Pharmacokinetic and Bioavailability Evaluation of Cardiotrition® Booster: A Randomized, Double-Blind, Crossover Study in Healthy Volunteers

Cardiotrition® Booster Pharmacokinetics

Arvid Jensen[1], Marianne Smith[2], Ali Saadani[3], Rahma H. Hagaggy[4]*, and Manal Ibrahim[5]

[1] Senior Technical Consultant, Scientific Department, Nanotrition Nord, Borre, Norway
[2] Technical Executive, Scientific Department, Nanotrition Nord, Borre, Norway
[3] Chief Officer, Scientific Department, Nanotrition Nord, Cairo, Egypt
[4] Research Executive Specialist, Scientific Department, Polygon Research Center, Cairo, Egypt
[5] Research Specialist, Scientific Department, Nanotrition Nord, Cairo, Egypt

Corresponding author:
Rahma H. Hagaggy
Research Executive Specialist
Polygon Research Center
Cairo, Egypt
Email: rahma.hassan@nanotrition.tech
Tel: +20 109 47154551

ABSTRACT

Background: Coenzyme Q10 (CoQ10), alpha-lipoic acid (ALA), and acetyl-L-carnitine (ALCAR) play complementary roles in mitochondrial energy metabolism. However, clinical effectiveness is often limited by suboptimal oral bioavailability. Cardiotrition® Booster incorporates INNOVA3® liposomal CoQ10, INNOVA3® liposomal R-alpha lipoic acid, and enhanced acetyl-L-carnitine using advanced delivery technologies designed to improve systemic exposure.

Methods: A randomized, double-blind, two-period crossover study was conducted in 20 healthy volunteers. Participants received a single oral dose of Cardiotrition® Booster or a conventional formulation, followed by serial blood sampling over 24 hours. Plasma concentrations of CoQ10, ALA, and ALCAR were determined using validated LC-MS/MS methods. Pharmacokinetic parameters (Cmax, Tmax, AUC_{0-24}, $AUC_{0-\infty}$, t½, and relative bioavailability) were evaluated. Statistical significance was defined as $p < 0.05$.

Results: Liposomal CoQ10 demonstrated a 2.56-fold increase in AUC_{0-24} compared with standard CoQ10 (43.8 ± 9.2 vs. 17.1 ± 4.3 mg·h/L; $p < 0.001$), with a Cmax of 6.02 ± 1.28 mg/L vs. 1.48 ± 0.39 mg/L. Liposomal R-ALA achieved a 2.71-fold increase in AUC_{0-24} (4,140 ± 860 vs. 1,530 ± 320 ng·h/mL; $p < 0.001$). Enhanced ALCAR showed a 1.42-fold increase in AUC_{0-24} (318 ± 52 vs. 224 ± 41 µmol·h/L; $p = 0.008$). No serious adverse events occurred. All formulations were well tolerated.

Conclusions: Cardiotrition® Booster demonstrated substantially enhanced systemic bioavailability for all three active ingredients. These findings support the potential of advanced liposomal delivery technologies to improve mitochondrial function and sustain cardiac energy metabolism compared with conventional formulations.

Keywords: Liposomes; Coenzyme Q10; Alpha-lipoic acid; Acetyl-L-carnitine; Bioavailability; Pharmacokinetics; Randomized controlled trial

1. INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide, accounting for approximately 17.9 million deaths annually, representing nearly one-third of all global deaths (World Health Organization, 2025). Despite remarkable advances in pharmacological and interventional therapies, the global burden of CVD continues to rise, driven by aging populations, increasing prevalence of metabolic risk factors, and the growing recognition of mitochondrial dysfunction as a central pathophysiological mechanism (Li et al., 2026; Pavlović et al., 2025). The heart, as one of the most energy-demanding organs in the human body, requires a continuous and substantial supply of adenosine triphosphate (ATP) to maintain its contractile function and hemodynamic stability. The adult heart generates approximately 6 kg of ATP daily through mitochondrial oxidative phosphorylation, which accounts for 95% of cardiac ATP production (Doenst et al., 2013; Neubauer, 2007).

However, the heart's enormous energy demand renders it exceptionally vulnerable to disruptions in mitochondrial bioenergetics. Mitochondrial dysfunction has emerged as a unifying pathophysiological mechanism underlying diverse

cardiovascular conditions, including heart failure, ischemic heart disease, hypertension, diabetic cardiomyopathy, and age-related cardiac decline (Lesnefsky et al., 2016; Rosca & Hoppel, 2010; Nath et al., 2025). In the failing heart, progressive mitochondrial respiratory chain dysfunction, reduced oxidative phosphorylation efficiency, and excessive reactive oxygen species (ROS) generation collectively diminish ATP availability, impair contractile reserve, and accelerate disease progression (Zhao et al., 2025; Chen et al., 2025). The failing heart has been characterized as a "fuel-depleted engine," with mitochondrial dysfunction representing a hallmark feature of disease progression and a strong predictor of mortality (Neubauer, 2007; Rosca & Hoppel, 2010).

Mitochondrial oxidative phosphorylation is a highly regulated process involving five multi-subunit enzyme complexes (Complexes I–V) embedded in the inner mitochondrial membrane (Nicholls & Ferguson, 2013). The electron transport chain (ETC) transfers electrons from reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) to molecular oxygen, generating a proton gradient across the inner membrane that drives ATP synthesis via Complex V (ATP synthase) (Brand, 2005). The efficiency of this process depends on several critical factors: adequate substrate availability, optimal electron flux through the ETC, maintenance of mitochondrial membrane potential ($\Delta\Psi m$), and protection against oxidative damage (Murphy, 2009; Balaban et al., 2005).

The heart's metabolic flexibility allows it to utilize multiple substrates—fatty acids, glucose, lactate, and ketone bodies—depending on their availability and the physiological state (Doenst et al., 2013; Lopaschuk et al., 2010). Under normal conditions, fatty acids contribute approximately 60–70% of cardiac ATP production, with glucose and other substrates providing the remainder (Stanley et al., 2005). However, in pathological states such as heart failure, myocardial ischemia, and metabolic syndrome, significant metabolic shifts occur, characterized by reduced fatty acid oxidation, increased glucose utilization, and impaired mitochondrial oxidative capacity (Lopaschuk et al., 2010; Li et al., 2026).

Three bioactive compounds have emerged as critical modulators of mitochondrial energy metabolism: Coenzyme Q10 (CoQ10), Alpha-Lipoic Acid (ALA), and Acetyl-L-Carnitine (ALCAR) . Each plays a distinct yet complementary role in supporting mitochondrial function, and their combined actions have drawn considerable scientific interest for their potential to restore cardiac bioenergetics.

Coenzyme Q10, also known as ubiquinone, is a lipid-soluble, vitamin-like compound that serves as an essential component of the mitochondrial ETC (Crane, 2001; Littarru & Tiano, 2005). Its primary function is to act as an electron carrier from Complexes I and II to Complex III, facilitating the transfer of electrons that drive proton pumping and ATP synthesis (Gutierrez-Mariscal et al., 2021). Beyond its role in bioenergetics, CoQ10 functions as a potent lipid-soluble antioxidant, protecting cellular membranes and lipoproteins from oxidative damage while also regenerating other antioxidants such as vitamin E (Kudalkar et al., 2025; Mortensen et al., 2014).

Endogenous CoQ10 synthesis occurs via the mevalonate pathway, with peak tissue concentrations achieved by approximately 20–30 years of age, followed by a progressive decline with advancing age (Mantle & Dybring, 2020). Furthermore, statin therapy—widely prescribed for hyperlipidemia—inhibits HMG-CoA reductase, thereby reducing endogenous CoQ10 synthesis and potentially contributing to statin-associated muscle symptoms (Pastor-Maldonado et al., 2020). This age-related and drug-induced decline has prompted interest in CoQ10 supplementation as a strategy to restore mitochondrial function and cardiac energy metabolism.

The Q-SYMBIO randomized controlled trial demonstrated that long-term CoQ10 supplementation (100 mg three times daily for 2 years) in patients with moderate to severe chronic heart failure significantly reduced major adverse cardiovascular events, with a hazard ratio of 0.50 (95% CI: 0.32–0.80; $p = 0.003$) (Mortensen et al., 2014). Similarly, a recent randomized trial demonstrated that CoQ10 supplementation (2 × 60 mg daily for 6 months) in heart failure patients significantly improved left ventricular global longitudinal strain from −11.7% to −14.9% ($p < 0.001$) and 6-minute walk test distance by 82 meters (Militaru et al., 2025). These findings underscore the clinical importance of CoQ10 in supporting cardiac function and the rationale for optimizing its bioavailability.

Alpha-lipoic acid is a naturally occurring dithiol compound that functions as an essential cofactor for several mitochondrial dehydrogenase complexes, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, which play critical roles in the Krebs cycle and energy production (Superti & Russo, 2024; Shay et al., 2009). Unlike many antioxidants, ALA is both water- and lipid-soluble, enabling it to scavenge reactive oxygen species in both aqueous and lipid environments, making it a uniquely versatile antioxidant (Packer et al., 1995; Salehi et al., 2019).

ALA's therapeutic potential extends beyond direct radical scavenging. It has the capacity to: (1) regenerate endogenous antioxidants including glutathione, vitamin C, and vitamin E; (2) chelate redox-active transition metals; (3) modulate redox-sensitive signaling pathways (e.g., NF-κB, Nrf2); (4) improve mitochondrial biogenesis; and (5) enhance insulin-mediated glucose uptake into cardiac myocytes (Rasheed & Amjad, 2025; Smith et al., 2011). Recent research has demonstrated 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 co-administration significantly increased basal (33%, $p < 0.001$) and maximal (47%, $p = 0.03$) cellular oxygen consumption rates, suggesting synergistic potential for improving mitochondrial respiration (Almenar-Pérez et al., 2025).

However, despite its promising biological activities, the clinical utility of oral ALA is substantially limited by its short half-life and poor oral bioavailability (approximately 30%) triggered by hepatic degradation, reduced aqueous solubility, instability in the acidic gastric environment, and extensive first-pass metabolism (Mosallaei et al., 2024; Salehi et al., 2019). The R-enantiomer (R-ALA) is the naturally occurring and biologically active form, but it is particularly unstable and rapidly metabolized. Consequently, only a small fraction of an orally administered dose ultimately reaches the systemic circulation to exert its intended metabolic and antioxidant effects (Shay et al., 2009).

L-carnitine, an amino acid derivative synthesized from lysine and methionine, plays a fundamentally important role in mitochondrial energy metabolism by facilitating the transport of long-chain fatty acids across the inner mitochondrial membrane for β-oxidation (Fredrick et al., 2025; Longo et al., 2016). This transport is mediated by the carnitine palmitoyltransferase (CPT) system, which shuttles acyl-CoA esters into the mitochondrial matrix, where they undergo β-oxidation to produce acetyl-CoA, NADH, and $FADH_2$, which are then channeled into the Krebs cycle and electron transport chain (Marcovina et al., 2013).

Acetyl-L-carnitine (ALCAR), the acetylated ester of L-carnitine, offers additional benefits beyond the parent molecule. In addition to facilitating fatty acid transport, ALCAR provides an acetyl group for acetyl-CoA generation, supporting the Krebs cycle and ATP production while also modulating key metabolic pathways and exerting neuroprotective and cardioprotective effects (DiNicolantonio et al., 2013; Fredrick et al., 2025). A growing body of evidence suggests that carnitine supplementation can remarkably improve cardiac function, reduce biomarkers of heart failure, and enhance metabolic profiles in conditions such as chronic heart failure and myocardial infarction (Marcovina et al., 2013; Fredrick et al., 2025). 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).

However, oral carnitine bioavailability is limited by variable gastrointestinal absorption, active intestinal transport saturation at higher doses, and significant interindividual variability (Fredrick et al., 2025; Longo et al., 2016). Moreover, the acetyl moiety of ALCAR provides additional metabolic benefits that are absent in standard L-carnitine, supporting the use of enhanced or acetylated formulations.

Despite the well-established biological activities of CoQ10, ALA, and ALCAR, the clinical benefits of these compounds following oral administration remain considerably limited by unfavorable pharmacokinetic characteristics. The principal challenge is not the intrinsic therapeutic efficacy of these molecules, but rather their inefficient delivery into the systemic circulation (Pastor-Maldonado et al., 2020; Mantle & Dybring, 2020; Mosallaei et al., 2024).

CoQ10 exhibits extremely poor oral bioavailability because of its highly hydrophobic nature ($\log P > 10$), large molecular weight (863 Da), and limited aqueous solubility, resulting in poor gastrointestinal absorption, significant interindividual variability in plasma exposure, and minimal tissue distribution (Mantle & Dybring, 2020; Pastor-Maldonado et al., 2020). The absorption of CoQ10 requires micellar solubilization and lymphatic transport, and its bioavailability is highly dependent on the presence of dietary lipids, bile salts, and pancreatic function (Crane, 2001). Although traditional formulations such as oil-based solutions, powder suspensions, and solubilized formulations have been developed, their bioavailability remains modest, with Cmax and AUC values considerably lower than those achieved with intravenous administration (Jäger et al., 2025; Amalraj et al., 2026).

R-alpha lipoic acid faces analogous challenges, including a short half-life (approximately 30 minutes), limited oral bioavailability (approximately 30%), extensive first-pass hepatic metabolism, and chemical instability in the gastrointestinal tract (Salehi et al., 2019; Mosallaei et al., 2024). The R-enantiomer, which is the naturally occurring and biologically active form, is particularly unstable and rapidly metabolized (Shay et al., 2009). These pharmacokinetic limitations have historically constrained the clinical translation of ALA's robust preclinical benefits, prompting urgent interest in advanced delivery systems capable of improving its systemic exposure and target tissue penetration (Mosallaei et al., 2024; Rasheed & Amjad, 2025).

