-state validation.
Holloway 2010 [5]	C2C12 myotubes	72 h sodium pyruvate	Pyruvate increased cytochrome c oxidase subunit 1, cytochrome c, CD36, and HAD while suppressing PGC1alpha expression and changing substrate-use markers.	Respiratory-chain content and substrate-use quality diverged; content alone is not proof of improved mito. function.
Pilegaard 2003 [6]	Human vastus lateralis biopsies	Acute and trained-leg knee-extensor exercise	Exercise induced transient PGC-1a transcription and mRNA increases peaking early during recovery.	Transcriptional activation was observed, but lactate and pyruvate routes were not isolated.
Little 2010 [7]	Human skeletal muscle after low-volume HIT	Six training sessions over 2 weeks	Training increased exercise capacity, citrate synthase, COX activity, COX proteins, Tfam, SIRT1, and nuclear PGC-1a abundance.	Integrated training response prevents assignment to lactate, pyruvate, redox, or mechanical signals alone.
Wright 2007 [8]	Rat skeletal muscle after acute exercise	Exercise-induced PGC-1alpha activation	NRF binding and mito. mRNAs increased before total PGC-1a protein increased, consistent with activation or relocalization as an early route.	Animal exercise model; monocarboxylate specificity was not tested.
Rowe 2012 [9]	Muscle-specific PGC-1alpha knockout mice	Voluntary wheel running	Exercise capacity and exercise-induced mito. biogenesis were retained despite muscle PGC-1a loss.	Compensation by related coactivators or developmental context remains possible.
Park 2021 [10]	Mouse hippocampus	High-intensity exercise, lactate injection, UK5099	Blood lactate or lactate injection increased hippocampal PGC-1a and mtDNA copy number; UK5099 attenuated lactate and exercise effects.	Brain result does not establish the same route in skeletal muscle.
Wu 2026 [11]	Ovarian cells	HSP90alpha lactylation mechanism	HSP90alpha lactylation promoted PGC1alpha/LRPGC1 nuclear translocation, NRF1/2 target expression, mito. mass expansion, and estrogen biosynthesis.	Tissue and steroidogenic context should be treated as mechanism-generating evidence for muscle hypotheses.
Wu 2026 [12]	Granulosa cells and FSH-treated mice	FSH-driven lactate metabolism, P300/CBP-dependent H4K5 lactylation, HDAC4, and PGC-1alpha acetylation-state perturbation	FSH increased intracellular lactate and H4K5 lactylation; the H4K5la/HDAC4 axis deacetylated PGC-1a and supported NRF1/2 target expression, TFAM/TFB regulators, mito. biogenesis, follicular growth, and estradiol production.	Reproductive granulosa-cell context; it strengthens lactylation-to-PGC-1 routing as a comparator but does not establish the same mechanism in skeletal muscle.
Cluntun 2024 [13]	Mouse heart, isolated mitochondria, and adult cardiomyocytes	Mpc1 deletion, MCT1 loss/inhibition, isotope tracing, and cardiac stress	Lactate carbon entered TCA metabolites despite impaired mito. pyruvate carrier function; mito. lactate oxidation depended on MCT1 and supported respiration and stress resilience.	Cardiac tissue and carbohydrate oxidation study; it supports lactate transport/redox routing but does not directly test biogenesis transcription.
Cai 2023 [14]	Cancer cell lines, proliferating primary cells, primary T cells, and isolated mitochondria	L- and D-lactate exposure with metabolism-independent ETC assays	Lactate entered the mito. matrix and activated ETC-linked oxidative phosphorylation even when lactate metabolism was bypassed by D-lactate.	The study tested acute bioenergetic signaling rather than mito. biogenesis and was not performed in skeletal muscle.

Study	System	Perturbation	Observed evidence	Interpretive limit
Gomez-Cabrera 2008 [15]	Humans and rats undergoing endurance training	Oral vitamin C during training	Vitamin C attenuated endurance-performance adaptation and reduced training-associated skeletal-muscle mito.-biogenesis markers in humans and rats.	Redox buffering was tested in an integrated exercise setting; the study does not isolate lactate, pyruvate, or transporter directionality.
Sharma 2019 [16]	Skeletal-muscle-specific mitochondrial pyruvate carrier deletion in mice	Muscle Mpc1 deletion with metabolic flux and whole-body phenotyping	Impaired muscle mito. pyruvate uptake diverted pyruvate into circulating lactate, increased Cori cycling, disinhibited fatty-acid oxidation, increased energy expenditure, and reduced adiposity while preserving muscle mass and strength.	Chronic genetic deletion may include developmental or compensatory adaptations and does not directly measure PGC-1-family routing.
Horikoshi 2024 [17]	Primary hepatocytes and skeletal-muscle cell line live-cell imaging	Lactate overload with genetically encoded lactate, pyruvate, and ATP indicators	Skeletal-muscle cells showed increased lactate and pyruvate in cytoplasm and mito. matrix, but mito. ATP remained unaffected.	Lactate overload in cultured cells is a compartmental falsifier rather than a biogenesis experiment.

