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Association of Cardiomyocyte Mitochondrial Ultrastructure Features with the Severity of Clinical Manifestations in Heart Failure

Aim	To study the quantitative characteristics of cardiomyocyte mitochondrial ultrastructure using electron microscopy data of patients with chronic heart failure (CHF) and to analyze the association of these characteristics with CHF clinical parameters and severity.
Material and methods	The study analyzed a total of 180 micrographs of right atrial appendage cardiomyocytes from 30 patients with CHF and reduced or mid-range left ventricular ejection fraction (LVEF). Standard laboratory and instrumental tests, including echocardiography, a 6-minute walk test (6MWT), and a cardiorespiratory exercise test, were performed in all patients. Biopsy samples were collected during coronary artery bypass grafting. Electron microscopy was performed with a JEM-1400 transmission electron microscope. The total interfibrillar mitochondrial area (Smtx) was calculated at a magnification of ×5,000. Also, the ratio of the outer membrane length of an individual mitochondrion to the length of its inner membrane at a magnification of ×15,000 was calculated.0.
Results	The Smtx positively correlated with the exercise tolerance (r=0.593; p=0.033), peak oxygen consumption during exercise (r=0.395; p=0.012), and the distance covered in the 6MWT (r=0.483; p=0.002). A negative correlation was found between Smtx and the concentration of N-terminal fragment of pro-brain natriuretic peptide (NT-proBNP) (r= –0.472; p=0.017). The ratio of the outer mitochondrial membrane length to the inner membrane length inversely correlated with LVEF (r= –0.593; p=0.033).
Conclusion	The total area of cardiomyocyte interfibrillar mitochondria correlated with exercise tolerance, peak oxygen consumption, and NT-proBNP concentration, while the mitochondrial membrane length ratio correlated with left ventricular ejection fraction. This suggests an association between quantitative parameters of mitochondrial ultrastructure and clinical manifestations of CHF.

Keywords	Chronic heart failure; chronic heart failure with reduced and mid-range left ventricular ejection fraction; electron microscopy, mitochondria; bicycle spiroergometry
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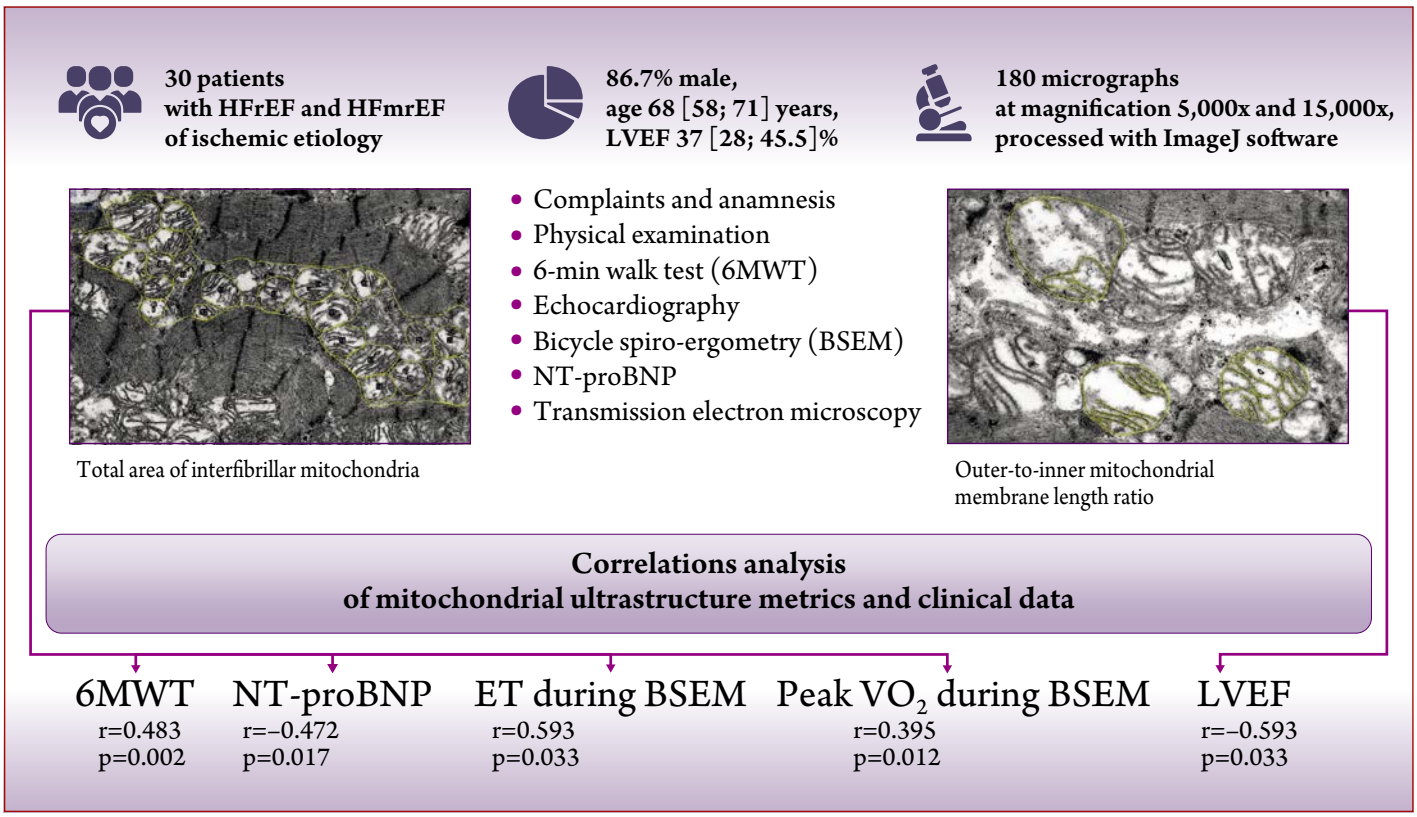
Introduction