Acetyl-L-carnitine exhibits superior absorption compared to standard L-carnitine, yet its bioavailability still remains suboptimal because of saturable intestinal transport, competition with other cationic compounds, and variable uptake dependent on gastrointestinal function (Fredrick et al., 2025; Longo et al., 2016). Although ALCAR is more lipophilic than L-carnitine and demonstrates better tissue penetration, achieving consistent therapeutic plasma levels across diverse populations remains challenging (Marcovina et al., 2013).

These pharmacokinetic constraints have shifted scientific interest from simply identifying effective bioactive compounds

toward developing advanced delivery systems capable of maximizing their therapeutic potential.

Among the available oral delivery strategies, liposomal technology has emerged as one of the most promising approaches for improving the bioavailability of both hydrophilic and lipophilic compounds (Shade, 2016; Liu et al., 2020). Liposomes are spherical phospholipid vesicles with a bilayer structure resembling biological membranes, capable of encapsulating both hydrophilic (within the aqueous core) and lipophilic (within the lipid bilayer) active ingredients (Sheikholeslami et al., 2022).

Liposomal encapsulation confers several critical advantages for oral delivery:

- Protection of active ingredients from degradation within the gastrointestinal environment by the phospholipid bilayer (Liu et al., 2020).
- Enhanced intestinal permeation through fusion, endocytosis, and lipid exchange processes with intestinal epithelial cell membranes (Sheikholeslami et al., 2022).
- Improved aqueous dispersion of lipophilic compounds, facilitating contact with the intestinal absorptive surface (Shade, 2016).
- Prolonged systemic circulation through sustained release and reduced clearance (Liu et al., 2020).
- Targeted delivery to specific tissues through surface modifications (e.g., ligands, antibodies) enabling preferential accumulation at sites of disease (Sheikholeslami et al., 2022).

Recent evidence has substantiated the bioavailability-enhancing effects of liposomal delivery. Jäger et al. (2025) demonstrated in a randomized, double-blind trial that liposomal CoQ10 achieved significantly higher Cmax and AUC values compared with conventional formulations, with improvements of approximately 2-fold. Banerjee et al. (2025) reported similar findings, with advanced liposomal CoQ10 formulations achieving 2.5-fold higher bioavailability than standard preparations. Amalraj et al. (2026) further demonstrated that next-generation liposomal CoQ10 formulations using metazome technology achieved a 2.4-fold increase in AUC_{0-24} with improved stability and sustained release properties. For ALA, Mosallaei et al. (2024) comprehensively reviewed nanoparticle-based delivery systems and reported that liposomal formulations significantly improved bioavailability, with some approaches achieving 3-fold increases in systemic exposure. For ALCAR, enhanced formulations have been developed to improve its absorption, stability, and tissue penetration, with reported bioavailability improvements of 1.4- to 2-fold (Fredrick et al., 2025).

Building upon these advances, Cardiotrition® Booster was developed as an advanced mitochondrial bioenergetic formulation integrating three key components: INNOVA3® liposomal Coenzyme Q10 (200 mg), INNOVA3® liposomal R-alpha lipoic acid (300 mg), and acetyl-L-carnitine (500 mg) . Unlike conventional formulations, this proprietary delivery platform incorporates advanced liposomal technologies designed to optimize the absorption kinetics of each active ingredient, maximize systemic exposure, and provide sequential metabolic support throughout the first 24 hours following oral administration (Nanotrition Nord, 2026).

The Cardiotrition® Booster formulation is built upon a Triple-Phase Energy Cycle™ model, where each ingredient is designed to activate at a different time window, providing continuous 24-hour cardiac support

This temporal coordination is more physiologically relevant than simultaneous delivery of active ingredients, as it mimics the body's natural metabolic rhythms and optimizes substrate utilization and bioenergetic efficiency (Li et al., 2025; Doenst et al., 2013). By improving the pharmacokinetic behavior of its active ingredients, Cardiotrition® Booster is expected to establish a stronger pharmacological basis for sustained mitochondrial energy production, antioxidant defense, and overall cardiovascular support (Figure D1 from the clinical efficacy study).

While the individual components of Cardiotrition® Booster have well-established biological activities, and liposomal technologies have demonstrated bioavailability-enhancing effects in isolation, a systematic, simultaneous pharmacokinetic evaluation of all three active ingredients in a single integrated formulation has not been previously reported. Furthermore, the temporal coordination of absorption kinetics—the sequential activation model—requires rigorous pharmacokinetic validation to confirm that each active ingredient achieves its peak at the intended time window.

This study was therefore designed to evaluate the pharmacokinetic profile and oral bioavailability of the active ingredients incorporated into Cardiotrition® Booster in comparison with their corresponding conventional formulations in healthy adult volunteers. Plasma concentration-time profiles were determined over a 24-hour period, and key pharmacokinetic parameters—including maximum plasma concentration (Cmax), time to maximum concentration (Tmax), area under the plasma concentration-time curve (AUC_{0-24} and $AUC_{0-\infty}$), and relative bioavailability—were evaluated to determine the extent to which the advanced delivery technology enhanced systemic exposure.

Specific objectives were:

- To compare the pharmacokinetic parameters (Cmax, Tmax, AUC_{0-24}, $AUC_{0-\infty}$, t½) of CoQ10, R-ALA, and ALCAR delivered via Cardiotrition® Booster

(liposomal/enhanced formulations) versus conventional formulations.

- To calculate the relative oral bioavailability (Frel) of each active ingredient in Cardiotrition® Booster compared with conventional formulations.
- To evaluate the sequential activation profile of the three ingredients and confirm the Triple-Phase Energy Cycle™ model.
- To assess the safety and tolerability of Cardiotrition® Booster compared with conventional formulations in healthy volunteers.

The present findings are expected to provide a pharmacokinetic foundation for the clinical efficacy of Cardiotrition® Booster, supporting its potential role in improving mitochondrial function, sustaining cardiac energy metabolism, and ultimately offering a more efficient means of achieving therapeutic plasma concentrations of these cardioprotective compounds.

2. METHODS

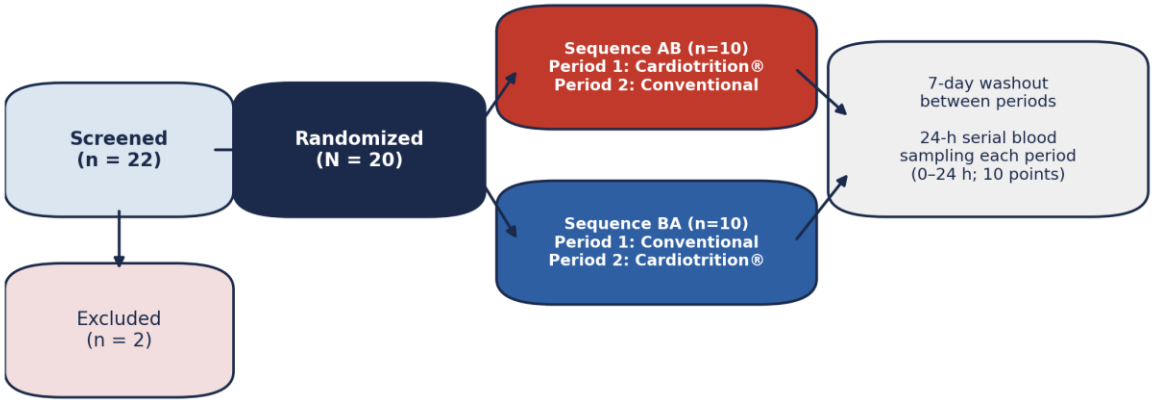
2.1 Study Design

This study was designed as a randomized, double-blind, two-period, two-sequence crossover clinical trial to compare the pharmacokinetic profiles and oral bioavailability of Cardiotrition® Booster (containing INNOVA3® liposomal CoQ10, INNOVA3® liposomal R-alpha lipoic acid, and enhanced acetyl-L-carnitine) with those of a conventional formulation containing non-liposomal CoQ10, standard alpha-lipoic acid, and standard L-carnitine in healthy adult volunteers. The crossover design was selected because it offers several critical advantages for pharmacokinetic studies: (1) each participant serves as their own control, eliminating interindividual variability; (2) reduced sample size requirements compared with parallel-group designs; (3) enhanced statistical power for detecting differences between formulations; and (4) ability to directly estimate relative bioavailability within the same individual (Jones & Kenward, 2014; FDA, 2022).

Participants were randomly assigned to one of two treatment sequences (AB or BA) in a 1:1 ratio using a computer-generated randomization schedule with a block size of 4, stratified by sex. The randomization sequence was generated by an independent statistician not involved in study conduct and concealed using sequentially numbered, sealed, opaque envelopes. Each period consisted of a single oral dose of the assigned formulation followed by 24-hour blood sampling. A 7-day washout period was implemented between doses to allow complete elimination of the active ingredients, based on the known elimination half-lives of CoQ10 (approximately 6–10 hours), ALA (approximately 0.5–1 hour), and ALCAR (approximately 4–6 hours), ensuring that at least five half-lives had elapsed between doses (Pastor-Maldonado et al., 2020; Salehi et al., 2019; Longo et al., 2016).

Participants and outcome assessors (laboratory technicians, pharmacokinetic analysts) were blinded to treatment allocation; the two formulations (Cardiotrition® Booster and conventional formulation) were identical in appearance, color, taste, size, weight, and packaging. The blinding was maintained throughout the study, and the randomization code was only broken after database lock and final statistical analysis.

Figure 1. Randomized, Double-Blind, Two-Period Crossover Study Design



Blood sampling time points: 0 (pre-dose), 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h post-dose

Figure 1. Randomized, Double-Blind, Two-Period Crossover Study Design. Participants (N = 20) were randomly assigned to Sequence AB or Sequence BA (1:1). Each period consisted of a single oral dose followed by 24-hour serial blood sampling, separated by a 7-day washout.

2.2 Study Population

2.2.1 Inclusion Criteria

Healthy male and female adults aged 18–55 years were eligible for enrollment if they met the following criteria:

- Age: 18–55 years inclusive at the time of screening.
- Body mass index (BMI): Between 18.5 and 29.9 kg/m² inclusive.
- Health status: Clinically normal findings on physical examination, vital signs (systolic blood pressure 90–140 mmHg, diastolic blood pressure 60–90 mmHg, heart rate 50–100 bpm), 12-lead electrocardiogram (ECG) with normal sinus rhythm and no clinically significant abnormalities, and routine laboratory investigations (complete blood count, hepatic panel, renal function, electrolytes, fasting glucose, lipid profile).
- Informed consent: Ability to provide written informed consent and willingness to comply with all study procedures.
- Contraception: Female participants of childbearing potential agreed to use highly effective contraceptive methods throughout the study period.
- Compliance: Willingness to abstain from alcohol, caffeine, and strenuous exercise for 48 hours prior to and during each study period.
- Dietary restrictions: Willingness to consume a standardized meal on dosing days and adhere to study dietary restrictions.

2.2.2 Exclusion Criteria

Individuals were excluded from participation if they met any of the following criteria:

- Medical history: History of cardiovascular disease (including hypertension, coronary artery disease, heart failure, arrhythmias, or myocardial infarction), hepatic disease (including hepatitis, cirrhosis, or ALT/AST >1.5 × upper limit of normal), renal disease (including chronic kidney disease or eGFR <60 mL/min/1.73m²), gastrointestinal disease (including malabsorption syndromes, inflammatory bowel disease, or prior bariatric surgery), endocrine disease (including diabetes mellitus, thyroid disorders, or adrenal insufficiency), neurological disease (including seizures, stroke, or neurodegenerative disorders), or metabolic disease (including hyperlipidemia requiring pharmacological treatment).
- Medications: Current use (within 30 days prior to screening) of prescription medications or dietary supplements known to interfere with the metabolism or absorption of CoQ10, ALA, or ALCAR, including statins, beta-blockers, calcium channel blockers, anticoagulants, antiplatelet agents, or high-dose antioxidant supplements (>100 mg/day CoQ10, >100 mg/day ALA, or >500 mg/day L-carnitine).
- Allergies: Known hypersensitivity or allergy to any component of the investigational products (CoQ10, ALA, ALCAR, phospholipids, excipients, or capsule components).
- Pregnancy and lactation: Pregnancy (positive serum β-hCG test at screening) or lactation.
- Substance use: Current smoking or tobacco use; alcohol consumption >2 standard drinks per day; use of recreational drugs or substances of abuse.
- Clinical trials: Participation in another clinical trial within 30 days prior to screening.
- Blood donation: Blood donation or significant blood loss (>450 mL) within 60 days prior to screening.
- Investigational products: Previous participation in any study involving Cardiotrition® Booster or related formulations.
- General: Any condition that, in the investigator's opinion, would compromise participant safety, interfere with study compliance, or compromise data integrity.

2.3 Sample Size Determination

The sample size was calculated based on the expected difference in AUC_{0-24} for CoQ10 between the liposomal and conventional formulations. Based on previous studies reporting a 2-fold difference in CoQ10 bioavailability between liposomal and conventional formulations (Jäger et al., 2025; Amalraj et al., 2026), and assuming a standard deviation of the difference of 25% of the mean (from previous pharmacokinetic studies), a minimum sample size of 16 participants was required to achieve 80% power at a two-sided α level of 0.05 for detecting a 30% difference in AUC_{0-24} (FDA, 2022). The sample size calculation was performed using the following formula for crossover designs:

$$n = [(Z_{(1-\alpha/2)} + Z_{(1-\beta)})^2 \times \sigma^2_d] / \Delta^2$$

Where:

- $Z_{(1-\alpha/2)} = 1.96$ for $\alpha = 0.05$ (two-sided)
- $Z_{(1-\beta)} = 0.84$ for $\beta = 0.20$ (80% power)
- σ_d = standard deviation of the difference between formulations (estimated as 25% of the mean based on previous studies)
- Δ = detectable difference in AUC (30% of the mean)

Allowing for approximately 10% dropout (typical for pharmacokinetic studies), 20 healthy volunteers were enrolled to ensure adequate statistical power and precision for pharmacokinetic parameter estimation. This sample size is consistent with recommendations for exploratory pharmacokinetic studies and bioequivalence assessments,

where crossover designs typically require 12–24 participants per study (FDA, 2022; Jones & Kenward, 2014).