Ordinal evidence analysis

The evidence-code totals were highest for test-discrimination value, functional metabolic output, mitochondrial content, and PGC-1-family routing. Axis totals were: test discrimination 28, functional metabolic output 27, mitochondrial content 22, PGC-1-family route 14, redox signal 14, lactate transport 13, and PGC-1alpha-independent branch 8. Positive codes occurred in 9 of 17 studies for lactate transport, 11 of 17 for redox signaling, 9 of 17 for PGC-1-family routing, 5 of 17 for the PGC-1alpha-independent branch, 13 of 17 for mitochondrial content, and all 17 for functional output and test discrimination. Three studies carried negative PGC-1-family-route codes; those counterexamples were preserved because they define the proposed route separation rather than weakening it by default. Lactate-transport support came from direct transport or MCT-sensitive designs [1,2,10,13] plus indirect support from tissue contexts where lactate was experimentally administered, accumulated, or imaged [3,11,12,14,17]. PGC-1alpha-independent support was concentrated in pyruvate, MPC, and knockout studies [4,5,9,16]. No single study carried the full model. Leave-one-study totals ranged from 115 to 122 compared with 126 for the complete evidence set, retaining 91.3% to 96.8% of the total ordinal support after any single study was removed. Spearman-style correlations between the complete axis ranking and each leave-one-study ranking ranged from 0.908 to 1.000 (mean 0.968), showing that the ordering of the mechanistic axes was also stable rather than merely the grand total. A fixed-seed, 10,000-iteration nonparametric study-resampling analysis then sampled 17 evidence rows with replacement and recalculated every axis total. Figure 2 reports the resulting 2.5th and 97.5th percentiles as descriptive evidence-set stability intervals, not effect-size confidence intervals. The qualitative conclusion therefore did not depend on one evidence row: the strongest remaining support still separated lactate-linked routing from pyruvate-linked PGC-1alpha-independent content or partitioning signals.

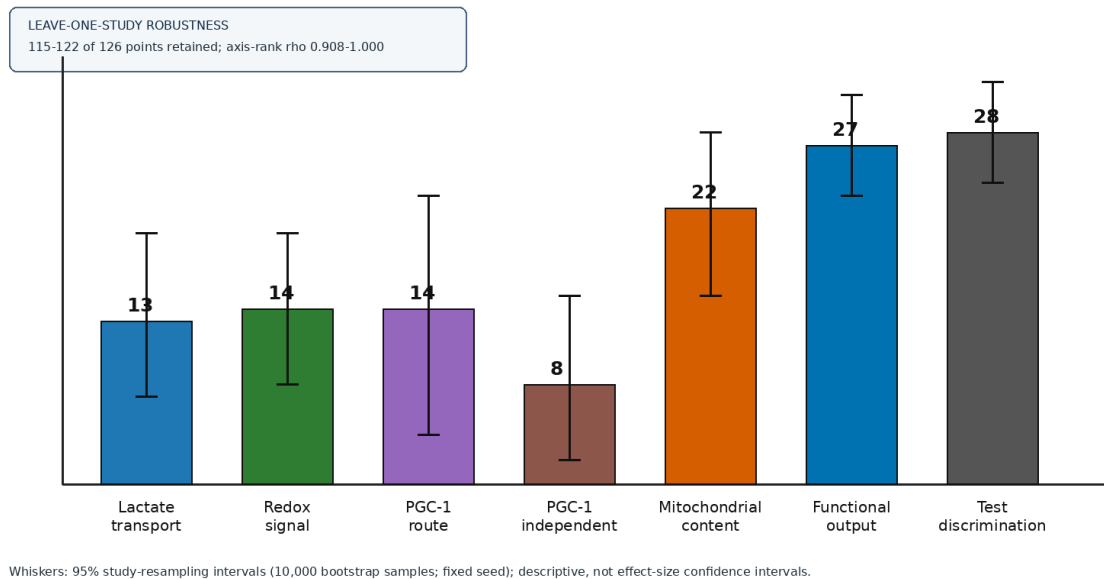


Figure 2. Evidence-code totals by mechanistic axis with leave-one-study robustness and 95% study-resampling intervals from 10,000 fixed-seed bootstrap samples. Intervals describe evidence-set stability, not pooled effect sizes.