Chronic heart failure (CHF) remains a serious problem in modern cardiology, characterized by a high prevalence, poor prognosis, and significant socioeconomic burden [1]. Despite advancements in pharmacological therapy and introduction of new treatment methods, the mechanisms driving the development and progression of CHF remain elusive. Recently, research has increasingly focused on mitochondrial dysfunction as a key factor in CHF pathogenesis, given the crucial role of mitochondria in cardiomyocyte energy metabolism and function [2].

Mitochondria, the primary "energy plants" of the cell, synthesize adenosine triphosphate (ATP) through oxidative phosphorylation. In CHF, when energy balance is disrupted, mitochondrial dysfunction can lead to impaired myocardial contractility, increased oxidative stress, disturbed calcium regulation, and activation of cardiomyocyte apoptosis [3].

Studying mitochondria in CHF is a relevant and expanding field, reflected by the steady growth of publications in major citation databases. However, most existing studies rely on in vitro or in vivo animal models, while morphological studies into the ultrastructure of

Central illustration. Association of cardiomyocyte mitochondrial ultrastructure with clinical severity of chronic heart failure



CHFrEF and CHFrEF, chronic heart failure with reduced and mid-range left ventricular ejection fraction; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; peak VO_2, peak oxygen consumption during exercise; 6MWT, 6-minute walk test; BSEM, bicycle spiro-ergometry; ET, exercise tolerance; r, Spearman's correlation coefficient; p, statistical significance value.

mitochondria in human cardiomyocytes are extremely scarce, primarily due to inherent challenges of obtaining clinical biopsy material.

CHF triggers systemic neurohumoral activation under conditions of impaired organ perfusion, affecting all cellular tissues. Consequently, morphological examination can be performed on biopsy specimens from any accessible cardiac section with minimal risk of perioperative complications. Current literature indicates that myocardial changes in the right atrial appendage reflect global processes of cardiomyocyte remodeling and correlate with the prognosis of patients with ischemic CHF [4].

