lek. Karol Momot

Parametry stresu oksydacyjnego, stresu nitrozacyjnego oraz stresu siateczki śródplazmatycznej w przewlekłej niewydolności serca z zachowaną oraz obniżoną frakcją wyrzutową

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

Promotor: prof. dr hab. n. med. Agnieszka Cudnoch-Jędrzejewska
Promotor pomocniczy: dr n. med. Małgorzata Wojciechowska

Katedra i Zakład Fizjologii Doświadczalnej i Klinicznej
Warszawski Uniwersytet Medyczny

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Słowa kluczowe

niewydolność serca, stres oksydacyjny, stres nitrozacyjny, stan zapalny,
stres siateczki śródplazmatycznej, odpowiedź na niesfałdowane białka

Keywords

heart failure, oxidative stress, nitrosative stress, inflammation,
endoplasmic reticulum stress, unfolded protein response

Badania stanowiące podstawę rozprawy doktorskiej zostały zrealizowane w ramach projektów naukowych:

1. „Odkrycie nowych potencjalnych kierunków terapeutycznych w leczeniu niewydolności serca”
   (SKN/SP/534125/2022)

2. „iNOS expression and IRE1alpha-XBP1 pathway activity in Sprague Dawley rats with Post-Myocardial Infarction Heart Failure and/or after High-Fat Diet”
   (41/M/MG/N/21)

3. „The levels of myeloperoxidase and 3-nitrotyrosine in post-myocardial infarction heart failure and high-fat diet in the Sprague Dawley rat model”
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Wykaz publikacji stanowiących podstawę pracy doktorskiej

Lp.	Opis bibliograficzny	Impact Factor	MNiSW
Artykuły			
1.	**Momot K**, Wojciechowska M, Krauz K, Czarzasta K, Puchalska L, Zarębiński M, Cudnoch-Jędrzejewska A. Endoplasmic reticulum stress and expression of nitric oxide synthases in heart failure with preserved and with reduced ejection fraction — pilot study. Cardiology Journal. 2024 Oct 3. doi: 10.5603/cj.97962 (ahead of print) [Rodzaj publikacji: praca oryginalna]	2,5	100
2.	**Momot K**, Krauz K, Czarzasta K, Tomaszewski J, Dobruch J, Żera T, Zarębiński M, Cudnoch-Jędrzejewska A, Wojciechowska M. Post-myocardial infarction heart failure and long-term high-fat diet: Cardiac endoplasmic reticulum stress and unfolded protein response in Sprague Dawley rat model. PLoS One. 2024;19(9):1-13 [Rodzaj publikacji: praca oryginalna]	2,9	100
3.	**Momot K**, Krauz K, Czarzasta K, Zarębiński M, Puchalska L, Wojciechowska M. Evaluation of Nitrosative/Oxidative Stress and Inflammation in Heart Failure with Preserved and Reduced Ejection Fraction. International Journal of Molecular Sciences. 2023;24(21):1-11 [Rodzaj publikacji: praca oryginalna]	4,9	140
	Łącznie:	**10,3**	**340**
Książki			
1.	-		
Rozdziały w książkach			
1.	-		

DYREKTOR
Biblioteki Uczelnianej
Warszawskiego Uniwersytetu Medycznego
mgr Agnieszka Czarnecka

Spis treści

Wykaz skrótów

3-NT - 3-nitrotyrozyna (ang. 3-nitrotyrosine)
ATF6 - Aktywujący czynnik transkrypcyjny 6 (ang. activating transcription factor 6)
CHOP - Białko homologiczne C/EBP (ang. C/EBP homologous protein)
eNOS - Śródbłonkowa syntaza tlenku azotu (ang. endothelial nitric oxide synthase)
ELISA - Enzymatyczny test immunoabsorpcyjny
(ang. enzyme-linked immunosorbent assay)
ER - Siateczka śródplazmatyczna (ang. endoplasmic reticulum)
GRP78 - Białko regulowane przez glukozę 78 (ang. glucose-regulated protein 78)
HF - Niewydolność serca (ang. heart failure)
HFmrEF - Niewydolność serca z łagodnie zmniejszoną frakcją wyrzutową
(ang. heart failure with mildly reduced ejection fraction)
HFpEF - Niewydolność serca z zachowaną frakcją wyrzutową
(ang. heart failure with preserved ejection fraction)
HFrEF - Niewydolność serca ze zmniejszoną frakcją wyrzutową
(ang. heart failure with reduced ejection fraction)
HFD - Dieta wysokotłuszczowa (ang. high-fat diet)
iNOS - Indukowana syntaza tlenku azotu (ang. inducible nitric oxide synthase)
IRE1α - Enzym 1α wymagający inozytolu (ang. inositol-requiring enzyme 1α)
LVEF - Frakcja wyrzutowa lewej komory (ang. left ventricular ejection fraction)
MI - Zawał mięśnia sercowego (ang. myocardial infarction)
MPO - Mieloperoksydaza (ang. myeloperoxidase)
nNOS - Neuronalna syntaza tlenku azotu (ang. neuronal nitric oxide synthase)
UPR - Odpowiedź na niesfałdowane białka (ang. unfolded protein response)
PERK - Kinaza białkowa aktywowana przez stres ER
(ang. protein RNA-like endoplasmic reticulum kinase)
PCR - Reakcja łańcuchowej polimerazy (ang. polymeraze chain reaction)

Streszczenie w języku polskim

Niewydolność serca (HF) to złożony zespół kliniczny, który pozostaje jednym z największych wyzwań współczesnej medycyny, a badania nad różnicami w patofizjologii poszczególnych typów HF stają się coraz istotniejsze. Mogą one przyczynić się do opracowania nowych metod diagnostycznych oraz bardziej precyzyjnych i skutecznych strategii terapeutycznych.

Niniejsza praca doktorska miała na celu ocenę nasilenia procesów patofizjologicznych, takich jak stres oksydacyjny, stres nitrozacyjny oraz stres siateczki śródplazmatycznej (ER) w różnych typach HF. Praca została podzielona na dwie główne części. W pierwszej porównałem stężenia markerów powyższych procesów w osoczu pacjentów z HF z zachowaną (HFpEF) oraz z HF z obniżoną frakcją wyrzutową (HFrEF). Druga część dotyczyła analizy wpływu zawału serca (MI) oraz diety wysokotłuszczowej (HFD) na nasilenie powyższych procesów w modelu zwierzęcym pozawałowej HF.

Wyniki pierwszej części wykazały istotne różnice w nasileniach stresu nitrozacyjnego i stresu ER, sugerując różne mechanizmy patofizjologiczne w obu typach HF. U pacjentów z HFpEF zaobserwowałem wyższe poziomy stresu nitrozacyjnego (podwyższone stężenia iNOS oraz 3-NT w osoczu), co sugeruje istotną rolę tego procesu w patogenezie HFpEF, natomiast wydaje się, że w HFrEF dominuje stres ER (podwyższone stężenia GRP78 w osoczu). W drugiej części pracy doktorskiej wykazałem, że HFD w połączeniu z MI prowadzi do nasilenia stresu nitrozacyjnego (podwyższony poziom iNOS oraz 3-NT) oraz stresu ER w mięśniu sercowym (podwyższony poziom GRP78). Zarówno HFD, jak i MI nasilają stan zapalny wraz ze stresem oksydacyjnym, mierzony za pomocą poziomu MPO.

W pracy wykazałem, że różne mechanizmy patofizjologiczne odgrywają kluczową rolę w rozwoju HFpEF i HFrEF. Wyniki sugerują ponadto, że wystąpienie MI przy stosowaniu HFD nasila w sercu wyżej wymienione patologiczne procesy. Te obserwacje otwierają nowe możliwości dla ukierunkowanych strategii diagnostycznych i terapeutycznych oraz wskazują na potencjalne korzyści płynące z interwencji dietetycznych w prewencji progresji pozawałowej HF.

Streszczenie w języku angielskim

Parameters of oxidative stress, nitrosative stress, and endoplasmic reticulum stress in chronic heart failure with preserved and reduced ejection fraction

Heart failure (HF) is a complex clinical syndrome and remains one of the greatest challenges in modern medicine. Research into the pathophysiological differences between various types of HF is becoming increasingly important. Such research can contribute to the development of new diagnostic methods and more precise, effective therapeutic strategies.

This doctoral dissertation aimed to assess the intensity of pathophysiological processes, such as oxidative stress, nitrosative stress, and endoplasmic reticulum (ER) stress, in different types of HF. The study was divided into two main areas. In the first, I compared the concentrations of markers of these processes in the plasma of patients with preserved ejection fraction HF (HFpEF) and reduced ejection fraction HF (HFrEF). The second area focused on analyzing the effects of myocardial infarction (MI) and a high-fat diet (HFD) on the intensity of these processes in a post-MI HF animal model.

The results of the first part demonstrated significant differences in the intensities of nitrosative stress and ER stress, suggesting different underlying pathophysiological mechanisms in each type of HF. I found that patients with HFpEF exhibited higher levels of nitrosative stress (elevated iNOS and 3-NT concentrations in plasma), indicating a critical role for this process in the pathogenesis of HFpEF. In contrast, ER stress appeared to dominate in HFrEF (elevated GRP78 levels in plasma). In the second part of the dissertation, I demonstrated that HFD combined with MI led to increased nitrosative stress (elevated iNOS and 3-NT levels) and ER stress (elevated GRP78 levels) in myocardial tissue. Both HFD and MI intensified inflammation and oxidative stress, as measured by MPO levels.

The study revealed that different pathophysiological mechanisms play a crucial role in HFpEF and HFrEF. Moreover, the findings suggest that MI in the presence of HFD exacerbates the aforementioned pathological processes in the heart. These observations open new possibilities for targeted diagnostic and therapeutic strategies and indicate potential benefits of dietary interventions in preventing post-MI HF progression.

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Wstęp

Niewydolność serca (ang. heart failure; HF) jest jednym z najpoważniejszych problemów zdrowotnych w Polsce charakteryzującym się wysoką umieralnością, znacznym pogorszeniem jakości życia oraz stanowiącym ogromne obciążenie dla systemów opieki zdrowotnej (1). Szacuje się, że w naszym kraju na HF cierpi około 1 miliona osób (2). Liczba ta stale rośnie wraz ze starzeniem się populacji i wzrostem częstości występowania chorób współistniejących, takich jak nadciśnienie tętnicze, cukrzyca, choroba wieńcowa i otyłość, które przyczyniają się do rozwoju HF (3).

HF to złożony zespół kliniczny, wynikający z zaburzeń strukturalnych i funkcjonalnych serca. Zmiany te prowadzą do upośledzenia funkcji skurczowej i/lub rozkurczowej lewej komory (4). Główne objawy HF to duszność i pogorszenie tolerancji wysiłku fizycznego. W przebiegu HF mogą również pojawiać się obrzęki obwodowe, nykturia czy też kaszel, jak i zaburzenia snu.

HF dzieli się na trzy główne typy w zależności od funkcji lewej komory ocenianej frakcją wyrzutową (ang. left ventricular ejection fraction; LVEF): niewydolność serca z zachowaną frakcją wyrzutową (ang. heart failure with preserved ejection fraction; HFpEF), niewydolność serca z łagodnie zmniejszoną frakcją wyrzutową (ang. heart failure with mildly reduced ejection fraction; HFmrEF) oraz niewydolność serca ze zmniejszoną frakcją wyrzutową (ang. heart failure with reduced ejection fraction; HFrEF) (5). HFpEF, definiowana jako LVEF $\geq$ 50%, występuje częściej u osób starszych, kobiet oraz pacjentów z otyłością i/lub zespołem metabolicznym (6). Z kolei HFrEF, charakteryzująca się LVEF $\leq$ 40%, najczęściej jest wtórna do choroby niedokrwiennej serca, zwykle stanowi powikłanie zawału serca (ang. myocardial infarction; MI) (7). HFmrEF, z LVEF 41-49%, jest pośrednią kategorią, charakteryzującą się cechami obu pozostałych typów HF (8). Zrozumienie różnic patofizjologicznych między poszczególnymi typami HF jest kluczowe dla opracowania skutecznych metod diagnostycznych, strategii terapeutycznych i poprawy rokowania pacjentów.

Oba typy HF łączy obecność ogólnoustrojowego i miejscowego (sercowego) stanu zapalnego, dysfunkcji śródbłonka, uszkodzenia kardiomiocytów oraz przebudowy mięśnia sercowego (9). Mimo tych podobieństw, występują istotne różnice w mechanizmach patofizjologicznych. W HFrEF procesy zapalne są wynikiem bezpośredniego uszkodzenia kardiomiocytów, głównie w wyniku niedokrwienia mięśnia sercowego (10, 11). W HFpEF natomiast stan zapalny jest efektem pozasercowych czynników metabolicznych, takich jak

otyłość, cukrzyca czy choroba nerek i/lub płuc (12). Dysfunkcja śródbłonka w HFpEF pojawia się na wcześniejszych etapach patologii, podczas gdy w HFrEF jest to zjawisko późniejsze, związane z postępującym przebiegiem choroby (13). W HFrEF utrata kardiomiocytów i pozawałowe włóknienie serca w konsekwencji prowadzi zazwyczaj do ekscentrycznego remodelingu (14, 15). W HFpEF z kolei dochodzi najczęściej do koncentrycznego remodelingu serca, poprzez przerost kardiomiocytów i wzrost ich sztywności (16).

Stres oksydacyjny, stres nitrozacyjny oraz stres siateczki śródplazmatycznej (ang. endoplasmic reticulum; ER) są procesami, które znacząco wpływają na zaburzenia funkcjonowania kardiomiocytów, choć ich nasilenie wydaje się różnić w przebiegu poszczególnych typów HF (9). Stres oksydacyjny polega na nadmiernej produkcji reaktywnych form tlenu, które prowadzą między innymi do uszkodzenia białek w kardiomiocytach i do dysfunkcji śródbłonka (17). Z kolei stres nitrozacyjny, który jest pochodną stresu oksydacyjnego, może stanowić efekt nadmiernej aktywności i ekspresji indukowanej syntazy tlenku azotu (ang. inducible nitric oxide synthase; **iNOS**) oraz obecności reaktywnych form azotu (18, 19). W rezultacie może prowadzić on do nitracji białek, co wtórnie powoduje dysfunkcje i uszkodzenia kardiomiocytów (20). Markerem stresu nitrozacyjnego (pośrednio również stresu oksydacyjnego) jest również 3-nitrotyrozyna (ang, 3-nitrotyrosine; **3-NT**), która jest produktem reakcji tyrozyny z wyżej wspomnianymi reaktywnymi formami azotu (21). Wykazano również, że dysfunkcja dwóch pozostałych izoform NOS – neuronalnej (ang. neuronal nitric oxide synthase; **nNOS**) oraz śródbłonkowej (ang. endothelial nitric oxide synthase; **eNOS**) może doprowadzać do zwiększenia stresu oksydacyjnego i zaburzeń rozkurczu serca (22, 23). W przebiegu stanu zapalnego istotną rolę odgrywa mieloperoksydaza (ang. myeloperoxidase; **MPO**), która może być używana jako marker zarówno nasilenia stanu zapalnego, jak i stresu oksydacyjnego, ponieważ wykazuje również silne działanie oksydacyjne (24, 25). Wykazano, że MPO może być wytwarzana nie tylko przez neutrofile czy monocyty, ale także przez komórki śródbłonka. MPO dodatkowo aktywuje stres nitrozacyjny, obniżając poziom tlenku azotu i zwiększając ilość rodników azotowych (26, 27). To z kolei może doprowadzić do stresu ER poprzez uszkodzenie enzymów zaangażowanych w regulację fałdowania białek w ER (28). Markerem stresu ER jest min. podwyższony poziom białka regulowanego przez glukozę 78 (ang. glucose regulated protein 78; **GRP78**) (29), które pełni jednocześnie kluczową rolę w ochronie komórek przed uszkodzeniami spowodowanymi przez stres ER, biorąc udział w eliminacji niesfałdowanych białek jako białko opiekuńcze (chaperon). W odpowiedzi na stres ER uruchamiany jest mechanizm zwany odpowiedzią na niesfałdowane białka (ang. unfolded protein response; UPR), który ma na celu przywrócenie homeostazy białkowej (30). Warto zwrócić uwagę, że w przebiegu stresu nitrozacyjnego, poprzez

zwiększoną aktywność i ekspresję iNOS, dochodzi do upośledzenia funkcji UPR wskutek zahamowania jednego z jego szlaków, w którym uczestniczy enzym wymagający inozytolu 1α (ang. inositol-requiring enzyme 1α; **IRE1α**) (31). W modelu zwierzęcym wykazano, że zaburzenie funkcji tego szlaku stanowi kluczowy element patogenezy HFpEF (32, 33). Inne szlaki inicjujące UPR są związane z kinazą białkową aktywowaną przez stres ER (ang. protein RNA-like endoplasmic reticulum kinase **PERK**) oraz aktywującym czynnikiem transkrypcyjnym 6 (ang. activating transcription factor 6; **ATF6**). Złożoność wymienionych powyżej procesów podkreśla potrzebę kontynuowania zarówno badań podstawowych, jak i klinicznych, które mogłyby dostarczyć istotnych wyników do opracowania metod diagnostycznych oraz skutecznych strategii terapeutycznych w leczeniu HF.