Discussion

Figure 3 presents the resulting conceptual model. The diagram is labeled conceptual because it integrates observed links from heterogeneous primary studies into a testable mechanism rather than reporting a single experiment. Solid arrows represent evidence-supported relationships: lactate exposure, accumulation, transport context, or mitochondrial import can affect MCT-linked signaling, mitochondrial lactate oxidation, ETC-linked signaling, lactylation-linked PGC-1 regulation, and PGC-1-family routing [1-3,10-14]. Pyruvate can increase mitochondrial content or respiratory-chain markers without proportional PGC-1alpha induction [4,5]. Dashed arrows represent hypotheses requiring direct discriminant experiments.

The model's main contribution is a stricter routing framework rather than a claim that lactate signals or that PGC-1alpha regulates mitochondrial adaptation. Its novel unit of analysis is endpoint decoupling: transport, nuclear routing, organelle content, respiratory quality, and whole-body carbon partitioning are treated as separable outcomes that can be experimentally crossed rather than as interchangeable synonyms for biogenesis. This converts a broad pathway narrative into a falsifiable perturbation matrix. Lactate-biased mitochondrial adaptation is predicted to be most vulnerable to transport blockade, redox buffering, mitochondrial lactate-oxidation disruption, ETC-signaling separation with nonmetabolized lactate analogs, and lactylation-site perturbation when the relevant tissue expresses the required machinery. Pyruvate-biased adaptation is predicted to preserve selected mitochondrial-content or carbon-partitioning outputs under PGC-1alpha loss or MPC perturbation, while still requiring separate tests of respiratory quality, ATP yield, substrate preference, stress tolerance, and mitophagic competence.

This distinction has practical value for experimental design. A study measuring only mtDNA copy number or OXPHOS protein abundance may classify both lactate and pyruvate as mitochondrial-biogenesis stimuli, even if routes and functional consequences differ. A decisive design should include matched lactate and pyruvate dosing, D-lactate or equivalent nonmetabolized lactate controls, LDH-direction perturbation, MCT inhibition or MCT1 loss, mitochondrial pyruvate-carrier perturbation, redox buffering, PGC-1alpha perturbation, and separate readouts for mtDNA copy number, OXPHOS protein abundance, oxygen consumption, isotope-resolved lactate and pyruvate carbon entry, substrate preference, mitochondrial ATP, membrane potential, ROS, stress tolerance, and mitophagy. Such a design would test whether transport-sensitive PGC-1 routing, direct mitochondrial lactate oxidation, ETC-linked lactate sensing, MPC-gated pyruvate partitioning, and PGC-1alpha-independent content changes are separable within the same cellular background.

