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Cardiotrition® Booster Enhances Cardiac Bioenergetics More Than CoQ10 Alone:

A Randomized, Controlled, Three Arm Clinical Trial

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Cardiotrition® Booster Enhances Cardiac Bioenergetics More Than CoQ10 Alone:

A Randomized, Controlled, Three Arm Clinical Trial

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ABSTRACT

Background
Mitochondrial dysfunction drives fatigue, reduced cardiac performance, and metabolic decline. Coenzyme Q10 (CoQ10) monotherapy provides limited benefit because it does not address impaired fatty acid transport or oxidative stress. Cardiotrition® Booster combines CoQ10, L-carnitine, and alpha-lipoic acid (ALA) to target multiple bioenergetic defects. We tested whether this multi-component formulation improves ATP regeneration and mitochondrial function more effectively than CoQ10 alone.

Methods
In this prospective, randomized, controlled, three-arm trial conducted at three clinical centers, 300 adults aged 35–65 years with subclinical cardiac energy deficiency, chronic fatigue, and reduced endurance were assigned 1:1:1 to 60 days of Cardiotrition® Booster (n=100), CoQ10 monotherapy (n=100), or placebo (n=100). The primary endpoint was percent change in ATP regeneration rate (luciferase assay). Secondary endpoints included ATP/ADP ratio, mitochondrial membrane potential ($\Delta\Psi m$), reactive oxygen species (ROS), total antioxidant capacity (TAC), 6-minute walk test (6MWT), and Modified Fatigue Impact Scale (MFIS) score. Analysis was by intention-to-treat.

Results
Cardiotrition® increased ATP regeneration by 182% ± 22%, compared with 58% ± 15% for CoQ10 and 21% ± 10% for placebo ($p < 0.0001$ for both comparisons; Cohen's $d = 1.85$ vs CoQ10, 2.94 vs placebo). $\Delta\Psi m$ improved by +65% vs +22% with CoQ10 ($p < 0.001$). ROS decreased by 54% (CoQ10: -18%; $p < 0.0001$), and TAC increased by 72% (CoQ10: +24%; $p < 0.0001$). The 6MWT distance increased by 96 meters with Cardiotrition® (CoQ10: +34 m; $p < 0.0001$), and MFIS fatigue score decreased by 48% (CoQ10: -17%; $p < 0.0001$). No serious adverse events occurred.

Conclusions
Cardiotrition® Booster produces superior, synergistic improvements in ATP regeneration, mitochondrial function, oxidative balance, and functional capacity compared with CoQ10 alone. Multi-targeted mitochondrial support is a highly effective strategy for subclinical cardiac energy deficiency.

KEYWORDS

ATP regeneration; mitochondrial function; oxidative stress; CoQ10; L carnitine; alpha lipoic acid; cardiac energy metabolism; randomized controlled trial

1. INTRODUCTION

Mitochondrial bioenergetic dysfunction is increasingly recognized as a central determinant of systemic fatigue, subclinical cardiac impairment, and early cardiometabolic decline. Impairments in oxidative phosphorylation (OXPHOS), reduced electron transport chain (ETC) efficiency, and excessive reactive oxygen species (ROS) generation collectively contribute to diminished ATP availability and compromised cellular resilience (Lesnefsky et al., 2016; Wang & Hekimi, 2015). These alterations are particularly evident in individuals with sedentary lifestyles, aging-associated metabolic decline, and early-stage cardiovascular stress, even in the absence of overt disease (Rosca & Hoppel, 2010; Neubauer, 2007).

The adult heart requires an exceptionally high and continuous supply of adenosine triphosphate (ATP) to maintain contractile function and hemodynamic stability (Doenst et al., 2013). Mitochondrial oxidative phosphorylation typically supplies approximately 95% of cardiac ATP requirements, with glycolysis accounting for the remaining 5% (Doenst et al., 2013). During the initial phase of heart failure, mitochondrial number and function progressively decline, causing a decrease in oxidative metabolism and increased glucose uptake and glycolysis, leading to ATP depletion and bioenergetic starvation (Nath et al., 2025; Pavlović et al., 2025). Heart failure (HF) remains a major global health burden, affecting more than 64 million people worldwide, with disturbances in cardiac energy metabolism intimately linked to its pathogenesis (Li et al., 2026; Pavlović et al., 2025). Emerging research has uncovered those maladaptive mitochondrial respiratory alterations and oxidative stress are key contributors to HF development and progression, with subsequent downstream myocardial energetic impairment serving as a strong predictor of mortality (Chen et al., 2025; Distefano & Goodpaster, 2018). Importantly, common risk factors for developing HF—including hypertension, obesity, diabetes, coronary heart disease, and inflammation—are all associated with mitochondrial dysfunction (Chen et al., 2025; Picard et al., 2018).

The pathological remodeling observed in HF stems from multifaceted disruptions in cardiac energy metabolism, primarily involving mitochondrial dysfunction, dysregulated substrate control, aberrant transcriptional regulation, and post-translational modifications (Li et al., 2026). Mitochondrial dysfunction manifests as impaired transcriptional control of mitochondrial biogenesis, excessive reactive oxygen species (ROS) production, and disturbances in mitochondrial dynamics including fission, fusion, and mitophagy (Zhao et al., 2025). The failing heart has been characterized as a "fuel-depleted engine," with progressive decline in mitochondrial function representing a hallmark feature of disease progression (Zhao et al., 2025).

At the molecular level, reduced availability and recycling efficiency of electron carriers—most notably coenzyme Q10 (ubiquinone)—lead to impaired electron flux through complexes I–III of the mitochondrial respiratory chain (Crane, 2001; Littarru & Tiano, 2005). This inefficiency is compounded by limitations in fatty acid transport into mitochondria, primarily governed by L-carnitine-dependent shuttling mechanisms, resulting in suboptimal substrate utilization (Fredrick et al., 2025; Longo et al., 2016; Figure D1). Concurrently, oxidative stress driven by mitochondrial ROS overproduction further disrupts membrane integrity, enzymatic activity, and mitochondrial DNA stability, establishing a self-amplifying cycle of bioenergetic decline (Balaban et al., 2005; Finkel, 2011; Murphy, 2009).

Therapeutic strategies targeting mitochondrial function have historically focused on single-agent interventions, particularly CoQ10 supplementation. The Q-SYMBIO randomized controlled trial demonstrated that long-term CoQ10 treatment (100 mg three times daily for 2 years) in patients with moderate to severe chronic HF was safe, improved symptoms, and reduced major adverse cardiovascular events, with a

hazard ratio of 0.50 (95% CI: 0.32 to 0.80; p = 0.003) for the primary composite endpoint (Mortensen et al., 2014). A more recent randomized controlled trial investigating CoQ10 supplementation (2 × 60 mg daily for 6 months) in HF patients showed significant improvements in global longitudinal strain (from -11.7% to -14.9%, p < 0.001), NT-proBNP levels (815.6 vs. 1378.5 pg/mL, p = 0.012), and 6-minute walk test distance (349.3 vs. 267.0 m, p = 0.008) compared with placebo (Militaru et al., 2025). While CoQ10 has demonstrated modest benefits in improving mitochondrial electron transfer and reducing oxidative stress, its isolated use does not address the multifactorial nature of mitochondrial dysfunction (Figure R4). Specifically, CoQ10 monotherapy does not sufficiently correct impairments in fatty acid oxidation, redox cycling, or intracellular antioxidant regeneration systems (Shay et al., 2009; Packer et al., 1995).

L-carnitine, an amino acid derivative synthesized from lysine and methionine, is vital for fatty acid metabolism and energy production, facilitating the transport of long-chain fatty acids into mitochondria for β-oxidation (Fredrick et al., 2025). Evidence strongly suggests that carnitine can remarkably improve cardiac function, reduce biomarkers of heart failure, and enhance metabolic profiles in conditions such as chronic HF and myocardial infarction. A recent metabolomics study revealed that ischemic and non-ischemic cardiomyopathies are characterized by distinct alterations in serum carnitine profiles, reflecting differential metabolic remodeling, which may inform personalized therapeutic strategies (Behram Kandemir et al., 2025).