W kontekście leczenia HFrEF, istnieje szeroki wachlarz sprawdzonych interwencji terapeutycznych, opartych na wytycznych, które znacząco poprawiają rokowanie pacjentów (34). Natomiast w przypadku HFpEF, możliwości leczenia poprawiającego rokowanie są ograniczone. Obecnie opierają się one głównie na flozynach (inhibitory kotransportera sodowo-glukozowego 2), które zostały włączone do wytycznych terapii tego typu HF dopiero w 2023 roku (5).

Dlatego też, identyfikacja markerów zaangażowanych w procesy patofizjologiczne, takie jak stres nitrozacyjny, stres oksydacyjny czy stres ER, u pacjentów z HFrEF i HFpEF mogłaby odegrać kluczową rolę w dokładniejszym scharakteryzowaniu fenotypów obu tych typów HF. To z kolei pozwoliłoby na ocenę, który z procesów dominuje w danym przypadku, co mogłoby prowadzić do opracowania bardziej precyzyjnych strategii leczenia.

Ostatnie badania wykazały, że hamowanie stanu zapalnego może być ważnym elementem w terapii HFpEF (35). Inhibitor MPO (mitiperstat) wykazał zdolność do redukcji stanu zapalnego u pacjentów z HFpEF, choć jego pełna skuteczność w tym zakresie wciąż wymaga dalszych badań (36). Ponadto, zahamowanie nadmiernej aktywności i ekspresji iNOS, a tym samym zmniejszenie stresu nitrozacyjnego, mogłoby prowadzić do poprawy funkcji UPR. W modelach zwierzęcych HFpEF, farmakologiczna inhibicja iNOS lub zahamowanie ekspresji genu iNOS - zmniejszały objawy kliniczne HF (32, 33), co sugeruje, że interwencje ukierunkowane na te mechanizmy mogą mieć potencjał terapeutyczny.

Dodatkowym istotnym czynnikiem, który może wpływać na przebieg HF, zwłaszcza u pacjentów po przebytym MI, jest wysoka zawartość tłuszczów nasyconych w diecie (37-39). Spożywanie wysokotłuszczowych pokarmów, szczególnie w okresie okołozawałowym, może prowadzić do nasilenia powyższych procesów patofizjologicznych zachodzących w sercu. Model zwierzęcy szczura Sprague Dawley z indukowanym MI poprzez podwiązanie lewej tętnicy wieńcowej, w połączeniu ze stosowaniem diety wysokotłuszczowej (ang. high-fat diet;

HFD), stanowi wartościowe narzędzie do badania wpływu HFD na procesy patofizjologiczne w kontekście pozawałowej HF serca (40). Dokładniejsze poznanie mechanizmów wpływu diety na rozwój HF może umożliwić rozwój nowych strategii terapeutycznych opartych na interwencjach dietetycznych.

Cele i założenia prac

Dotychczasowe wyniki badań dotyczących HF wskazują na konieczność dokładniejszej analizy roli procesów takich jak stres oksydacyjny, stres nitrozacyjny i stres ER w przebiegu wspomnianej choroby. Dlatego też wymieniony cykl prac stanowiący podstawę dysertacji doktorskiej koncentruje się na badaniu oraz porównaniu nasilenia opisanych wyżej procesów u pacjentów z HFpEF i HFrEF. W pracy doktorskiej ponadto oceniłem wpływ zastosowania HFD na pozawałową HF w modelu zwierzęcym.

Publikacje

1. **„Evaluation of Nitrosative/Oxidative Stress and Inflammation in Heart Failure with Preserved and Reduced Ejection Fraction"**
2. **„Endoplasmic Reticulum Stress and Expression of Nitric Oxide Synthases in Heart Failure with Preserved and with Reduced Ejection Fraction – Pilot Study"**

Celem prac było porównanie nasilenia stresu nitrozacyjnego (ocenianego na podstawie stężenia **3-NT** oraz **iNOS**), stresu ER (**GRP78**), nasilenia stanu zapalnego oraz stresu oksydacyjnego (**MPO**) w osoczu pacjentów z HFpEF i HFrEF.

Publikacja

3. **„Post-myocardial infarction heart failure and long-term high-fat diet cardiac endoplasmic reticulum stress and unfolded protein response in Sprague Dawley rat model"**

Celem pracy było zbadanie wpływu MI oraz zastosowania HFD na stres nitrozacyjny (oceniany na podstawie poziomu **3-NT** oraz **iNOS**), stan zapalny wraz ze stresem oksydacyjnym (**MPO**), stres ER (**GRP78**) oraz na aktywność szlaków UPR, które są odpowiedzią na ten stres (**IRE1α, ATF6, PERK**) w mięśniu lewej komory serca szczurów Sprague Dawley.

Kopie opublikowanych prac

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Article

Evaluation of Nitrosative/Oxidative Stress and Inflammation in Heart Failure with Preserved and Reduced Ejection Fraction

Karol Momot [1], Kamil Krauz [1], Katarzyna Czarzasta [1], Maciej Zarębiński [2], Liana Puchalska [1] and Małgorzata Wojciechowska [1,*]

[1] Chair and Department of Experimental and Clinical Physiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, 02-097 Warsaw, Poland; karolmomot@icloud.com (K.M.); kamilkrauz01@gmail.com (K.K.); katarzyna.czarzasta@wum.edu.pl (K.C.); liana.puchalska@wum.edu.pl (L.P.)
[2] Department of Invasive Cardiology, Independent Public Specialist Western Hospital John Paul II, Lazarski University, 05-825 Grodzisk Mazowiecki, Poland; maciej@zarebinski.pl
* Correspondence: malgorzata.wojciechowska2@wum.edu.pl

Abstract: Heart failure (HF) is a complex syndrome characterized by impaired cardiac function. Two common subtypes of HF include heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF). In this study, we aimed to evaluate and compare the plasma levels of 3-nitrotyrosine (3-NT)—as a marker of nitrosative/oxidative stress and myeloperoxidase (MPO)—as an indicator of inflammation between HFpEF and HFrEF. Twenty-seven patients diagnosed with HFpEF and twenty-two with HFrEF were enrolled in this study. Additionally, forty-one patients were recruited for the control group. An echocardiographic assessment was conducted, followed by the collection of blood samples from all participants. Subsequently, the levels of 3-NT and MPO were quantified using the ELISA method. Comprehensive clinical characteristics and medical histories were obtained. Circulating levels of 3-NT were significantly higher in the HFpEF patients than in the control and the HFrEF groups. Nitrosative/oxidative stress is significantly intensified in HFpEF but not in HFrEF.

Keywords: heart failure pathogenesis; nitric oxide; nitrosative stress; oxidative stress; inflammation

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1. Introduction

Heart failure (HF) remains a severe problem for individual patients and public health. Approximately 1–20 new cases are diagnosed per 1000 persons every year [1]. In 2017, around 64.3 million people worldwide had HF [2]. However, its prevalence depends on the geographical area. HF occurs more often in older adults and is related to high mortality in this group [3]. About half of all cases of HF constitute HF with preserved ejection fraction (HFpEF) [4].

HFpEF and heart failure with reduced ejection fraction (HFrEF) have different pathogeneses. Nevertheless, chronic inflammation plays a crucial role in both types of HF. In HFpEF, the systemic and cardiac inflammation results from metabolic risk factors (type 2 diabetes, obesity, and hypertension) and causes the activation of the endothelium in myocardial microcirculation, leading to the upregulation of NADPH oxidase 2 (NOX2) [5]. This results in oxidative stress, increased levels of H_2O_2, the uncoupling of endothelial nitric oxide synthase (eNOS), and the decreased production of nitric oxide (NO). In cardiomyocytes, diminished NO bioavailability leads to the lower stimulation of soluble guanylate cyclase (sGC), reduced formation of cyclic guanosine monophosphate (cGMP), and reduced protein kinase G (PKG) activity. The latter is associated with decreased titin phosphorylation and increased passive stiffness of cardiomyocytes [6]. The inflammatory state in HFrEF may be triggered by myocardial infarction (MI) [7]. This inflammation is sustained throughout heart tissue regeneration, remodeling, and scar formation, and is

associated with the infiltration of neutrophils, followed by monocytes and macrophages [8]. In this process, the renin-angiotensin-aldosterone system plays a significant role [9].

Recently, Schiattarella et al. established the critical role of nitrosative stress in the pathogenesis of HFpEF. The enhanced activity of inducible nitric oxide synthase leads to the improper functioning of the Inositol-Requiring Enzyme 1α - X-Box Binding Protein 1 (IRE1α-XBP1) pathway, playing a role in unfolded protein response [10]. 3-nitrotyrosine (3-NT) is a marker of nitro/oxidative stress. It is produced from tyrosine in the presence of reactive nitrogen species. Nitration can occur for free tyrosine and amino acids in the polypeptide chain [11]. Loek van Heerebeek et al. discovered that 3-NT expression was elevated in myocardial homogenates from patients with HFpEF compared to HFrEF. However, no studies have been conducted to assess and compare the circulating levels of 3-NT in patients with HFpEF and HFrEF or healthy individuals [12].

Myeloperoxidase (MPO) is a heme protein derived from leukocytes, which is related to vascular dysfunction and is a prognostic factor in cardiovascular pathologies [13]. It has been implicated in the pathogenesis of several inflammatory conditions, including HFpEF [14]. However, some authors have demonstrated a strong relationship between higher levels of MPO and the incidence of HFrEF but not HFpEF [15]. Increased circulating MPO plasma concentrations are associated with increased fibrosis and structural remodeling of the myocardium [16]. Under oxidative stress, human endothelial cells express MPO, which consumes NO and converts it to a nitrogen radical, causing protein nitration and tissue damage [17]. This also results in the reduction in NO levels and correlates with microvascular dysfunction [18]. NO deficiency causes the dysregulation of the NO-sGC-cGMP signaling pathway, which plays a role in development of endothelial dysfunction and hypertension. Decreased cGMP concentrations lead to the inadequate activation of its signaling targets and contribute to aberrant vascular toning [19,20].

2. Results

The baseline characteristics of patients are presented in Table 1. Echocardiographic features are displayed in Table 2. The results of the measurements for 3-NT, MPO, and NT-proBNP are depicted in Figure 1.

Table 1. Baseline characteristics of patients.

Characteristics	HFpEF (N = 27)	HFrEF (N = 22)	Control (N = 41)	*p*-Value
Male gender, n (%)	15 (56)	16 (73)	15 (37)	0.0203
Age, years, median (IQR)	72.00 (64.00–76.00)	74.50 (61.00–79.00)	70.00 (64.00–73.00)	ns
BMI, kg/m^2, median (IQR)	29.00 (26.37–32.65)	28.39 (25.39–31.25)	29.32 (26.81–32.47)	ns
NYHA II, n (%)	25 (93)	12 (55)	-	0.0021
NYHA III, n (%)	2 (7)	10 (45)	-	
H2FPEF score, median (IQR)	5 (4–6)	4.5 (3–6)	5 (3–6)	ns
HFA-PEFF score, median (IQR)	4 (4–6) [1]	5 (4–6) [2]	3 (2–4)	<0.0001
Medical History				
Hypertension, n (%)	23 (85)	15 (68)	33 (80)	ns
Diabetes or prediabetes, n (%)	4 (15)	11 (50)	8 (20)	0.0094
Lipid disorders, n (%)	4 (15)	2 (9)	13 (32)	ns
Renal dysfunction, n (%)	4 (15)	7 (32)	6 (15)	ns
Anemia, n (%)	4 (15)	5 (23)	6 (15)	ns

Table 1. *Cont.*

Characteristics	HFpEF (N = 27)	HFrEF (N = 22)	Control (N = 41)	*p*-Value
Obesity, n (%)	11 (41)	9 (41)	18 (44)	ns
Thyroid disease, n (%)	5 (19)	5 (23)	16 (39)	ns
Chronic obstructive pulmonary disease, n (%)	1 (4)	1 (5)	0 (0)	ns
Currently smoking, n (%)	2 (7)	3 (14)	1 (2)	ns
CAD, n (%)	16 (59)	16 (73)	9 (22)	0.0001
History of MI, n (%)	6 (22)	10 (45)	3 (7)	0.0018
History of PCI, n (%)	8 (30)	9 (41)	3 (7)	0.0051
History of CABG, n (%)	2 (7)	3 (14)	2 (5)	ns
Cardiomyopathy	0 (0)	6 (28)	2 (5)	0.0058
History of AF, n (%)	19 (70)	13 (59)	19 (46)	ns
AF at the time of enrollment, n (%)	4 (15)	5 (23)	1 (4)	0.0380
Implanted ICD/pacemaker, n (%)	2 (7)	16 (73)	4 (10)	<0.0001
Documented VF/VT, n (%)	0 (0)	4 (18)	2 (5)	0.0329
Selected Medications				
Diuretics	23 (85)	15 (68)	33 (80)	ns
ACEI/ARB	24 (89)	18 (82)	37 (90)	ns
Beta-blocker	24 (89)	19 (86)	34 (83)	ns
SGLT2i	5 (19)	20 (91)	11 (27)	<0.0001
Statin	17 (63)	17 (77)	19 (46)	ns
NOAC	17 (63)	13 (59)	10 (24)	0.0021

[1] HFpEF vs. control, $p < 0.001$, [2] HFrEF vs. control, $p < 0.001$. Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin-receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; ICD, implantable cardioverter-defibrillator; IQR, interquartile range; MI, myocardial infarction; NOAC, non-vitamin K antagonist oral anticoagulants; ns, non-significant; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SGLT2i, sodium-glucose cotransporter-2 inhibitor; VF, ventricular fibrillation; VT, ventricular tachycardia.

Table 2. Echocardiographic characteristics.