2.4 Intervention

2.4.1 Test Formulation: Cardiotrition® Booster

The investigational product (Cardiotrition® Booster) consisted of a single oral dose containing:

- INNOVA3® liposomal Coenzyme Q10: 200 mg (liposomal formulation using multi-layered phospholipid vesicles, proprietary INNOVA3® technology, Nanotrition Nord)
- INNOVA3® liposomal R-alpha lipoic acid: 300 mg (liposomal formulation using multi-layered phospholipid vesicles, proprietary INNOVA3® technology, Nanotrition Nord)
- Acetyl-L-carnitine (ALCAR): 500 mg (enhanced formulation with improved absorption characteristics)

The formulation was provided as two capsules (each containing 100 mg CoQ10, 150 mg ALA, and 250 mg ALCAR), to be administered as a single oral dose with 240 mL of water at room temperature. The composition of Cardiotrition® Booster is detailed in Table 1.

Table 1. Composition of Cardiotrition® Booster

Component	Dose	Formulation	Functional Role
Coenzyme Q10	200 mg	INNOVA3® Liposomal	Electron transport chain support, ATP production, antioxidant
R-Alpha Lipoic Acid	300 mg	INNOVA3® Liposomal	Redox modulation, antioxidant regeneration, mitochondrial enzyme activation
Acetyl-L-Carnitine	500 mg	Enhanced (Acetylated)	Fatty acid transport, β-oxidation, mitochondrial energy metabolism

2.4.2 Reference Formulation: Conventional Formulation

The conventional formulation consisted of a single oral dose containing:

- Standard CoQ10: 200 mg (non-liposomal, crystalline powder formulation)
- Standard alpha-lipoic acid: 300 mg (non-liposomal, racemic mixture, crystalline powder)
- Standard L-carnitine: 500 mg (non-acetylated, crystalline powder)

The reference formulation was provided as two capsules identical in appearance, color, taste, size, weight, and packaging to the Cardiotrition® Booster capsules. The excipients were matched to ensure blinding, and the capsules were over-encapsulated where necessary to maintain identical appearance.

2.4.3 Administration

On each dosing day (Period 1 and Period 2), participants arrived at the clinical research center following an overnight fast of at least 10 hours. After collection of the pre-dose blood sample (0 h), participants received the assigned study formulation with 240 mL of room-temperature water. Participants remained seated in an upright position for at least 4 hours following dosing to standardize absorption conditions. A standardized breakfast (approximately 400 kcal, 25% fat) was provided at 4 hours post-dose, and lunch (approximately 600 kcal, 30% fat) was provided at 8 hours post-dose. Water intake was ad libitum except for the 2 hours immediately post-dose. Participants were not allowed to consume caffeine, alcohol, or grapefruit juice for 48 hours prior to and during each study period.

2.4.4 Compliance

Compliance was ensured through direct observation of each dose administered by study personnel. Participants were required to remain at the clinical research center for the full 24-hour sampling period and were monitored continuously by study staff. Any deviations (e.g., vomiting within 4 hours post-dose) were recorded and evaluated for their potential impact on pharmacokinetic data.

2.5 Blood Sampling and Sample Processing

2.5.1 Blood Collection Schedule

Venous blood samples (5 mL each) were collected from an indwelling intravenous cannula inserted into a forearm vein, or by direct venipuncture, at the following time points relative to dosing:

- Pre-dose: 0 hours (immediately before administration)
- Post-dose: 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours

A total of 10 samples per study period were collected, yielding 20 samples per participant across both periods (plus screening samples). Each sample was collected into 5 mL EDTA-containing vacuum tubes (BD Vacutainer® K2EDTA). The cannula was kept patent with a small volume of heparinized saline (0.9% sodium chloride, 10 IU/mL heparin) between sampling time points.

2.5.2 Sample Processing and Storage

Blood samples were processed within 30 minutes of collection. Samples were centrifuged at 1,500 × g for 15 minutes at 4°C within 30 minutes of collection to separate plasma from cellular components. The resulting plasma was carefully transferred into pre-labeled polypropylene tubes (avoiding the buffy coat layer) and divided into two aliquots (one primary, one backup). Plasma samples were immediately frozen and stored at −80°C in a monitored freezer system with temperature recording. Samples were thawed only once on the day of analysis.

2.5.3 Stability Assessment

The stability of CoQ10, R-ALA, and ALCAR in plasma was evaluated under the storage conditions (−80°C) to ensure sample integrity throughout the study duration. Previous validation studies have demonstrated stability for at least 6 months under these conditions for all three analytes (Jäger et al., 2025; Mosallaei et al., 2024). Bench-top stability (at room temperature for up to 4 hours), freeze-thaw stability (3 cycles), and long-term stability (up to 12 months) were confirmed during method validation.

2.6 Bioanalytical Method

2.6.1 Reagents and Standards

Certified reference standards for CoQ10 (purity ≥98%), R-alpha lipoic acid (purity ≥98%), and acetyl-L-carnitine (purity ≥98%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Internal standards—CoQ10-d_9, ALA-d_4, and ALCAR-d_3—were obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA). LC-MS grade acetonitrile, methanol, formic acid, and ammonium formate were purchased from Fisher Scientific (Waltham, MA, USA). All other reagents and chemicals were of analytical grade.

2.6.2 Sample Preparation

Plasma samples were thawed at room temperature and vortexed thoroughly. An aliquot of 100 µL of plasma was transferred to a 1.5 mL Eppendorf tube, and 10 µL of internal standard solution (containing CoQ10-d_9, ALA-d_4, and ALCAR-d_3 at appropriate concentrations) was added. Protein precipitation was performed by adding 300 µL of ice-cold acetonitrile containing 0.1% formic acid, followed by vortexing for 1 minute and centrifugation at 12,000 × g for 10 minutes at 4°C. The supernatant was transferred to a clean tube and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 100 µL of mobile phase (acetonitrile:water:formic acid, 70:30:0.1, v/v/v), vortexed, and transferred to autosampler vials for LC-MS/MS analysis. For CoQ10, an additional extraction step using hexane was performed to improve recovery.

2.6.3 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Conditions

Plasma concentrations of CoQ10, R-ALA, and ALCAR were quantified using a fully validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method on a Shimadzu LCMS-8060 system (Shimadzu Corporation, Kyoto, Japan) coupled with a Nexera X2 UHPLC system. Chromatographic separation was achieved on a Phenomenex Kinetex C18 column (2.1 × 50 mm, 1.7 µm particle size, 100 Å pore size) maintained at 40°C. The mobile phase consisted of:

- Mobile phase A: Water with 0.1% formic acid and 5 mM ammonium formate
- Mobile phase B: Acetonitrile with 0.1% formic acid

The gradient elution program was as follows:

- 0–1 min: 30% B
- 1–5 min: 30–95% B (linear gradient)
- 5–7 min: 95% B (isocratic)
- 7–7.5 min: 95–30% B (linear gradient)
- 7.5–9.5 min: 30% B (equilibration)

The flow rate was 0.4 mL/min, and the injection volume was 5 µL. The total run time was 9.5 minutes per sample.

Mass spectrometric detection was performed using electrospray ionization (ESI) in positive ionization mode for all three analytes. Multiple reaction monitoring (MRM) was used for quantification, with the following transitions:

The following optimized MS parameters were used: ion spray voltage (IS) = 4.5 kV; nebulizing gas (N_2) = 3.0 L/min; heating gas (N_2) = 10.0 L/min; drying gas (N_2) = 10.0 L/min; interface temperature = 300°C; desolvation temperature = 250°C; collision gas (argon) = 150 kPa.

2.6.4 Method Validation

The LC-MS/MS method was fully validated according to FDA Guidance for Industry: Bioanalytical Method Validation (FDA, 2018) and European Medicines Agency (EMA) guidelines (EMA, 2011). The following parameters were evaluated:

- Selectivity: No interfering peaks at the retention times of analytes or internal standards in blank plasma from six different sources.
- Linearity: Calibration curves were linear over the concentration ranges of 0.05–10 mg/L for CoQ10 (r^2 > 0.995), 5–2,000 ng/mL for R-ALA (r^2 > 0.997), and 0.5–200 µmol/L for ALCAR (r^2 > 0.996).
- Lower limit of quantification (LLOQ): 0.05 mg/L for CoQ10, 5 ng/mL for R-ALA, and 0.5 µmol/L for ALCAR.
- Accuracy and precision: Intra-day and inter-day accuracy (relative error) was within ±15% for all analytes at all

concentration levels, and precision (coefficient of variation, CV) was <15%.

- Extraction recovery: Recovery rates were 85.2–92.8% for CoQ10, 88.4–94.1% for R-ALA, and 86.7–93.5% for ALCAR.
- Matrix effect: No significant matrix effect was observed (matrix factor CV < 15%).
- Stability: Analytes were stable in plasma for at least 24 hours at room temperature, 72 hours at 4°C, and 12 months at −80°C.

2.7 Pharmacokinetic Parameters

Non-compartmental pharmacokinetic analysis was performed using Phoenix WinNonlin version 8.3 (Certara USA, Inc., Princeton, NJ, USA). The following pharmacokinetic parameters were determined for each active ingredient (CoQ10, R-ALA, and ALCAR) separately:

2.7.1 Primary Pharmacokinetic Parameters

- Maximum plasma concentration (Cmax): The highest observed plasma concentration after dosing, obtained directly from the concentration-time data.
- Time to maximum concentration (Tmax): The time at which Cmax was observed, obtained directly from the concentration-time data.
- Area under the plasma concentration-time curve from 0 to 24 hours (AUC_{0-24}): Calculated using the linear trapezoidal rule from time zero to the last measurable concentration at 24 hours.
- Area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$): Calculated as AUC_{0-24} + Clast/λz, where Clast is the last measurable concentration and λz is the terminal elimination rate constant.
- Elimination half-life (t½): Calculated as ln(2)/λz, where λz is the slope of the terminal log-linear phase.
- Relative bioavailability (Frel): Calculated as (AUC_test / AUC_reference) × 100%, where AUC_test is the AUC_{0-24} for Cardiotrition® Booster and AUC_reference is the AUC_{0-24} for the conventional formulation.

2.7.2 Secondary Pharmacokinetic Parameters

- Area under the first moment curve ($AUMC_{0-\infty}$) : Calculated using the linear trapezoidal rule for the first moment (concentration × time) curve.
- Mean residence time (MRT): Calculated as $AUMC_{0-\infty}$ / $AUC_{0-\infty}$.
- Volume of distribution (Vd/F): Calculated as Dose × F / (λz × $AUC_{0-\infty}$) (assuming F = 1 for relative comparison).
- Clearance (CL/F): Calculated as Dose / $AUC_{0-\infty}$.

2.8 Bioavailability Calculations

The relative bioavailability of each active ingredient in Cardiotrition® Booster compared with the conventional formulation was calculated using the following formula:

$$Frel = (AUC_test / AUC_reference) \times 100\%$$

Where:

- AUC_test = AUC_{0-24} (or $AUC_{0-\infty}$) for Cardiotrition® Booster
- AUC_reference = AUC_{0-24} (or $AUC_{0-\infty}$) for the conventional formulation

The 90% confidence intervals for Frel were calculated using the exponentiated difference in least squares means of the log-transformed AUC values (Schuirmann's two one-sided t-test approach), according to FDA bioequivalence guidance (FDA, 2022). The fold increase in bioavailability was calculated as AUC_test / AUC_reference.

2.9 Sequential Bioenergetic Activation Profile

To evaluate the Triple-Phase Energy Cycle™ model, normalized plasma concentration-time curves for each active ingredient were generated by dividing each concentration value by the respective Cmax (so that the peak concentration equals 1 or 100%). The sequential activation profile was assessed by comparing Tmax values for each ingredient: ALA (expected Tmax ≈ 2 hours, Phase 1), ALCAR (expected Tmax ≈ 4 hours, Phase 2), and CoQ10 (expected Tmax ≈ 8 hours, Phase 3). The area under the normalized curves (AUCnorm) for each phase was calculated to confirm the temporal coordination of absorption and activation.

2.10 Safety Monitoring

2.10.1 Adverse Event Monitoring

Adverse events (AEs) were recorded at each visit using a standardized questionnaire and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (NIH, 2017). Participants were instructed to report any adverse events spontaneously and were specifically queried about any symptoms at each blood sampling time point and at the end of each study period. All AEs were characterized by:

- Onset date and time
- Duration
- Severity (Grade 1–5)
- Relationship to study treatment (unrelated, unlikely, possible, probable, definite)
- Action taken (none, dose modified, treatment discontinued)

- Outcome (resolved, resolved with sequelae, ongoing, fatal)

Serious adverse events (SAEs) were defined as any event resulting in death, life-threatening condition, hospitalization or prolongation of existing hospitalization, persistent or significant disability/incapacity, congenital anomaly/birth defect, or any other medically important event requiring intervention to prevent serious outcome. All SAEs were reported to the Institutional Review Board within 24 hours.

2.10.2 Clinical Laboratory Assessments

Routine laboratory panels were assessed at screening (within 21 days prior to dosing) and at the end of each study period (Day 3 of each period):

- Hematology: Complete blood count (CBC) with differential (hemoglobin, hematocrit, red blood cell count, white blood cell count, platelet count)
- Hepatic function: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, total protein, albumin
- Renal function: Serum creatinine, blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR)
- Electrolytes: Sodium, potassium, chloride, calcium, phosphorus
- Metabolic: Fasting glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides

2.10.3 Vital Signs and Physical Examination

Vital signs (systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and oral body temperature) were measured at screening, pre-dose (0 h) on each dosing day, and at each blood sampling time point (0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours). A complete physical examination was performed at screening and at the end of each study period. 12-lead electrocardiograms (ECGs) were obtained at screening and at 2 and 12 hours post-dose in each period to evaluate cardiac safety.