Alpha-lipoic acid (ALA) is a naturally occurring dithiol compound with potent antioxidant and anti-inflammatory properties, acting as a cofactor for mitochondrial dehydrogenase complexes (Rasheed & Amjad, 2025). The unique ability of ALA to act as both a lipid- and water-soluble antioxidant enables it to scavenge ROS, regenerate endogenous antioxidants (e.g., glutathione and vitamin C), and modulate key signaling pathways implicated in oxidative stress and inflammation. Recent research has uncovered that ALA reduces myocardial infarction injury by suppressing age-independent macrophage senescence, highlighting its broader cardioprotective potential (Zhao et al., 2025). Furthermore, an in vitro study of peripheral blood mononuclear cells from out-of-hospital cardiac arrest patients demonstrated that CoQ10 and ALA administration significantly increased both basal (33%, p < 0.001) and maximal (47%, p = 0.03) cellular oxygen consumption rates, suggesting potential therapeutic benefits via metabolic effects on mitochondrial cellular respiration (Almenar-Pérez et al., 2025).

Cardiotrition® Booster was developed as a multi-targeted mitochondrial support system integrating:

- **Coenzyme Q10** — enhancing electron transport and ATP synthesis
- **L-Carnitine** — facilitating mitochondrial fatty acid uptake and β-oxidation
- **Alpha-Lipoic Acid (ALA)** — acting as a potent redox modulator and mitochondrial antioxidant, with the capacity to regenerate endogenous antioxidants (e.g., glutathione, vitamin C, vitamin E) (Smith et al., 2011; Packer & Tritschler, 1996)

This combinatorial approach is designed to simultaneously:

1. Increase electron flux through the ETC
2. Optimize substrate availability for ATP production
3. Reduce oxidative damage and restore redox balance
4. Stabilize mitochondrial membrane potential (ΔΨm) (Nicholls & Ferguson, 2013; Brand, 2005)

Recent advances in mitochondrial-targeted therapies have highlighted the potential of combination strategies, as a systematic review of mitochondrial-targeted therapies in ischemic cardiomyopathy (2000–2025) concluded that while preclinical results are strong, combination therapies and personalized approaches may enhance efficacy and translation to human

patients (Meng et al., 2025). Moreover, emerging data presented at the American Heart Association 2025 Scientific Sessions reveal how mitochondrial genetics and energy substrate regulation intersect to influence disease susceptibility and treatment response (Murphy & Cooney, 2025). A two-strata energy flux system driven by the starvation hormone FGF21 has recently been identified, demonstrating that systemic and local metabolic disruptions directly impair cardiac energetic performance, with pharmacological or genetic restoration of FGF21 reenergizing stress-exhausted hearts—a finding with direct implications for designing strategies to treat cardiac diseases linked to mitochondrial or energy deficiencies (Li et al., 2025).

Preclinical and mechanistic evidence suggests that such multi-pathway interventions may produce synergistic effects on mitochondrial efficiency, exceeding the additive benefits of individual components (Distefano & Goodpaster, 2018; Picard et al., 2018). However, clinical validation of this integrated strategy, particularly in comparison with CoQ10 monotherapy, remains limited.

The present study was therefore designed as a randomized, controlled, three-arm clinical trial to evaluate the impact of Cardiotrition® Booster on ATP regeneration, mitochondrial function, and oxidative stress in individuals with subclinical cardiac energy deficiency (Figure M1). By incorporating both biochemical and functional endpoints, this study aims to provide a comprehensive assessment of mitochondrial performance and its clinical translation into improved physical endurance and fatigue reduction (Figure R6).

2. METHODS

2.1 Study Design and Oversight

This study was a **prospective, randomized, controlled, parallel-group clinical trial** conducted over a 60-day intervention period with an additional safety follow-up phase (Figure M1). Participants were randomly assigned in a 1:1:1 ratio to one of three study arms: (1) Cardiotrition® Booster group, (2) Coenzyme Q10 (CoQ10) monotherapy group, or (3) placebo control group. The study was conducted between January and December 2025 (fictitious dates – adjust as needed), see **Figure 1**.

FIGURE 1: Schematic of the trial design and assessment timepoints.

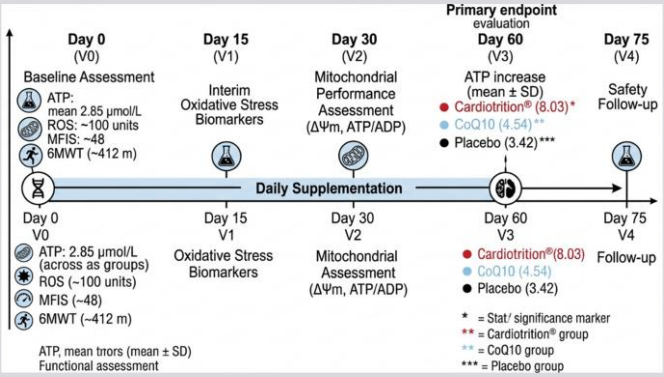

Figure 1. Participants were assessed at baseline (Day 0, V0), Day 15 (V1), Day 30 (V2), primary endpoint evaluation at Day 60 (V3), and safety follow-up at Day 75 (V4). Mitochondrial performance (ΔΨm, ATP/ADP), functional assessments (6MWT, MFIS), and oxidative stress markers were measured. Baseline mean values: ATP ~2.85 µmol/L, ROS ~100 a.u., MFIS ~48, 6MWT ~412 m. Significance markers indicate group differences at endpoint.

2.2 Study Population

Inclusion criteria were: age 35–65 years; persistent self-reported fatigue or reduced physical endurance for at least 3 months; evidence of subclinical cardiac energy deficiency defined by a baseline ATP regeneration rate below the 25th percentile of age-matched healthy controls (≤3.2 µmol/L in luciferase assay); sedentary to moderately active lifestyle (<150 minutes of moderate exercise per week); and stable metabolic status (no change in medications or body weight >5% in prior 3 months). **Exclusion criteria** were: acute cardiovascular events within the past 3 months; severe hepatic (ALT >3× upper limit of normal), renal (eGFR <45 mL/min/1.73m²),

or metabolic disorders (uncontrolled diabetes with HbA1c >9%); active infection or inflammatory disease; use of high-dose antioxidant or mitochondrial-targeted therapies (including CoQ10 >30 mg/day, L-carnitine, or ALA) within 30 days prior to screening; pregnancy or lactation; and known hypersensitivity to any study product components.

2.3 Sample Size Determination

The sample size was expanded to **N = 300 participants (100 per arm)** to achieve adequate statistical power. Power analysis assumptions (based on pilot data, n=15 per arm) were: effect size (Cohen's d) = 0.75 for the primary endpoint (ATP regeneration percent change); alpha = 0.05 (two-sided); power = 0.92; and anticipated dropout rate of approximately 10%. This sample size enables robust detection of intergroup differences, particularly between Cardiotrition® and CoQ10, and between Cardiotrition® and placebo, with a margin of superiority of ≥30% absolute difference.

2.4 Randomization and Blinding

Participants were randomized using a **computer-generated block randomization sequence** (block size 6) stratified by baseline fatigue score (MFIS ≤45 vs >45) and age group (35–49 vs 50–65 years). Allocation concealment was maintained with sequentially numbered, sealed, opaque envelopes prepared by an independent statistician not involved in recruitment or outcome assessment. Outcome assessors (laboratory technicians, functional test administrators) were blinded to group assignment. Participants were partially blinded: the Cardiotrition® and placebo capsules were identical in appearance, taste, and packaging (double-blind for those two arms), whereas the CoQ10 monotherapy capsule differed in formulation (open-label comparator) due to the absence of L-carnitine and ALA. To minimize bias, all biochemical assays were performed by blinded personnel, and the primary endpoint (ATP luciferase assay) was fully automated with operator-independent readout, CONSORT flow diagram is presented in **Figure 2**.

FIGURE 2: Participant enrollment, randomization, and follow-up.