Ultrastructural changes in mitochondria, such as fragmentation, cristae destruction, and disrupted network organization, are key markers of mitochondrial dysfunction [5]. An imbalance in mitochondrial dynamics, the equilibrium between fusion and division, alters mitochondrial shape and size and compromises their function [6].

Beyond the invasive requirement of biopsy sampling, a major limitation in evaluating cardiomyocyte mitochondrial ultrastructure via electron microscopy is the lack of standardized, generally accepted quantitative morphological assessment methods. Such standardization is essential for robust statistical analysis.

Most research into mitochondrial dysfunction in CHF has focused on biochemical and molecular markers. However, the assessment of mitochondrial ultrastructural alterations, which reflect profound structural and functional disorganization, remains relatively underexplored. Specifically, there is a lack of evidence regarding the correlation between mitochondrial ultrastructure and the clinical profiles of patients with CHF across the left ventricular ejection fraction (LVEF) spectrum, including CHF with reduced (HFrEF) and mid-range (HFmrEF) ejection fractions. Furthermore, the relationship between these structural parameters and clinical markers of CHF severity, such as NYHA functional class, N-terminal pro-brain natriuretic peptide (NT-proBNP) levels, 6-minute walk test (6MWT) distance, exercise tolerance (ET) metrics, and peak oxygen consumption during bicycle spiro-ergometry (BSEM), requires further investigation.

Accordingly, the present study aimed to quantify the characteristics of cardiomyocyte mitochondrial ultrastructure of cardiomyocytes in patients with CHF using electron microscopy, as well as to analyze their relationship with clinical parameters and the severity of heart failure.

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Material and methods

A cohort study was conducted involving patients with CHF with reduced (HFrEF) and mid-range (HFmrEF) LVEF and ischemic heart disease (IHD) scheduled for coronary artery bypass grafting (CABG). The study analyzed 180 micrographs of right atrial appendage cardiomyocytes obtained from biopsies of 30 patients with HFrEF and HFmrEF.

Inclusion criteria: CHF with LVEF <50% [7], obstructive multivessel coronary atherosclerosis as an indication for CABG [8]; signed informed consent for participation in the study and collection of biomaterial.