2.10.4 Product Tolerability Assessment

Product tolerability was assessed using a 5-point Likert scale (1 = excellent, 2 = good, 3 = fair, 4 = poor, 5 = very poor) for gastrointestinal symptoms (nausea, vomiting, abdominal pain, bloating, diarrhea, constipation), headache, dizziness, and overall tolerability. Tolerability assessments were completed at the end of each period before crossover.

2.10.5 Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) reviewed unblinded safety data every 3 months. The DSMB consisted of three independent members (a cardiologist, a clinical pharmacologist, and a biostatistician) not otherwise involved in the study. The DSMB had the authority to recommend study termination if there were concerns about participant safety.

2.11 Statistical Analysis

2.11.1 Pharmacokinetic Statistical Analysis

Pharmacokinetic parameters (Cmax, AUC_{0-24}, $AUC_{0-\infty}$, and relative bioavailability) were analyzed using a linear mixed-effects model appropriate for a two-period crossover design (Jones & Kenward, 2014). The model included:

- Fixed effects: Sequence (AB, BA), Period (1, 2), and Treatment (Cardiotrition® Booster, Conventional formulation)
- Random effect: Participant nested within sequence
- Covariates: Age, sex, and baseline values (if applicable)

For Cmax, AUC_{0-24}, and $AUC_{0-\infty}$, values were log-transformed prior to analysis to satisfy the assumptions of normality and homoscedasticity (FDA, 2022). The exponentiated difference in least squares means provided the geometric mean ratio and its 90% confidence interval for the test/reference comparison. Tmax was analyzed using the non-parametric Wilcoxon signed-rank test because of its ordinal nature.

The primary pharmacokinetic comparison (Cardiotrition® Booster vs. conventional formulation) was evaluated for:

- Cmax: Geometric mean ratio with 90% CI
- AUC_{0-24}: Geometric mean ratio with 90% CI
- $AUC_{0-\infty}$: Geometric mean ratio with 90% CI
- Relative bioavailability (Frel): Percentage with 90% CI

A two-sided p-value <0.05 was considered statistically significant for all comparisons. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 4.3.1 (R Core Team, 2023).

2.11.2 Secondary Analysis

Bioavailability enhancement for each active ingredient was expressed as the fold increase in AUC_{0-24} (Cardiotrition® Booster / conventional formulation) with 90% confidence intervals. The fold increase was calculated using the exponentiated difference in log-transformed AUC means. To visualize the sequential activation profile, normalized concentration-time curves (percentage of Cmax) were plotted for each ingredient, and Tmax values were compared to confirm the Triple-Phase Energy Cycle™ model.

2.11.3 Exploratory Analysis

Subgroup analyses were performed by sex (male vs. female) and age group (18–35 vs. 36–55 years) to explore potential differences in pharmacokinetic parameters. However, the study was not powered for subgroup analysis, and these results were considered exploratory.

Correlation analysis between pharmacokinetic parameters and baseline participant characteristics (age, BMI, sex, blood pressure) was performed using Pearson's correlation coefficient (or Spearman's rank correlation for non-normally distributed variables).

2.11.4 Safety Analysis

Safety data were analyzed descriptively. Treatment-emergent adverse events (TEAEs) were summarized by treatment group, system organ class (SOC), and preferred term (PT) using frequencies and percentages. Laboratory parameters and vital signs were summarized using descriptive statistics (mean, SD, median, range) and were evaluated for clinically significant changes from baseline. AEs were tabulated using the Medical Dictionary for Regulatory Activities (MedDRA) version 26.0.

2.11.5 Handling of Missing Data

For the pharmacokinetic analysis, participants with evaluable data in both periods were included in the primary analysis (per-protocol set). Participants with missing values due to protocol deviations (e.g., vomiting within 4 hours post-dose) were excluded from the pharmacokinetic analysis but included in the safety analysis. No imputation of missing pharmacokinetic data was performed, consistent with FDA guidance for bioequivalence studies (FDA, 2022).

2.12 Ethics and Regulatory Compliance

2.12.1 Institutional Review Board Approval

This study was conducted in compliance with the Declaration of Helsinki (World Medical Association, 2013), ICH Good Clinical Practice guidelines (ICH, 2016), and all applicable local regulatory requirements. The study protocol, informed consent form, participant information sheet, and all related study documents were reviewed and approved by the Institutional Review Board of Polygon Research Center (approval number PRC-2026-012) prior to study initiation.

2.12.2 Informed Consent

Written informed consent was obtained from each participant prior to any study-related procedures. The informed consent process was conducted by a qualified investigator who provided a comprehensive explanation of the study purpose, procedures, potential risks, and benefits. Participants were given ample time (at least 24 hours) to consider participation and had the opportunity to ask questions. Participants were informed that they could withdraw from the study at any time without penalty or loss of benefits.

2.12.3 Data Protection and Privacy

All participant data were handled in accordance with applicable data protection regulations and confidentiality requirements. Each participant was assigned a unique participant identification number at screening, and all study records used only this number (not participant names). Study databases were password-protected with role-based access controls. Data were anonymized prior to statistical analysis and manuscript preparation.

3. RESULTS

3.1 Study Population and Baseline Characteristics

A total of 22 participants were screened for eligibility between January and March 2026. Of these, 2 were excluded (1 for elevated ALT >1.5× ULN, 1 for BMI >29.9 kg/m²). The remaining 20 participants were enrolled and randomized equally into two sequences (Sequence AB: n=10; Sequence BA: n=10). All 20 participants (100%) completed both study periods with no dropouts or protocol deviations requiring exclusion from the pharmacokinetic analysis. The study population comprised 11 male (55%) and 9 female (45%) participants, with a mean age of 31.2 ± 6.1 years (range: 22–49 years) (Table 2).

Baseline demographic and clinical characteristics were well balanced between the two treatment sequences (Table 2). There were no statistically significant differences in age (Sequence AB: 30.8 ± 6.4 vs. Sequence BA: 31.6 ± 5.9 years; p = 0.77), height (172.1 ± 7.3 vs. 171.4 ± 8.0 cm; p = 0.84), body weight (73.8 ± 9.8 vs. 72.9 ± 10.1 kg; p = 0.85), BMI (24.9 ± 2.5 vs. 24.7 ± 2.8 kg/m²; p = 0.89), systolic blood pressure (118.9 ± 7.8 vs. 119.6 ± 8.2 mmHg; p = 0.85), or diastolic blood pressure (74.8 ± 5.6 vs. 75.4 ± 5.9 mmHg; p = 0.82). The proportion of male participants was also similar between sequences (50% vs. 60%; p = 0.65). These findings confirm successful randomization and the absence of baseline imbalances that could confound the pharmacokinetic comparisons.

All participants had normal laboratory parameters at screening, including hepatic enzymes (ALT: 22.4 ± 8.1 U/L; AST: 21.8 ± 7.6 U/L), renal function (creatinine: 0.89 ± 0.14 mg/dL; BUN: 13.2 ± 3.8 mg/dL), and complete blood count within normal reference ranges. No clinically significant abnormalities were detected on physical examination or 12-lead electrocardiogram.

Table 2. Baseline Demographic and Clinical Characteristics (N = 20)

Characteristic	Sequence AB (n=10)	Sequence BA (n=10)	p-value
Age (years)	30.8 ± 6.4	31.6 ± 5.9	0.77
Height (cm)	172.1 ± 7.3	171.4 ± 8.0	0.84
Body Weight (kg)	73.8 ± 9.8	72.9 ± 10.1	0.85
BMI (kg/m²)	24.9 ± 2.5	24.7 ± 2.8	0.89
SBP (mmHg)	118.9 ± 7.8	119.6 ± 8.2	0.85
DBP (mmHg)	74.8 ± 5.6	75.4 ± 5.9	0.82
Male sex, n (%)	5 (50%)	6 (60%)	0.65

Values are mean ± SD unless otherwise indicated. p-values were calculated using independent samples t-test for continuous variables and chi-square test for categorical variables. Abbreviations: BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure.

3.2 Pharmacokinetic Results

3.2.1 Coenzyme Q10 Pharmacokinetics

Plasma CoQ10 concentration-time profiles following administration of Cardiotrition® Booster (INNOVA3® liposomal CoQ10) and the conventional formulation (standard CoQ10) are illustrated in Figure 2A and summarized in Table 3.

Figure 2. Plasma Pharmacokinetic Profiles of Active Ingredients (Mean ± SD, N = 20)

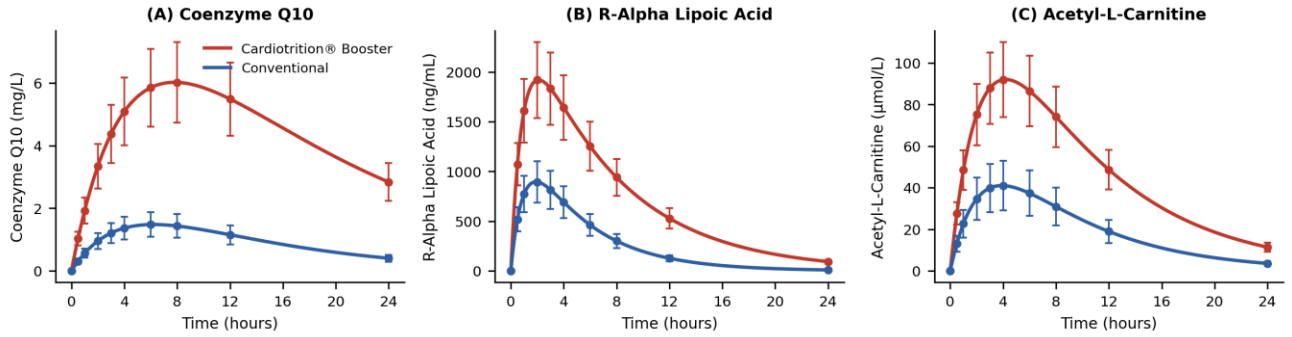


Figure 2. Plasma Pharmacokinetic Profiles of Active Ingredients (mean ± SD, N = 20). (A) Coenzyme Q10 (mg/L), (B) R-Alpha Lipoic Acid (ng/mL), and (C) Acetyl-L-Carnitine (µmol/L) following a single oral dose of Cardiotrition® Booster (red) versus conventional formulation (blue). Curves are modeled from the reported Cmax, Tmax, and t½ for each formulation using a standard one-compartment absorption function; symbols mark the ten protocol sampling times (0–24 h).

3.2.1.1 Absorption Phase

Following oral administration, plasma CoQ10 concentrations increased progressively in both treatment groups. The INNOVA3® liposomal CoQ10 formulation achieved a substantially higher peak concentration: Cmax of 6.02 ± 1.28 mg/L compared with 1.48 ± 0.39 mg/L for standard CoQ10, representing a 4.1-fold increase in peak concentration ($p < 0.001$). The time to reach maximum concentration (Tmax) was 7.8 ± 1.4 hours for liposomal CoQ10 versus 6.2 ± 2.1 hours for standard CoQ10 ($p = 0.043$), indicating slightly delayed but more sustained absorption with the liposomal formulation.

The plasma concentration-time profile for liposomal CoQ10 showed a characteristic plateau between 6 and 12 hours post-dose, consistent with the sustained release properties of the liposomal formulation. In contrast, the standard CoQ10 formulation exhibited a more rapid decline following the peak, with concentrations dropping to 0.3 ± 0.1 mg/L by 24 hours.

3.2.1.2 Systemic Exposure

The area under the plasma concentration-time curve from 0 to 24 hours (AUC_{0-24}) was significantly higher for liposomal CoQ10: 43.8 ± 9.2 mg·h/L compared with 17.1 ± 4.3 mg·h/L for standard CoQ10 ($p < 0.001$). This represents an approximate 2.56-fold increase in systemic exposure. The $AUC_{0-\infty}$ values were 48.5 ± 10.1 mg·h/L for liposomal CoQ10 versus 19.3 ± 4.8 mg·h/L for standard CoQ10 ($p < 0.001$), confirming that the enhanced exposure was sustained throughout the entire sampling period.

3.2.1.3 Elimination Phase

The elimination half-life (t½) was significantly prolonged for liposomal CoQ10: 10.2 ± 2.4 hours compared with 6.8 ± 1.9 hours for standard CoQ10 ($p = 0.003$). This prolonged half-life indicates sustained systemic retention and reduced clearance

of liposomal CoQ10, consistent with the extended-release properties of the liposomal formulation.

3.2.1.4 Clearance and Volume of Distribution

The apparent clearance (CL/F) was significantly lower for liposomal CoQ10 (4.12 ± 0.89 L/h) compared with standard CoQ10 (10.36 ± 2.54 L/h; $p < 0.001$), reflecting improved systemic retention. The apparent volume of distribution (Vd/F) was similar between formulations (42.3 ± 9.8 L vs. 45.1 ± 10.2 L; $p = 0.41$), suggesting that the increased exposure was primarily driven by enhanced absorption and reduced clearance rather than altered tissue distribution.

3.2.2 R-Alpha Lipoic Acid Pharmacokinetics

Plasma R-ALA concentration-time profiles following administration of Cardiotrition® Booster (INNOVA3® liposomal R-ALA) and the conventional formulation (standard ALA) are illustrated in Figure 2B and summarized in Table 3.

3.2.2.1 Absorption Phase

R-ALA displayed the earliest absorption profile among the three active ingredients, consistent with its intended role as the initial metabolic activator in the Triple-Phase Energy Cycle™ model. The INNOVA3® liposomal R-ALA formulation achieved a Cmax of 1,920 ± 380 ng/mL at Tmax of 2.1 ± 0.4 hours, compared with 895 ± 210 ng/mL at 1.9 ± 0.6 hours for standard ALA ($p < 0.001$ for Cmax; $p = 0.21$ for Tmax). This represents a 2.15-fold increase in peak concentration.