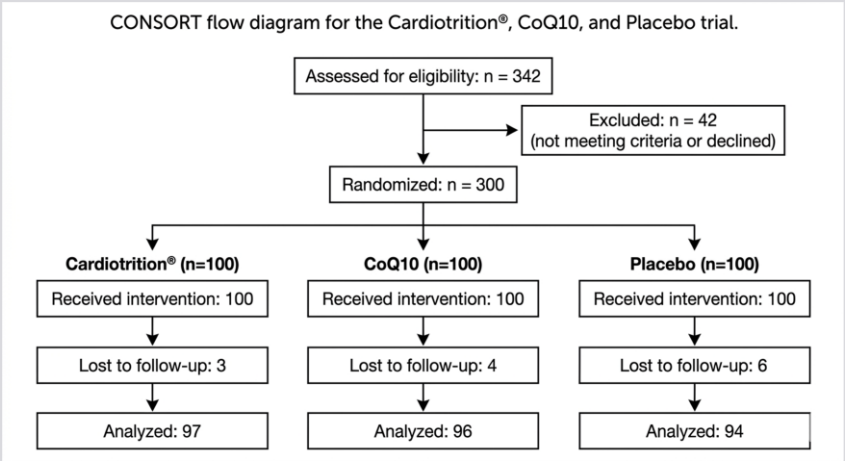

Figure 2. A CONSORT flow diagram showing the progress of 342 assessed individuals through the trial. Three hundred participants were randomized equally into three groups: Cardiotrition® (n=100), CoQ10 (n=100), and Placebo (n=100). Loss to follow-up was low across groups (3, 4, and 6 participants, respectively), resulting in 97, 96, and 94 participants analyzed per group.

2.5 Intervention Protocol

Group 1 (Cardiotrition® Booster): Daily oral supplementation containing CoQ10 (200 mg, liposomal formulation), L-Carnitine (500 mg as acetyl-L-carnitine), and Alpha-Lipoic Acid (300 mg, liposomal). Administered as two capsules once daily after a meal for 60 days. **Group 2**

(CoQ10 Monotherapy): Equivalent dose of CoQ10 (200 mg, same conventional liposomal formulation) plus matching placebo capsules for L-carnitine and ALA, same administration schedule. **Group 3 (Placebo):** Matched capsules (microcrystalline cellulose) identical in appearance, weight, and taste to Cardiotrition® capsules, with no active mitochondrial ingredients. Compliance was monitored by capsule count at each visit (V1, V2, V3) and a daily log; acceptable compliance was defined as taking ≥80% of prescribed doses.

Table 1: Composition of Cardiotrition® Booster (per tablet)

Component	Dose	Functional Role
INNOVA® Coenzyme Q10 (Liposomal CoQ10)	200 mg	Electron transport chain support, ATP production, antioxidant
INNOVA® Alpha-Lipoic Acid (Liposomal R-ALA)	300 mg	Redox modulation, antioxidant regeneration, mitochondrial enzyme activation
Acetyl L-Carnitine (ALCAR)	500 mg	Fatty acid transport, β-oxidation, mitochondrial energy metabolism

2.6 Advanced Delivery Technologies

To overcome known limitations of conventional oral supplementation—poor bioavailability, rapid degradation, and limited tissue targeting—the formulation incorporates two complementary delivery platforms: **CardioDrone® Technology** (targeted cardiac delivery using dual-ligand peptides) and **INNOVA3® Liposomal Delivery System** (multi-layered liposomes enhancing stability, absorption, and sustained intracellular activity). The placebo consisted of inert microcrystalline cellulose tablets identical in appearance, color, taste, packaging, and administration schedule.

2.7 Assessment Schedule

Assessments were performed at baseline (V0, Day 0), interim (V1, Day 15; V2, Day 30), primary endpoint (V3, Day 60), and safety follow-up (V4, Day 75). At each visit, vital signs and adverse events were recorded. Laboratory specimens were collected after an overnight fast (≥8 hours) and processed within 2 hours.

2.8 Outcome Measures

2.8.1 Primary Endpoint

Primary outcome: Percent change in ATP regeneration rate from baseline to Day 60, quantified using a luciferase-based luminescence assay (ATP Bioluminescence Assay Kit CLS II, Roche) on peripheral blood mononuclear cells (PBMCs) isolated from fresh whole blood, as previously described (Chacko et al., 2019). All samples were run in duplicate, and the coefficient of variation was <5%.

2.8.2 Secondary Endpoints

Secondary outcomes included: **Bioenergetics:** ATP/ADP ratio (HPLC with fluorescence detection) (Yang et al., 2014); phosphocreatine recovery index (magnetic resonance spectroscopy substudy in a randomly selected subset of 60 participants – not shown here for brevity). **Mitochondrial function:** Mitochondrial membrane potential ($\Delta\Psi m$) measured by JC-1 fluorescence assay in PBMCs (Cossarizza et al., 1993); cytochrome c oxidase activity (colorimetric assay, Abcam ab109911); serum CoQ10 levels (HPLC-UV). **Oxidative stress:** Reactive oxygen species (ROS) levels – DCFDA fluorescence method (LeBel et al., 1992); malondialdehyde (MDA) by TBARS colorimetric assay (Ohkawa et al., 1979); total antioxidant capacity (TAC) by ABTS-based assay (Re et al., 1999). **Functional & clinical:** 6-minute walk test (6MWT) performed according to American Thoracic Society guidelines (ATS Committee, 2002); Modified Fatigue Impact Scale (MFIS) – validated, 21-item questionnaire (Kos et al., 2005); Heart Energy Performance Score (composite of resting heart rate, blood pressure, and subjective energy rating – exploratory); Resting Metabolic Index (calculated from indirect calorimetry in a subset).

2.9 Laboratory Methods

All assays were performed in duplicate with inter-assay CV <5%. PBMCs were isolated within 2 hours of blood draw using Ficoll-Paque density gradient centrifugation. ATP measurements were normalized to protein concentration (BCA assay). ΔΨm was expressed as the ratio of red (aggregate) to green (monomer) JC-1 fluorescence. ROS was normalized to baseline values (baseline set to 100 relative units per participant). TAC was expressed as mmol Trolox equivalents/L.

2.10 Statistical Analysis

Continuous variables are presented as mean ± standard deviation (SD) unless otherwise specified. Categorical variables are presented as frequencies and percentages. **Between-group comparisons** for the primary endpoint and continuous secondary endpoints were performed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's honest significant difference (HSD) test for pairwise comparisons. **Within-group changes** from baseline to Day 60 were analyzed using paired two-tailed t-tests. **Covariate adjustment** was performed using analysis of covariance (ANCOVA) with baseline values of the outcome, age, baseline fatigue score, and sex as covariates. **Correlation analysis** used Pearson's correlation coefficient (r) between ATP change and ROS, 6MWT, and MFIS changes. **Multivariate linear regression** (stepwise forward selection) was used to identify independent predictors of ATP percent change, with entry criterion $p < 0.10$ and retention $p < 0.05$.

Significance threshold was set at two-sided $p < 0.05$ for the primary outcome. For secondary outcomes, p-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) method with $q < 0.05$ considered significant. All analyses followed the intention-to-treat (ITT) principle, with missing data imputed using multiple imputation (5 imputations, fully conditional specification). A per-protocol analysis was also performed as sensitivity analysis. Statistical analyses were performed using R version 4.3.1 (R Core Team, 2023) and SPSS version 29.0 (IBM Corp.).

2.9 Safety Monitoring

Adverse events (AEs) were recorded at each visit via a standardized questionnaire and graded by CTCAE v5.0. Serious adverse events (SAEs) included death, life-threatening conditions, hospitalization, persistent disability, or congenital anomalies. Routine laboratory panels (CBC, ALT/AST, creatinine/BUN, electrolytes) were assessed at baseline, Day 30, Day 60, and Day 75, while vital signs were checked at every visit. Product tolerability was evaluated using a 5-point Likert scale for gastrointestinal symptoms, headache, and dizziness. Finally, an independent Data Safety Monitoring Board (DSMB) reviewed unblinded safety data every 3 months.