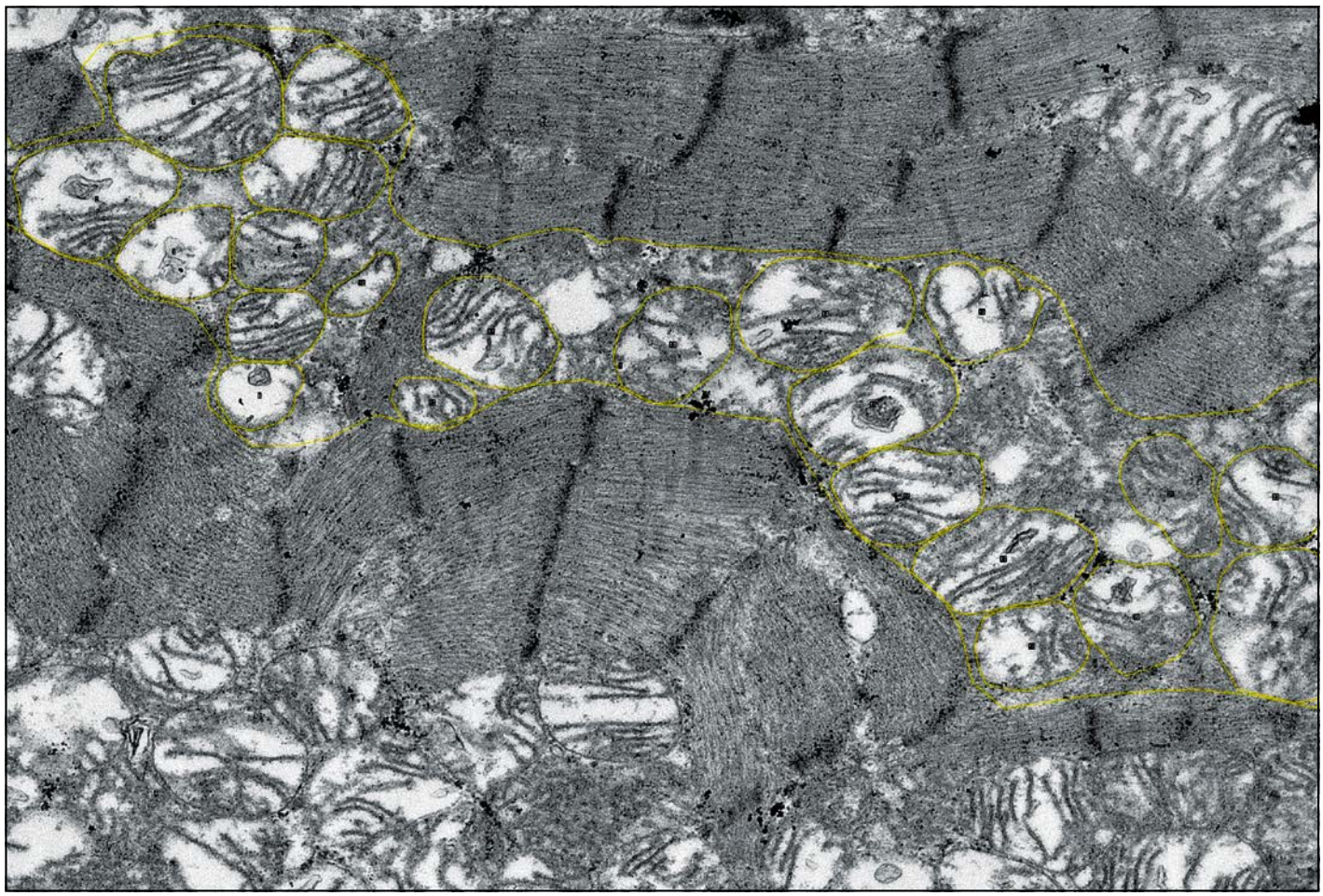
Exclusion criteria: refusal of revascularization or participation in the study, requirement for concurrent cardiac surgical procedures other than CABG, active malignancy, presence of implanted devices, estimated glomerular filtration rate <30 mL/min/1.73 m^2, infiltrative heart diseases, autoimmune diseases, acute infectious and exacerbations of chronic somatic diseases, severe chronic obstructive pulmonary disease, bronchial asthma, diabetes mellitus, anemia.

The study protocol complies with the principles of the Declaration of Helsinki and was approved by the local ethics committee (Protocol No. 241, dated March 9, 2023). All patients signed a voluntary informed consent form prior to any study procedures. The study protocol is registered at ClinicalTrials.gov: NCT05770349.

All patients received a standard laboratory and instrumental evaluation, including echocardiography (performed by a single specialist using a Philips HD 15 system), the 6MWT, and a cardiopulmonary exercise test [9] on a Schiller ergospirometry system (Switzerland) to measure exercise tolerance and peak oxygen consumption.