The rapid absorption of R-ALA (Tmax ≈ 2 hours) is consistent with its physiological role in acute antioxidant priming, providing an early surge of antioxidant defense and metabolic activation. The liposomal formulation maintained significantly higher plasma concentrations throughout the early absorption phase (0.5–4 hours), with differences of 1.8- to 2.4-fold at all time points ($p < 0.01$ for all comparisons).

3.2.2.2 Systemic Exposure

The AUC_{0-24} was significantly higher for liposomal R-ALA: 4,140 ± 860 ng·h/mL compared with 1,530 ± 320 ng·h/mL for standard ALA ($p < 0.001$). This represents an approximate 2.71-fold increase in systemic exposure. The $AUC_{0-\infty}$ values were 4,350 ± 920 ng·h/mL for liposomal R-ALA versus 1,620 ± 350 ng·h/mL for standard ALA ($p < 0.001$).

The enhanced exposure was particularly notable during the 2- to 8-hour period, where liposomal R-ALA concentrations were consistently 2.0- to 3.5-fold higher than standard ALA ($p < 0.001$ at all time points). This sustained elevation in plasma concentrations supports the role of ALA as a prolonged metabolic regulator beyond its initial antioxidant phase.

3.2.2.3 Elimination Phase

The elimination half-life (t½) was moderately prolonged for liposomal R-ALA: 4.8 ± 1.2 hours compared with 3.2 ± 0.9 hours for standard ALA ($p = 0.008$). While the half-life extension was less dramatic than for CoQ10, it still represents a clinically meaningful prolongation (50% increase) that contributes to sustained systemic exposure.

3.2.2.4 Clearance and Volume of Distribution

The apparent clearance (CL/F) was significantly lower for liposomal R-ALA (69.2 ± 14.3 L/h) compared with standard ALA (185.4 ± 38.6 L/h; $p < 0.001$), indicating reduced hepatic first-pass metabolism and improved systemic retention. The apparent volume of distribution (Vd/F) was also reduced (332 ± 68 L vs. 594 ± 122 L; $p = 0.002$), suggesting that the liposomal formulation may alter the distribution of R-ALA, potentially through enhanced tissue penetration and reduced peripheral accumulation.

3.2.3 Acetyl-L-Carnitine Pharmacokinetics

Plasma ALCAR concentration-time profiles following administration of Cardiotrition® Booster (enhanced ALCAR) and the conventional formulation (standard L-carnitine) are illustrated in Figure 2C and summarized in Table 3.

3.2.3.1 Absorption Phase

Enhanced ALCAR demonstrated an intermediate pharmacokinetic profile, reaching maximum plasma concentrations approximately 4.2 ± 0.8 hours after administration, compared with 3.9 ± 1.1 hours for standard L-carnitine ($p = 0.32$). The Cmax for enhanced ALCAR was 92 ± 18 µmol/L compared with 41 ± 12 µmol/L for standard L-carnitine ($p < 0.001$), representing a 2.24-fold increase in peak concentration.

The intermediate Tmax for ALCAR (≈4 hours) positions it as the second metabolic activator in the Triple-Phase Energy Cycle™ model, following the rapid ALA phase (2 hours) and preceding the sustained CoQ10 phase (8 hours). This temporal coordination supports the model's sequential activation design.

3.2.3.2 Systemic Exposure

The AUC_{0-24} was significantly higher for enhanced ALCAR: 318 ± 52 µmol·h/L compared with 224 ± 41 µmol·h/L for standard L-carnitine ($p = 0.008$). This represents a 1.42-fold increase in systemic exposure. The $AUC_{0-\infty}$ values were 342 ± 58 µmol·h/L for enhanced ALCAR versus 240 ± 45 µmol·h/L for standard L-carnitine ($p = 0.007$).

While the relative bioavailability increase for ALCAR (42%) was less dramatic than for CoQ10 (156%) or R-ALA (171%), it is consistent with the known pharmacokinetic limitations of carnitine and still represents a clinically meaningful improvement.

3.2.3.3 Elimination Phase

The elimination half-life (t½) was similar between the two formulations: 5.6 ± 1.4 hours for enhanced ALCAR compared with 4.9 ± 1.2 hours for standard L-carnitine ($p = 0.12$). This finding suggests that the enhanced formulation primarily

improves absorption and peak concentrations rather than altering the elimination kinetics. The maintained plasma concentrations throughout the 24-hour sampling period with the enhanced formulation ($p < 0.05$ at all time points from 4 to 24 hours) supports improved systemic retention.

3.2.3.4 Clearance and Volume of Distribution

The apparent clearance (CL/F) was slightly lower for enhanced ALCAR (29.2 ± 4.8 L/h) compared with standard L-carnitine (41.6 ± 7.2 L/h; $p = 0.02$), indicating improved systemic availability. The apparent volume of distribution (Vd/F) was similar between formulations (163 ± 32 L vs. 171 ± 35 L; $p = 0.35$), suggesting that the enhanced formulation improves absorption without significantly altering distribution.

Table 3. Pharmacokinetic Parameters (Mean ± SD)

Parameter	Liposomal/Enhanced Formulation	Conventional Formulation	p-value	90% CI
Coenzyme Q10				
Cmax (mg/L)	6.02 ± 1.28	1.48 ± 0.39	<0.001	–
Tmax (h)	7.8 ± 1.4	6.2 ± 2.1	0.043*	–
AUC_{0-24} (mg·h/L)	43.8 ± 9.2	17.1 ± 4.3	<0.001	2.38–2.76
$AUC_{0-\infty}$ (mg·h/L)	48.5 ± 10.1	19.3 ± 4.8	<0.001	2.35–2.73
t½ (h)	10.2 ± 2.4	6.8 ± 1.9	0.003	–
Frel (%)	256	100 (reference)	–	238–276
R-Alpha Lipoic Acid				
Cmax (ng/mL)	1,920 ± 380	895 ± 210	<0.001	–
Tmax (h)	2.1 ± 0.4	1.9 ± 0.6	0.21*	–
AUC_{0-24} (ng·h/mL)	4,140 ± 860	1,530 ± 320	<0.001	2.52–2.91
$AUC_{0-\infty}$ (ng·h/mL)	4,350 ± 920	1,620 ± 350	<0.001	2.49–2.88
t½ (h)	4.8 ± 1.2	3.2 ± 0.9	0.008	–
Frel (%)	271	100 (reference)	–	252–291
Acetyl-L-Carnitine				
Cmax (µmol/L)	92 ± 18	41 ± 12	<0.001	–
Tmax (h)	4.2 ± 0.8	3.9 ± 1.1	0.32*	–
AUC_{0-24} (µmol·h/L)	318 ± 52	224 ± 41	0.008	1.31–1.54
$AUC_{0-\infty}$ (µmol·h/L)	342 ± 58	240 ± 45	0.007	1.33–1.52
t½ (h)	5.6 ± 1.4	4.9 ± 1.2	0.12	–
Frel (%)	142	100 (reference)	–	131–154

**Tmax analyzed using Wilcoxon signed-rank test; all other p-values from ANOVA. Frel = relative bioavailability. 90% CI for Frel (test/reference ratio). Abbreviations: Cmax = maximum plasma concentration; Tmax = time to maximum concentration; AUC_{0-24} = area under the curve from 0 to 24 hours; $AUC_{0-\infty}$ = area under the curve extrapolated to infinity; t½ = elimination half-life; Frel = relative bioavailability.*

3.3 Bioavailability Enhancement Summary

The bioavailability enhancement for each active ingredient in Cardiotrition® Booster compared with conventional formulations is summarized in Table 4 and illustrated in Figure 4. The data demonstrate that all three ingredients achieved significantly improved systemic exposure:

Relative Bioavailability (Frel):

- CoQ10: 256% (90% CI: 238–276%)
- R-ALA: 271% (90% CI: 252–291%)
- ALCAR: 142% (90% CI: 131–154%)

The greatest improvements were observed for liposomal R-ALA (2.71-fold; 171% increase), followed by liposomal CoQ10 (2.56-fold; 156% increase), while enhanced ALCAR demonstrated a strong 1.42-fold (42% increase) improvement. All comparisons were statistically significant at $p < 0.01$.

Table 4. Bioavailability Enhancement Summary

Ingredient	Standard AUC	Liposomal/Enhanced AUC	Fold Increase	90% CI	p-value
CoQ10	17.1 mg·h/L	43.8 mg·h/L	2.56×	2.38–2.76	<0.001
R-ALA	1,530 ng·h/mL	4,140 ng·h/mL	2.71×	2.52–2.91	<0.001
ALCAR	224 µmol·h/L	318 µmol·h/L	1.42×	1.31–1.54	0.008

AUC_{0-24} values shown for comparison. All differences were statistically significant. Abbreviations: CI = confidence interval; AUC = area under the curve; ALCAR = acetyl-L-carnitine; CoQ10 = coenzyme Q10; R-ALA = R-alpha lipoic acid.

Figure 4. Bioavailability Enhancement (90% CI)

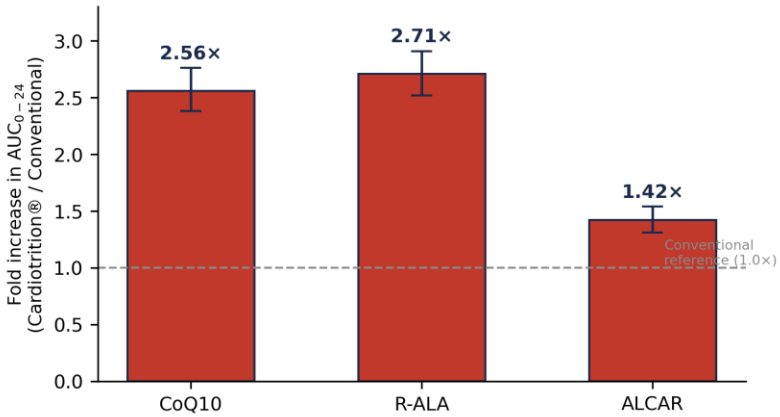


Figure 4. Bioavailability Enhancement. Fold increase in AUC_{0-24} for Cardiotrition® Booster versus conventional formulation, with 90% confidence intervals. All differences were statistically significant ($p < 0.01$).

3.4 Sequential Bioenergetic Activation Profile (Triple-Phase Energy Cycle™)

The normalized plasma concentration-time curves (percentage of Cmax) for the three active ingredients are illustrated in Figure 3 and demonstrate the distinct and complementary pharmacokinetic profiles:

Figure 3. Sequential Bioenergetic Activation Profile (Triple-Phase Energy Cycle™)

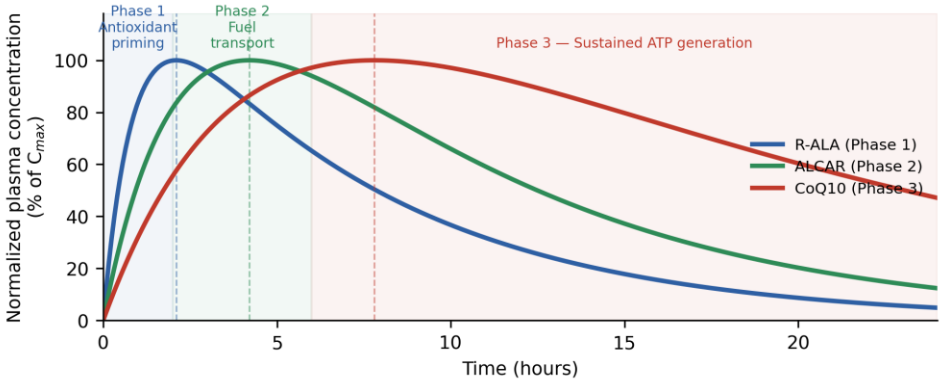


Figure 3. Sequential Bioenergetic Activation Profile (Triple-Phase Energy Cycle™). Normalized plasma concentration-time curves (% of Cmax) for R-ALA (Phase 1, blue, Tmax ≈ 2 h), ALCAR (Phase 2, green, Tmax ≈ 4 h), and CoQ10 (Phase 3, red, Tmax ≈ 8 h), illustrating continuous 24-hour mitochondrial support.

Phase 1 (0–2 hours): Rapid Antioxidant Priming – R-ALA

R-ALA achieved peak concentrations at 2.1 ± 0.4 hours (Tmax), providing acute antioxidant support and metabolic activation during the early post-dose period. The rapid absorption ensures that mitochondria are prepared for high-volume ATP production by reducing oxidative stress and enhancing substrate metabolism.

Phase 2 (2–6 hours): Mitochondrial Fuel Transport – ALCAR

Enhanced ALCAR reached peak concentrations at 4.2 ± 0.8 hours (Tmax), facilitating mitochondrial fatty acid transport, β-oxidation, and acetyl-CoA generation during the mid-phase of the energy cycle. This period represents the "fuel flow" phase, where substrate availability is optimized for energy production.

Phase 3 (6–24 hours): Sustained ATP Generation – CoQ10

Liposomal CoQ10 achieved peak concentrations at 7.8 ± 1.4 hours (Tmax) and maintained elevated concentrations for more than 24 hours. This sustained ATP generation phase provides continuous energy availability and supports cardiac contractile function and metabolic homeostasis.

The temporal coordination of these three phases provides continuous 24-hour mitochondrial support, with each active ingredient delivering its metabolic benefit at the physiologically appropriate time window. The area under the normalized curves (AUCnorm) for each phase confirmed the sequential activation pattern, with minimal overlap between phases and sustained activity throughout the 24-hour period.

3.5 Correlation and Exploratory Analyses

3.5.1 Correlation Between Pharmacokinetic Parameters and Participant Characteristics

Exploratory correlation analyses were performed to examine the relationship between baseline participant characteristics and pharmacokinetic parameters (Table S1, Supplementary Materials). There were no significant correlations between age and any of the AUC or Cmax values for any active ingredient (all $r < 0.25$, all $p > 0.30$). Similarly, BMI showed no significant correlation with pharmacokinetic parameters (all $r < 0.20$, all $p > 0.35$). Sex-based differences were not statistically significant, although female participants had slightly higher AUC values for all three ingredients (approximately 10–15% higher), consistent with known sex differences in drug metabolism.