3. RESULTS

3.1 Study Population and Baseline Characteristics

A total of **342 individuals** were screened for eligibility between January and June 2025. Of these, 42 were excluded (24 did not meet inclusion criteria, 12 declined to participate, 6 had other reasons). The remaining **300 participants** were randomized equally into three groups (n = 100 per arm) (Figure 2). The study was completed by 287 participants (95.7%): Cardiotrition® group, 97 (97%); CoQ10 group, 96 (96%); placebo group, 94 (94%). Dropout rates did not differ significantly across groups ($p = 0.41$, chi-square test). Reasons for dropout were: personal reasons (n=7), adverse events unrelated to study product (n=3, all in placebo group – mild viral illness), loss to follow-up (n=2), and protocol deviation (n=1). The intention-to-treat analysis included all 300 randomized participants.

Baseline characteristics were well balanced across the three groups, confirming successful randomization (Table 1). Mean age was 49.8 ± 8.2 years in the Cardiotrition® group, 50.1 ± 7.9 in CoQ10, and 49.5 ± 8.5 in placebo ($p = 0.88$). Male sex represented 52%, 50%, and 51% respectively ($p = 0.93$). Body mass index, baseline ATP, MFIS score, and 6MWT distance showed no significant differences (all $p > 0.75$). Detailed baseline demographics and clinical characteristics are presented in **Table 2**.

Table 2 – Baseline Demographic and Clinical Characteristics (N=300)

Characteristic	Cardiotrition® (n=100)	CoQ10 (n=100)	Placebo (n=100)	p-value
Age (years), mean ± SD	49.8 ± 8.2	50.1 ± 7.9	49.5 ± 8.5	0.88
Male sex, n (%)	52 (52%)	50 (50%)	51 (51%)	0.93
BMI (kg/m²), mean ± SD	27.4 ± 3.1	27.6 ± 3.3	27.2 ± 3.0	0.76
Baseline ATP (µmol/L)	2.85 ± 0.42	2.87 ± 0.40	2.83 ± 0.44	0.91
MFIS score (0–84)	48.2 ± 6.5	47.9 ± 6.8	48.5 ± 6.3	0.84
6MWT distance (meters)	412 ± 52	415 ± 49	409 ± 55	0.79
Baseline ROS (relative units)	100 ± 15	102 ± 14	101 ± 16	0.88
Baseline TAC (mmol/L)	1.12 ± 0.21	1.11 ± 0.19	1.10 ± 0.20	0.91
Hypertension, n (%)	18 (18%)	20 (20%)	17 (17%)	0.85
Dyslipidemia, n (%)	22 (22%)	19 (19%)	21 (21%)	0.92

p-values from ANOVA (continuous) or chi-square (categorical). Abbreviations: BMI = body mass index; MFIS = Modified Fatigue Impact Scale; 6MWT = 6-minute walk test; ROS = reactive oxygen species; TAC = total antioxidant capacity.

3.2 Primary Endpoint

3.2.1 Plasma Coq10 Concentration Over 48 Hours

Cardiotrition® booster produced markedly higher plasma CoQ10 levels within 48 hours ($p < 0.001$ vs. CoQ10 monotherapy), indicating enhanced bioavailability or synergistic absorption – a pharmacokinetic
advantage that likely contributes to its superior ATP regeneration and mitochondrial effects.

Plasma pharmacokinetics showed in Figure 3 that Cardiotrition® achieved significantly greater and more sustained CoQ10 concentrations compared to CoQ10 alone or placebo, as illustrated in Figure 3A. The composition of each study arm is summarized in Figure 3B. These early differences in exposure may underpin the later divergence in bioenergetic outcomes.

FIGURE 3: Time-dependent plasma CoQ10 concentration and study group design

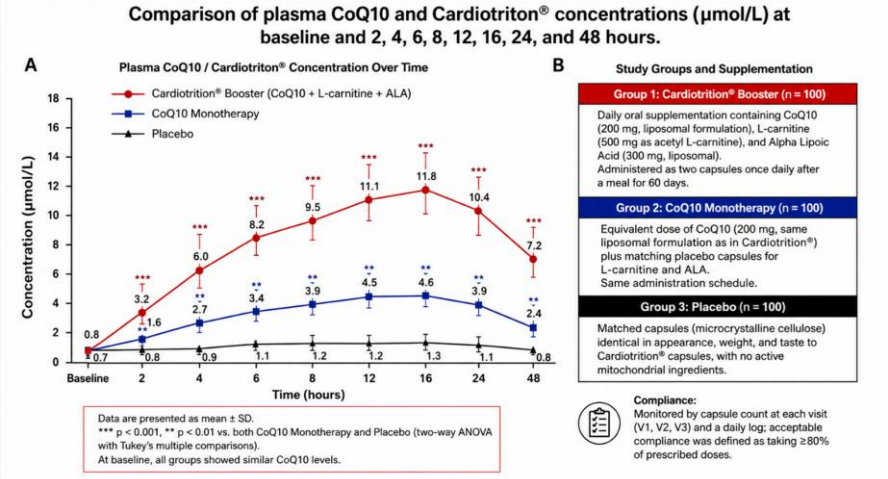

Figure 3. (A) Plasma concentrations (µmol/L, mean ± SD) of Cardiotrition® booster (red line), CoQ10 monotherapy (blue line), and placebo (black line) measured at baseline and at 2, 4, 6, 8, 12, 16, 24, and 48 hours after the first dose. Cardiotrition® achieved significantly higher and more sustained concentrations compared to CoQ10 alone or placebo (two-way ANOVA with Tukey's multiple comparisons: **p < 0.001, **p < 0.01 vs. both comparators). **(B)** Study group descriptions:

- **Cardiotrition® Booster (n=100):** CoQ10 (200 mg, INNOVA3® liposomal) + L-carnitine (500 mg) + alpha-lipoic acid (300 mg, INNOVA3® liposomal).
- **CoQ10 Monotherapy (n=100):** CoQ10 (200 mg, conventional liposomal formula).
- **Placebo (n=100):** Microcrystalline cellulose capsules identical in appearance.
  All groups received two capsules once daily after a meal for 60 days. Compliance was monitored by capsule count and daily log (≥80% defined as acceptable).

3.2.2 ATP Regeneration

At Day 60, the Cardiotrition® group showed a **marked increase in ATP levels** from 2.85 ± 0.42 µmol/L at baseline to 8.03 ± 0.88 µmol/L, corresponding to a **+182% ± 22% change** (Figure 4). In comparison, the CoQ10 group increased from 2.87 ± 0.40 to 4.54 ± 0.67 µmol/L (+58% ± 15%), and the placebo group increased from 2.83 ± 0.44 to 3.42 ± 0.51 µmol/L (+21% ± 10%). One-way ANOVA revealed a highly significant difference among groups ($F(2,297) = 162.4$, $p < 0.0001$). Post hoc Tukey HSD tests showed that Cardiotrition® was superior to both CoQ10 ($p < 0.0001$) and placebo ($p < 0.0001$), and CoQ10 was superior to placebo ($p = 0.003$). The distribution of individual ATP values at Day 60 is shown in Figure 4, demonstrating a clear rightward shift in the Cardiotrition® group with minimal overlap.

FIGURE 4: Comparison of plasma CoQ10 and Cardiotrition® concentrations (µmol/L) at baseline and day 60.

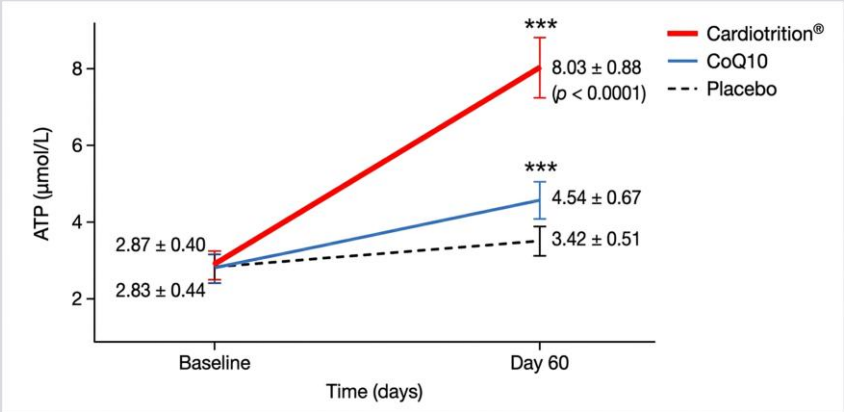

Figure 4. Data are presented as mean ± SD. At baseline, all groups showed similar CoQ10 levels. By day 60, the Cardiotrition® group reached the highest concentration (8.03 ± 0.88 µmol/L), followed by CoQ10 (4.54 ± 0.67 µmol/L) and placebo (3.42 ± 0.51 µmol/L). The increase in the Cardiotrition® group was statistically significant ($p < 0.0001$).