Characteristics	HFpEF (N = 27)	HFrEF (N = 22)	Control (N = 41)	*p*-Value
LVEF, %, median (IQR)	55 (50–60) [1]	37.5 (30–40) [2]	55 (50–60)	<0.0001
E velocity, cm/s, mean (SE)	76.15 (4.57)	62.36 (4.43)	69.45 (2.21)	ns
A velocity, cm/s, mean (SE)	70.62 (3.87)	75.41 (4.15)	75.90 (3.36)	ns
s' septal, cm/s, median (IQR)	6.5 (6–8)	5 (4–6.75) [2]	8 (7–9)	<0.0001
e' septal, cm/s, median (IQR)	7 (5–8)	4 (3.75–6.25) [2]	7 (6–8)	0.0023
s' lateral, cm/s, median (IQR)	7.5 (6–8.25) [3]	5.5 (4–8) [2]	8 (7–9)	0.0067
e' lateral, cm/s, median (IQR)	9.5 (7–11) [1]	6 (5–9.25)	8 (6–11)	0.0309
E/A, ratio, median (IQR)	1.17 (0.76–1.59)	0.74 (0.58–1.30)	0.91 (0.80–1.17)	ns

Table 2. *Cont.*

Characteristics	HFpEF (N = 27)	HFrEF (N = 22)	Control (N = 41)	*p*-Value
E/e', ratio, median (IQR)	9.54 (7.69–12.22)	8.06 (6.67–18.00)	8.88 (7.30–11.34)	ns
IVS, cm median (IQR)	1.20 (1.10–1.33)	1.35 (1.12–1.5) [2]	1.2 (1.05–1.30)	0.0472
LVEDD, cm median (IQR)	4.65 (4.25–5.03) [1]	5.63 (4.98–6.05) [2]	4.6 (4.25–5.00)	<0.0001
PW, cm median (IQR)	1.1 (0.90–1.33) [1]	1.3 (1.2–1.50) [2]	1.1 (0.95–1.30)	0.0015
LAVI, mL/m^2, mean (SE)	44.23 (3.14)	53.60 (5.25) [2]	35.53 (1.62)	0.0003
LVMI, g/m^2, median (IQR)	103.10 (84.10–110.60) [1]	192.35 (145.50–222.00) [2]	97.50 (80.70–116.50)	<0.0001
RWT, median (IQR)	0.50 (0.40–0.60)	0.50 (0.40–0.50)	0.50 (0.40–0.60)	ns
IVCC, %, mean (SE)	45 (3)	41 (4)	49 (4)	ns
Moderate valve disease, n (%)	3 (11)	1 (5)	3 (7)	ns
Severe valve disease, n (%)	3 (11)	6 (27)	0 (0)	0.0026

[1] HFpEF vs. HFrEF, $p < 0.001$, [2] HFrEF vs. control, $p < 0.001$, [3] HFpEF vs. control. Abbreviations: HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IQR, interquartile range; IVCC, inferior vena cava collapsibility; IVS, intraventricular septum; LAVI, left atrial volume index; LVEDD, left ventricle end-diastolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; ns, non-significant; PW, posterior wall; RWT, relative wall thickness; SE, standard error.

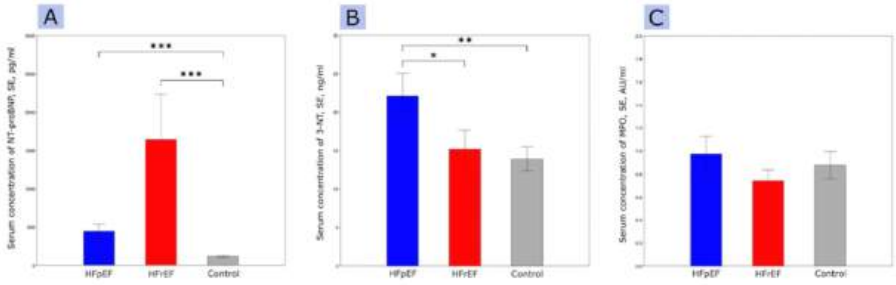

Figure 1. NT-pro-BNP levels (**A**), 3-NT circulating levels (**B**), MPO circulating levels (**C**); abbreviations: 3-NT, 3-nitrotyrosine; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; MPO, myeloperoxidase; SE, standard error. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The linear and rank correlation charts are presented in Figure 2. Regardless of the group, age negatively correlates with Hemoglobin (Hgb) levels ($r = -0.28$, $p = 0.0081$) and estimated Glomerular Filtration Rate (eGFR) ($r = -0.36$, $p = 0.0006$). The control group had no correlations between MPO and 3-NT and any other variables.

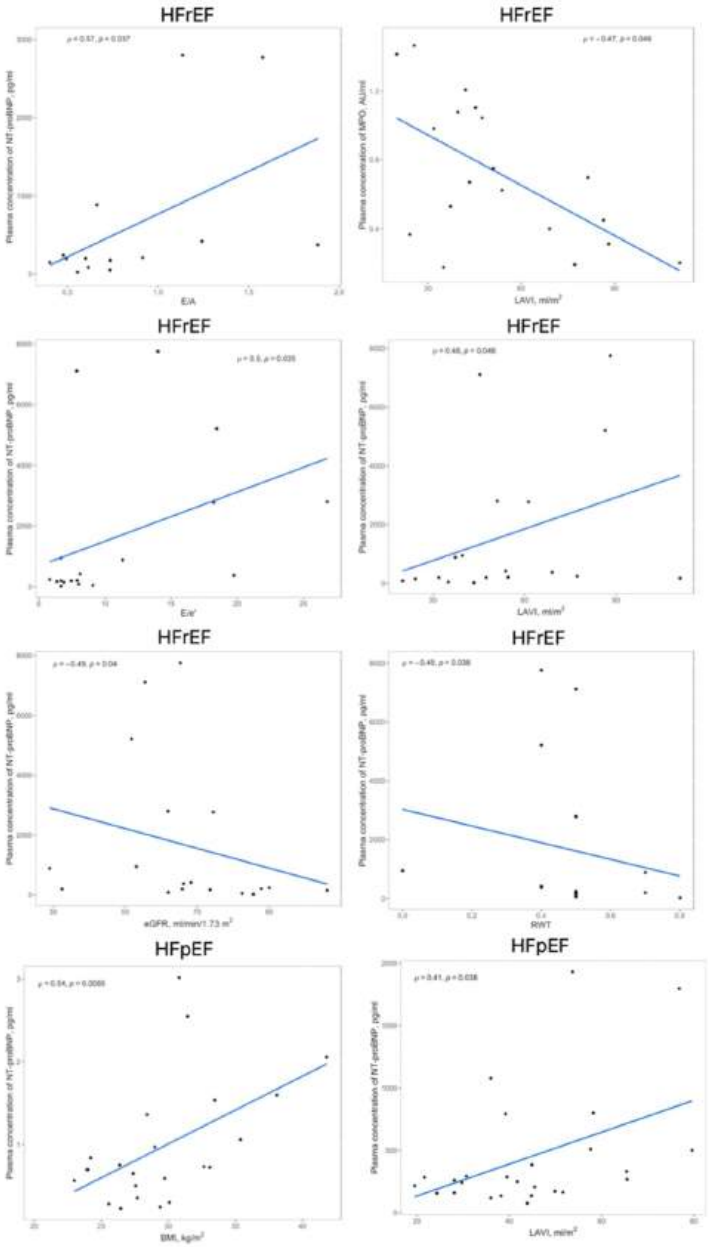

Figure 2. Significant correlations within the HFrEF and HFpEF groups between circulating levels of MPO, 3-NT, or NT-proBNP and echocardiographic findings, BMI or eGFR; abbreviations: 3-NT, 3-nitrotyrosine; BMI, body mass index; eGFR, estimated glomerular filtration rate; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LAVI, left atrial volume index; MPO, myeloperoxidase; RWT, relative wall thickness.

Obese patients have greater MPO levels than the non-obese patients (0.91 (0.45–1.41) vs. 0.64 (0.39–0.97), AU/mL, $p = 0.049$). Patients with thyroid disease present higher 3-NT concentrations compared to healthy subjects (9.42 (7.55–12.14) vs. 14.22 (10.09–22.05), ng/mL, $p = 0.008$). Patients with renal dysfunction present higher NT-proBNP levels than those with normal eGFR (270 (172.5–1014) vs. 159 (77–267), pg/mL, $p = 0.0123$).

CAD and history of MI are the states related to higher NT-proBNP levels (242 (157–794) and 273 (154–863), pg/mL, respectively) when compared to the healthy state (120.5 (73.5–234.75) and 163 (77–268), pg/mL, respectively) ($p = 0.0016$ and $p = 0.0311$, respectively). Regardless of the group, therapy with Sodium-Glucose Co-Transporter 2 Inhibitors (SGLT2i), Angiotensin-Converting Enzyme Inhibitors (ACEI), Angiotensin II Receptor Blockers (ARB), Statins, Beta-blockers, or non-vitamin K antagonist oral anticoagulants (NOAC) does not significantly impact levels of MPO and 3-NT.

3. Discussion

In this study, circulating levels of MPO and 3-NT as markers of inflammation and oxidative/nitrosative stress, respectively, were assessed in patients with HFpEF and HFrEF and healthy controls. Our results revealed that HFpEF is associated with significantly increased plasma concentration of 3-NT compared to other groups, suggesting that the pathogenesis of HFpEF is associated with oxidative/nitrosative stress. Using quantitative immunohistochemistry, van Heerebeek et al. showed that myocardial 3-NT levels were significantly higher in samples obtained from patients with HFpEF than HFrEF, which aligns with our results [12]. Kolijn et al. observed higher concentrations of 3-NT in the left ventricular myocardium from HFpEF patients than from healthy donors. Moreover, the skinned fibers obtained from the biopsies were treated in vivo with empagliflozin. This resulted in a significant reduction in 3-NT levels, suggesting that the medication attenuated nitrosative stress [21].

Shishehbor et al. revealed significantly increased levels of 3-NT in patients with coronary artery disease compared to healthy controls [22]. Moreover, statin therapy significantly reduced circulating levels of 3-NT, which suggests that it could also be beneficial in HFpEF [23,24]. In the rat model of diabetic cardiomyopathy, treatment with irisin significantly reduces cardiac 3-NT levels [25]. However, no other research has explored irisin's impact on plasma 3-NT levels in the HFpEF model.

Within the scope of our investigation, the use of medications such as NOACs, statins, and SGLT2 inhibitors did not exhibit a significant correlation with the levels of 3-NT and MPO. However, it is important to note that investigating the impact of these medications on these markers' levels was not our study's primary objective. To assess the influence of therapy with these drugs on nitrosative/oxidative stress or inflammation, it would be necessary to measure these markers' levels before initiating treatment.

In our study, MPO concentrations do not differ between the three groups, which is contrary to the study of Hage et al., who described significantly higher levels of MPO in HFpEF patients compared to healthy controls. Another study, which compared 33 biomarkers between HFpEF and HFrEF, showed that circulating levels of MPO were similar in both groups [14]. Similar to our results, body mass index (BMI) positively correlated with MPO among the HFpEF population [13]. In our research, MPO levels were positively associated with BMI in both HF groups, but not in the control group.

We did not find any difference in circulating levels of MPO between the HFpEF population and healthy individuals. This remains an exciting finding because the previous studies suggest that the MPO levels should rather be elevated in the HFpEF group. However, Kao et al. identified subgroups among HFpEF patients with distinct clinical characteristics and prognoses [26]. It is possible that the HFpEF population in our study belonged to a specific subpopulation that does not have raised circulating levels of inflammatory markers. This finding could potentially be essential information in developing personalized therapies for HFpEF. Standard treatments do not sufficiently target inflammation, which may play a key role in this disease. Recent research showed that an MPO inhibitor-AZD4831

(mitiperstat) decreased inflammation in HFpEF patients, but the efficacy findings were inconclusive [27–29]. The Phase 2b-3 clinical trial ENDEAVOR, to investigate the use of the MPO inhibitor AZD483, is ongoing [30]. In the HFrEF group, there were significantly more cases of patients with CAD, a history of MI, and a history of percutaneous coronary intervention (PCI). This is not surprising, as these factors are fundamental to HFrEF etiology. Additionally, these patients had a higher incidence of implanted implantable cardioverter-defibrillator (ICD) or pacemakers, which results from guidelines about primary prevention of sudden cardiac death. Furthermore, this group exhibited a greater prevalence of documented ventricular fibrillation/ventricular tachycardia (VF/VT), which is also associated with MI and reperfusion following PCI.

Our study has several limitations. First, we analyzed levels of markers in peripheral blood rather than directly from the heart. Therefore, the results may be influenced by the fact that these markers could originate from other compartments. However, it is known that HFpEF is a result of the overlap of coexisting extracardiac conditions, and measured nitrosative/oxidative stress may affect the entire body. In general, measuring MPO and MPO-derived products remains expensive and time-consuming. These products are usually found in low concentrations that affect the measurement accuracy. Recently, ELISA kits have been more commonly used, making testing more affordable [8]. Lastly, the study lacked a long-term follow-up of the participants, so we cannot analyze the impact of variables on survival.

4. Materials and Methods

This was an observational cohort study conducted at a single cardiac center. A total of 90 patients identified from the hospital medical records were invited to participate in the study. HF was confirmed based on the patient's symptoms, signs, and transthoracic echocardiography (TTE) results. Preserved ejection fraction was defined as left ventricular ejection fraction (LVEF) $\geq$ 50%, and reduced ejection fraction was defined as LVEF $\leq$ 40%. The study population was divided into three groups: HFpEF (n = 27), HFrEF (n = 22), and patients without HF (control, n = 41). The exclusion criteria included heart failure with mid-range ejection fraction (HFmrEF) to precisely differentiate both types of HF (HFpEF and HFrEF). Exclusion criteria also included refusal to participate in the study, an active neoplastic process, an active inflammatory process, a recent history (3 months) of acute MI or cardiac surgery, and new onset and/or exacerbation of any HF. All patients included in the study were in stable condition at the time of enrollment. Comprehensive clinical characteristics and medical records were obtained. An electrocardiogram (ECG) examination was conducted to confirm or rule out the presence of atrial fibrillation (AF). Height and weight were measured, and BMI was calculated. The New York Heart Association (NYHA) functional class for each patient was determined. Based on the echocardiography results, biomarker levels, and clinical assessment, the scores of the HFAPEFF and H2FPEF scales were quantified [31,32].

4.1. Blood Sample Collection and Biomarkers Assessment

Peripheral venous blood was collected from each patient from the cephalic vein into citrate tubes. The samples were centrifuged and stored at –80 °C until laboratory assay, not exceeding a storage duration of 6 months. The plasma concentrations of the biomarkers were assessed with the 3-Nitrotyrosine ELISA Kit (Colorimetric) [Novusbio, Littleton, CO, USA, NBP2-66363] and the Myeloperoxidase ELISA Kit (Colorimetric) [Novusbio, NBP2-60581], strictly according to instructions provided by the manufacturer. MPO antibody was used to quantitatively determine autoimmune response to the target antigen. The eGFR was calculated utilizing the CKD-EPI equation based on plasma creatinine levels. Renal dysfunction was characterized by an eGFR of less than 60 mL/min/1.73 m^2. Anemia was identified by Hgb concentrations below 12 g/dL in females and below 13 g/dL in males.

4.2. Echocardiography

TTE was performed by experienced cardiac sonographers using a Philips Affinity 70 Ultrasound machine. All procedures were conducted in accordance with European Society of Cardiology (ESC) guidelines. LV diameters and wall thickness were measured using a parasternal long-axis view. LVEF was determined using Simpson's method by measuring LV volume in systole and diastole obtained from apical four- and two-chamber views. LV mass index (LVMI) was calculated using the formula for estimation of LV mass from LV linear dimensions and indexed to body surface area (BSA). LV geometry was classified based on relative wall thickness (RWT). LA volume was assessed using the biplane area–length method from apical 2- and 4-chamber views at end-diastole from the frame preceding mitral valve opening and was indexed to BSA (LA volume index, LAVI). The early diastolic peak flow velocity (E velocity) and late diastolic peak flow velocity (A velocity) were assessed through pulsed wave Doppler from the apical 4-chamber view by positioning the sample volume at the tip of the mitral leaflets. Peak early diastolic tissue velocity (e') and peak systolic tissue velocity (s') were measured from the septal and lateral aspects of the mitral annulus. The E/e' ratio was calculated as E velocity divided by mean e' velocity (average value of the lateral and the septal velocity). The inferior vena cava (IVC) diameter was measured using M-mode echocardiography in subcostal view at end-expiration (IVCmax) and at inspiration (IVCmin). The IVC collapsibility (IVCC) was calculated as (IVCmax − IVCmin)/IVCmax and was expressed as a ratio. Sample echocardiographic measurements from selected patients are presented in Figure 3.