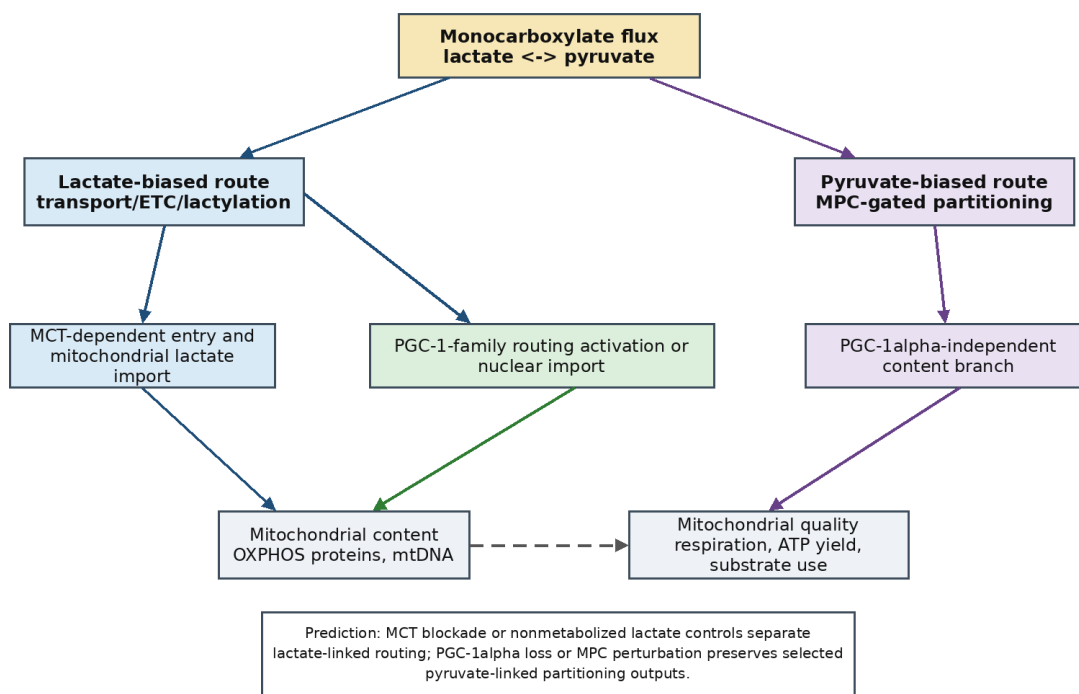


Figure 3. Concept-summary diagram. Solid arrows indicate evidence-supported links from included studies; dashed arrows indicate hypotheses requiring direct perturbation tests.

Decisive experiment and preregisterable predictions

A decisive first experiment would use donor-matched human primary myotubes or ex vivo skeletal-muscle preparations in a factorial design. Carbon source would be randomized between uniformly labeled lactate and pyruvate at matched carbon exposure; MCT1 would be intact or acutely inhibited; PGC-1 α would be intact or attenuated; and a parallel MPC perturbation arm would distinguish mitochondrial pyruvate entry from extracellular monocarboxylate transport. Vehicle, osmolality, pH, exposure time, differentiation state, and substrate concentration would be matched. The primary mechanistic endpoints would be TCA-cycle isotopologue labeling, mitochondrial oxygen consumption, ATP production, and nuclear PGC-1-family abundance measured in the same samples. Secondary endpoints would include NAD⁺/NADH, SIRT1 activity, mtDNA copy number, OXPHOS-protein synthesis, membrane potential, ROS, stress tolerance, and mitophagy flux.

Three directional predictions would be preregistered. First, MCT1 inhibition should preferentially suppress lactate-linked carbon entry or signaling while leaving an MPC-dependent pyruvate response distinguishable. Second, PGC-1 α attenuation should reduce the nuclear-routing signature more strongly than selected pyruvate-linked content or partitioning outputs. Third, any rise in mtDNA or OXPHOS proteins without concordant respiration, ATP yield, or stress tolerance would count as content-quality dissociation rather than successful biogenesis. The two-route model would be rejected if matched lactate and pyruvate exposures produced indistinguishable perturbation-response surfaces across MCT1, MPC, PGC-1 α , redox, content, and respiratory-quality endpoints. This design turns the synthesis into an explicit decision experiment rather than an open-ended pathway narrative.

Several falsifiers follow directly. If MCT blockade suppresses both lactate- and pyruvate-induced content changes to the same degree, then the transport-specific lactate branch would be weakened. If PGC-1 α loss eliminates all pyruvate-induced mitochondrial-content markers, then the independence branch would be falsified. If MPC perturbation produces no shift in lactate release, Cori cycling, TCA entry, fatty-acid oxidation, or mitochondrial readouts, then the pyruvate-partitioning gate would be unnecessary. If nonmetabolized lactate changes acute ETC activity but never changes PGC-1-family routing or mitochondrial-content endpoints, then acute lactate bioenergetic sensing should be separated from biogenesis signaling. If content, mitochondrial ATP, and respiratory quality always move together under matched lactate and pyruvate exposure, then the content-quality distinction would be unnecessary. If mitochondrial pyruvate-carrier impairment abolishes lactate carbon entry to TCA intermediates in the presence of intact MCT1, then the direct mitochondrial-lactate-import comparator would not transfer beyond the cardiac evidence base. Conversely, observation of lactate-sensitive PGC-1-family nuclear routing with pyruvate-preserved content under PGC-1 α knockdown would support the two-route model.

Table 2. Claim-boundary and falsification matrix.