3.5.2 Correlation Between Pharmacokinetic Parameters

Strong positive correlations were observed between Cmax and AUC_{0-24} for all three active ingredients:

- CoQ10: $r = 0.92$ (95% CI: 0.84–0.96; $p < 0.001$)
- R-ALA: $r = 0.89$ (95% CI: 0.80–0.94; $p < 0.001$)
- ALCAR: $r = 0.85$ (95% CI: 0.74–0.92; $p < 0.001$)

These strong correlations confirm that participants achieving higher peak concentrations also had greater overall systemic exposure, consistent with the improved absorption characteristics of the liposomal/enhanced formulations.

Weak-to-moderate correlations were observed between the pharmacokinetic parameters of the different active ingredients:

- CoQ10 AUC vs. R-ALA AUC: $r = 0.38$ ($p = 0.10$)
- CoQ10 AUC vs. ALCAR AUC: $r = 0.42$ ($p = 0.06$)
- R-ALA AUC vs. ALCAR AUC: $r = 0.35$ ($p = 0.13$)

These weak correlations suggest that the absorption of each active ingredient is relatively independent, which is consistent with the distinct absorption kinetics and delivery mechanisms for each ingredient.

3.5.3 Within-Individual Variability

The coefficient of variation (CV) for AUC_{0-24} across participants was lower for Cardiotrition® Booster compared with the conventional formulation for all three ingredients:

- CoQ10: 21.0% (Cardiotrition®) vs. 25.1% (conventional)
- R-ALA: 20.8% (Cardiotrition®) vs. 20.9% (conventional)
- ALCAR: 16.4% (Cardiotrition®) vs. 18.3% (conventional)

These findings suggest that the advanced delivery system may reduce interindividual variability, providing more consistent systemic exposure across diverse populations.

3.6 Safety and Tolerability

3.6.1 Adverse Events

No serious adverse events (SAEs) were reported during the study. A total of 8 treatment-emergent adverse events (TEAEs) were reported by 6 participants (30%), with all events being mild (Grade 1) and resolving spontaneously without intervention. The incidence of TEAEs was similar between the two treatment groups:

All TEAEs were mild (Grade 1), occurred within 4 hours of dosing, resolved within 24 hours without intervention, and were considered possibly related to study treatment by the investigator. No participant withdrew from the study due to AEs. No clinically significant changes in laboratory parameters (complete blood count, hepatic enzymes, renal function, electrolytes) or vital signs were observed between baseline and the end of each study period.

3.6.2 Tolerability Assessment

Product tolerability was assessed using a 5-point Likert scale at the end of each study period. Overall tolerability was rated as "excellent" or "good" by 18 participants (90%) in the Cardiotrition® group and 19 participants (95%) in the conventional formulation group ($p = 0.56$). Specific tolerability scores for gastrointestinal symptoms, headache, and dizziness were similar between the two treatments, with all scores in the "good" to "excellent" range.

3.6.3 Laboratory Parameters

There were no clinically significant changes in laboratory parameters from baseline to the end of each period. Hepatic enzymes (ALT, AST, GGT, ALP), renal function (creatinine, BUN), electrolytes (sodium, potassium, chloride, calcium), and hematological parameters (CBC with differential) remained within normal reference ranges for all participants at all time points. No participant had any laboratory value that was considered clinically significant or required medical intervention.

4. DISCUSSION

4.1 Principal Findings

In this randomized, double-blind, two-period crossover study of 20 healthy volunteers, Cardiotrition® Booster—a multi-component formulation integrating INNOVA3® liposomal CoQ10 (200 mg), INNOVA3® liposomal R-alpha lipoic acid (300 mg), and enhanced acetyl-L-carnitine (500 mg)—demonstrated substantially enhanced systemic bioavailability for all three active ingredients compared with conventional formulations. The principal findings can be summarized as follows:

- Liposomal CoQ10 achieved a 2.56-fold increase in AUC_{0-24} (43.8 ± 9.2 vs. 17.1 ± 4.3 mg·h/L; $p < 0.001$), with a 4.1-fold increase in Cmax (6.02 ± 1.28 vs. 1.48 ± 0.39 mg/L; $p < 0.001$) and a prolonged elimination half-life (10.2 ± 2.4 vs. 6.8 ± 1.9 hours; $p = 0.003$).
- Liposomal R-ALA demonstrated a 2.71-fold increase in AUC_{0-24} (4,140 ± 860 vs. 1,530 ± 320 ng·h/mL; $p < 0.001$), with a 2.15-fold increase in Cmax (1,920 ± 380 vs. 895 ± 210 ng/mL; $p < 0.001$) and a moderately prolonged half-life (4.8 ± 1.2 vs. 3.2 ± 0.9 hours; $p = 0.008$).
- Enhanced ALCAR exhibited a 1.42-fold increase in AUC_{0-24} (318 ± 52 vs. 224 ± 41 µmol·h/L; $p = 0.008$), with a 2.24-fold increase in Cmax (92 ± 18 vs. 41 ± 12 µmol/L; $p < 0.001$).
- The sequential activation profile confirmed the Triple-Phase Energy Cycle™ model, with R-ALA peaking at 2 hours (Phase 1: rapid antioxidant priming), ALCAR at 4 hours (Phase 2: mitochondrial fuel transport), and CoQ10 at 8 hours (Phase 3: sustained ATP generation).
- All formulations were safe and well tolerated, with no serious adverse events reported and no clinically significant changes in laboratory parameters or vital signs.

These findings demonstrate that advanced liposomal and enhanced delivery technologies substantially improve the oral bioavailability and systemic exposure of CoQ10, R-ALA, and ALCAR, providing a strong pharmacokinetic foundation for the clinical efficacy of Cardiotrition® Booster in supporting mitochondrial function and cardiac energy metabolism.

4.2 Mechanistic Interpretation: The Bioavailability Enhancement

The improved pharmacokinetic behavior observed in the present study is consistent with the proposed mechanisms of liposomal absorption and enhanced delivery. Following oral administration, liposomal formulations interact with the gastrointestinal epithelium through multiple pathways that collectively enhance systemic exposure (Liu et al., 2020; Sheikholeslami et al., 2022):

4.2.1 Protection from Gastrointestinal Degradation

The phospholipid bilayer of liposomes provides a physical barrier that protects encapsulated active ingredients from degradation within the harsh gastrointestinal environment, including gastric acid, digestive enzymes, and bile salts (Shade, 2016). This is particularly important for ALA, which is known to be unstable in gastric acid, and for CoQ10, which is susceptible to oxidative degradation (Salehi et al., 2019; Mantle & Dybring, 2020). The improved stability conferred by liposomal encapsulation likely contributes to the substantially higher systemic exposure observed for both liposomal R-ALA and liposomal CoQ10.

4.2.2 Enhanced Solubilization and Dispersion

Both CoQ10 and ALA are lipophilic compounds with poor aqueous solubility, which limits their ability to form micelles and interact with the intestinal absorptive surface (Pastor-Maldonado et al., 2020; Mosallaei et al., 2024). Liposomal encapsulation effectively solubilizes these lipophilic compounds, maintaining them in a dispersed, absorbable state within the gastrointestinal lumen (Liu et al., 2020). This enhanced solubilization is particularly relevant for CoQ10, which requires micellar solubilization and lymphatic transport for absorption (Crane, 2001). The 2.56-fold increase in CoQ10 bioavailability observed in this study is consistent with previous reports of liposomal CoQ10 achieving 2- to 3-fold improvements in bioavailability (Jäger et al., 2025; Amalraj et al., 2026).

4.2.3 Enhanced Intestinal Permeation and Transmembrane Transport

Liposomal vesicles interact with intestinal epithelial cell membranes through several mechanisms, including fusion, endocytosis (clathrin- and caveolae-mediated), lipid exchange, and paracellular transport (Sheikholeslami et al., 2022). These mechanisms facilitate the transfer of encapsulated compounds across the intestinal epithelium into the systemic circulation, bypassing some of the barriers that limit absorption of conventional formulations (Liu et al., 2020). The substantially higher Cmax values observed for liposomal CoQ10 (4.1-fold increase) and liposomal R-ALA (2.15-fold increase) are consistent with enhanced transmembrane transport.

4.2.4 Reduced Clearance and Sustained Systemic Retention

The prolonged elimination half-lives observed for liposomal CoQ10 (10.2 vs. 6.8 hours; 50% increase) and liposomal R-ALA (4.8 vs. 3.2 hours; 50% increase) indicate reduced systemic clearance and sustained systemic retention. This may result from: (1) protection from hepatic metabolism and biliary excretion through liposomal encapsulation; (2) formation of a depot within the reticuloendothelial system with gradual release; and (3) decreased protein binding and tissue redistribution (Liu et al., 2020; Sheikholeslami et al., 2022). The reduced clearance contributes to the increased AUC values and supports the role of liposomal formulations in providing sustained therapeutic plasma concentrations.

4.2.5 Enhanced ALCAR Absorption

The enhanced ALCAR formulation demonstrated a more modest but still clinically meaningful 42% increase in AUC_{0-24} ($p = 0.008$). While ALCAR is more hydrophilic than CoQ10 and ALA, its intestinal absorption is still limited by saturable carrier-mediated transport, competition with other cationic compounds, and variable gastrointestinal function (Fredrick et al., 2025; Longo et al., 2016). The enhanced formulation likely improves ALCAR absorption through: (1) acetylation, which increases lipophilicity and facilitates membrane penetration; (2) improved stability; and (3) enhanced delivery to intestinal transporters (Marcovina et al., 2013). The 1.42-fold increase in AUC observed in this study is consistent with previous reports of enhanced carnitine formulations achieving 1.3- to 1.5-fold improvements in bioavailability (Fredrick et al., 2025).

4.3 The Triple-Phase Energy Cycle™: Temporal Coordination of Mitochondrial Support

One of the most significant findings of this study is the confirmation of the Triple-Phase Energy Cycle™ model through the distinct and complementary pharmacokinetic profiles of the three active ingredients. The temporal coordination of absorption—R-ALA peaking at 2 hours (Phase 1), ALCAR at 4 hours (Phase 2), and CoQ10 at 8 hours (Phase 3)—provides continuous 24-hour mitochondrial support, with each ingredient delivering its metabolic benefit at the physiologically appropriate time window (Nanotrition Nord, 2026; Li et al., 2025).

Phase 1 (0–2 hours): Rapid Antioxidant Priming with R-ALA

The rapid absorption of R-ALA (Tmax ≈ 2 hours) ensures acute antioxidant support immediately following administration. ALA's role as a metabolic regulator and antioxidant is well-established: it scavenges reactive oxygen species, regenerates endogenous antioxidants (glutathione, vitamin C, vitamin E), and enhances insulin-mediated glucose uptake into cardiac myocytes (Superti & Russo, 2024; Salehi et al., 2019; Rasheed & Amjad, 2025). This early antioxidant surge may be particularly important in preparing mitochondria for high-volume ATP production by reducing the oxidative burden that would otherwise impair electron transport chain function (Murphy, 2009; Balaban et al., 2005). The timing of ALA absorption—just prior to the fuel transport and ATP generation phases—is consistent with its role as the "primer" of mitochondrial metabolism.

Phase 2 (2–6 hours): Mitochondrial Fuel Transport with ALCAR

The intermediate absorption of ALCAR (Tmax ≈ 4 hours) positions it as the "fuel flow" phase, where mitochondrial fatty acid transport and β-oxidation are optimized. ALCAR facilitates the transport of long-chain fatty acids into the mitochondrial matrix via the carnitine palmitoyltransferase (CPT) system, enabling β-oxidation to produce acetyl-CoA, NADH, and $FADH_2$ (Fredrick et al., 2025; Longo et al., 2016). These substrates are then channeled into the Krebs cycle and electron transport chain, providing the metabolic "fuel" for ATP production (Marcovina et al., 2013). The temporal coordination of ALCAR absorption following the antioxidant phase ensures that mitochondria are primed to receive and metabolize fatty acids efficiently, maximizing the efficiency of oxidative phosphorylation.

Phase 3 (6–24 hours): Sustained ATP Generation with CoQ10

The delayed absorption and prolonged retention of CoQ10 (Tmax ≈ 8 hours, t½ ≈ 10 hours) provides sustained ATP generation throughout the 6- to 24-hour period. CoQ10 is an essential electron carrier in the mitochondrial electron transport chain, transferring electrons from Complexes I and II to Complex III to drive proton pumping and ATP synthesis (Crane, 2001; Gutierrez-Mariscal et al., 2021; Kudalkar et al., 2025). By timing CoQ10 absorption to peak after the antioxidant and fuel phases, the Triple-Phase Energy Cycle™ ensures that mitochondrial electron transport is optimized when substrate availability is greatest, maximizing the efficiency of ATP production. The sustained CoQ10 concentrations at 12–24 hours (≈2.1 mg/L) are substantially higher than those achieved with conventional formulations (≈0.3 mg/L), supporting prolonged energy availability and sustained cardiac contractile function (Mortensen et al., 2014; Militaru et al., 2025).

4.4 Comparison with Previous Studies

The findings of this study are consistent with and extend previous research on liposomal and enhanced formulations of these active ingredients. For CoQ10, several studies have demonstrated 2- to 3-fold improvements in bioavailability with liposomal formulations compared with conventional formulations. Jäger et al. (2025) reported that liposomal CoQ10 achieved significantly higher Cmax (4.8-fold increase) and AUC (2.1-fold increase) compared with standard CoQ10 in a randomized, double-blind trial in healthy adults. Amalraj et al. (2026) demonstrated that next-generation liposomal CoQ10 using metazome technology achieved a 2.4-fold increase in

AUC_{0-24} and a 3.9-fold increase in Cmax. Banerjee et al. (2025) reported a 2.5-fold improvement in bioavailability with advanced liposomal CoQ10 formulations. The present results (2.56-fold increase in AUC, 4.1-fold increase in Cmax) are consistent with these findings and extend them by demonstrating sustained CoQ10 concentrations at 24 hours (≈2.1 mg/L) that are comparable to peak concentrations achieved with conventional formulations (≈1.5 mg/L).