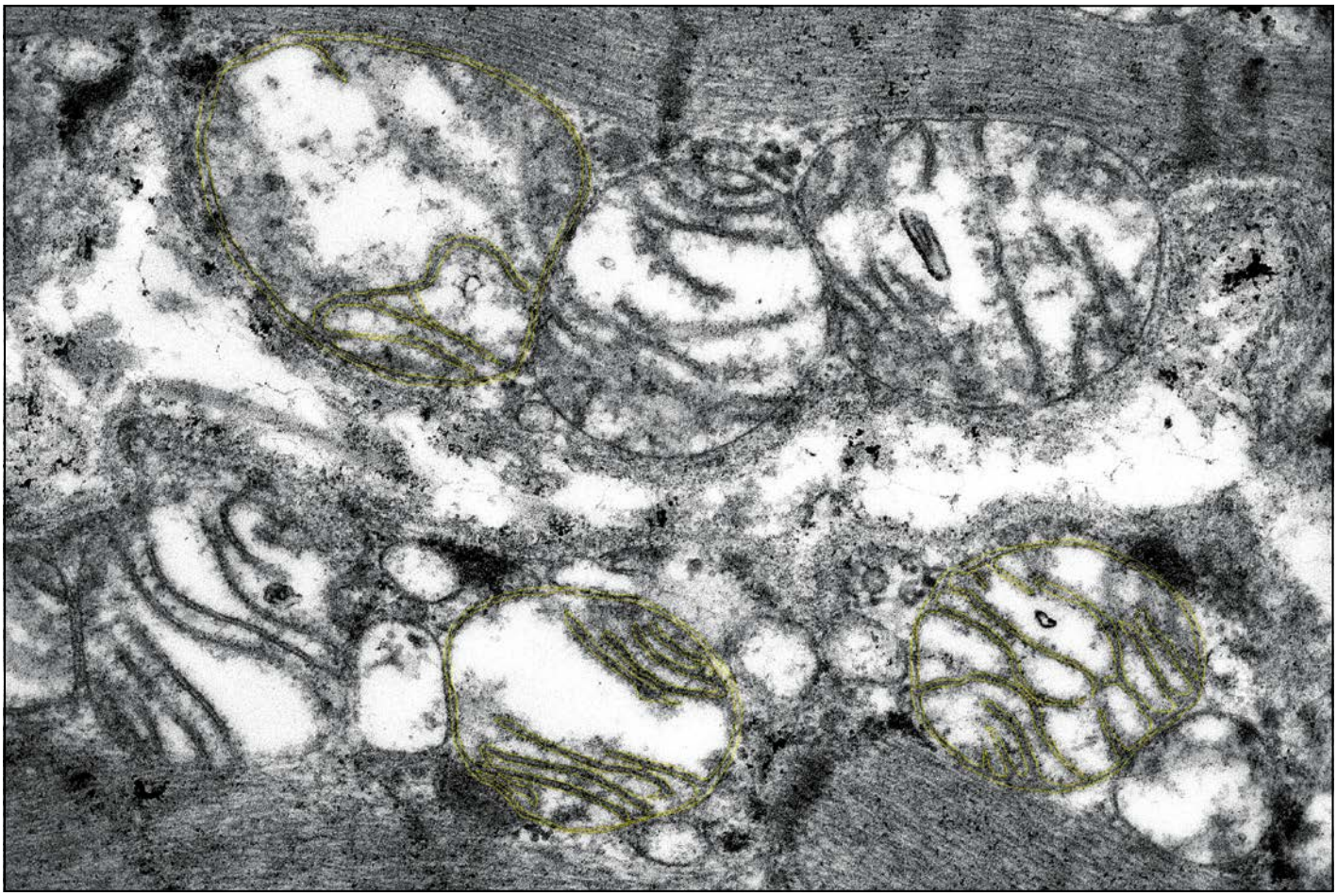
Right atrial appendage biopsies were obtained during CABG. Ultrastructural analysis was performed using a JEM-1400 transmission electron microscope (TEM) (Core Facilities Center 'Subdiffraction Microscopy' at the Department of Electron Microscopy of the Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University). For each patient, 3 to 5 longitudinally oriented sections were

Figure 1. Sample calculation of the total area of interfibrillar mitochondria. Electron micrograph, magnification ×5,000



Electron micrograph of a right atrial appendage cardiomyocyte. The interfibrillar mitochondria and interfibrillar spaces are demarcated to calculate cumulative interfibrillar mitochondrial surface area.

Figure 2. Sample calculation of the outer-to-inner mitochondrial membrane length ratio. Electron micrograph, magnification ×15,000



Electron micrograph of a right atrial appendage cardiomyocyte with demarcated membranes of 3 mitochondria for calculation of the outer-to-inner mitochondrial membrane length ratio.

examined at magnifications of ×5,000 and ×15,000, yielding a total of 180 micrographs for analysis.

