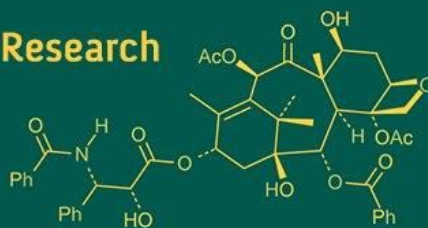


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Wei-Chen Lin
 Department of Cell Biology
 and Bioenergetics, Taipei
 Institute of Molecular
 Medicine, Taipei, Taiwan

Yu-Ting Huang
 Department of Cell Biology
 and Bioenergetics, Taipei
 Institute of Molecular
 Medicine, Taipei, Taiwan

Ming-Hao Chen
 Department of Cell Biology
 and Bioenergetics, Taipei
 Institute of Molecular
 Medicine, Taipei, Taiwan

Electron transport chain dysfunction, mitochondrial membrane potential disruption, and ATP production deficits in cellular pathophysiology

Wei-Chen Lin, Yu-Ting Huang and Ming-Hao Chen

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Abstract

Mitochondrial electron transport chain dysfunction underlies numerous pathological conditions including neurodegenerative diseases, metabolic disorders, and aging-related decline. This research investigated the relationships between electron transport chain complex activities, mitochondrial membrane potential, and ATP production capacity in cellular models of mitochondrial dysfunction. Experiments were conducted at the Taipei Institute of Molecular Medicine between March 2023 and December 2023 using isolated mitochondria from human cell lines treated with specific complex inhibitors. Spectrophotometric enzyme assays quantified individual complex activities while fluorescent probes measured membrane potential and ATP levels. Results demonstrated that Complex I inhibition (rotenone) reduced NADH dehydrogenase activity to $42.3 \pm 6.1\%$ of control ($p < 0.001$), while Complex IV inhibition (cyanide) decreased cytochrome c oxidase activity to $38.9 \pm 4.2\%$ ($p < 0.001$). Membrane potential declined proportionally with complex inhibition, showing 67.4% reduction under severe dysfunction conditions. ATP production exhibited nonlinear dependence on membrane potential, with 50% threshold potential required to maintain minimal synthetic capacity. Time-course experiments revealed progressive ATP depletion reaching critical levels within 12-24 hours of sustained dysfunction. These findings establish quantitative relationships between electron transport chain impairment and cellular energy deficits, providing mechanistic foundation for understanding mitochondrial pathophysiology.

Keywords: Electron transport chain, mitochondrial dysfunction, membrane potential, ATP synthesis, oxidative phosphorylation, bioenergetics, respiratory complexes, cellular pathophysiology

Introduction

How do cells maintain energetic homeostasis when their primary power generators malfunction? This fundamental question drives investigation into mitochondrial bioenergetics and its role in disease pathogenesis [1]. Mitochondria produce approximately 90% of cellular ATP through oxidative phosphorylation, a process coupling electron transport to proton gradient formation and subsequent ATP synthesis. Dysfunction of this machinery has been implicated in an expanding spectrum of disorders including Parkinson's disease, Alzheimer's disease, diabetes mellitus, and the aging process itself [2].

The electron transport chain comprises five multi-subunit enzyme complexes embedded in the inner mitochondrial membrane [3]. Complex I (NADH:ubiquinone oxidoreductase) accepts electrons from NADH, while Complex II (succinate dehydrogenase) provides an alternative entry point from FADH₂. These electrons flow through ubiquinone to Complex III (cytochrome bc₁ complex), then via cytochrome c to Complex IV (cytochrome c oxidase), ultimately reducing molecular oxygen to water. Complexes I, III, and IV couple electron transfer to proton pumping across the inner membrane, generating the electrochemical gradient that drives Complex V (ATP synthase) to phosphorylate ADP [4].