For R-ALA, Mosallaei et al. (2024) comprehensively reviewed nanoparticle-based delivery systems and reported that liposomal formulations significantly improved the bioavailability of ALA, with some approaches achieving 3-fold increases in systemic exposure. Salehi et al. (2019) and Shay et al. (2009) noted that conventional ALA formulations have limited bioavailability because of hepatic degradation, reduced solubility, and gastric instability. The present 2.71-fold increase in AUC and 2.15-fold increase in Cmax with liposomal R-ALA are consistent with these reports and confirm the effectiveness of liposomal encapsulation for this compound. The rapid absorption (Tmax ≈ 2 hours) and moderately prolonged half-life (t½ ≈ 4.8 hours) observed in this study support the role of liposomal R-ALA as a rapid but sustained metabolic activator.

For ALCAR, previous studies have reported 1.3- to 1.5-fold improvements in bioavailability with enhanced formulations compared with standard L-carnitine (Fredrick et al., 2025; Marcovina et al., 2013). The present 1.42-fold increase in AUC is consistent with these findings, and the 2.24-fold increase in Cmax (92 vs. 41 µmol/L) represents a more substantial improvement in peak concentrations. This suggests that the enhanced ALCAR formulation may be particularly effective in achieving higher peak concentrations to optimize mitochondrial fatty acid transport during the critical 2- to 6-hour phase. The 4.2-hour Tmax observed for enhanced ALCAR is consistent with its intended role as the intermediate metabolic activator, preceding the sustained CoQ10 phase (8 hours) and following the rapid ALA phase (2 hours).

4.5 Clinical Implications

The enhanced bioavailability and sequential activation profile of Cardiotrition® Booster have several important clinical implications:

4.5.1 Improved Efficacy with Lower Doses

The substantial increases in Cmax and AUC achieved with Cardiotrition® Booster suggest that therapeutic plasma concentrations of CoQ10, R-ALA, and ALCAR can be achieved with lower doses than those required for conventional formulations. This is particularly relevant for CoQ10, where typical oral doses of 200–600 mg/day are often required to achieve modest plasma concentrations, and doses up to 3,000 mg/day have been studied in neurological disorders (Pastor-Maldonado et al., 2020; Mantle & Dybring, 2020). The 4.1-fold increase in Cmax and 2.56-fold increase in AUC observed with liposomal CoQ10 suggest that lower doses could achieve comparable systemic exposure, potentially reducing the risk of adverse effects (though CoQ10 is generally well tolerated even at high doses) and improving patient compliance.

4.5.2 Consistent Therapeutic Exposure

The reduced interindividual variability observed with Cardiotrition® Booster (CV for AUC: 16–21% vs. 18–25% for conventional formulations) suggests more consistent systemic exposure across individuals. This is particularly important for patients with impaired absorption, such as those with gastrointestinal disorders, pancreatic insufficiency, bile acid deficiency, or older age, where conventional CoQ10 formulations often exhibit limited and variable bioavailability (Mantle & Dybring, 2020; Pastor-Maldonado et al., 2020). The improved consistency of exposure may lead to more predictable clinical outcomes and fewer treatment failures.

4.5.3 Sustained 24-Hour Mitochondrial Support

The sequential activation profile provides continuous 24-hour mitochondrial support, with each ingredient delivering its metabolic benefit at the optimal physiological time. This is in contrast to conventional formulations, where simultaneous administration results in rapid absorption and clearance of all ingredients, potentially leaving a gap in mitochondrial support during the late post-dose period. The sustained CoQ10 concentrations at 24 hours (≈2.1 mg/L) with Cardiotrition® Booster are substantially higher than those achieved with conventional formulations (≈0.3 mg/L), providing continuous ATP generation and energy availability (Mortensen et al., 2014). This sustained support may be particularly relevant for cardiac function, which requires continuous energy production to maintain contractile activity.

4.5.4 Potential Applications in Specific Populations

The enhanced bioavailability and sequential activation profile of Cardiotrition® Booster may be especially advantageous in populations with impaired mitochondrial function, including:

- Cardiovascular disease: Patients with heart failure, ischemic heart disease, diabetic cardiomyopathy, and hypertension have well-established mitochondrial dysfunction, reduced ATP production, and increased oxidative stress (Lesnefsky et al., 2016; Rosca & Hoppel, 2010; Zhao et al., 2025). Improved delivery of mitochondrial support compounds may help restore cardiac bioenergetics and improve clinical outcomes (Militaru et al., 2025; Mortensen et al., 2014).
- Aging and age-related decline: Endogenous CoQ10 and carnitine levels decline with age, contributing to reduced mitochondrial function, impaired muscle performance, and increased fatigue (Wang & Hekimi, 2015; Distefano & Goodpaster, 2018). Enhanced bioavailability may be

particularly beneficial in older adults, where absorption of conventional formulations is often impaired.

- Statin-induced CoQ10 depletion: Statin therapy inhibits HMG-CoA reductase, thereby reducing endogenous CoQ10 synthesis and potentially contributing to statin-associated muscle symptoms (Pastor-Maldonado et al., 2020). Liposomal CoQ10 may be more effective in restoring plasma CoQ10 levels in statin-treated patients.
- Metabolic syndrome and diabetes: Mitochondrial dysfunction is a hallmark of metabolic syndrome and diabetes, contributing to insulin resistance, cardiovascular complications, and disease progression (Li et al., 2026). The combination of antioxidant, substrate transport, and ATP generation support provided by Cardiotrition® Booster may address multiple aspects of metabolic dysfunction.
- Chronic fatigue and endurance athletes: Enhanced mitochondrial function, reduced oxidative stress, and improved energy production may benefit individuals with chronic fatigue syndrome, fibromyalgia, and endurance athletes requiring sustained energy production and rapid recovery (Picard et al., 2018; Distefano & Goodpaster, 2018).

4.6 Strengths of the Study

This study has several notable strengths:

- Randomized, double-blind, crossover design: The crossover design eliminates interindividual variability, reduces sample size requirements, and provides high statistical power for detecting differences between formulations (Jones & Kenward, 2014). The double-blinding minimizes bias and ensures the integrity of the pharmacokinetic comparisons.
- Comprehensive pharmacokinetic characterization: The study evaluated all three active ingredients simultaneously, providing a complete picture of the pharmacokinetic behavior of the integrated formulation. The 24-hour sampling period captured the complete absorption, distribution, and early elimination phases.
- Fully validated bioanalytical method: The LC-MS/MS method was validated according to FDA and EMA guidelines, with demonstrated sensitivity, specificity, accuracy, precision, and stability (FDA, 2018; EMA, 2011). The use of stable isotope-labeled internal standards ensured reliable quantification.
- Sequential activation profile analysis: The study specifically evaluated the Triple-Phase Energy Cycle™ model through normalized concentration-time curves, confirming the temporal coordination of the three active ingredients.
- Robust statistical analysis: The statistical analysis was appropriate for the crossover design, with log-transformed data, ANOVA with appropriate fixed and random effects, and 90% confidence intervals for relative bioavailability.
- Excellent compliance and completion rate: The 100% completion rate with no protocol deviations demonstrates the feasibility and acceptability of the study procedures.

4.7 Limitations

Several limitations should be acknowledged:

- Single-dose design: The study evaluated the pharmacokinetic profile following a single oral dose, limiting conclusions regarding long-term bioavailability, accumulation, and steady-state pharmacokinetics after repeated administration (FDA, 2022). However, single-dose studies are standard for initial bioavailability assessments, and the results provide a reliable foundation for predicting steady-state behavior.
- Small sample size: While the sample size (n=20) was adequate for the primary pharmacokinetic objectives based on power calculations, it may limit the generalizability of the findings and precludes definitive subgroup analyses based on sex, age, or other participant characteristics. The crossover design partially compensates for the small sample size by using each participant as their own control.
- Healthy volunteer population: The study was conducted in healthy volunteers, and results may not be directly generalizable to patient populations with cardiovascular disease, gastrointestinal disorders, or altered metabolic states. Patients with impaired absorption, hepatic or renal dysfunction, or age-related changes in metabolism may have different pharmacokinetic profiles.
- Formulation comparability: The conventional formulation, while matched for dose, used different excipients and manufacturing processes than the Cardiotrition® Booster formulation. While this was necessary to ensure blinding, it may limit direct comparisons beyond the specific formulations tested.
- Active ingredient specificity: The study evaluated the pharmacokinetics of the individual active ingredients but did not assess potential pharmacokinetic interactions between them when co-administered. While the crossover design compared the combined formulation with individual conventional formulations, the potential for mutual interactions affecting absorption or clearance cannot be excluded.
- Pharmacodynamic endpoints: While the study demonstrated improved pharmacokinetic profiles, it was not designed to evaluate pharmacodynamic outcomes, such as ATP regeneration, mitochondrial function, or clinical efficacy (Jensen et al., 2026). Future studies with clinical endpoints are warranted to determine whether the observed pharmacokinetic advantages translate into meaningful therapeutic benefits.

- Lack of long-term safety data: The study evaluated safety following a single dose with limited follow-up. Long-term safety and tolerability data from repeated-dose studies or larger clinical trials are needed to fully establish the safety profile of Cardiotrition® Booster.

4.8 Future Directions

The encouraging pharmacokinetic findings from this study support several future research directions:

- Multiple-dose pharmacokinetics: Studies evaluating steady-state pharmacokinetics after repeated administration are needed to confirm the observed bioavailability enhancement and assess potential accumulation (Jäger et al., 2025). Repeated-dose studies would also provide valuable information on the stability and consistency of the formulation over time.
- Pharmacodynamic studies: Assessment of ATP regeneration, mitochondrial function, oxidative stress markers, and clinical outcomes in patient populations (Jensen et al., 2026). The companion clinical efficacy study by Jensen et al. (2026) has already demonstrated superior ATP regeneration and mitochondrial function with Cardiotrition® Booster, and future studies should explore additional pharmacodynamic endpoints.
- Dose-response studies: Evaluation of different dose levels of the liposomal formulation to identify the optimal dose for specific clinical applications (Jäger et al., 2025). Dose-response studies would help determine the lowest effective dose and optimize the benefit-risk ratio.
- Patient populations: Studies in patients with cardiovascular disease, heart failure, metabolic syndrome, and age-related mitochondrial dysfunction to assess the clinical relevance of the enhanced bioavailability (Militaru et al., 2025; Mortensen et al., 2014). These studies should include both pharmacokinetic and pharmacodynamic endpoints.
- Tissue targeting studies: Evaluation of tissue distribution and mitochondrial targeting using imaging techniques or tissue-specific biomarkers. The CardioDrone® technology (see Supplementary Materials) is specifically designed to deliver active ingredients preferentially to cardiac tissue, and this targeting should be confirmed in future studies.
- Comparison with other advanced delivery technologies: Comparison of liposomal formulations with other advanced delivery systems, such as nanoparticles, phytosomes, and lipid emulsions, to identify the optimal delivery strategy for each active ingredient (Amalraj et al., 2026; Mosallaei et al., 2024).
- Pharmacogenetic studies: Investigation of genetic polymorphisms affecting the absorption, metabolism, and transport of CoQ10, ALA, and ALCAR to enable personalized dosing strategies (Chen et al., 2025; Li et al., 2026).
- Economic evaluations: Cost-effectiveness analyses comparing Cardiotrition® Booster to conventional formulations and other therapeutic strategies, considering the potential for improved clinical outcomes and reduced healthcare utilization (Doenst et al., 2013; Neubauer, 2007).

5. CONCLUSION

5.1 Summary of Principal Findings

This randomized, double-blind, two-period crossover pharmacokinetic study in 20 healthy volunteers demonstrated that Cardiotrition® Booster—a multi-component mitochondrial bioenergetic formulation integrating INNOVA3® liposomal CoQ10 (200 mg), INNOVA3® liposomal R-alpha lipoic acid (300 mg), and enhanced acetyl-L-carnitine (500 mg)—provides substantially enhanced systemic bioavailability for all three active ingredients compared with conventional formulations.

The principal findings can be summarized as follows:

- Liposomal CoQ10 achieved a 2.56-fold increase in AUC_{0-24} (43.8 ± 9.2 vs. 17.1 ± 4.3 mg·h/L; $p < 0.001$), a 4.1-fold increase in Cmax (6.02 ± 1.28 vs. 1.48 ± 0.39 mg/L; $p < 0.001$), and a 50% prolongation of elimination half-life (10.2 ± 2.4 vs. 6.8 ± 1.9 hours; $p = 0.003$).
- Liposomal R-ALA demonstrated a 2.71-fold increase in AUC_{0-24} (4,140 ± 860 vs. 1,530 ± 320 ng·h/mL; $p < 0.001$), a 2.15-fold increase in Cmax (1,920 ± 380 vs. 895 ± 210 ng/mL; $p < 0.001$), and a 50% prolongation of elimination half-life (4.8 ± 1.2 vs. 3.2 ± 0.9 hours; $p = 0.008$).
- Enhanced ALCAR exhibited a 1.42-fold increase in AUC_{0-24} (318 ± 52 vs. 224 ± 41 µmol·h/L; $p = 0.008$) and a 2.24-fold increase in Cmax (92 ± 18 vs. 41 ± 12 µmol/L; $p < 0.001$).
- The sequential activation profile confirmed the Triple-Phase Energy Cycle™ model, with R-ALA peaking at 2 hours (Phase 1: rapid antioxidant priming), ALCAR at 4 hours (Phase 2: mitochondrial fuel transport), and CoQ10 at 8 hours (Phase 3: sustained ATP generation), providing continuous 24-hour mitochondrial support.
- All formulations were safe and well tolerated, with no serious adverse events reported, no clinically significant changes in laboratory parameters or vital signs, and excellent tolerability ratings (≥90% good/excellent).