4.3. Statistical Analysis

Categorical variables are expressed as numbers and percentages and compared using the χ^2 test. Quantitative variables are presented as means (standard deviation [SD]) or medians (interquartile range [IQR]). The ANOVA test (normal distribution) and the Kruskal–Wallis H test (non-normal distribution) were performed to compare variables between the three groups. Appropriate post hoc tests were performed (Dunn and Tukey tests). The Shapiro–Wilk test was used to assess the normality of data. The equality of variances was assessed using the Levene test. Statistical data were considered significant with a p-value < 0.05. All statistical analyses were performed using RStudio software (version 2022.12.0, Posit Software). Depending on whether the distribution of the quantitative data was normal or not, either the Pearson correlation method or the Spearman analysis was employed to assess linear correlation.

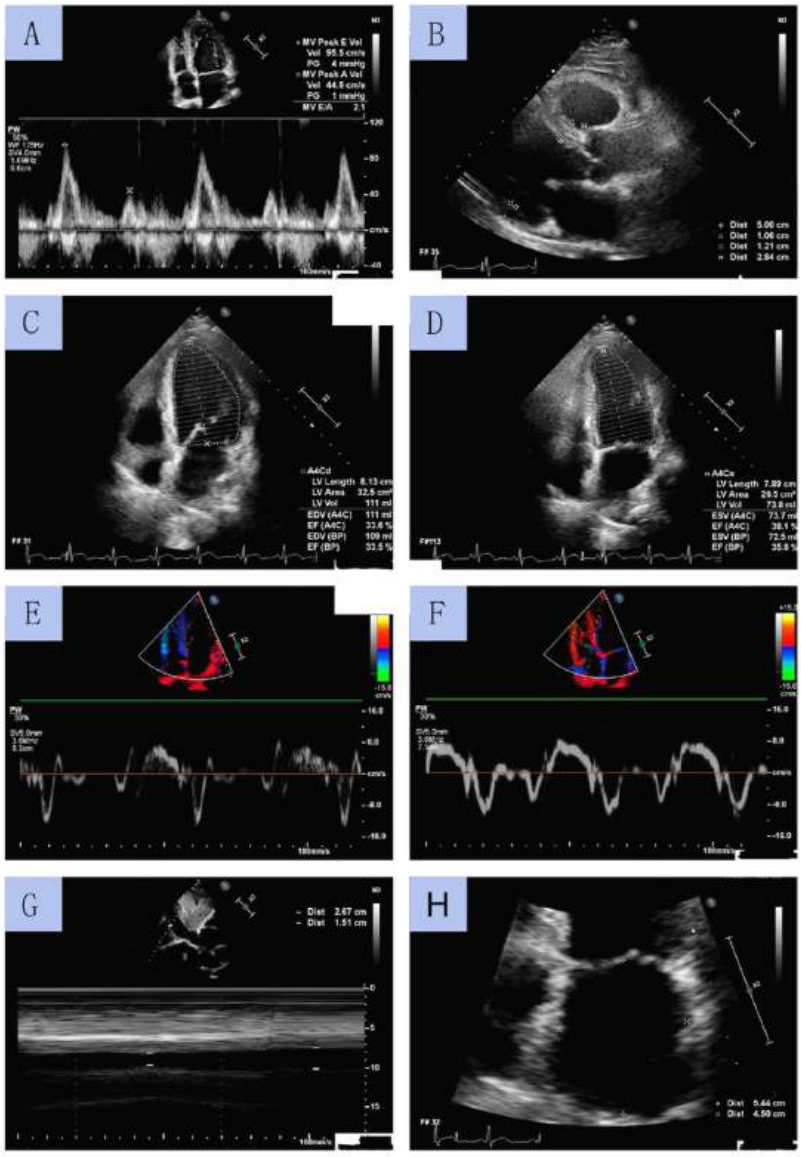

Figure 3. Sample echocardiographic measurements: mitral diastolic peak flow velocities by pulsed wave Doppler (**A**); left ventricle diameters in long axis parasternal view (**B**); left ventricle geometry on end-diastole and end-systole (**C**,**D**); Tissue Doppler Imaging of tissue velocities of the lateral and septal aspects of the mitral annulus (**E**,**F**); the inferior vena cava collapsibility (**G**); left atrium diameters (**H**).

5. Conclusions

Nitrosative/oxidative stress measured via circulating levels of 3-NT is significantly intensified in HFpEF but not in HFrEF. The extent of inflammation assessed by MPO concentration is comparable between patients with two types of HF and the healthy population. These findings provide input to the current discussion about the pathogenesis of HF, diagnostic markers, and new therapeutic targets.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Lazarski University (LU no. 02/11/22).

Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: This study's data cannot be shared publicly for privacy reasons. The data will be shared upon request to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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Endoplasmic reticulum stress and expression of nitric oxide synthases in heart failure with preserved and with reduced ejection fraction — pilot study

Authors: Karol Momot, Małgorzata Wojciechowska, Kamil Krauz, Katarzyna Czarzasta, Liana Puchalska, Maciej Zarębiński, Agnieszka Cudnoch-Jędrzejewska

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ORIGINAL ARTICLE

Endoplasmic reticulum stress and expression of nitric oxide synthases in heart failure with preserved and with reduced ejection fraction — pilot study

Running title: GRP78 and Nitric Oxide Synthases Levels in HFpEF and HFrEF

Karol Momot[1] https://orcid.org/0000-0001-8659-2948, Małgorzata Wojciechowska[1], Kamil Krauz[1], Katarzyna Czarzasta[1], Liana Puchalska[1], Maciej Zarębiński[2], Agnieszka Cudnoch-Jędrzejewska[1]

[1]Department of Experimental and Clinical Physiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Poland

[2]Department of Invasive Cardiology, Independent Public Specialist Western Hospital John Paul II Lazarski University, Grodzisk Mazowiecki, Poland

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Address for correspondence:

Małgorzata Wojciechowska MD PhD,

Laboratory of Centre for Preclinical Research, Medical University of Warsaw,

Department of Experimental and Clinical Physiology, 02–097 Warsaw, Poland; tel: (0-22) 572 07 08, e mail: malgorzata.wojciechowska2@wum.edu.pl

Keywords: nitrosative stress, oxidative stress, endoplasmic reticulum stress, heart failure with preserved ejection fraction, heart failure with reduced ejection fraction

ABSTRACT

Background: Unfolded Protein Response (UPR), endoplasmic reticulum (ER) stress, and inducible nitric oxide synthase (iNOS) overexpression have been found to influence heart failure with preserved ejection fraction (HFpEF) pathogenesis. Their importance in heart failure with reduced ejection fraction (HFrEF) is not entirely established; there is little data involving a detailed comparison between HFpEF and HFrEF from this perspective. This pilot study aimed to compare circulating levels of Glucose-regulated protein 78kDa (GRP78) (ER — stress marker) and all NOS isoforms between both HFpEF and HFrEF and to analyze the correlation between these markers and the clinical characteristics of the patients.

Methods: Forty-two patients with HFpEF and thirty-eight with HFrEF were involved in this study. Clinical characteristics and echocardiographic data were obtained. Basic laboratory tests were performed and ELISA tests for iNOS, endothelial NOS (eNOS), neuronal NOS (nNOS), and GRP78.

Results: Patients with HFpEF had lower circulating levels of GRP78 and higher iNOS concentrations when compared to HFrEF patients ($P = 0.023$, $P < 0.0001$, accordingly). The subgroup of the HFpEF population with eGFR < 60 mL/min/1.73m2 had higher nNOS and eNOS levels than HFpEF patients with normal GFR ($P = 0.049$, $P = 0.035$, respectively). In the HFrEF subgroup, patients with coexistent diabetes mellitus had elevated concentrations of nNOS compared to the subpopulation without diabetes mellitus ($P = 0.041$). There was a positive correlation between eNOS and nNOS concentrations ($\rho = 0.86$, $P < 0.0001$).

Conclusions: In HFpEF, there is a more intensified iNOS overexpression, while in HFrEF, ER stress is more prominent.

Introduction

Heart failure (HF) is a clinical syndrome characterized by symptoms and signs arising from structural and functional changes in the heart, which results in elevated cardiac pressures and abnormal cardiac output. The cardinal symptoms of HF include shortness of breath, fatigue, swelling (edema), and impaired exercise tolerance. Considering the left ventricular ejection fraction (LVEF), HF can be divided into three types: heart failure with preserved ejection fraction (HFpEF; LVEF ≥ 50%), heart failure with mildly reduced ejection fraction (HFmrEF;

LVEF: 41 — 49%) and heart failure with reduced ejection fraction (HFrEF; LVEF ≤ 40%) [1]. The estimated occurrence of HF among adults from developed countries ranges from 1 to 3%. Approximately 50% of them have HFrEF. However, the population diagnosed with HFpEF is rising [2].

There are several differences and similarities between HFpEF and HFrEF concerning their pathogenesis, progression, and abnormalities at the molecular level [3]. Both types are associated with systemic and cardiac inflammation, endothelial dysfunction, cardiac injury [3–5]. However, even within their similarities, differences can be observed. For example, inflammatory response in HFrEF results rather from cardiomyocyte damage, inflammation in HFpEF arises from extra-cardiac metabolic and inflammatory risk factors. In HFpEF, endothelial dysfunction mainly precedes its progression, whereas in HFrEF, endothelial dysfunction may rather be a late-stage consequence. Furthermore, there are differences in etiology between these types. For instance, a history of myocardial infarction and ischemic heart disease is more common in HFrEF [3], while obesity, kidney disease, and old age are more likely to contribute to the development of HFpEF [6].

Schiattarella et al., using preclinical models of HFpEF and human myocardial samples, presented the important role of meta-inflammation, inducible nitric oxide synthase (iNOS), nitrosative stress, and alterations in the unfolded protein response (UPR) in the pathogenesis of the disease [7]. On the contrary, data about iNOS expression and UPR in HFrEF pathophysiology are currently very limited.

UPR plays a crucial role in maintaining endoplasmic reticulum (ER) homeostasis. When the protein-folding capacity in the ER is impaired, it results in the accumulation of unfolded and

misfolded proteins. This disruption in ER homeostasis is known as ER stress. Glucose-regulated protein 78 kDa (GRP78) is involved in this process. This protein functions as a quality control system [8] by monitoring the proteins' folding process and ensuring they are transported only when properly folded. Induction of this protein causes a reduction in ER stress and has cardioprotective effects [8, 9]. Overexpression of GRP78 reduces ER stress and cardiac damage by inducing UPR. A study conducted on muscle cell lines found that GRP94, similar to GRP78, reduced cardiomyocyte necrosis in ischemia conditions [10].

The enzyme nitric oxide synthase (NOS) produces nitric oxide (NO) from the amino acid L-arginine. This enzyme exists in three distinct isoforms: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) [11]. The constitutive expression of nNOS was found in neurons and endothelial cells and has a role in regulating blood pressure. nNOS is also the source of myocardial NO, which takes part in cardiac relaxation and contraction [12], and both of these functions are disrupted in HF. eNOS is constitutively expressed in every endothelial cell, including heart vessels. eNOS is a dimer consisting of two identical monomers. In a coupled state (dimer), eNOS typically produces NO. Uncoupled eNOS (monomer) shifts to produce dangerous cytotoxic superoxide anions instead of NO, which causes oxidative stress and endothelial dysfunction, leading to cardiovascular diseases, including HF [13, 14]. Finally, recently popular- iNOS is usually expressed in the human heart. However, this process in cardiac tissue can be intensified after relevant triggers such as inflammation, hypoxia, or excessive oxidative stress. All of the mentioned triggers are present in cardiovascular pathologies [15, 16]. iNOS-derived NO causes S-nitrosylation of proteins and, in this way, disrupts their activity. The role of other abnormalities related to NO metabolism is broadly explained in cardiovascular pathologies [17]. This pilot, cross-sectional study provides a novel approach to HFrEF and HFpEF. It compares the circulating serum levels of GRP78 (a

marker of endoplasmic reticulum stress) and all nitric oxide synthases between HFpEF and HFrEF, aiming to identify potential differences or similarities in the pathogenesis of these conditions. Herein, the correlation between these markers and the clinical and echocardiographic characteristics of the patients is analyzed.

Methods

The study was conducted according to the principles outlined in the Declaration of Helsinki, reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement, and received approval from the Local Ethics Committee (LU no. 02/11/22). Before recruitment, all participating patients were asked to sign a written informed consent form.

Study Population

Forty-two patients with HFpEF and thirty-eight with HFrEF, identified from their hospital medical records, were invited to this pilot, cross-sectional study conducted at a single tertiary cardiac center. The patients with mid-range EF (HFmrEF) were not included to avoid bias in the characterization of HFpEF and HFrEF. All enrolled patients were diagnosed with HF at least 3 months prior and were treated according to the current guidelines. Exclusion criteria were refusal to participate in the study, an active neoplastic process, and an active inflammatory process. Patients with a myocardial infarction, exacerbation of HF, or cardiac surgery within the prior 3 months were not included.

Serum biomarker assessment

Venous blood samples from the cephalic vein were collected in citrate tubes and then centrifuged. Samples with obtained serum were promptly frozen at –80°C and preserved for a maximum duration of four months prior to the biochemical assessment. Serum concentrations of markers were analyzed using ELISA kits: iNOS [NBP2–80255, Novusbio], eNOS [NBP2–

80134, Novusbio], nNOS [NBP2–80252, Novusbio], GRP78 [NBP2–82201, Novusbio]. Each ELISA test was conducted following the manufacturer's instructions. The assessment also included evaluating NT–proBNP and the concentration of serum creatinine. Estimated GFR (eGFR) was calculated by an equation developed by the Chronic Kidney Disease Epidemiology Collaboration (EPI-CKD). Renal dysfunction was defined by an eGFR below 60 mL/min/1.73m².

Transthoracic echocardiography examination

Echocardiography was conducted following the European Society of Cardiology guidelines, using a Philips Epiq Ultrasound machine by a single experienced sonographer, to reduce the risk of bias in measurements. LVEF was measured using Simpson's method by obtaining LV volume in systole and diastole through apical four- and two-chamber views. Other echocardiographic characteristics were evaluated: peak early diastolic transmitral flow velocity (E), peak late diastolic transmitral flow velocity (A), left ventricular end-diastolic dimension (LVEDD), intraventricular septal thickness (IVS), posterior wall thickness (PW), relative wall thickness (RWT), left ventricular mass index (LVMI), left atrial (LA) volume, LA volume index (LAVI) and the inferior vena cava (IVC) collapsibility (IVCC). Tissue Doppler imaging (TDI) was performed (both the septal and lateral aspects of the mitral annulus): peak systolic mitral annular tissue velocity (s'), peak early diastolic mitral annular tissue velocity (e'), peak late diastolic mitral annular tissue velocity (a'). Based on the measurements, the E/e' ratio was assessed.

Statistical analysis

Statistical analysis was carried out with the Statistica software, version 13.3. Quantitative variables are presented as means with standard deviation (SD) or medians with interquartile range (IQR). The T-test was performed with data following a normal distribution, while the Mann-Whitney U test was done with data that did not conform to a normal distribution. These

tests were used to compare quantitative variables between the groups. Categorical variables are presented as numbers with percentages (%), and their comparison was conducted using the $\chi 2$ test. The normality of the data was assessed using the Shapiro-Wilk test, while the equality of variances was evaluated using the Levene test. The correlation between the quantitative variables was assessed using Spearman's rank correlation coefficient, and Spearman's rho is labeled as ρ. Statistical significance was determined when the P-value was less than 0.05. The figures were created with Past4 and RStudio.