The total area of interfibrillar mitochondria (S_{mtx}) was calculated as the ratio of the total area of mitochondria located between the cardiomyocyte contractile fibers to the total interfibrillar space area at ×5,000 magnification. Measurements were performed on three micrographs per patient to calculate the arithmetic mean, with values expressed as percentages [10] (Figure 1). Furthermore, the ratio of the outer membrane length to the inner membrane length of individual mitochondria was analyzed at ×15,000 magnification. This analysis involved measuring three individual mitochondria across three separate micrographs per patient to derive the arithmetic mean value [11] (Figure 2). All image processing and morphometric measurements were performed using ImageJ software.

Statistical analysis was performed using IBM SPSS Statistics version 21. Continuous variables are expressed as median and interquartile range (Me [Q1; Q3]), while categorical variables are reported as absolute frequencies and percentages (n, %). Pearson's chi-square test or Fisher's exact test (two-tailed) were used to compare categorical data. Correlations were evaluated using Spearman's rank correlation coefficient. Statistical significance was defined as $p<0.05$.

This study was supported by the Russian Science Foundation grant No. 23-75-00009.

Results

The study enrolled 30 patients with HFrEF or HFmrEF secondary to IHD scheduled for elective CABG. The median age was 68 (58; 71) years. The study cohort was predominantly male (86.7%; n=26), with the majority of patients classified as NYHA functional class II–III. The median LVEF was 37 [28; 45.5]%, and the median preoperative NT-proBNP level was 171.15 [73.6–326.6] pg/mL. Comprehensive clinical characteristics are summarized in Table 1.

Quantitative ultrastructural analysis of mitochondria yielded the following median values: interfibrillar mitochondrial area of 40.5 [34; 51.2]% and an outer-to-inner membrane length ratio of 0.29 [0.23; 0.36].

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Table 1. Clinical and anamnestic characteristics and key laboratory and instrumental findings in patients with CHF and multivessel obstructive coronary atherosclerosis.

Parameters	Abs. number	%
Males	26	86.7
Females	4	13.3
History of myocardial infarction	26	86.7
Obesity	16	53.3
History of acute cerebrovascular accident	2	6.7
Femoral artery atherosclerosis	29	96.7
Carotid artery atherosclerosis	11	36.7
Arrhythmias (total)	14	46.7
Atrial fibrillation	11	36.7
Premature ventricular complexes, Lown-Wolf grade IV–V	4	13.3
CHF functional class I II III IV	 3 14 12 1	 10 46.7 40.0 3.3
Current smoking	12	40
6MWT distance, m (Me [Q1; Q3])	305 [250; 400]	–

Laboratory and instrumental findings

Variable	Median	Percentile	
		25th	75th
NT-proBNP concentrations before surgery, pg/mL	171.15	73.6	326.6
LVEF, %	37	28	45.5
LAVI, mL/m²	44	34	54
E/A	0.79	0.67	1.25
E/e'	10.9	8.9	15.44
PASP, mm Hg	32	27.5	42
SV, mL	65	58	75
LVMI, g/m²	116	102	145
Peak VO_2 during BSEM, mL/kg/min	8.79	6.8	11.5
ET during BSEM, W	60	50	90

CHF, chronic heart failure; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEF, left ventricular ejection fraction; 6MWT, 6-minute walk test; LAVI, left atrial volume index; E / A, ratio of early and late diastolic filling velocities; E / e', ratio of mitral inflow velocity to mitral annular relaxation velocity; PASP, pulmonary artery systolic pressure; SV, stroke volume; LVMI, left ventricular mass index; BSEM, bicycle spiro-ergometry; peak VO2, peak oxygen consumption during exercise; ET, exercise tolerance.

Correlation analysis was performed between clinical indices of CHF severity and quantitative metrics of mitochondrial ultrastructure (Table 2).