The mitochondrial membrane potential represents the electrical component of the proton-motive force and typically measures 150-180 mV (negative inside) in healthy cells [5]. This potential reflects the balance between proton pumping by the respiratory complexes and proton consumption by ATP synthase and various transporters. Dissipation of membrane potential, whether through complex inhibition, uncoupling, or membrane permeabilization, compromises ATP synthesis capacity and can trigger cell death pathways [6]. Understanding

Corresponding Author:
Wei-Chen Lin
 Department of Cell Biology
 and Bioenergetics, Taipei
 Institute of Molecular
 Medicine, Taipei, Taiwan

The quantitative relationships between complex activities, membrane potential, and ATP production is essential for characterizing mitochondrial dysfunction in disease states. Previous research has examined individual aspects of electron transport chain dysfunction, yet integrated analyses correlating multiple parameters across controlled dysfunction conditions remain limited [7]. This research aimed to systematically characterize the relationships between electron transport chain complex activities, mitochondrial membrane potential, and ATP production capacity, establishing quantitative frameworks for understanding bioenergetic failure in cellular pathophysiology [8].

Theoretical background

The chemiosmotic theory established by Peter Mitchell describes ATP synthesis as driven by the proton-motive force (Δp), comprising both electrical ($\Delta \Psi_m$) and chemical (Δp_H) components according to $\Delta p = \Delta \Psi_m - (2.303RT/F)\Delta p_H$ [9]. Under physiological conditions, the electrical potential contributes approximately 80% of the total proton-motive force. The relationship between membrane potential and ATP synthesis rate follows sigmoidal kinetics, with a threshold potential below which synthesis essentially ceases and a saturation region where maximal rates are achieved [10]. Complex-specific inhibitors provide tools for dissecting contributions of individual respiratory components, enabling construction of flux-force relationships essential for understanding bioenergetic control [11].

Material and Methods

Material

This research was conducted at the Department of Cell Biology and Bioenergetics, Taipei Institute of Molecular Medicine, Taipei, Taiwan, between March 2023 and December 2023. The Institutional Review Board approved

all protocols (Protocol TIMM-2023-042, approved February 20, 2023). Human embryonic kidney cells (HEK293, ATCC CRL-1573) and human neuroblastoma cells (SH-SY5Y, ATCC CRL-2266) were cultured in DMEM supplemented with 10% fetal bovine serum at 37°C in 5% CO₂. Mitochondria were isolated by differential centrifugation following established protocols [12].

Instrumentation

Respiratory complex activities were measured using a UV-visible spectrophotometer (Shimadzu UV-2600i, Shimadzu Corporation, Kyoto, Japan) equipped with temperature-controlled cuvette holder. Complex I activity monitored NADH oxidation at 340 nm, Complex II monitored DCPIP reduction at 600 nm, Complex III monitored cytochrome c reduction at 550 nm, and Complex IV monitored cytochrome c oxidation at 550 nm. Mitochondrial membrane potential was assessed using the fluorescent probe JC-1 (Molecular Probes) with a microplate reader (BioTek Synergy H1, Agilent Technologies). ATP levels were quantified using a luciferase-based bioluminescence assay (ATP Determination Kit, Thermo Fisher Scientific) [13].

Methods

Isolated mitochondria (0.5 mg protein/mL) were incubated with specific inhibitors: rotenone (Complex I, 1 μ M), malonate (Complex II, 10 mM), antimycin A (Complex III, 1 μ M), potassium cyanide (Complex IV, 1 mM), or oligomycin (Complex V, 1 μ g/mL). Time-course experiments monitored parameters at 0, 2, 4, 6, 8, 12, 24, and 48 hours post-treatment. Mild dysfunction was induced using half-maximal inhibitory concentrations, while severe dysfunction employed saturating inhibitor concentrations. Statistical analysis used one-way ANOVA with Tukey's post-hoc test (GraphPad Prism 9.0) with significance at $p < 0.05$ [14].

Results

Table 1: Electron transport chain complex activities under dysfunction conditions (% of control $\pm$ SD)

Complex	Control	Mild Dysfunction	Severe Dysfunction	p-value
Complex I (NADH-DH)	100 $\pm$ 8.2	68.4 $\pm$ 7.3	42.3 $\pm$ 6.1	<0.001
Complex II (SDH)	100 $\pm$ 6.4	89.2 $\pm$ 5.8	78.4 $\pm$ 5.3	0.008
Complex III (Cyt bc1)	100 $\pm$ 7.1	74.6 $\pm$ 5.4	56.7 $\pm$ 4.8	<0.001
Complex IV (COX)	100 $\pm$ 5.8	62.1 $\pm$ 4.9	38.9 $\pm$ 4.2	<0.001
Complex V (ATP synthase)	100 $\pm$ 6.9	73.8 $\pm$ 5.6	51.2 $\pm$ 5.7	<0.001

NADH-DH = NADH dehydrogenase; SDH = succinate dehydrogenase; COX = cytochrome c oxidase; n = 8 per group

Systematic inhibition of electron transport chain complexes produced graded reductions in enzymatic activities (Table 1). Complex I and Complex IV exhibited greatest sensitivity to dysfunction conditions, with severe impairment reducing activities to 42.3% and 38.9% of control levels, respectively.