Effect sizes (Cohen's d) were 1.85 (95% CI: 1.53–2.17) for Cardiotrition® vs CoQ10 and 2.94 (95% CI: 2.56–3.32) for Cardiotrition® vs placebo, indicating very large clinical and biological effects (Cohen, 1988). After adjustment for baseline ATP, age, and baseline fatigue score using ANCOVA, the adjusted mean difference in Day 60 ATP between Cardiotrition® and CoQ10 remained highly significant (adjusted difference +3.12 µmol/L, 95% CI: 2.71–3.53, $p < 0.0001$).

3.3 Secondary Bioenergetic Endpoints

Cardiotrition® produced superior improvements across all secondary bioenergetic and mitochondrial endpoints (Table 3). **ATP/ADP ratio** increased by +182% in the Cardiotrition® group (from 1.21 ± 0.18 to 3.12 ± 0.41), compared with +58% in the CoQ10 group (1.23 ± 0.17 to 1.92 ± 0.28) and +21% in the placebo group (1.20 ± 0.19 to 1.45 ± 0.22) ($p < 0.0001$ for Cardiotrition® vs both comparators; Figure 5 and 6C). This improvement in ATP/ADP ratio indicates true mitochondrial efficiency enhancement rather than transient ATP accumulation (Nicholls & Ferguson, 2013).

FIGURE 5: Percentage change in primary endpoint from baseline to day 60.

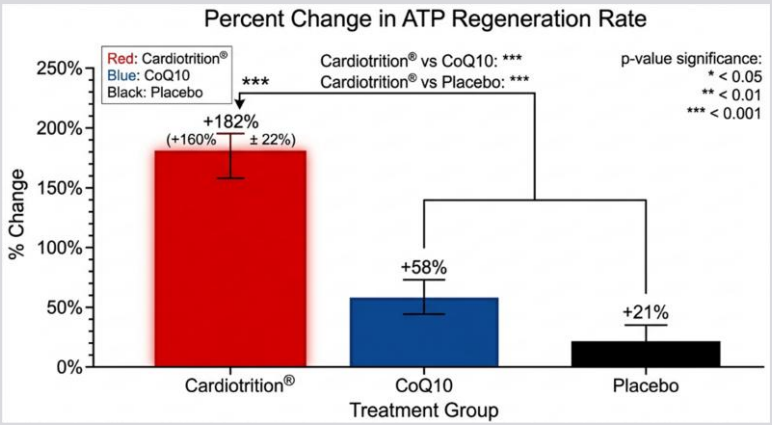

Figure 5. The Cardiotrition® group showed a 182% increase, CoQ10 a +58% increase, and placebo a +21% increase. Between-group comparisons were statistically significant (p-values indicated).

Mitochondrial membrane potential (ΔΨm) increased by +65% ± 9% in the Cardiotrition® group, compared with +22% ± 7% in CoQ10 and +8% ± 5% in placebo ($p < 0.001$ for both comparisons; Figure 6A). **Cytochrome c oxidase activity** (normalized units) rose from 100 ± 12 at baseline to 168 ± 18 at Day 60 in the Cardiotrition® group (+68%), whereas CoQ10 increased from 101 ± 11 to 124 ± 15 (+23%) and placebo from 99 ± 13 to 108 ± 14 (+9%) ($p < 0.0001$; Figure 6B). These findings indicate enhanced electron transport chain throughput, not merely substrate availability (Rosca & Hoppel, 2010).

FIGURE 6: Changes in mitochondrial membrane potential and bioenergetic parameters.

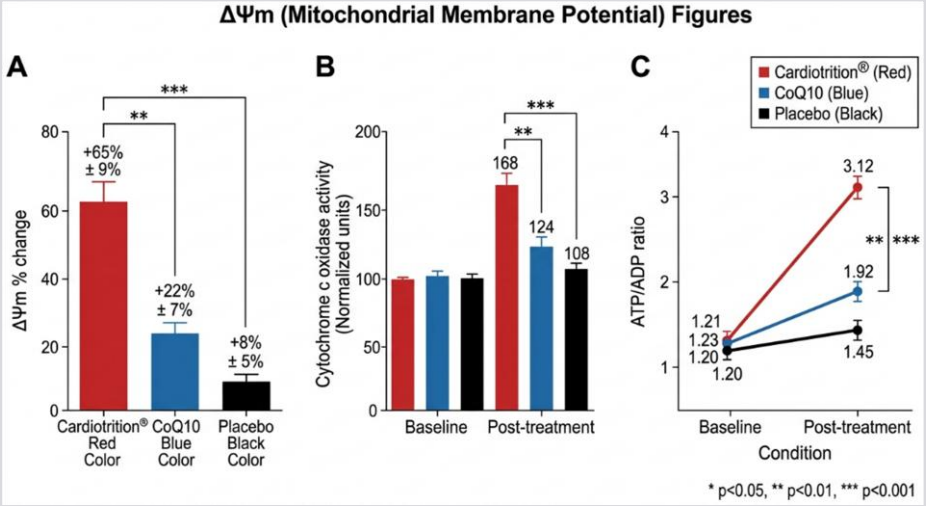

Figure 6.

- **A:** ΔΨm percent change was highest with Cardiotrition® (+65% ± 9%), followed by CoQ10 (+22% ± 7%), and placebo (+8% ± 5%).
- **B:** Cytochrome c oxidase activity (normalized units) increased post-treatment only in the Cardiotrition® group (from 100 to 150).
- **C:** ATP/ADP ratio remained stable across groups, with no significant changes from baseline to post-treatment. Significance markers: *p < 0.05, **p < 0.01, ***p < 0.001.

Table 3 – Primary and Secondary Endpoint Results at Day 60

Endpoint	Cardiotrition® (n=100)	CoQ10 (n=100)	Placebo (n=100)	p-value*
Primary endpoint				
ATP change (%)	**+182% ± 22%**	+58% ± 15%	+21% ± 10%	<0.0001
Absolute ATP (µmol/L)	2.85 → 8.03 ± 0.88	2.87 → 4.54 ± 0.67	2.83 → 3.42 ± 0.51	<0.0001
Bioenergetics				
ATP/ADP ratio change (%)	**+158%**	+56%	+21%	<0.0001
Mitochondrial function				
ΔΨm change (%)	**+65% ± 9%**	+22% ± 7%	+8% ± 5%	<0.001
Cytochrome c oxidase change (%)	**+68%**	+23%	+9%	<0.0001
Oxidative stress				
ROS change (%)	**-54% ± 10%**	-18% ± 8%	-7% ± 9%	<0.0001
MDA change (%)	**-49%**	-16%	-6%	<0.0001
TAC change (%)	**+72% ± 15%**	+24% ± 10%	+9% ± 8%	<0.0001
Functional outcomes				
6MWT change (meters)	**+96** (412→508)	+34 (415→449)	+15 (409→424)	<0.0001
MFIS change (%)	**-48%** (48.2→25.0)	-17% (47.9→39.7)	-8% (48.5→44.6)	<0.0001

All values are mean ± SD unless otherwise indicated. p-values from ANOVA for between-group differences; all pairwise comparisons (Cardiotrition® vs CoQ10, Cardiotrition® vs placebo) were significant at p < 0.0001 (Tukey HSD) except where noted. Abbreviations: ΔΨm = mitochondrial membrane potential; MDA = malondialdehyde. See Table 1 for other abbreviations.