Spearman's correlation analysis revealed a positive correlation between S_{mtx} and exercise tolerance, peak oxygen consumption (measured during BSEM), and the distance covered in the 6MWT. Additionally, the total area of intermyofibrillar mitochondria was

Table 2. Correlations between mitochondrial ultrastructure metrics and CHF clinical picture and severity

Metrics	6MWT	NT-proBNP level before surgery	ET during BSEM	Peak VO_2 during BSEM	LVEF
Total area of interfibrillar mitochondria, r	0.483	–0.472	0.593	0.395	0.173
p	0.002	0.017	0.033	0.012	0.418
Outer-to-inner membrane length ratio in a single mitochondrion, r	–0.192	–0.429	0.514	0.028	–0.593
p	0.510	0.397	0.068	0.931	0.033

LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; peak VO2, peak oxygen consumption during exercise; 6MWT, 6-minute walk test; BSEM, bicycle spiro-ergometry; ET, exercise tolerance; r, Spearman's correlation coefficient; p, statistical significance value.

negatively correlated with NT-proBNP concentrations. Furthermore, the ratio of the outer mitochondrial membrane length to the inner membrane length was inversely correlated with LVEF.

No statistically significant correlation was identified between the mitochondrial ultrastructure parameters and CHF NYHA functional class.

Discussion

The role of structural and functional alterations in cardiomyocyte mitochondria during the development and progression of heart failure is currently a subject of intensive research. However, there is scarce data in the available literature regarding the ultrastructural changes of mitochondria in patients with HFrEF and HFmrEF. Specifically, the relationship between these mitochondrial changes and clinical markers of CHF severity, such as NYHA functional class, NT-proBNP levels, 6MWT distance, exercise tolerance, and peak oxygen consumption during BSEM, remains largely unexplored.

A hypothesis was proposed that the total area of interfibrillar mitochondria [10] defined as the ratio of the total area of all interfibrillar mitochondria to the total interfibrillar space area, is associated with the clinical features of CHF. It is suggested that S_{mtx} may be an important morphological parameter reflecting the density of mitochondrial distribution between cardiomyocyte myofibrils. This hypothesis is supported by significant positive correlations between S_{mtx} and exercise tolerance, assessed by BSEM and the 6MWT, as well as peak oxygen consumption during exercise. Consequently, a higher S_{mtx} correlates with superior exercise tolerance in CHF patients. The identified correlation is supported by Russian and

international studies emphasizing the role of intermitochondrial junctions, formed at the points of mitochondrial contact, in the formation of structural-functional clusters that enhance overall mitochondrial efficiency [12].

The study identified an inverse correlation between the outer-to-inner mitochondrial membrane length ratio and LVEF. This finding supports the hypothesis that mitochondrial ultrastructure is critical for maintaining cardiac contractile function in CHF. This relationship stems from the fact that the ratio serves as an indicator of cristae integrity. These inner membrane structures house the respiratory chain proteins essential for oxidative phosphorylation and the synthesis of ATP, the primary energy source for cardiomyocyte contraction [13]. Our findings align with previous research indicating that reduced cristae density and their ultrastructural remodeling are hallmarks of mitochondrial dysfunction, leading to diminished ATP output and elevated reactive oxygen species production, which ultimately damages cardiomyocytes and impairs their contractility [14].

Study limitations

The primary limitation of this study is the relatively small sample size (n=30). However, the analysis of 180 cardiomyocyte micrographs provided a robust dataset for calculating mean values of the parameters under investigation. Furthermore, ultrastructural studies of human cardiomyocytes are inherently limited by their complex design and the requirement for invasive myocardial biopsies. Consequently, our findings are of high scientific significance.

Conclusion

The study demonstrated that the total area of interfibrillar mitochondria in cardiomyocytes correlates with exercise tolerance, peak oxygen consumption, and NT-proBNP concentrations. Furthermore, the mitochondrial membrane length ratio was associated with left ventricular ejection fraction. These findings suggest a clear link between quantitative mitochondrial ultrastructure parameters and the clinical manifestations of chronic heart failure.

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Conflict of interest: None.

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