Proposed control layer	Discriminating perturbation	Anchoring evidence	Prespecified falsifier
Lactate transport or import	Labeled lactate with acute MCT1 inhibition or loss	MCT-sensitive skeletal muscle, hippocampus, and cardiac import [2,10,13]	MCT1 perturbation changes neither lactate entry nor downstream routing
Metabolism-independent ETC sensing	D-lactate or equivalent nonmetabolized control with ETC readouts	Metabolism-independent ETC activation [14]	The signal disappears whenever lactate carbon oxidation is prevented
PGC-1-family nuclear routing	PGC-1 α attenuation plus localization and target-expression assays	Transcription, nuclear routing, and lactylation comparators [1,6-8,11,12]	Nuclear-routing and target signatures persist unchanged after PGC-1-family attenuation
Pyruvate-linked content branch	Matched pyruvate under PGC-1 α loss and parallel MPC perturbation	PGC-1 α -independent or MPC-gated findings [4,5,9,16]	PGC-1 α loss eliminates every pyruvate-linked content or partitioning output
Content-quality dissociation	Measure mtDNA and OXPHOS proteins with respiration, ATP, and stress tolerance in the same samples	Content, substrate-use, and ATP counterexamples [5,17]	Content and functional-quality endpoints remain obligatorily concordant

Limitations

The synthesis is qualitative and hypothesis-generating. The ordinal codes are transparent but not equivalent to effect sizes, and no formal meta-analysis was applied. The certainty notes should be treated as a lightweight transfer assessment rather than a replacement for a full risk-of-bias instrument. Included studies span cell lines, mouse skeletal muscle, human exercise biopsies, rat exercise, mouse hippocampus, ovarian cells, granulosa cells, mouse heart, isolated mitochondria, adult cardiomyocytes, hepatocytes, primary T cells, cancer cells, and live-cell imaging in cultured muscle cells. Therefore, tissue transfer is a hypothesis rather than an observed fact. Several lactate and pyruvate exposures were supraphysiological or experimentally artificial, and dose-response work remains necessary. The two 2026 lactylation mechanisms provide valuable evidence for lactate-linked PGC-1-family routing, but their ovarian and granulosa-cell contexts should not be generalized to skeletal muscle without direct testing [11,12]. The cardiac lactate-import study strongly supports MCT1-dependent mitochondrial lactate oxidation, but it does not show that this mechanism drives skeletal-muscle biogenesis [13]. The lactate-ETC study supports metabolism-independent lactate sensing but does not establish biogenesis or skeletal-muscle transfer [14]. The vitamin C exercise trial supports redox-sensitive adaptation but does not establish lactate as the upstream signal [15]. The MPC-deletion and live-cell-imaging studies add valuable discriminators, but the former is a chronic genetic mouse model and the latter is a lactate-overload cell system rather than an adult-muscle training experiment [16,17].

Conclusions

Available primary evidence supports a bounded monocarboxylate-routing hypothesis. Lactate and pyruvate can converge on mitochondrial adaptation, but the routes are not interchangeable. Lactate-biased conditions preferentially implicate transport context, mitochondrial lactate oxidation, metabolism-independent ETC-linked sensing, redox or lactylation signaling, and PGC-1-family routing. Pyruvate-biased conditions can increase mitochondrial content, respiratory-chain abundance, or systemic carbon partitioning even when PGC-1 α expression is reduced or absent. The highest-value next step is a single controlled perturbation matrix that tests lactate, pyruvate, nonmetabolized lactate controls, MCTs, LDH directionality, mitochondrial pyruvate-carrier status, redox buffering, and PGC-1 α status against separate content, ATP-yield, and quality endpoints.

Data and code availability

All data and code required to reproduce the evidence table, ordinal analysis, and figures are included in the publication bundle. Primary files are `analysis.py`, `evidence_table.csv`, `source_data.json`, `analysis_results.json`, `paper_meta.json`, and `render_publication.py`. The manuscript was rendered to PDF and DOCX with the bundle-local renderer using the repository paper-rendering environment, and page images plus a contact sheet were generated with Ghostscript and PIL for visual QA.

Ethics statement

This study used public literature records and generated no new human-participant, animal, or identifiable patient data. No ethics-board review was required for the literature synthesis.

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Competing interests

The author reports no competing interests for this working paper.

Author contributions

Andrew Friedl: conceptualization, project direction, evidence review, methodology, supervision, writing review and editing, and approval of the manuscript for human review.

AI assistance

AI systems assisted with literature triage, evidence-table structuring, draft preparation, figure generation, formatting, and quality-control checks. AI systems were not authors. Andrew Friedl remains responsible for the submitted content.

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