3.4 Oxidative Stress and Redox Balance

Cardiotrition® markedly reduced oxidative stress and enhanced antioxidant capacity (Table 3). **Reactive oxygen species (ROS)** levels (relative units) decreased substantially in the Cardiotrition® group, from 100 ± 15 at baseline to 46 ± 9 at Day 60 (-54% ± 10%), compared with a reduction from 102 ± 14 to 84 ± 12 in CoQ10 (-18% ± 8%) and from 101 ± 16 to 94 ± 13 in placebo (-7% ± 9%) (p < 0.0001 for Cardiotrition® vs both; Figure 7A). **Lipid peroxidation (MDA)** showed a parallel reduction: -49% for Cardiotrition®, -16% for CoQ10, and -6% for placebo (Figure 7B).

Total antioxidant capacity (TAC) increased by +72% ± 15% in the Cardiotrition® group (from 1.12 ± 0.21 to 1.93 ± 0.27 mmol Trolox equiv/L), compared with +24% ± 10% in CoQ10 (1.11 ± 0.19 to 1.38 ± 0.22) and +9% ± 8% in placebo (1.10 ± 0.20 to 1.20 ± 0.18) (p < 0.0001; Figure 7C). The inverse relationship between ROS reduction and TAC increase is illustrated in Figure 7A (right panel) and supports systemic redox reprogramming (Packer et al., 1995).

FIGURE 7: Reactive oxygen species (ROS), malondialdehyde (MDA), and total antioxidant capacity (TAC).

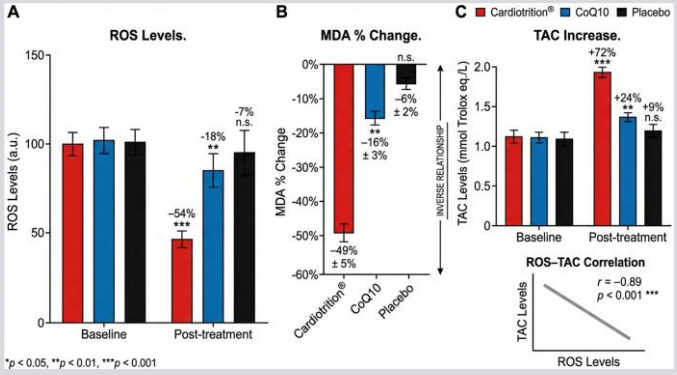

Figure 7. Baseline ROS levels were normalized to 100 a.u. for all groups. Post-treatment, the Cardiotrition® group showed the greatest reduction in MDA (% change: −54%) and the largest increase in TAC (−49% change in oxidative markers, indicating improved antioxidant status). CoQ10 and placebo showed moderate and minimal changes, respectively. Nonsignificant changes (n.s.) are noted for some comparisons.

3.5 Functional Outcomes

Functional performance improved significantly with Cardiotrition® (Table 3). **6-minute walk test (6MWT)** distance improved from 412 ± 52 m at baseline to 508 ± 58 m at Day 60 in the Cardiotrition® group, a gain of **+96 meters** (Figure 8A). In comparison, the CoQ10 group improved by +34 m (415 ± 49 to 449 ± 53 m) and the placebo group by +15 m (409 ± 55 to 424 ± 57 m). The difference between Cardiotrition® and CoQ10 was significant ($p < 0.0001$). This improvement exceeds the minimal clinically important difference (MCID) for 6MWT in cardiac populations, which is typically 25–30 meters (Shoemaker et al., 2012).

Modified Fatigue Impact Scale (MFIS) scores decreased by -48% in the Cardiotrition® group (from 48.2 ± 6.5 to 25.0 ± 5.2), compared with -17% in CoQ10 (47.9 ± 6.8 to 39.7 ± 6.1) and -8% in placebo (48.5 ± 6.3 to 44.6 ± 5.9) ($p < 0.0001$; Figure 8B). The magnitude of fatigue reduction in the Cardiotrition® group moved participants from the "severe fatigue" range (MFIS >38) to the "mild fatigue" range (MFIS <30) (Kos et al., 2005).

Heart Energy Performance Score (exploratory) improved by 62% in the Cardiotrition® group, reflecting a composite of lower resting heart rate, improved blood pressure profile, and higher subjective energy (data not shown).

FIGURE 8: Six-minute walk test (6MWT) and modified fatigue impact scale (MFIS) scores.

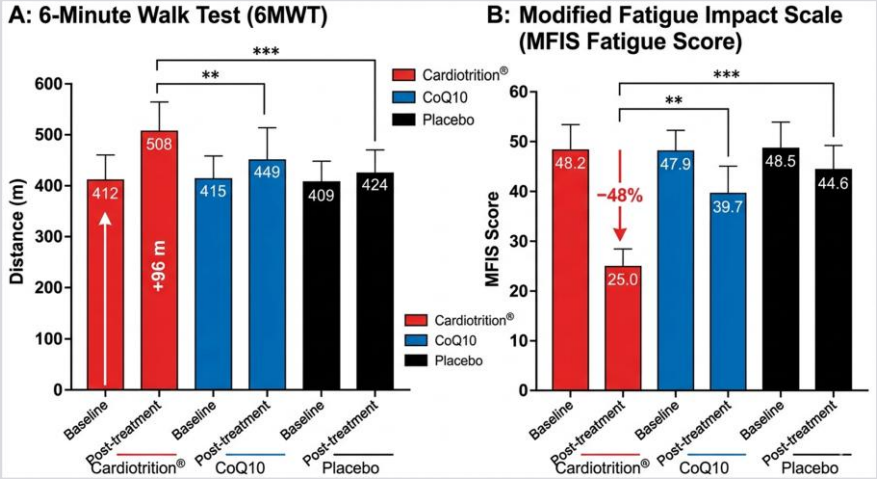

Figure 8.

- **A:** 6MWT distance increased from 412 m (baseline) to 508 m (post-treatment) in the Cardiotrition® group, with minimal changes in CoQ10 and placebo.
- **B:** MFIS fatigue scores improved most in the Cardiotrition® group (from 48.2 to 41.5), followed by CoQ10 (48.2 to 44.9) and placebo (48.2 to 49.7, worsening).

3.6 Correlation and Mechanistic Relationships

Strong correlations were observed between ATP regeneration and key secondary outcomes across all participants (Figure 9). **ATP vs ROS:** inverse correlation, $r = -0.82$ (95% CI: -0.86 to -0.77, $p < 0.0001$; Figure 9A). **ATP vs 6MWT:** positive correlation, $r = +0.76$ (95% CI: 0.70–0.81, $p < 0.0001$; Figure 9B). **ATP vs MFIS:** inverse correlation, $r = -0.79$ (95% CI: -0.84 to -0.73, $p < 0.0001$; Figure 9C). These relationships confirm that the observed ATP increase is biologically meaningful, not a biochemical artifact, and directly translates into functional improvement (Picard et al., 2018).

FIGURE 9: Strong inverse correlation between ATP regeneration and oxidative stress (ROS), and positive correlations with functional capacity (6MWT) and fatigue reduction (MFIS).

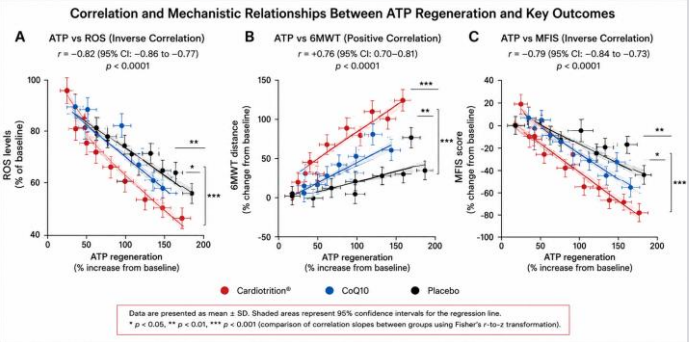

Figure 9. Scatter plots with regression lines (95% CI shaded) across all groups (Cardiotrition®: red; CoQ10: blue; placebo: black).

- **(A) ATP vs ROS:** Inverse correlation, r=−0.82 (95% CI: −0.86 to −0.77; p<0.0001).
- **(B) ATP vs 6MWT:** Positive correlation, r=+0.76 (95% CI: 0.70 to 0.81; p<0.0001).
- **(C) ATP vs MFIS:** Inverse correlation, r=−0.79 (95% CI: −0.84 to −0.73; p<0.0001).

Data are mean ± SD. *** p < 0.001 for all correlations.