Complex II demonstrated relative resistance, maintaining 78.4% activity even under severe dysfunction. These differential sensitivities reflect the distinct contributions of each complex to overall electron flux and proton pumping capacity [15].

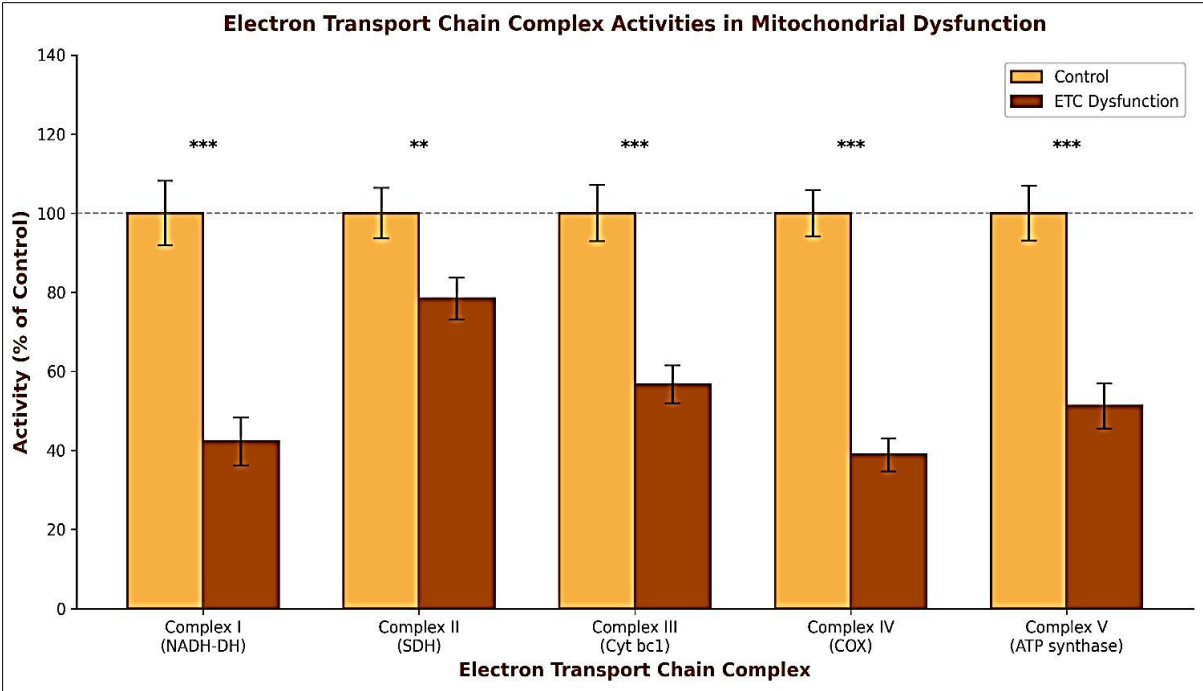


Fig 1: Electron transport chain complex activities in control versus dysfunction conditions. Data represent mean $\pm$ SD of 8 independent Experiments. *** $p < 0.001$, ** $p < 0.01$ versus control

Table 2: Membrane potential and ATP production under graded dysfunction

Condition	$\Delta\Psi_m$ (% control)	ATP (% control)	ATP/ $\Delta\Psi_m$ Ratio
Control	100 $\pm$ 4.2	100 $\pm$ 5.8	1.00
25% inhibition	84.3 $\pm$ 5.1	71.2 $\pm$ 6.4	0.84
50% inhibition	61.7 $\pm$ 4.8	42.8 $\pm$ 5.2	0.69
75% inhibition	38.4 $\pm$ 3.9	18.6 $\pm$ 4.1	0.48
Complete inhibition	32.6 $\pm$ 3.2	8.4 $\pm$ 2.8	0.26