Results

Table 1 presents the baseline characteristics of the study population. As shown in this table, patients with HFpEF were older than those with HFrEF (mean age: 73 *vs.* 68, P = 0.020). In this study, it was more common for individuals with HFpEF to be female (57% *vs.* 21%, P = 0.001). Most of the subjects from both groups presented NYHA II class symptoms. In the HFrEF group, there were more cases of NYHA III manifestations (7% vs. 21%, P = 0.071). As shown in Table 2, all of the LV dimensions were greater in the HFrEF group (LVEDD: 4.88 *vs.* 5.86, cm, P < 0.001; IVS: 1.11 *vs.* 1.19, cm, P = 0.210; PW: 1.14 *vs.* 1.33, cm, P = 0.002). LVMI and LAVI were greater in the HFrEF group than in the HFpEF (106.47 *vs.* 154.79, g/m^2, P < 0.001 and 39.37 *vs.* 49.68, ml/m^2, P = 0.014; accordingly).

Figure 1 shows the differences between HFpEF and HFrEF groups in blood serum concentrations of nitric oxide synthases and GRP78. Patients with HFpEF presented lower GRP78 serum concentrations than those with HFrEF [0.44 (0.33) *vs.* 0.84 (0.75), respectively, ng/ml, P = 0.008]. The HFpEF group had greater iNOS serum concentrations than the HFrEF group [605.13 (399.13) *vs.* 402.49 (266.06), respectively, pg/ml, P < 0.0001]. The serum concentrations of nNOS and eNOS did not differ significantly between groups.

A subdomain analysis revealed that patients with HFrEF with the coexistence of DM2 presented significantly higher serum concentrations of nNOS than those without DM2 [1.06 (0.68–1.77) *vs.* 0.55 (0.35–1.23), ng/mL, P = 0.041].

In the HFpEF group, patients with renal dysfunction, when compared to those without decreased eGFR, had significantly greater blood serum concentrations of nNOS [1.13 (0.94–1.24) *vs.* 0.69 (0.56–1.12), ng/mL, P = 0.049] and eNOS [424.63 (358.05–498.72) *vs.* 313.96 (233.86–380.40), pg/mL, P = 0.035].

Other comorbidities, including CAD, COPD, or hypertension, were not found to be related to changes in serum concentrations of GRP78 and all NOS.

Age-related significance in GFR decrease in the population with confirmed HF regardless of LVEF is presented in Figure 2A (ρ = - 0.34, P = 0.0022). The concentration of both eNOS (Figure 2B) and nNOS (Figure 2C) were negatively correlated with GFR regardless of the group (ρ = –0.39, p = 0.00034 and ρ = –0.35, p = 0.0014 respectively). The correlation comparing eNOS and nNOS concentrations, regardless of the group, was positive (ρ = 0.86, P < 2.2e-16) (figure 2D). Among the general population, iNOS concentration was negatively related to LVMI (Figure 2E) and GRP 78 (Figure 2F) (ρ = –0.3, P = 0.0064 and ρ = –0.25, P = 0.036 respectively). In the HFpEF group, eNOS concentration was positively correlated with GRP78 concentration (ρ = 0.33, P = 0.046) (Figure 2G). Concentrations of iNOS, eNOS, and nNOS were not influenced by age, BMI, or NT-proBNP concentration and other echocardiography findings. No linear and rank correlations, apart from the ones listed above, were found.

Discussion

The current results revealed that HFpEF is associated with significantly higher iNOS overexpression than in the HFrEF (figure 1D), suggesting its essential role in the pathogenesis

of HFpEF but not in HFrEF. According to available research, there are currently no studies comparing circulating serum iNOS between those types of HF.

Low levels of iNOS are expressed in normal, healthy heart tissue. However, during cell stress, especially chronic inflammation, iNOS becomes activated, and large quantities of NO are generated [15]. Induction of iNOS is also present in conditions characterized by reactive oxygen species overproduction [18], such as metabolic diseases, and kidney disorders [19, 20].

Even though NO plays a crucial role in regulating vascular tone as well as in inhibiting platelet aggregation and adhesion, significantly excessive NO levels can exacerbate inflammation and cytotoxic injury [21]. Furthermore, iNOS-induced nitrosative stress contributes to the progression of HFpEF, and inhibiting the synthesis or activity of iNOS improves the HFpEF phenotype in an animal mouse model [7]. iNOS gene knockout improves cardiac diastolic function in this HFpEF model. Furthermore, iNOS overexpression leads to cardiac nitrosative stress by upregulating Akt S-nitrosylation in cardiomyocytes [22]. While the significance of iNOS in HF development appears pivotal in animal models, no investigations have assessed iNOS levels in the blood of human individuals with either HFpEF or HFrEF.

The present research revealed higher concentrations of GRP78 in patients with HFrEF than those with HFpEF (figure 1A), which is consistent with the conclusions of Zhao et al. [23]. According to conjecture herein, lower concentrations of GRP78 in HFpEF may be attributed to a disrupted and downregulated UPR. In the present study, serum concentration of GRP78 was not associated with other comorbidities such as CAD, hypertension, and especially DM2,

contrary to other studies [24]. GRP78 concentrations were negatively correlated with LVEF, which confirms the differences in pathogenesis in HFpEF and HFrEF. Additionally, the concentration of GRP78 was significantly lower in the elderly patients.

The ER stress leads to upregulating GRP78 expression, which is crucial for maintaining the ER homeostasis [25]. The ER stress can be advantageous under specific circumstances; however, it can result in cell death via apoptosis when it becomes severe and persists for an extended duration. The ER stress is present during cardiac hypoxia and acute or chronic inflammation, which are also closely linked to HF pathogenesis [26]. The disruption of ER homeostasis results in HF, aggravating ER stress even more [27]. GRP78 is a protein that inhibits apoptotic signaling and protects cells from apoptosis caused by ER stress [28].
In the current research, serum concentrations of nNOS did not differ between the HFrEF and HFpEF groups (Figure 1B). Although Dumy et al. pointed out overexpression in myocardial nNOS in HFrEF patients [29]. Similarly to eNOS, the concentration of nNOS was significantly greater in the HFpEF subpopulation with renal dysfunction. In the HFrEF group, serum concentration of nNOS was substantially higher among patients with DM2 compared to individuals without this disease. Surprisingly, a recent study showed that elevated expression of nNOS in cardiac tissue acted as a protective factor against cardiac hypertrophy and HF [30]. Also, nNOS gene knockout in mice raised oxidative stress in the heart [28], suggesting its protective role in oxidative stress.

In this study, the serum concentrations of eNOS did not differ between the two groups (figure 1C). At this moment, only one study evaluating eNOS concentrations in HF patients was conducted. Results revealed that in left ventricular tissue samples from humans, eNOS levels were significantly higher in HFpEF, but they compared it to samples from healthy donors, not

from donors with HFrEF [31]. Nevertheless, the present study found that eNOS overexpression was more significant in the HFpEF subpopulation with decreased GFR. It has been shown that eNOS can be involved in renal vascular damage by inducing nodular glomerulosclerosis, which ultimately declines GFR [32].

Recent findings on HFpEF pathophysiology, regarding intensified pro-inflammatory state, elevated iNOS activity, and also nitrosative stress [3], showed why previous attempts of treatment based on elevating NO levels (for example, phosphodiesterase-5 inhibition) were unsuccessful in HFpEF management. Future studies aiming to find HFpEF treatment should focus on inhibiting nitrosative stress, ER stress, and S-nitrosylation. The research for any future treatments for HFpEF should concentrate on decreasing iNOS activity specifically, not increasing it [33]. A recent study showed that both pharmacological inhibition of iNOS with L-NIL administration and iNOS gene knockout improved the phenotype of HFpEF in mice, mitigating mitochondrial dysfunction, oxidative stress, and S-nitrosylation [22]. GW274150 and GW273629 are two highly selective iNOS inhibitors that have the potential to be promising therapeutic agents. They were discovered about 20 years ago, and their role was studied in human breast tumors or renal ischemia/reperfusion injury [34–36]. However, GW274150 and GW273629 were not tested for HFpEF management in any animal model of this disease. iNOS expression is not limited to the cardiovascular system. Therefore, its inhibition may potentially cause serious adverse events. Further studies regarding the safety and efficacy of iNOS inhibitors are needed.

Despite the fact that all NOS enzymes are intracellular, cell death leads to their release into the bloodstream. As a result, they become detectable in ELISA tests, and it has been found that plasma levels of certain NOS enzymes can be elevated in depression following a stroke [37].

Limitations of the study

The study has some limitations. Firstly, the current investigation is only theoretical, and further exploration of specific approaches is necessary to discover therapeutic benefits in the future. The measurements of selected markers were related to plasma rather than cardiac tissue, which may reduce their sensitivity and specificity. However, it is worth emphasizing that the results remain statistically significant even after accounting for comorbidities. Although these markers may originate from different compartments, nitrosative stress is known to affect the entire organism, including the heart. This systemic impact can contribute to conditions like HFpEF, which is characterized as a clinical syndrome rather than a distinct disease. Secondly, in the HFrEF group, there were significantly more cases of patients with CAD. This is not surprising because, as previously mentioned, this factor is fundamental to HFrEF etiology. The HFrEF patients also had more implanted ICD/pacemakers, which can be explained by current guidelines about primary prevention of sudden cardiac death in individuals with LVEF below 35%.

Conclusions

The present study reveals that nitrosative stress, as assessed by iNOS levels, appears to be more pronounced in HFpEF, whereas endoplasmic reticulum stress, measured by GRP78 concentration, seems to be more intensified in HFrEF. While these observations may not offer definitive insights into the pathophysiology of heart failure, they do serve as a valuable starting point for future research and also hold promise for potential future applications in differential diagnostics.

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Conflict of interest: None declared.

Table 1. Baseline characteristics of patients

Characteristics	HFpEF (N = 42)	HFrEF (N = 38)	*P*-value
Female gender, n (%)	24 (57.14%)	8 (21.05%)	0.001
Age, years, mean (SD)	73 (7.14)	68.26 (9.74)	0.020
BMI, kg/m^2, median (IQR)	29.49 (25.77–32.02)	28.41 (24.54–30.49)	0.423
BMI > 30 kg/m^2, n (%)	20 (47.62%)	13 (34.21%)	0.223
NT-proBNP, pg/ml, mean, (IQR)	723 (430.5–1479.5)	1048.50 (592.25–2050.00)	0.136
NYHA II, n (%)	39 (92.86%)	30 (78.95%)	0.071
NYHA III, n (%)	3 (7.14%)	8 (21.05%)	0.071
H2FPEF score, median (IQR)	5 (3–8)	5 (3–6)	0.034
HFA-PEFF score, median (IQR)	6 (5–6)	6 (6–6)	0.818
Comorbidities			
Diabetes Mellitus, n (%)	13 (30.95%)	20 (52.63%)	0.051
Hypertension, n (%)	31 (73.81%)	30 (78.95%)	0.590
History of chronic kidney disease, n (%)	10 (23.81%)	8 (21.05%)	0.768
eGFR at the time of enrollment, ml/min/1,73 m^2, mean (SD)	73.81 (26.55)	67.66 (24.62)	0.287
Renal dysfunction, n (%)	10 (23.81%)	16 (42.11%)	0.081

Coronary Artery Disease, n (%)	21 (50.00%)	26 (68.42%)	0.049
History of MI, n (%)	16 (38.10%)	20 (52.63%)	0.192
Thyroid disease, n (%)	8 (19.05%)	10 (26.32%)	0.437
COPD, n (%)	4 (9.52%)	3 (7.89%)	0.797
Present AF at the time of enrollment, n (%)	20 (47.62%)	16 (42.11%)	0.168
Smoking, n (%)	3 (7.14%)	5 (13.16%)	0.370
Moderate valve disease, n (%)	8 (19.05%)	9 (23.68%)	0.700
Severe valve disease, n (%)	1 (2.38%)	4 (10.53%)	0.133
History of valve surgery, n (%)	2 (4.76%)	7 (18.42%)	0.054
Cardiomyopathy, n (%)	1 (2.38%)	13 (34.21%)	< 0.001
Implanted ICD/pacemaker, n (%)	14 (33.33%)	27 (71.05%)	< 0.001
Documented VF/VT, n (%)	2 (4.76%)	11 (28.95%)	0.003
Medications			
Statin	24 (57.14%)	29 (76.32%)	0.070
ACEI/ARB	36 (85.71%)	30 (78.95%)	0.426
NOAC	30 (71.43%)	21 (55.26%)	0.133

ACEI, AF — Atrial Fibrillation; ARB; BMI — Body Mass Index; CABG — Coronary Artery Bypass Grafting; COPD — Chronic Obstructive Pulmonary Disease; eGFR — estimated Glomerular Filtration Rate; ICD — Implantable Cardioverter-Defibrillator; IQR —

Interquartile Range; NYHA — New York Heart Association; NOAC, SD — Standard Deviation; VF — Ventricular Fibrillation; VT — Ventricular Tachycardia

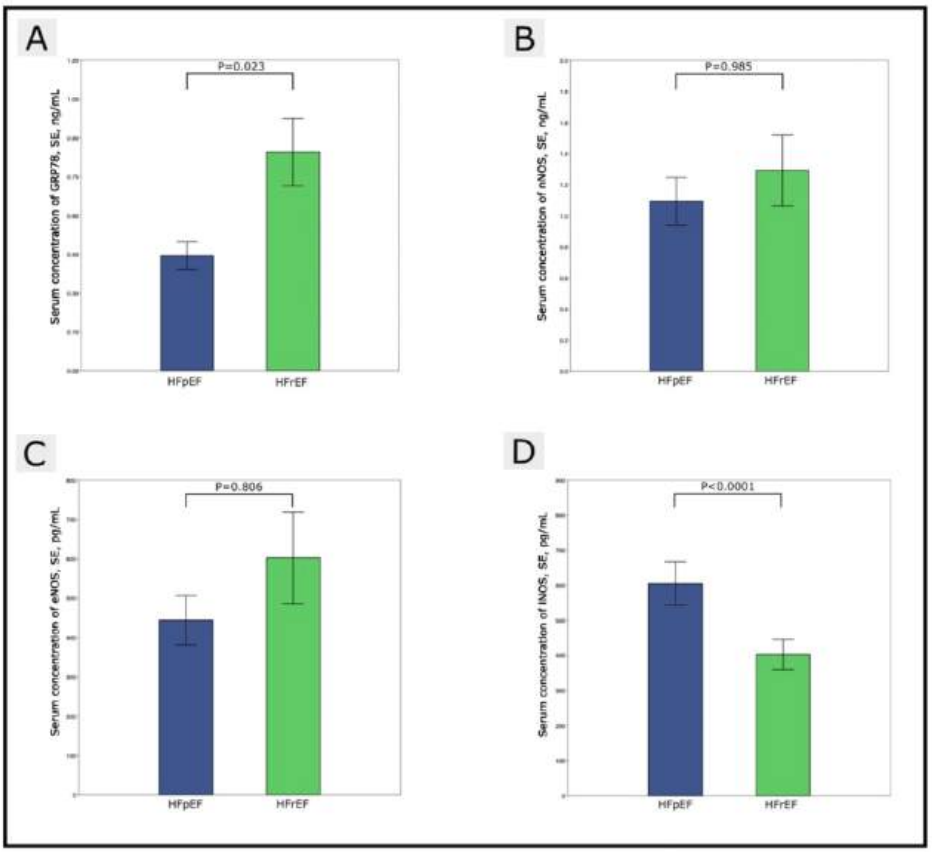
Table 2. Echocardiographic characteristics