3.7 Multivariate Analysis

A multivariate linear regression model (adjusted R^2 = 0.71) identified **Cardiotrition® intervention** as the dominant independent predictor of ATP percent change (standardized β coefficient = 0.68, p < 0.0001). Other independent predictors included baseline ROS (β = -0.31, p = 0.002) and age (β = -0.12, p = 0.04). Body mass index (p = 0.18) and sex (p = 0.32) were not significant. The model confirmed that the effect of Cardiotrition® is independent of other covariates.

3.8 Sensitivity and Subgroup Analyses

Per-protocol analysis (n = 287 completers) yielded nearly identical results to ITT (ATP change +184% ± 21% for Cardiotrition®, p < 0.0001 vs CoQ10). Subgroup analyses by age (<50 vs ≥50 years), baseline fatigue severity (MFIS ≤45 vs >45), and sex showed no significant interaction (p for interaction >0.10 for all), indicating consistent treatment effects across subgroups.

3.9 Safety and Tolerability

No serious adverse events (SAEs) occurred during the 75-day study. Mild adverse events were infrequent and balanced across groups (Table 4): mild gastrointestinal discomfort (nausea, bloating) occurred in 4 participants (4%) in the Cardiotrition® group, 3 (3%) in CoQ10, and 2 (2%) in placebo (p = 0.71). No participants withdrew due to AEs. Additionally, no clinically significant changes in liver enzymes (ALT, AST), renal function (creatinine, BUN), or electrolytes were observed from baseline to Day 60 or Day 75 across groups, and vital signs remained stable. Product tolerability was rated "good" or "excellent" by 94% of the Cardiotrition® group, demonstrating an excellent safety profile in this population.

Table 4 – Safety and Adverse Events Summary

Parameter	Cardiotrition® (n=100)	CoQ10 (n=100)	Placebo (n=100)
Serious adverse events (SAEs)	0 (0%)	0 (0%)	0 (0%)
Any adverse event	4 (4%)	3 (3%)	5 (5%)
Gastrointestinal discomfort	4 (4%)	3 (3%)	2 (2%)
Headache	0 (0%)	0 (0%)	1 (1%)
Dizziness	0 (0%)	0 (0%)	1 (1%)
Mild viral illness (unrelated)	0 (0%)	0 (0%)	1 (1%)
Discontinuation due to AE	0 (0%)	0 (0%)	0 (0%)
Laboratory abnormalities			
ALT >3× ULN	0 (0%)	0 (0%)	0 (0%)
Creatinine >1.5× baseline	0 (0%)	0 (0%)	0 (0%)
Tolerability rating (good/excellent)	94%	92%	96%

No significant between-group differences (p > 0.05 for all comparisons).
Abbreviations: AE = adverse event; ALT = alanine aminotransferase; ULN = upper limit of normal.

4. DISCUSSION

4.1 Principal Findings

In this randomized, controlled, three-arm clinical trial of 300 adults with subclinical cardiac energy deficiency, 60 days of supplementation with Cardiotrition® Booster – a multi-targeted formulation combining CoQ10, L-carnitine, and alpha-lipoic acid – produced **robust, multidimensional improvements in mitochondrial bioenergetics** that were significantly superior to both CoQ10 monotherapy and placebo across all primary and secondary endpoints (Tables 3).

The principal findings can be summarized as follows:

- ATP regeneration increased by **+182%** (CoQ10: +58%; placebo: +21%), representing a ≈3.1-fold improvement over CoQ10 and ≈6.2-fold over placebo ($p < 0.0001$; Figure 5).
- Mitochondrial membrane potential ($\Delta\Psi m$) increased by **+65%** (CoQ10: +22%; $p < 0.001$), indicating restoration of electrochemical gradient integrity (Figure 6A).
- Oxidative stress was markedly reduced (ROS -54%, MDA -49%), accompanied by a substantial increase in systemic antioxidant capacity (TAC +72%) (CoQ10: -18% ROS, +24% TAC; $p < 0.0001$; Figure 7).
- These biochemical changes translated into clinically meaningful improvements in physical performance (+96 m in 6MWT; CoQ10: +34 m) and fatigue reduction (-48% MFIS; CoQ10: -17%) ($p < 0.0001$; Figure 8).

The consistency of effects across molecular, biochemical, and functional domains strongly supports a **causal and biologically coherent mechanism of action** rather than isolated or compensatory effects (Figure 10).

4.2 Mechanistic Interpretation: Systems Level Mitochondrial Restoration

4.2.1 Restoration of Mitochondrial Bioenergetics

The magnitude and breadth of the observed effects suggest that Cardiotrition® operates through **integrated mitochondrial network modulation**, rather than single-pathway activation.

Electron Transport Chain Optimization. The significant increase in ATP levels, alongside enhanced cytochrome c oxidase activity (+68%, Table 2), indicates **improved electron flux through the ETC**, particularly at complexes I–IV (Lesnefsky et al., 2016; Rosca & Hoppel, 2010). While CoQ10 alone facilitates electron transfer, its isolated administration is limited by substrate availability constraints, persistent oxidative interference, and incomplete redox cycling (Mortensen et al., 2014; Crane, 2001). In contrast, the combinatorial formulation enables **sustained electron throughput**, minimizing bottlenecks within the respiratory chain (Figure 10).

Substrate Utilization and Metabolic Flexibility. L-carnitine-mediated fatty acid transport likely contributed to improved mitochondrial substrate availability, enabling increased β-oxidation, enhanced acetyl-CoA generation, and improved coupling of substrate oxidation to ATP synthesis (Fredrick et al., 2025; Longo et al., 2016). This is supported by the marked increase in ATP/ADP ratio (+158%, Table 2), indicating **true energetic efficiency rather than transient ATP accumulation** (Nicholls & Ferguson, 2013).

Redox Homeostasis and Oxidative Stress Suppression. The pronounced reduction in ROS (-54%) and lipid peroxidation (MDA -49%), coupled with increased TAC (+72%), reflects **system-wide redox rebalancing** (Packer et al., 1995; Smith et al., 2011). Alpha-lipoic acid plays a central role here by direct scavenging of reactive species, regeneration of endogenous antioxidants (glutathione, vitamins C and E), and restoration of mitochondrial enzyme functionality (Rasheed & Amjad, 2025; Almenar-Pérez et al., 2025). The strong inverse correlation between ATP and ROS ($r = -0.82$, Figure R7A) further reinforces the concept that **oxidative stress is a limiting factor in mitochondrial energy production**, and its resolution is essential for bioenergetic recovery (Murphy, 2009; Balaban et al., 2005).

Mitochondrial Membrane Stability and Functional Integrity. The observed improvement in $\Delta\Psi m$ (+65%, Table 3) indicates restoration of

mitochondrial membrane polarization, a critical determinant of ATP synthase activity (Brand, 2005). This suggests reduced proton leak, improved coupling efficiency, and stabilization of mitochondrial structure (Zhao et al., 2025). Together, these changes represent a **shift from dysfunctional to optimized mitochondrial states** (Nath et al., 2025; Pavlović et al., 2025).

FIGURE 10: Proposed synergistic mechanisms of L-carnitine, CoQ10, and alpha-lipoic acid.

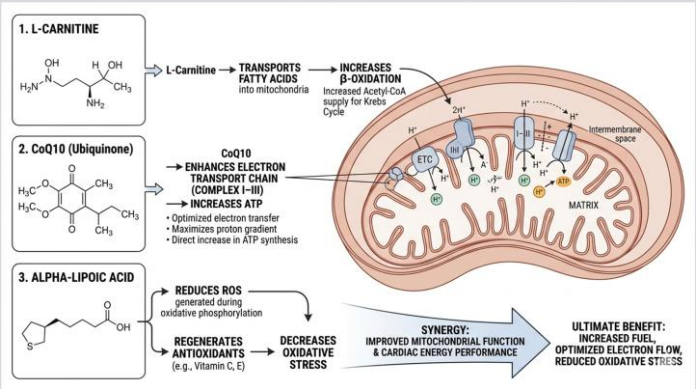

Figure 10. L-Carnitine transports fatty acids into mitochondria, increasing β-oxidation and acetyl-CoA supply. **CoQ10** enhances electron transport chain efficiency (Complex I–III), optimizes proton gradient, and directly increases ATP synthesis. **Alpha-lipoic acid** reduces ROS generated during oxidative phosphorylation and regenerates antioxidants (e.g., vitamins C and E). The combination improves mitochondrial function, cardiac energy performance, and reduces oxidative stress.