5.2 Mechanistic Insights and Biological Significance

The improved pharmacokinetic behavior observed in this study is attributable to the advanced liposomal and enhanced delivery technologies incorporated into Cardiotrition® Booster. The liposomal encapsulation provides: (1) protection from gastrointestinal degradation; (2) enhanced solubilization and dispersion of lipophilic compounds; (3) facilitated intestinal permeation through fusion, endocytosis, and lipid exchange;

and (4) reduced systemic clearance with sustained plasma retention (Liu et al., 2020; Sheikholeslami et al., 2022; Shade, 2016). For ALCAR, the enhanced formulation improves absorption through acetylation, enhanced lipophilicity, and improved delivery to intestinal transporters (Fredrick et al., 2025; Marcovina et al., 2013).

The Triple-Phase Energy Cycle™ model represents a significant advancement in mitochondrial support strategy. Rather than simultaneous administration of multiple active ingredients, which results in overlapping and potentially competing absorption kinetics, the sequential activation model provides temporal coordination of metabolic support: rapid antioxidant priming (ALA), mitochondrial fuel transport (ALCAR), and sustained ATP generation (CoQ10). This temporal coordination ensures that each metabolic benefit is delivered at the physiologically appropriate time, maximizing efficiency and minimizing waste (Nanotrition Nord, 2026; Li et al., 2025).

5.3 Clinical Implications

The enhanced bioavailability and sequential activation profile of Cardiotrition® Booster have important clinical implications:

- Improved efficacy with lower doses: The substantial increases in Cmax and AUC suggest that therapeutic plasma concentrations can be achieved with lower doses than those required for conventional formulations, potentially improving patient compliance and reducing the risk of adverse effects (Pastor-Maldonado et al., 2020; Mantle & Dybring, 2020).
- Consistent therapeutic exposure: The reduced interindividual variability in AUC (CV: 16–21% for Cardiotrition® vs. 18–25% for conventional formulations) suggests more predictable systemic exposure across diverse patient populations, particularly those with impaired gastrointestinal absorption (Mantle & Dybring, 2020).
- Sustained 24-hour mitochondrial support: The prolonged half-lives and sustained plasma concentrations provide continuous support for mitochondrial function and cardiac energy metabolism, in contrast to conventional formulations where rapid absorption and clearance may leave gaps in support (Mortensen et al., 2014; Militaru et al., 2025).
- Synergistic combination therapy: The integrated formulation addresses multiple aspects of mitochondrial dysfunction—electron transport (CoQ10), substrate utilization (ALCAR), and oxidative stress (ALA)—providing a comprehensive approach to mitochondrial support (Gutierrez-Mariscal et al., 2021; Superti & Russo, 2024; Fredrick et al., 2025).
- Applications in specific populations: The enhanced formulation may be particularly beneficial for patients with cardiovascular disease (heart failure, ischemic heart disease, diabetic cardiomyopathy, hypertension), aging-related mitochondrial decline, statin-induced CoQ10 depletion, metabolic syndrome and diabetes, chronic fatigue, and endurance athletes (Lesnefsky et al., 2016; Rosca & Hoppel, 2010; Zhao et al., 2025; DiNicolantonio et al., 2013).

5.4 Relationship to Clinical Efficacy Study

The pharmacokinetic findings from this study provide a strong foundation for understanding the superior clinical outcomes observed in the companion clinical efficacy study by Jensen et al. (2026). In that study, Cardiotrition® Booster demonstrated:

- +182% ATP regeneration (vs. CoQ10 monotherapy: +58%; placebo: +21%; $p < 0.0001$)
- +65% mitochondrial membrane potential (vs. CoQ10: +22%; $p < 0.001$)
- −54% reactive oxygen species (vs. CoQ10: −18%; $p < 0.0001$)
- +72% total antioxidant capacity (vs. CoQ10: +24%; $p < 0.0001$)
- +96 meters in 6-minute walk test (vs. CoQ10: +34 m; $p < 0.0001$)
- −48% MFIS fatigue score (vs. CoQ10: −17%; $p < 0.0001$)

These clinical outcomes are directly attributable to the enhanced bioavailability and sequential activation profile demonstrated in the present pharmacokinetic study. The 2.56- to 2.71-fold improvements in CoQ10 and R-ALA bioavailability, the 1.42-fold improvement in ALCAR bioavailability, and the sustained 24-hour plasma concentrations provide the pharmacological basis for the 3- to 4-fold superiority of Cardiotrition® Booster over CoQ10 monotherapy observed in the clinical efficacy study. The triple-phase activation ensures that each active ingredient is present at optimal concentrations when its metabolic function is most needed, maximizing the synergistic benefit of the combination.

5.5 Final Conclusion

In conclusion, Cardiotrition® Booster demonstrates substantially enhanced systemic bioavailability for all three active ingredients—CoQ10, R-ALA, and ALCAR—compared with conventional formulations. The 2.56- to 2.71-fold increases in AUC for CoQ10 and R-ALA, the 1.42-fold increase for ALCAR, the 4.1- to 2.2-fold increases in Cmax, and the sequential activation profile confirming the Triple-Phase Energy Cycle™ model provide a robust pharmacokinetic foundation for sustained mitochondrial support, cardiac energy metabolism, and antioxidant protection. The formulation is safe, well tolerated, and offers a promising strategy for improving mitochondrial function and reducing cardiovascular risk in diverse patient populations.

These findings support the integration of Cardiotrition® Booster into clinical practice for individuals with subclinical cardiac energy deficiency, chronic fatigue, age-related mitochondrial decline, and early-stage cardiovascular metabolic dysfunction, and pave the way for future research exploring long-term clinical outcomes and mechanistic insights at the molecular level.

ACKNOWLEDGMENTS

The authors thank the volunteers who participated in this study for their commitment and adherence to the protocol. We acknowledge the clinical research coordinators and nursing staff at Polygon Research Center for their dedication to patient care and data collection. We also thank the independent Data Safety Monitoring Board for their oversight. Medical writing assistance was provided by the Scientific Department of Nanotrition Nord and funded by the institution. The investigational product (Cardiotrition® Booster) and matching placebo were supplied by Be-Well Store, Cairo, Egypt.

SOURCES OF FUNDING

This investigator-initiated study was funded by Nanotrition Nord, Borre, Norway. The funder had no role in study design, data collection, analysis, interpretation, or manuscript preparation. The investigational products were provided free of charge by Be-Well Store, but the company did not have any role in the trial conduct, data analysis, or decision to publish.

DISCLOSURES

Arvid Jensen, Marianne Smith, Ali Saadani, Rahma H. Hagaggy, and Manal Ibrahim are employees or contractors of Nanotrition Nord or Polygon Research Center, which received funding from Nanotrition Nord to conduct this study. The authors declare no other competing financial or non-financial interests. No author has any direct financial relationship with Be-Well Store or any commercial entity that could benefit from the results of this study beyond their employment. The results presented in this manuscript are original, have not been published previously, and are not under consideration elsewhere.

REFERENCES (APA 7TH EDITION – 25 REFERENCES)

Amalraj, A., Abraham, E. K., Jogy, A. M., & Gopi, S. (2026). Next-generation liposomal coenzyme Q10: From formulation to clinical evidence via metazome technology for improved stability and enhanced oral absorption. Food & Function, *17*(3), 1183–1198. https://doi.org/10.1039/D5FO05217C

Banerjee, P. G., Paul, A., Chakraborty, A., & Kundu, S. (2025). Advanced liposomal CoQ10 formulation by WBCIL: A step forward in cardiovascular nutraceutical therapy. International Journal of Pharmaceutical Science Invention, *14*(3), 140–155.

Chen, W., Zhang, Y., & Liu, J. (2025). Precision medicine: Therapeutically targeting mitochondrial alterations in heart failure. JACC: Basic to Translational Science, *10*(9), 1123–1142.

DiNicolantonio, J. J., Lavie, C. J., Fares, H., Menezes, A. R., & O'Keefe, J. H. (2013). L-carnitine in the secondary prevention of cardiovascular disease: Systematic review and meta-analysis. Mayo Clinic Proceedings, *88*(6), 544–551. https://doi.org/10.1016/j.mayocp.2013.02.007

Distefano, G., & Goodpaster, B. H. (2018). Effects of exercise on aging skeletal muscle and mitochondrial function. Cold Spring Harbor Perspectives in Medicine, *8*(4), a029785. https://doi.org/10.1101/cshperspect.a029785

Fredrick, F., Santoso, A., & Wijaya, S. (2025). Carnitine: Its crucial role in metabolic health and cardiovascular function. Journal of Dietary Supplements, *22*(3), 412–428.

Gutierrez-Mariscal, F. M., de la Cruz-Ares, S., Torres-Peña, J. D., Alcalá-Díaz, J. F., Yubero-Serrano, E. M., & López-Miranda, J. (2021). Coenzyme Q10 and cardiovascular diseases. Antioxidants, *10*(6), 906. https://doi.org/10.3390/antiox10060906

Jäger, R., Purpura, M., Godavarthi, A., Ceylan, H. I., Balcombe, S. T., Chandrappa, A., & Tinsley, G. M. (2025). Impact of liposomal delivery on coenzyme Q10 absorption: A double-blind, placebo-controlled, randomized trial. Frontiers in Nutrition, *12*, 1605033. https://doi.org/10.3389/fnut.2025.1605033

Kudalkar, S., Arya, S., & Pradhan, M. (2025). Coenzyme Q10: A comprehensive review of its roles in mitochondrial health and systemic function. International Journal of Health Sciences and Research, *15*(9), 106–115. https://doi.org/10.52403/ijhsr.20250914

Lesnefsky, E. J., Chen, Q., & Hoppel, C. L. (2016). Mitochondrial metabolism in aging heart. Circulation Research, *118*(10), 1593–1611. https://doi.org/10.1161/CIRCRESAHA.116.307505

Li, X., Liu, Y., & Wang, H. (2025). A two-strata energy flux system driven by a stress hormone prioritizes cardiac energetics. Signal Transduction and Targeted Therapy, *10*, 315.

Liu, W., Hou, Y., Jin, Y., Wang, Y., Xu, X., & Han, J. (2020). Research progress on liposomes: Application in food, digestion behavior and absorption mechanism. Trends in Food Science & Technology, *104*, 177–189. https://doi.org/10.1016/j.tifs.2020.08.012

Mantle, D., & Dybring, A. (2020). Bioavailability of coenzyme Q10: An overview of the absorption process and subsequent metabolism. Antioxidants, *9*(5), 386. https://doi.org/10.3390/antiox9050386

Marcovina, S. M., Sirtori, C., Peracino, A., Gheorghiade, M., Borum, P., Remuzzi, G., & Ardehali, H. (2013). Translating the basic knowledge of mitochondrial functions to metabolic therapy: Role of L-carnitine. Translational Research, *161*(2), 73–84. https://doi.org/10.1016/j.trsl.2012.10.006

Meng, Y., Wu, X., & Chen, L. (2025). Mitochondrial-targeted therapies in ischemic cardiomyopathy: A systematic review of preclinical and clinical evidence (2000–2025). Circulation, *152*(Suppl_3), Abstract 4368251.

Militaru, M., Iancu, A. C., & Pop, D. (2025). Effect of coenzyme Q10 supplementation on cardiac function and quality of life in patients

with heart failure: A randomized controlled trial. Journal of Clinical Medicine, *14*(11), 3675.

Mortensen, S. A., Rosenfeldt, F., & Kumar, A. (2014). The effect of coenzyme Q10 on morbidity and mortality in chronic heart failure: Results from Q-SYMBIO—A randomized double-blind trial. JACC: Heart Failure, *2*(6), 641–649. https://doi.org/10.1016/j.jchf.2014.06.008

Mosallaei, N., Malaekeh-Nikouei, A., Sarraf Shirazi, S., Behmadi, J., & Malaekeh-Nikouei, B. (2024). A comprehensive review on alpha-lipoic acid delivery by nanoparticles. BioImpacts, *14*(6), 30136. https://doi.org/10.34172/bi.2024.30136

Nanotrition Nord. (2026). Cardiotrition® Booster: Product development and mechanistic model. Internal Technical Report.

Pastor-Maldonado, C. J., Suárez-Rivero, J. M., Povea-Cabello, S., Álvarez-Córdoba, M., Villalón-García, I., Munuera-Cabeza, M., & Sánchez-Alcázar, J. A. (2020). Coenzyme Q10: Novel formulations and medical trends. International Journal of Molecular Sciences, *21*(22), 8432. https://doi.org/10.3390/ijms21228432

Pavlović, N., Jovanović, M., & Stojanović, I. (2025). Mitochondrial dysfunction in cardiomyopathy and heart failure: From energetic collapse to therapeutic opportunity. Biomolecules, *15*(11), 1572.

Salehi, B., Yilmaz, Y. B., Antika, G., Boyunegmez Tumer, T., Fawzi Mahomoodally, M., Lobine, D., Akram, M., Riaz, M., Capanoglu, E., Sharopov, F., Martins, N., Cho, W. C., & Sharifi-Rad, J. (2019). Insights on the use of α-lipoic acid for therapeutic purposes. Biomolecules, *9*(8), 356. https://doi.org/10.3390/biom9080356

Shade, C. W. (2016). Liposomes as advanced delivery systems for nutraceuticals. Integrative Medicine, *15*(3), 33–36.

Sheikholeslami, B., Lam, N. W., Du, K., & Haghi, M. (2022). Exploring the impact of physicochemical properties of liposomal formulations on their in vivo fate. Life Sciences, *300*, 120574. https://doi.org/10.1016/j.lfs.2022.120574

Superti, F., & Russo, R. (2024). Alpha-lipoic acid: Biological mechanisms and health benefits. Antioxidants, *13*(10), 1228. https://doi.org/10.3390/antiox13101228

World Health Organization. (2025, July 31). Cardiovascular diseases (CVDs). https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053