	HFpEF (N = 42)	HFrEF (N = 38)	*P*-value
LVEF (%), median (IQR)	55 (51–62)	36 (31–39)	< 0.001
E, cm/s, mean (SD)	84.79 (23.21)	76.58 (36.01)	0.790
A, cm/s, mean (SD)	70.95 (21.83)	56.23 (24.52)	0.041
E/A, ratio, mean (SD)	1.12 (0.85–1.37)	1.33 (0.96–2.20)	0.234
E/e', ratio, median (IQR)	11 (10–16)	13 (9–21)	0.484
TDI septal			
s', cm/s, median (IQR)	6 (5–7)	5 (4–6)	< 0.001
e' cm/s, median (IQR)	6 (5–8)	5 (4–6)	0.004
a' cm/s, median (IQR)	7 (5–7.5)	6 (4–6)	0.015
TDI lateral			
s' cm/s, median (IQR)	7 (6–8)	5 (4–6)	< 0.001
e' cm/s, median (IQR)	8 (6–9)	6 (5–8)	0.016
a' cm/s, median (IQR)	7 (5–8.5)	4 (3–7)	0.004
RWT, mean (SD)	0.48 (0.13)	0.46 (0.09)	0.509
LVMI, g/m^2, median (IQR)	106.47 (85.77–131.58)	154.79 (132.24–187.56)	< 0.001
LAVI, ml/m^2, median (IQR)	39.37 (32.11–50.05)	49.68 (38.28–	0.014

		58.94)	
IVCC (%), median (IQR)	44.44 (35.49–50.00)	35.71 (26.67–40.00)	0.031

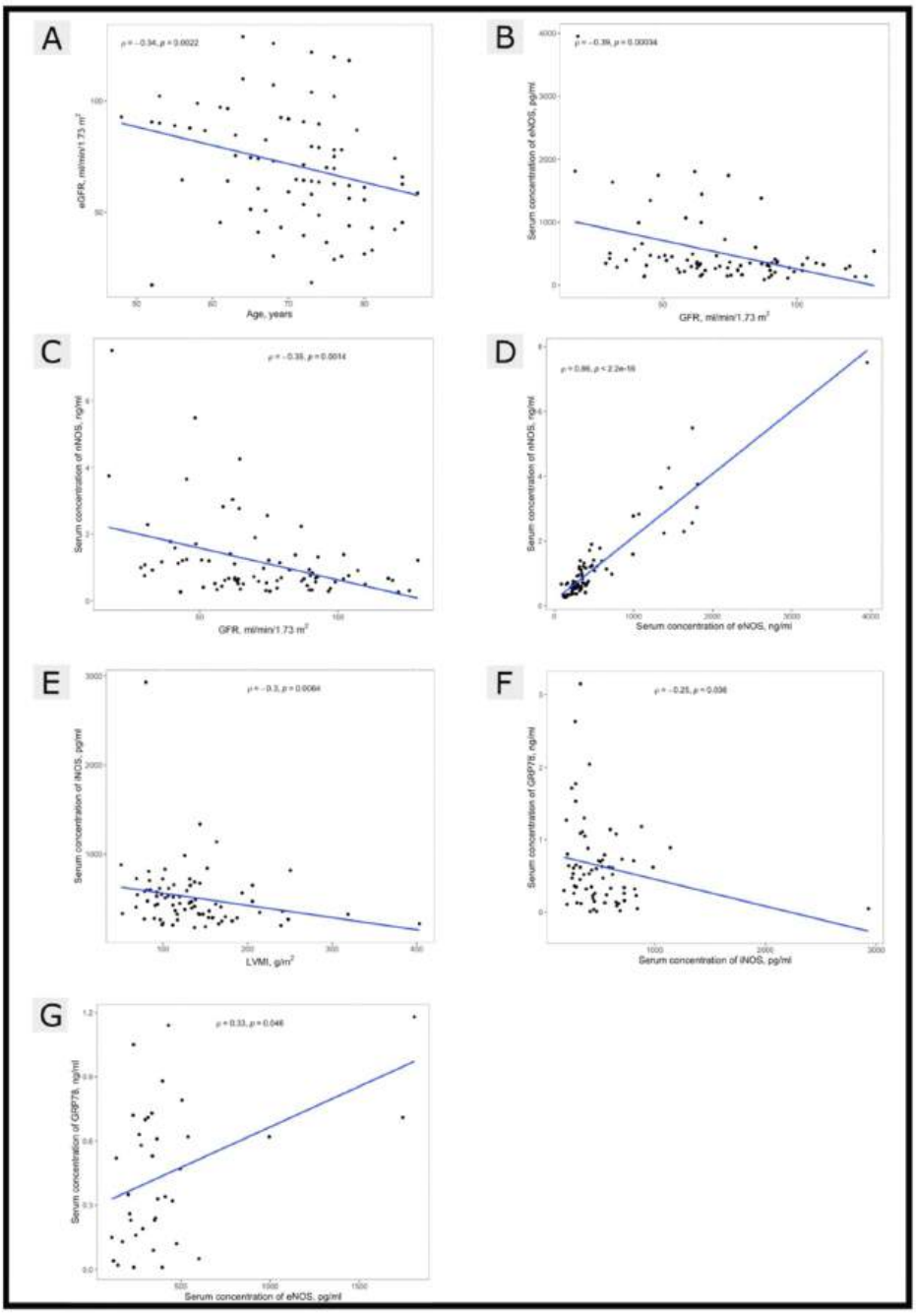
A — peak late diastolic transmitral flow velocity; a' — peak late diastolic mitral annular tissue velocity; E — peak early diastolic transmitral flow velocity; E/e' — the ratio of mitral peak velocity of early filling to early diastolic mitral annular velocity; e' — peak early diastolic mitral annular tissue velocity; IQR — Interquartile Range; IVCC — inferior vena cava collapsibility; LAVI — left atrial volume index; LVEF — left ventricular ejection fraction; LVMI — left ventricular mass index; RWT — relative wall thickness; s', peak systolic mitral annular tissue velocity; TDI — Tissue Doppler imaging

Figure 1. Comparison of serum concentrations of glucose-regulated protein 78 (**A**), neuronal nitric oxide synthase (**B**), endothelial nitric oxide synthase (**C**), inducible nitric oxide synthase (**D**)



eNOS — endothelial nitric oxide synthase; GRP78 — glucose-regulated protein 78; HFpEF — heart failure with preserved ejection fraction; HFrEF — heart failure with reduced ejection fraction; iNOS — inducible nitric oxide synthase; nNOS — neuronal nitric oxide synthase; SE — standard error

Figure 2. Rank correlations between quantitative variables in the general population (**A–F**) and in a population with Heart Failure with Preserved Ejection Fraction (**G**)


A
eGFR, ml/min/1.73 m²
Age, years
B
Serum concentration of eNOS, pg/ml
GFR, ml/min/1.73 m²
C
Serum concentration of nNOS, ng/ml
GFR, ml/min/1.73 m²
D
Serum concentration of nNOS, ng/ml
Serum concentration of eNOS, ng/ml
E
Serum concentration of iNOS, pg/ml
LVMI, g/m²
F
Serum concentration of GRP78, ng/ml
Serum concentration of iNOS, pg/ml
G
Serum concentration of GRP78, ng/ml
Serum concentration of eNOS, pg/ml

eGFR — estimated glomerular filtration rate; eNOS — endothelial nitric oxide synthase; GRP78 — glucose-regulated protein 78; HFpEF — heart failure with preserved ejection fraction; iNOS — inducible nitric oxide synthase; LVMI — left ventricular mass index; nNOS — neuronal nitric oxide synthase

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PLOS ONE

RESEARCH ARTICLE

Post-myocardial infarction heart failure and long-term high-fat diet: Cardiac endoplasmic reticulum stress and unfolded protein response in Sprague Dawley rat model

Karol Momot[1], Kamil Krauz[1], Katarzyna Czarzasta[1], Jakub Tomaszewski[1], Jakub Dobruch[2], Tymoteusz Żera[1], Maciej Zarębiński[3], Agnieszka Cudnoch-Jędrzejewska[1], Małgorzata Wojciechowska[1]*

1 Laboratory of Centre for Preclinical Research, Department of Experimental and Clinical Physiology, Medical University of Warsaw, Warsaw, Poland, **2** Centre of Postgraduate Medical Education, Department of Urology, Warsaw, Poland, **3** Department of Invasive Cardiology, Independent Public Specialist Western Hospital John Paul II, Lazarski University, Grodzisk Mazowiecki, Poland

* malgorzata.wojciechowska2@wum.edu.pl

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Abstract

Background

Myocardial infarction (MI) significantly contributes to the global mortality rate, often leading to heart failure (HF) due to left ventricular remodeling. Key factors in the pathomechanism of HF include nitrosative/oxidative stress, inflammation, and endoplasmic reticulum (ER) stress. Furthermore, while a high-fat diet (HFD) is known to exacerbate post-MI cardiac remodeling, its impact on these critical factors in the context of HF is not as well understood.

Aims

This study aimed to assess the impact of post-MI HF and HFD on inflammation, nitro-oxidative stress, ER stress, and unfolded protein response (UPR).

Methods

The study was performed on fragments of the left ventricle harvested from 30 male adult Sprague Dawley rats, which were divided into four groups based on diet (normal-fat vs. high-fat) and surgical procedure (sham operation vs. coronary artery ligation to induce MI). We assessed body weight, NT-proBNP levels, protein levels related to nitrosative/oxidative stress, ER stress, UPR, apoptosis, and nitric oxide synthases, through Western Blot and ELISA.

Results

HFD and MI significantly influenced body weight and NT-proBNP concentrations. HFD elevated 3-nitrotyrosine and myeloperoxidase levels and altered nitric oxide synthase levels. HFD and MI significantly affected ER stress markers and activated or inhibited UPR pathways.

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Competing interests: The authors have declared that no competing interests exist.

Conclusions

The study demonstrates significant impacts of post-MI HF and dietary fat content on cardiac function and stress markers in a rat model. The interaction between HFD and MI on UPR activation suggests the importance of dietary management in post-MI recovery and HF prevention.

Introduction

Myocardial infarction (MI) is a significant cause of death worldwide [1]. Due to modern treatment options, the mortality from MI has been decreasing, resulting in a growing population of MI survivors [2]. Many of these individuals subsequently develop symptoms of heart failure (HF) [3, 4]. After the cardiomyocytes' death due to ischemia, the development of HF is related to unfavorable left ventricular remodeling, leading to loss of function [5, 6]. Consumption of a high-fat diet (HFD) can intensify the remodeling after MI through mechanisms such ascardiac hypertrophy, cardiomyocyte apoptosis and interstitial fibrosis [7, 8].

Experimental studies have shown that HFD significantly exacerbates hypertensive heart disease in aging rats, leading to worsened atrial and ventricular remodeling and associated impairment of left ventricular systolic function [9]. Moreover, just 12 weeks of HFD can adversely affect cardiac function, as measured by the left ventricular speckle tracking imaging [10], a parameter capable of detecting subclinical left ventricular. Unfortunately, recent clinical studies have revealed that the consumption of high-fat products in the human population has been steadily increasing [11]. In the context of HF, there's much discussion about nitrosative/oxidative stress, inflammation, and ER stress [12–15]. However, little is known about the impact of HFD on these processes in HF.

Nitrosative/oxidative stress refers to the biochemical reaction between nitric oxide (NO) and reactive oxygen species when a disorder in oxygen metabolism is present. This process results in the generation of reactive nitrogen species (such as the peroxynitrite anion), which cause nitration and damage to proteins [16]. A marker of such a damage is the 3-nitrotyrosine (3-NT) [17].

Production of NO is catalyzed by NO synthase (NOS), which has three isoforms: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS) [18]. These isoforms play crucial roles in cardiovascular health and disease. iNOS is expressed in normal heart tissue at very low levels [19]. Inflammation results in iNOS activation and overexpression, which is linked with harmful effects on the heart, whereas overexpression of nNOS and eNOS in transgenic animals improves cardiac functions following MI [20]. Myeloperoxidase (MPO) plays a vital role in the inflammatory response [21]. It is expressed mainly in neutrophils and monocytes. MPO catalyzes the production of hypochlorous acid, a potent oxidative agent [22]. Moreover, this protein can also directly contribute to forming reactive nitrogen species. Enhanced circulating levels of MPO are related to inflammation and oxidative stress [23]. What is more, a recent meta-analysis suggests that MPO can be a valuable marker for HF diagnosis [24].

ER stress occurs when misfolded or unfolded proteins overwhelm the ER, a crucial cell organelle for protein folding and lipid biosynthesis. As previously mentioned, nitrosative/oxidative stress affects the protein folding process and contributes to ER stress [25, 26]. The latter activates the unfolded protein response (UPR), a complex signaling network aiming to restore proteostasis or promote apoptosis when it is not possible. This process is crucial in the

pathogenesis of HF [13]. Especially, UPR, ER stress, and iNOS overexpression have been found to influence heart failure with preserved ejection fraction (HFpEF) pathogenesis [12].

In physiological conditions, when ER stress is not exacerbated, 78-kDa glucose-regulated protein (GRP78) is attached to the ER stress sensors—inositol-requiring enzyme type 1 α (IRE1α), activating transcription factor 6 (ATF6), protein kinase R-like endoplasmic reticulum kinase (PERK) and, thus they remain inactive. When unfolded proteins excessively accumulate in the ER and ER stress exacerbates, GRP78 dissociates from these sensors, which activates them and initiates downstream signaling pathways. When ATF6 is released from GRP78, it migrates to the Golgi apparatus, where it is cleaved. Then, the cleaved ATF6 (ATF6c) is translocated to the nucleus and functions as an active transcription factor [27]. If severe ER stress persists, apoptotic pathways are activated. It has been reported that overexpression of CHOP leads to apoptosis due to ER stress [28].

This study aimed to assess the impact of post-MI HF and HFD on inflammation, nitro-oxidative stress, ER stress, and UPR.

Methods

Experimental procedures

The study was performed on fragments of the left ventricle harvested from 30 male adult Sprague Dawley rats, which underwent the following procedures (Fig 1, Table 1). This study is a continuation of previous experiments [29–31]. All experiments were approved by the Second Local Animal Research Ethics Committee of the Medical University of Warsaw and followed the European Communities Council Directive 2010/63/E.U. Rules of September 2010.

Starting from the fourth week of age, the animals were fed with HFD (31% fat, 17.1% protein, 35.5% carbohydrates, 0.18% sodium, and 3842 kcal/kg; Labofeed B, Kcynia, Poland) or a normal fat diet (NFD) (3.6% fat, 17.4% protein, 60% carbohydrates, 0.2% sodium, and 2864 kcal/kg; Labofeed B, Kcynia, Poland) for twelve weeks until the sixteenth week of age. The detailed composition of NFD and HFD is provided in Table 2. At the twelfth week of age, the surgical procedures were performed. During the surgeries, animals were under general anesthesia (Ketamine 10mg/100g body weight i.p., Xylazine 1mg/100g body weight i.p.). The MI model consists of a permanent ligation of the left coronary artery (LCA) with a suture thread (Ethicon 6.0). The sham surgeries (SO) were similar, but the pericardium was only touched with a needle, and the LCA was not ligated. Following surgery, the animals were administered analgesic medication (Buprenorphine chloride 3μg/100g body weight i.p.; 5.95nmol/ml, twice daily for 2–3 days) and an antibiotic (Penicillin, 10,000 IU/100g body weight i.m.; 0.047mmol/ml).

Four weeks following surgery were given to develop the post-MI HF in rats with the LCA ligation. After this period, the rats were anesthetized again. A 4 ml blood sample was taken

Fig 1. Graphical representation of the study design. Abbreviations: HFD, high-fat diet; MI, myocardial infarction; NFD, normal-fat diet; SO, sham surgery.

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Table 1. The experimental rat groups.

	Group 1 N = 7	Group 2 N = 9	Group 3 N = 6	Group 4 N = 8
Diet	Normal-fat Diet (NFD)	Normal-fat Diet (NFD)	High-fat Diet (HFD)	High-fat Diet (HFD)
Procedure	Sham operation (SO)	Ligation of the coronary artery (MI)	Sham operation (SO)	Ligation of the coronary artery (MI)
Survival rates at the end of the study	70% (7 of 10)	60% (9 of 15)	60% (6 of 10)	50% (8 of 16)

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from the right ventricle. The collected blood was immediately transferred into tubes containing Ethylenediaminetetraacetic acid (EDTA). The tubes were then centrifuged to separate the plasma from other blood components, and then levels of N-terminal prohormone of brain natriuretic peptide (NT-proBNP) were assessed using an enzyme-linked immunosorbent assay (ELISA).