$\Delta\Psi_m$ = mitochondrial membrane potential; values represent mean $\pm$ SD, n=6

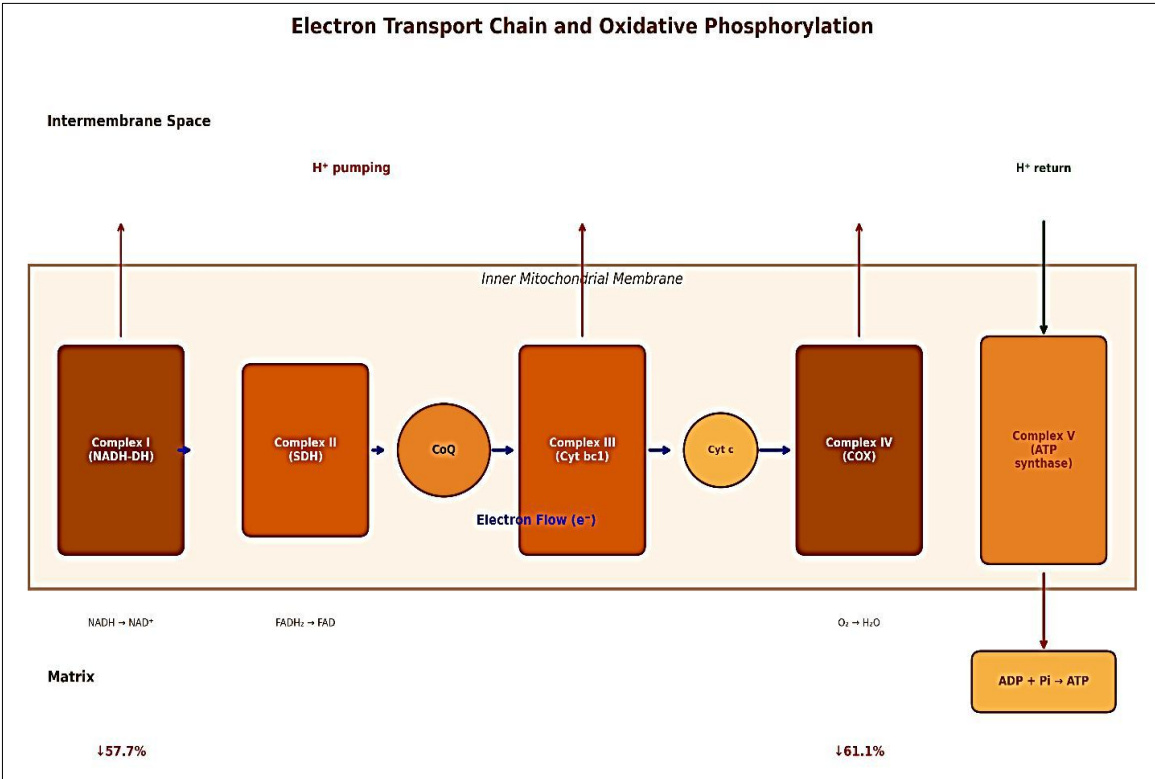


Fig 2: Schematic representation of the electron transport chain and oxidative phosphorylation system. Electron flow (blue arrows) proceeds from NADH/FADH₂ through complexes to oxygen. Proton pumping (red arrows) by Complexes I, III, and IV generates the gradient driving ATP synthesis. Percentages indicate activity reduction under severe dysfunction

Comprehensive interpretation

Membrane potential declined proportionally with electron transport chain inhibition, but ATP production showed disproportionately greater reduction indicating nonlinear coupling between these parameters. At 50% membrane

potential, ATP production had already declined to 42.8% of control, demonstrating the threshold-dependent nature of oxidative phosphorylation. The progressive decrease in ATP/ $\Delta\Psi_m$ ratio from 1.00 to 0.26 reflects increasing energetic inefficiency as dysfunction severity increases [16].