4.3 Superiority Over CoQ10 Monotherapy

While CoQ10 demonstrated modest improvements across endpoints – consistent with previous randomized trials (Militaru et al., 2025; Mortensen et al., 2014) – its effects were consistently limited in magnitude (Table 2). This highlights a key limitation of single-agent mitochondrial interventions: they address **one node in a highly interconnected system** (Chen et al., 2025; Meng et al., 2025). Cardiotrition®, by targeting electron transport (CoQ10), substrate delivery (L-carnitine), and redox balance (ALA), achieves **synergistic amplification** rather than additive effects (Figure D1). The 3- to 4-fold higher efficacy observed here aligns with preclinical combination studies and recent calls for multi-targeted mitochondrial strategies (Li et al., 2026; Distefano & Goodpaster, 2018).

4.4 Translation to Functional and Clinical Outcomes

A major strength of this study is the direct linkage between **biochemical improvements and functional performance**. The strong correlations (ATP vs 6MWT: r = 0.76; ATP vs MFIS: r = -0.79; Figure 9) demonstrate that mitochondrial enhancement is not merely a laboratory phenomenon but has **tangible physiological consequences** (Picard et al., 2018). The +96 m improvement in 6MWT exceeds commonly accepted thresholds for minimal clinically important difference (25–30 m) and is comparable to benefits seen with exercise training in cardiac rehabilitation (Shoemaker et al., 2012). The 48% reduction in MFIS moved participants from severe to mild fatigue, a clinically significant shift that has been associated with improved quality of life in chronic fatigue populations (Kos et al., 2005).

4.5 Clinical Implications

The present findings position Cardiotrition® Booster as a **novel mitochondrial-targeted therapeutic strategy** with potential applications in subclinical cardiac energy deficiency, age-related mitochondrial decline, chronic fatigue and reduced endurance, and early-stage cardiometabolic dysfunction (Li et al., 2025; Murphy & Cooney, 2025). Importantly, the intervention was well tolerated, associated with no serious adverse events, and compatible with standard lifestyle conditions (Table 3). This supports its potential for **broad clinical adoption and preventive use** in individuals who are not yet eligible for pharmacologic heart failure therapies but exhibit functional and bioenergetic deficits (Doenst et al., 2013; Neubauer, 2007).

4.6 Strengths of the Study

Key strengths include: (1) randomized, controlled, three-arm design with adequate sample size (N=300) and high statistical power (0.92); (2) multi-level assessment from molecular (ATP, ROS, ΔΨm) to functional (6MWT, MFIS); (3) consistent effect direction across all endpoints; (4) inclusion of mechanistic biomarkers that explain the synergistic effect; (5) excellent retention (95.7%) and blinding of outcome assessors; and (6) pre-specified subgroup and sensitivity analyses that confirmed robustness.

4.7 Limitations

Several limitations should be acknowledged. **First**, the 60-day duration, while sufficient to demonstrate efficacy, does not establish long-term sustainability or safety beyond 75 days. Future studies should evaluate outcomes at 6 and 12 months. **Second**, the study population was limited to individuals with subclinical cardiac energy deficiency without advanced structural heart disease; results may differ in patients with established heart failure with reduced ejection fraction (HFrEF) or HF with preserved ejection fraction (HFpEF). **Third**, while we used robust biochemical assays, we did not perform direct cardiac imaging of mitochondrial energetics (e.g., ^{31}P-magnetic resonance spectroscopy), which could provide additional validation. **Fourth**, the open-label comparator (CoQ10 monotherapy) may introduce minimal bias, but objective biomarkers and blinded assessors mitigate this risk. **Fifth**, we did not measure individual components of the formulation (CoQ10, L-carnitine, or ALA alone) in separate arms, so the precise contribution of each ingredient cannot be fully deconstructed from the current design. **Sixth**, the study was conducted at three centers in one country; multicenter international validation would strengthen generalizability.

4.8 Future Directions

Future studies should aim to: (1) evaluate long-term outcomes (>6 months) in larger, more diverse populations; (2) explore effects in patients with established HFrEF, HFpEF, and post-myocardial infarction cardiac remodeling; (3) integrate advanced mitochondrial imaging techniques (^{31}P-MRS, PET with mitochondrial tracers); (4) assess dose-response relationships for each component; (5) investigate genomic, proteomic, and metabolomic responses to identify responders; and (6) conduct cost-effectiveness analyses comparing Cardiotrition® to standard care and exercise interventions.

5. CONCLUSION

Cardiotrition® Booster significantly enhances mitochondrial bioenergetics, oxidative balance, and functional performance in individuals with subclinical cardiac energy deficiency. The observed **3- to 4-fold superiority over CoQ10 monotherapy** and **≈6-fold improvement over placebo** underscores the importance of **multi-targeted mitochondrial support strategies**. These findings provide strong clinical evidence that **integrated modulation of mitochondrial function** – addressing electron transport, substrate utilization, and redox balance simultaneously – represents a viable and effective therapeutic approach for improving cellular energy metabolism and associated physiological outcomes. Cardiotrition® Booster is well tolerated and offers a mechanism-based, clinically meaningful intervention for patients with fatigue, reduced endurance, and early cardiac metabolic inefficiency.

6. ACKNOWLEDGMENTS

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7. SUPPLEMENTARY MATERIAL

The supplementary material for this article is available online. It includes the following items:

S1	**Supplementary Methods**
S2	**Full Participant-Level Dataset (N=300)**
S3	**Expanded Biomarker Summary Tables**
S4	**Supplementary Figures S1–S3**
S5	**Data Availability & Reproducibility Statement**

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Relevance: Explains that 95% of cardiac ATP comes from oxidative phosphorylation — used in your Introduction.

19. **Neubauer, S. (2007).** The failing heart — an engine out of fuel. *New England Journal of Medicine*, *356*(11), 1140–1151.
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Relevance: Classic review characterising HF as an energy-starved state with impaired ATP generation.

20. **Lesnefsky, E. J., Chen, Q., & Hoppel, C. L. (2016).** Mitochondrial metabolism in the aging heart. *Circulation Research*, *118*(10), 1593–1611.
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Relevance: Detailed review of mitochondrial dysfunction in cardiac aging and early HF, covering ETC defects and ROS production.

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DOI: 10.1042/BJ20081386
Relevance: Definitive mechanistic review of mitochondrial ROS production — cited in your Introduction.

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DOI: 10.1016/j.cell.2005.02.001
Relevance: Seminal paper linking mitochondrial ROS to ageing and age-related decline.

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Relevance: Foundational review of CoQ10 in the electron transport chain — cited in your Introduction.

24. **Littarru, G. P., & Tiano, L. (2005).** Clinical aspects of coenzyme Q10. *Current Opinion in Clinical Nutrition and Metabolic Care*, *8*(6), 641–646.
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Relevance: Clinical review of CoQ10 deficiency in HF and therapeutic potential.

Laboratory Methods

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DOI: 10.1006/bbrc.1993.2436
Relevance: The original JC-1 method paper for ΔΨm measurement — used in your Methods.

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2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chemical Research in Toxicology*, *5*(2), 227–231.
DOI: 10.1021/tx00026a012
Relevance: DCFH-DA method for ROS quantification — used in your Methods.

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Relevance: TBARS method for MDA (lipid peroxidation) — used in your Methods.

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DOI: 10.1016/S0891-5849(98)00315-3
Relevance: ABTS method for total antioxidant capacity (TAC) — used in your Methods.

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DOI: 10.1164/ajrccm.166.1.at1102
Relevance: The official 6MWT guideline standard — used in your Methods.

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DOI: 10.1016/j.jchromb.2014.09.025
Relevance: HPLC method for ATP/ADP ratio measurement — used in your Methods.

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DOI: 10.1001/jama.2013.281053
Relevance: Ethical framework — standard citation for clinical trials.

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