At the end of the experiments, the rats were euthanized with an intraperitoneal injection of a lethal dose of Ketamine (300mg/100g body weight i.p.). The heart muscle tissue of the left ventricle was isolated, frozen in liquid nitrogen, and then stored at −80˚C.

Protein analysis

Collected tissues were homogenized using the RIPA buffer, whose composition was previously described [29]. Samples containing 10μg/μl of total protein were resolved by 8% SDS-polyacrylamide gels. Isolated proteins were transferred into PVDF membranes (#1704274, Bio-Rad) or nitrocellulose membranes using the Trans-Blot® TurboTM Transfer System (Bio-Rad). Then, blots were blocked with 5% nonfat dry milk buffered solution, immunoblotted for one hour with anti-IRE1α (NB100-2324, Novus-Biologicals), anti-p-IRE1α (NB100-2323), anti-iNOS (NB300-605), anti-eNOS (NB300-500), anti-nNOS (NBP1-39681), anti-GRP78 (NBP1-06274), anti-ATF6 (NBP1-76675), anti-PERK (NBP3-12891), anti-CHOP (NBP2-13172), anti-3NT (sc-32757, Santa Cruz Biotechnology), and anti-MPO (MPO-101AP, Thermo Fisher Scientific) antibodies. As a loading control, an anti-beta actin antibody was used (ab8226, Abcam). Incubation with secondary antibodies conjugated to horseradish peroxidase (ab205718, Abcam or sc-516102, Santa Cruz) was performed for one hour. The specific bands were detected and quantified with the ChemiDoc MP Imaginating System (Bio-Rad), and then protein expressions were normalized with β-actin and expressed as a relative ratio. Each measurement was repeated three times, and the final result was calculated as the average of these repetitions. The raw data images are provided as Supporting Information (S1 Raw images).

Table 2. Composition of diets.

	Normal-fat Diet	High-fat Diet
Energy (kcal per 100g)	286	382
Carbohydrates (g per 100g)	60.0	35.5
Proteins (g per 100g)	17.4	17.1
Fats (g per 100g)	3.2	28.0
Saturated (%)	13	49
Unsaturated (%)	37	44
Polyunsaturated (%)	50	7
Raw ash (g per 100g)	12	12
Water (g per 100g)	12	12

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Statistical analysis

Statistical analysis was carried out with the Statistica software, version 13.3. Normal distribution was tested using the Shapiro-Wilk test. The Levene test was used to assess the equality of variances. A two-way ANOVA was conducted to examine the effects of diet (Normal-fat Diet vs. High-fat Diet) and procedure (Sham operation vs. Myocardial infarction), as well as their interaction. Post-hoc comparisons were performed using Tukey's HSD test to identify significant differences between groups. For all the variables measured, outliers that were 1.5 interquartile ranges (IQRs) below the first quartile or 1.5 IQRs above the third quartile were removed from the analysis. Pearson's correlation coefficient was calculated to assess linear relationships between variables. Box plots were created using BioRender.com, and scatter plots with regression lines illustrating the relationships between variables were generated using an AI-based tool. All values presented in the text and figures are expressed as mean ± standard deviation (SD). All differences were considered significant if $P < 0.05$.

Results

Basic parameters

At the end of the experiment, significant differences in body weight were observed among the groups (Fig 2A). Notably, the group with post-MI HF, on an NFD, had the lowest body mass compared to other groups. A simple analysis of the main effects indicated that post-MI HF and diet significantly influenced the final body weight ($P = 0.028$ and $P = 0.031$, respectively).

NT-proBNP circulating levels differed significantly between groups (Fig 2B). Notably, higher levels of NT-proBNP were observed in groups with post-MI HF–NFD-MI and HFD-MI. A simple main effects analysis revealed that post-MI HF alone had a significant effect on serum NT-proBNP levels ($P < 0.0001$).

Nitrosative/oxidative stress and inflammation

The levels of 3-NT in the left ventricle tissue showed significant differences between the rat groups (Fig 3A). The group with post-MI HF and an implemented HFD exhibited the highest

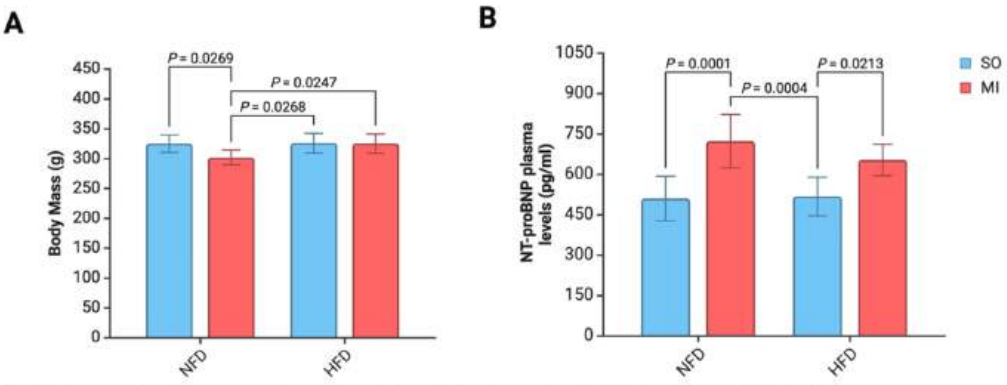

Fig 2. Basic parameters at the end of experiments. Abbreviations: HF, heart failure; HFD, high-fat diet; NFD, normal-fat diet; SO, sham surgery.

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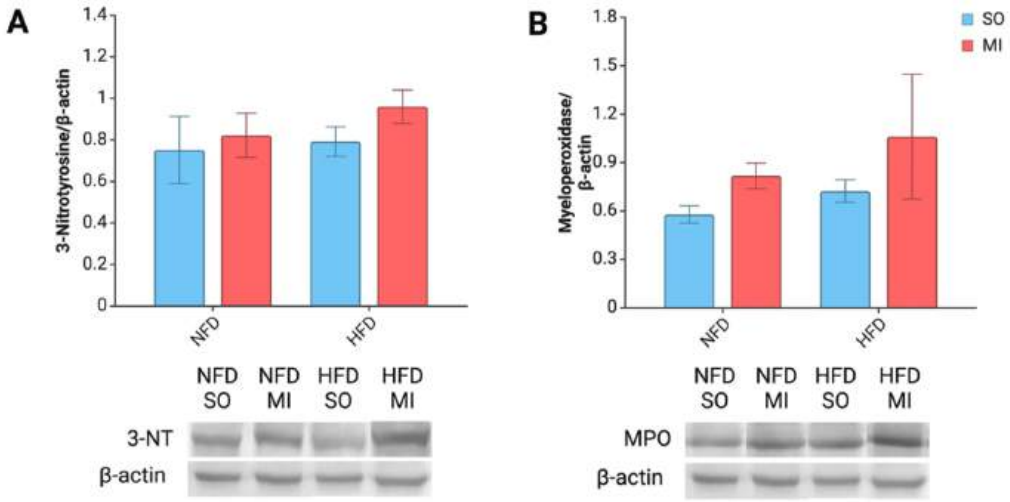

Fig 3. Bar graphs and representative Western blot images. 3-nitrotyrosine (A) and myeloperoxidase (B). Abbreviations: 3-NT, 3-nitrotyrosine; HF, heart failure; HFD, high-fat diet; MPO, myeloperoxidase; NFD, normal-fat diet; SO, sham surgery.

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3-NT levels. A simple main effects analysis showed that post-MI HF and HFD had a significant effect on 3-nitrotyrosine levels ($P = 0.021$ and $P = 0.048$, respectively).

The levels of MPO in the left ventricular tissue showed significant differences among the groups (Fig 3B). The group that developed HF after MI and was subjected to HFD exhibited the highest levels of MPO. A two-way ANOVA indicated that post-MI HF and diet significantly impacted MPO levels in the left ventricular tissue ($P = 0.004$ and $P = 0.028$, respectively).

Nitric oxide synthases levels

Levels of nNOS in the left ventricular tissue significantly differed between groups (Fig 4A). Simple main effects analysis showed that post-MI HF did have a significant effect on nNOS levels ($P < 0.001$). It was associated with reduced levels of eNOS independently of the type of diet implemented.

There was a significant difference in eNOS levels within the left ventricular tissue between groups (Fig 4B). eNOS levels were significantly reduced in other groups compared to the control group–NFD-SO. A two-way ANOVA revealed a significant interaction between the effects of post-MI HF and diet ($F(1,25) = 5.995$, $P = 0.022$).

The levels of iNOS in the left ventricle tissue showed significant differences between the groups (Fig 4C). The rats with post-MI HF and an implemented HFD exhibited the highest iNOS levels, significantly higher than other groups. Simple main effects analysis showed that both post-MI HF and diet did have a significant effect on iNOS levels ($P < 0.001$ and $P = 0.010$, respectively).

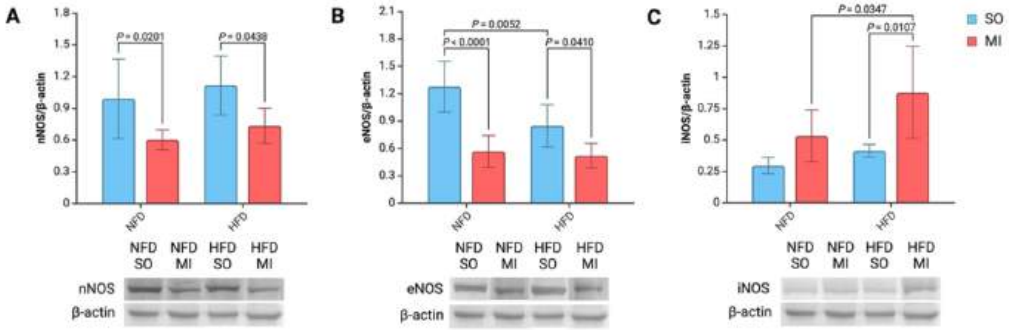

Fig 4. Bar graphs and representative Western blot images. nNOS (A), eNOS (B) and iNOS (C). Abbreviations: 3-NT, 3-nitrotyrosine; HF, heart failure; HFD, high-fat diet; MPO, myeloperoxidase; NFD, normal-fat diet; SO, sham surgery; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase.

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Endoplasmic reticulum stress, unfolded protein response, and apoptosis

There was a significant difference in GRP78 protein levels within left ventricular tissue among the various groups (Fig 5A). GRP78 levels were significantly higher in rats subjected to HFD than those on NFD across all combinations. Furthermore, a simple main effects analysis revealed that only diet statistically impacted GRP78 levels ($P < 0.0001$).

The activity of the IRE1α axis of the UPR, measured by the ratio of phosphorylated IRE1α to non-phosphorylated IRE1α, showed significant variation between groups (Fig 5B). The lowest activity was observed in rats subjected to HFD and after MI. A simple main effects analysis revealed that only diet affected the p-IRE1α/IRE1α ratio ($P = 0.026$). A two-way ANOVA indicated that the interaction between the effects of post-MI HF and diet on the activity of the IRE1α axis was significant ($F(1, 22) = 7.401$, $P = 0.012$).

The activity of the ATF6 axis of the UPR, measured by the ratio of cleaved ATF6 to non-cleaved ATF6, showed significant differences between groups (Fig 5C). The highest activity was observed in rats subjected to HFD after MI. This activity level was significantly higher than in other groups. Both post-MI HF and diet affected the activity of the ATF6 axis ($P < 0.0001$ and $P < 0.0001$). Furthermore, a two-way ANOVA revealed a significant interaction between the effects of post-MI HF and diet on the activity of the ATF6 axis ($F(1, 24) = 8.707$, $P = 0.007$).

PERK levels in the left ventricular tissue significantly differed between groups. The rats subjected to an NFD without post-MI HF exhibited the highest levels of PERK (Fig 5D). This difference was significant compared to other groups. Other groups showed similar levels of PERK, with no significant differences. A two-way ANOVA indicated a significant interaction between the effects of post-MI HF and diet on PERK levels ($F(1, 25) = 7.988$, $P = 0.009$).

CHOP levels in left ventricular tissue varied significantly between groups (Fig 5E). The highest level of CHOP was observed in the control group (NFD-SO). This difference was significant compared to other groups. Additionally, a two-way ANOVA demonstrated that post-MI HF and diet significantly affected CHOP levels in heart left ventricular tissue ($P < 0.0001$ and $P < 0.001$, respectively).

As depicted in Fig 6, the levels of 3-nitrotyrosine correlated with GRP78 levels ($\rho = 0.571$, $P = 0.002$).

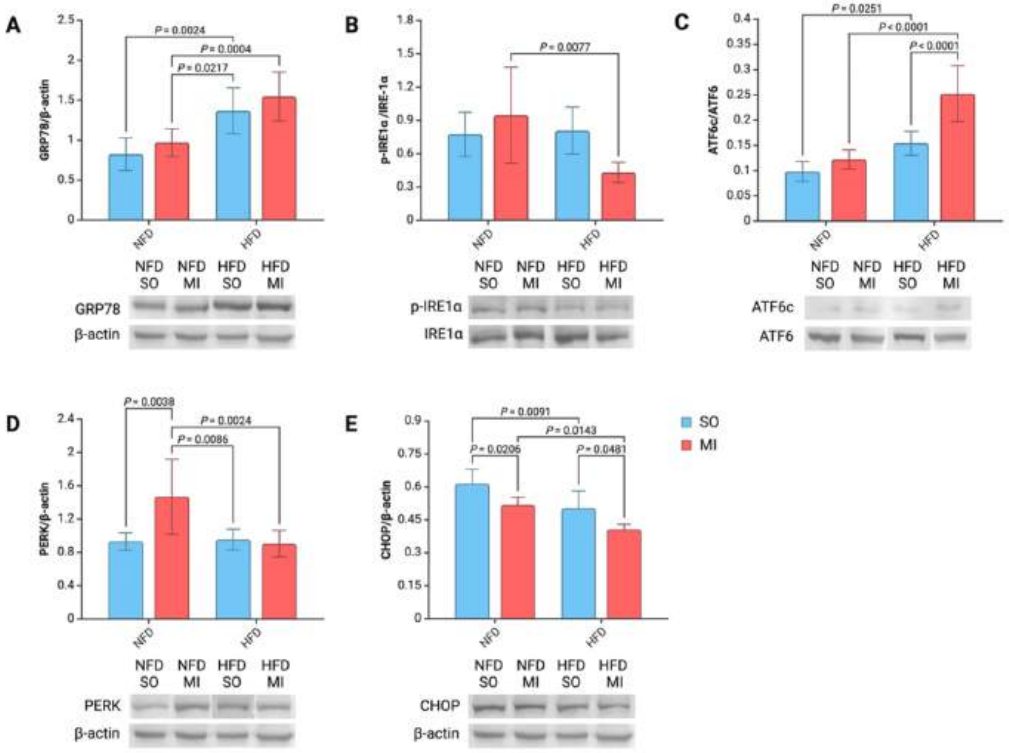

Fig 5. Bar graphs and representative Western blot images. GRP78 (A), p-IRE1α/IRE1α (B), ATF6c/ATF6 (C), PERK (D) and CHOP (E). Abbreviations: ATF6, activating transcription factor 6; ATF6c, activating transcription factor 6 (cleaved form); CHOP, C/EBP homologous protein; GRP78, 78-kDa glucose-regulated protein; HF, heart failure; HFD, high-fat diet; IRE1α, inositol-requiring enzyme type 1 α; NFD, normal-fat diet; PERK, protein kinase R-like endoplasmic reticulum kinase; SO, sham surgery; p-IRE1α, phosphorylated inositol-requiring enzyme type 1 α.

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Discussion

This preliminary study sheds light on the pathogenesis of post-MI HF and its exacerbation by HFD. We have demonstrated that in our model of post-MI HF with an implemented HFD, we can observe disturbances similar to those seen in HFpEF—specifically ER stress, nitrosative/oxidative stress, and disturbances in the UPR. Given that HFpEF is typically not associated with MI, this finding offers a new perspective on the mechanisms underlying HF development and progression.