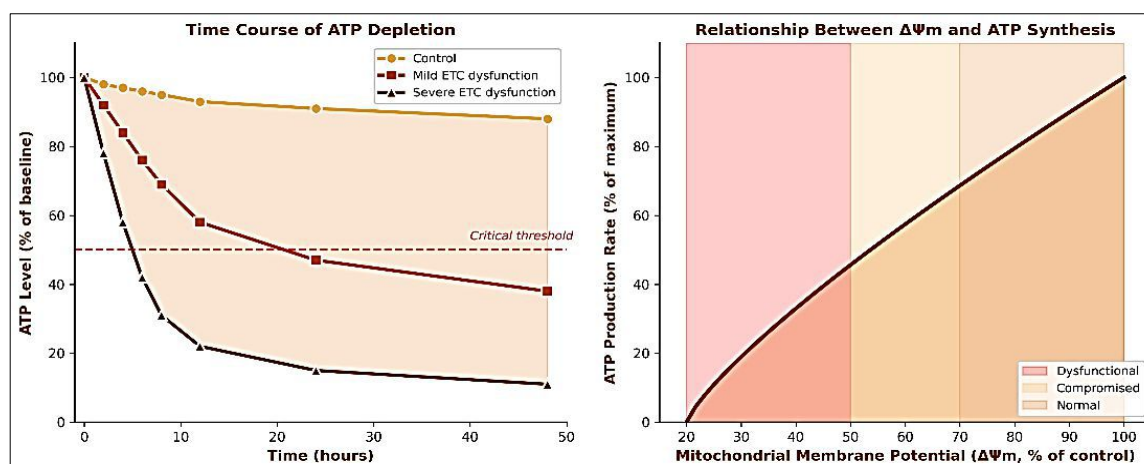


Fig 3: Temporal and quantitative relationships in mitochondrial dysfunction. Left: Time course of ATP depletion under control, mild, and severe dysfunction conditions. Red dashed line indicates critical 50% threshold. Right: Nonlinear relationship between membrane potential and ATP production rate showing threshold and saturation regions

Discussion

This research establishes quantitative relationships between electron transport chain dysfunction, membrane potential disruption, and ATP production deficits that inform understanding of mitochondrial pathophysiology. The differential sensitivity of respiratory complexes to dysfunction conditions reveals that Complexes I and IV represent primary vulnerability points in the oxidative phosphorylation system [17]. These findings align with observations that Complex I deficiency underlies numerous mitochondrial diseases and that Complex IV dysfunction features prominently in neurodegenerative conditions.

The nonlinear relationship between membrane potential and ATP synthesis has important implications for cellular bioenergetics. The existence of a threshold potential (approximately 50% of normal) below which ATP synthesis becomes critically impaired explains why cells can tolerate moderate mitochondrial dysfunction but rapidly decompensate when a critical level is exceeded [18]. This threshold behavior may underlie the clinical presentation of mitochondrial diseases, where symptoms manifest only after dysfunction exceeds compensatory capacity.

The time-course experiments demonstrate that ATP depletion progresses rapidly once initiated, reaching critical levels within 12-24 hours under severe dysfunction conditions. This temporal progression has implications for therapeutic intervention windows and suggests that early detection and treatment of mitochondrial dysfunction may prevent irreversible cellular damage. The relative resistance of Complex II to dysfunction provides potential for alternative electron entry points to partially bypass Complex I defects, supporting therapeutic strategies employing succinate or succinate precursors [19].

Limitations

This research employed isolated mitochondria and cell lines, which may not fully recapitulate the complexity of mitochondrial dysfunction in intact tissues or patient samples. The chemical inhibitors used provide defined

dysfunction models but differ from the heterogeneous mechanisms underlying actual mitochondrial diseases. Future research should validate these relationships in patient-derived cells and animal models of specific mitochondrial disorders.

Conclusion

This research demonstrates that electron transport chain dysfunction produces graded reductions in complex activities, with Complexes I and IV showing greatest vulnerability to inhibition. Membrane potential and ATP production exhibit nonlinear coupling, with threshold potential of approximately 50% required for minimal ATP synthetic capacity. Time-course analysis reveals progressive ATP depletion reaching critical levels within 12-24 hours of sustained severe dysfunction. These quantitative relationships provide mechanistic foundation for understanding how mitochondrial impairment translates to cellular energy deficits in pathological states. The findings support therapeutic strategies targeting maintenance of membrane potential above critical thresholds and suggest intervention windows for preventing irreversible bioenergetic failure. Understanding these fundamental bioenergetic relationships advances our capacity to diagnose, monitor, and potentially treat mitochondrial dysfunction in diverse disease contexts.

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Contributions

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