Our study found that HFD was associated with increased ER stress measured by GRP78 levels. Zhang et al. also revealed that GRP78 was highly expressed in the atrial myocardium of HFD mice [32]. Additionally, they demonstrated that GRP78 expression in the atrial myocardium of overweight patients was significantly higher. These data suggest that ER stress could be activated in the myocardium not only in the case of obesity but also in HFD. Interestingly, in our study, post-MI HF had no significant impact on GRP78 levels. Contrary to our findings, Mainali et al. demonstrated that after inducing MI, GRP78 was markedly upregulated in heart

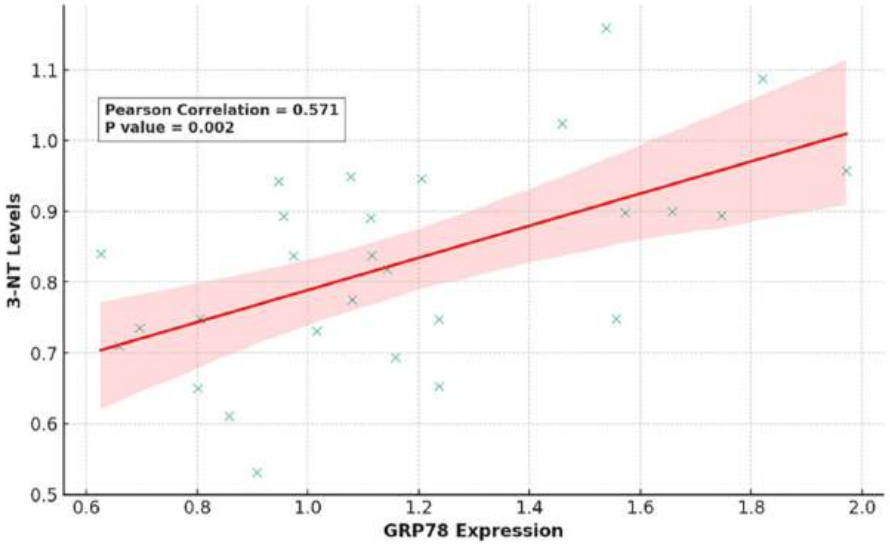

Fig 6. Pearson linear correlation between 3-NT levels GRP78 relative expression. Abbreviations: 3-NT, 3-nitrotyrosine; GRP78, 78-kDa glucose-regulated protein.

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tissue [33]. In the study by Mainali et al., GRP78 levels were measured one week after inducing MI, which could explain why our results differed. Additionally, exercise appears to reduce GRP78 expression, suggesting that the cardioprotective effect of exercise may be mediated through the reduction of ER stress, which is involved in the intrinsic apoptosis pathway [5, 34].

Furthermore, we discovered that the intensity of nitrosative/oxidative stress, measured by 3-NT, positively correlates with increased expression of GRP78. This indicates that these two components participate in the development of post-MI heart failure (HF).The study by Dickhout et al. observed that 3-NT could colocalize with GRP78 within early atherosclerotic lesions in the walls of arteries, suggesting that nitrosative/oxidative stress and ER stress also contribute to other diseases like coronary artery disease (CAD) [35].

Our findings indicate that iNOS protein levels are increased in cardiac tissue from rats with post-MI HF and with the implementation of HFD, suggesting the occurrence of nitrosative/oxidative stress under both conditions. This observation aligns with other studies demonstrating that the iNOS expression is up-regulated in association with obesity and after MI [19, 35, 36]. Furthermore, iNOS is found to be overexpressed in cardiac tissue in cases of HFpEF [37], which has recently been recognized as a foundation of this disease's pathomechanism [12].

Our study revealed that both nNOS and eNOS levels decrease in post-MI HF in the case of a high-fat and normal-fat diet. Furthermore, eNOS protein levels also decrease after implementing HFD, regardless of MI. Currently, there have been no studies analyzing the impact of HFD on eNOS expression in cardiac tissue. Interestingly, similar to HFD, a high-sugar diet also leads to a reduction in eNOS levels [38]. Moreover, in a model of spontaneously hypertensive rats, eNOS was downregulated explicitly in cardiomyocytes [39]. Similarly, eNOS levels are decreased in hypertrophic cardiomyopathy [40]. However, diabetes does not affect the overall eNOS protein level [41].

We report that both HFD and post-MI HF lead to the elevation of MPO levels. Moreover, studies indicate that even a single high-fat meal can elevate circulating MPO levels and contribute to oxidative stress [42], which could be detrimental if repeated frequently in the context of HF pathogenesis. Elevated MPO levels are present in CAD; also, its high levels may indicate a high risk of acute coronary syndrome (ACS) [43]. Increased level of MPO is related to oxidative stress and inflammatory state in chronic systolic HF. Elevated plasma MPO levels are also associated with an increased likelihood of more advanced HF [18, 44].

We demonstrated that both HFD and MI, as well as the combination of these two factors, significantly increase the activity of the ATF6 axis of the UPR. Interestingly, studies show that ATF6 is critical in protecting the heart after MI. Inhibiting ATF6 activity leads to dilatation of the left ventricle and depression of cardiac function [43]. ATF6 may also regulate the dynamics of CHOP induction [45]. In our study, the levels of CHOP were highest in the control group and both post-MI HF and HFD were associated with the decrease in its levels, which may suggest inhibition of apoptosis related to the ER stress. However, in other studies—HFD or cardiac injury are rather linked with elevated levels of CHOP [46, 47]. Thus, further research is needed to explore this phenomenon.

In our study, we demonstrated that the activity of the IRE1α branch of the UPR was lowest in rats subjected to both HFD and HF simultaneously. This suggests that in the event of an MI, HFD may exacerbate the accumulation of misfolded proteins in cardiomyocytes. Reduced activity of this branch plays a role in the pathogenesis of HFpEF [12]. Until now, no one has demonstrated that a similar reduction in activity can occur in cases where post-MI HF coincides with HFD.

Limitations

This study primarily relied on Western Blot assays without incorporating PCR assays, a decision driven by the limited budget and the exploratory nature of this project. Additionally, the lack of histopathological evaluation and immunohistochemical verification of the distribution of the studied markers in the left ventricle was significant limitations. The absence of echocardiographic assessment, in particular, was a major issue, as it restricted our ability to evaluate cardiac function. Given the limitations mentioned above, the findings must be interpreted with caution.

Conclusions

This preliminary study has several limitations, as mentioned above, but provides valuable insights. The findings elucidate the significant impacts of post-MI HF and dietary fat content on cardiac function and stress markers in a rat model. HFD, independently and in combination with post-MI HF, significantly influenced ER stress (as indicated by GRP78 levels), nitrosative/oxidative stress markers (3-NT, iNOS), and other forms of NOS in the heart. Notably, post-MI HF with HFD resulted in the most pronounced alterations in these markers, suggesting exacerbated cardiac dysfunction and stress responses.

The study highlights the interaction between post-MI HF and dietary factors in modulating the UPR pathways (PERK, ATF6, IRE1α) and MPO levels. What is more, HFpEF and the overlap of post-MI HF and HFD may have similarities in pathogenesis. This is a new perspective for future studies.

Supporting information

S1 Raw images.
(PDF)

Author Contributions

Conceptualization: Karol Momot, Jakub Tomaszewski.

Formal analysis: Karol Momot, Małgorzata Wojciechowska.

Funding acquisition: Maciej Zarębiński.

Investigation: Karol Momot, Kamil Krauz, Jakub Dobruch, Tymoteusz Żera.

Supervision: Agnieszka Cudnoch-Jędrzejewska.

Visualization: Karol Momot, Kamil Krauz.

Writing – original draft: Karol Momot, Kamil Krauz.

Writing – review & editing: Katarzyna Czarzasta, Małgorzata Wojciechowska.

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Wyniki

1. W pierwszej pracy wykazałem, że pacjenci z HFpEF charakteryzowali się wyższym osoczowym stężeniem 3-NT, co sugeruje większe nasilenie stresu nitrozacyjnego w tej grupie pacjentów w porównaniu do grupy z HFrEF i grupy kontrolnej. Stwierdziłem, że stężenie MPO w osoczu, związane z zapaleniem i stresem oksydacyjnym było porównywalne we wszystkich badanych grupach.

2. W drugiej pracy opisałem niższe stężenia GRP78 i wyższe stężenia iNOS w osoczu pacjentów z HFpEF w porównaniu do pacjentów z HFrEF. Sugeruje to, że stres ER jest bardziej nasilony w HFrEF, podczas gdy nadmierna ekspresja iNOS (która jest bezpośrednio powiązana ze stresem nitrozacyjnym) odgrywa większą rolę w patogenezie HFpEF.

3. W ostatniej pracy z cyklu doktorskiego wykazałem, że zarówno zastosowanie HFD, jak i wywołanie MI mają istotny wpływ na podwyższenie poziomu 3-NT oraz MPO w mięśniu lewej komory serca. Poziom iNOS w mięśniu lewej komory był również najwyższy w grupie, u której podano HFD oraz wywołano MI. Dodatkowo, wykazałem, że stres ER jest bardziej nasilony po zastosowaniu HFD niż po wystąpieniu MI. Aktywność jednego ze szlaków UPR - szlaku IRE1α - była najniższa u szczurów, u których jednocześnie zastosowano HFD i wyindukowano MI.

Wnioski

1. **Stres nitrozacyjny i stres ER różnicują HFpEF i HFrEF**

Różne mechanizmy patofizjologiczne dominują w poszczególnych typach HF. W HFpEF wydaje się przeważać stres nitrozacyjny, oznaczony za pomocą stężenia 3-NT oraz iNOS w osoczu. Z kolei w HFrEF wydaje się dominować stres ER, którego osoczowym markerem w pracy doktorskiej był GRP78.

2. **HFD i pozawałowa HF**

Jednoczasowa podaż HFD wraz z wywołaniem MI wydaje się nasilać stres ER, co zostało ocenione poprzez wzrost poziomu GRP78 w mięśniu sercowym. Dodatkowo w tej grupie zwierząt zaobserwowano również nasilenie stresu nitrozacyjnego, mierzonego za pomocą poziomów iNOS i 3-NT, a także wzrost stanu zapalnego i stresu oksydacyjnego, ocenianego na podstawie poziomu MPO.

3. **Interwencje dietetyczne w pozawałowej HF**

Wyniki badania przedklinicznego sugerują, że interwencje dietetyczne, polegające na zmniejszeniu zawartości tłuszczów nasyconych, mogą odgrywać istotną rolę w prewencji progresji pozawałowej HF, poprzez redukcję stresu oksydacyjnego i nitrozacyjnego, stresu ER oraz stanu zapalnego w mięśniu sercowym.

Dyskusja i podsumowanie

Uwagę zwracają moje wyniki opublikowane w ***International Journal of Molecular* Sciences**, które pokazują, że u pacjentów z HFpEF występuje wyższe stężenie 3-NT w osoczu w porównaniu z pacjentami z HFrEF oraz grupą kontrolną. Wynik ten sugeruje istotny udział stresu nitrozacyjnego w patogenezie HFpEF. Wyniki stanowią potwierdzenie wcześniejszych doniesień o roli stresu nitrozacyjnego w tym typie HF (33, 41). W zacytowanych badaniach autorzy analizowali poziom stresu nitrozacyjnego w homogenatach mięśnia sercowego pochodzących od szczurów ZSF-1 (model zwierzęcy HFpEF) oraz od pacjentów ze zdiagnozowanym HFpEF, u których podejrzewano pierwotnie zapalenie mięśnia sercowego (co uzasadniło decyzję o wykonaniu biopsji serca). Moje badania jednak jako pierwsze pokazały, że 3-NT może służyć jako osoczowy marker do oznaczania stresu nitrozacyjnego w HFpEF, co otwiera nowe możliwości w zakresie diagnostyki tej choroby. W pracy doktorskiej stężenie MPO, związane z zapaleniem i stresem oksydacyjnym, nie różniło się istotnie statystycznie pomiędzy HFpEF, HFrEF oraz grupą kontrolną. Otrzymany wynik jest interesujący, ponieważ w pozostałych badaniach wykazywano podwyższone stężenie MPO w grupie HFpEF (42, 43). To odkrycie może sugerować, że w naszej populacji pacjentów z HFpEF przeważa fenotyp, który nie cechuje się podwyższonym stanem zapalnym, przy czym obecność różnych fenotypów HFpEF została już wcześniej opisana w literaturze (44). W kolejnej pracy, opublikowanej w ***Cardiology Journal*** skoncentrowałem się na różnicach w stężeniach GRP78 i iNOS u pacjentów z HFrEF oraz HFpEF. Wykazałem, że pacjenci z HFpEF mieli niższe stężenie GRP78, co jest zgodne z wynikami z wcześniejszego badania (45) oraz wyższe stężenie iNOS w porównaniu do pacjentów z HFrEF. Chociaż znaczenie iNOS w rozwoju HFpEF wydaje się kluczowe w modelach zwierzęcych (32, 46) żadne dotychczasowe badania kliniczne nie oceniały stężenia iNOS we krwi u pacjentów z HFpEF i HFrEF. W pracy opublikowanej w ***PLOS ONE*** skoncentrowałem się na wpływie zastosowania HFD w pozawałowej HF u szczurów Sprague Dawley. Wyniki pokazują, że zarówno HFD, jak i MI znacząco wpływały na wzrost poziomów 3-NT oraz MPO w mięśniu sercowym, a także na zwiększenie poziomu iNOS, co może wskazywać na nasilony stres nitrozacyjny, stan zapalny wraz ze stresem oksydacyjnym w przypadku jednoczesnego występowania obu czynników. Aktywność jednego ze szlaków UPR - szlaku IRE1α - była najniższa u szczurów, u których jednocześnie zastosowano HFD i wyindukowano MI. Można przypuszczać, że jednoczasowe występowanie MI i HFD może prowadzić do zwiększonej akumulacji niesfałdowanych białek w kardiomiocytach, co wcześniej wykazano jako istotny element patogenezy HFpEF (47, 48).

Praca ta stanowi wstęp do dalszych badań nad poszukiwaniem skuteczniejszych metod diagnostyki i leczenia tej złożonej jednostki chorobowej. Moim zdaniem, istotnym kierunkiem przyszłych badań powinno być potwierdzenie znaczenia wybranych markerów (3-NT, GRP78) w diagnostyce HFpEF i HFrEF w większych badaniach klinicznych. Równie istotnym aspektem badań mógłby być rozwój terapii ukierunkowanych na zmniejszenie nasilenia stresu nitrozacyjnego w HFpEF oraz modulacji odpowiedzi na stres ER. Wydaje się, że zahamowanie nadmiernej aktywności iNOS ma potencjał, by stać się obiecującym celem terapeutycznym w leczeniu HF. W modelach zwierzęcych HFpEF, takie interwencje (farmakologiczne lub genetyczne) wykazywały skuteczność.

Przyszłe badania zarówno podstawowe, jak i kliniczne powinny koncentrować się również na HFmrEF, która stanowi formę pośrednią między HFpEF a HFrEF. Zbadanie HFmrEF pod kątem dominujących procesów patofizjologicznych, takich jak stres nitrozacyjny, stres oksydacyjny oraz stres ER, pozwoliłoby na opracowanie terapii celowanych, dostosowanych do przeważających mechanizmów w każdym przypadku.

Głębsze zrozumienie wspomnianych procesów mogłoby otworzyć drogę do bardziej spersonalizowanego leczenia HF, co przyczyni się do poprawy rokowania oraz jakości życia pacjentów zmagających się z tą chorobą.

Ograniczenia pracy

Dane od pacjentów analizowane w artykułach opublikowanych w ***International Journal of Molecular Sciences*** oraz ***Cardiology Journal*** pochodziły z niewielkich grup o zróżnicowanej charakterystyce, a pomiar stężenia markerów był wykonany jedynie w osoczu chorych, a nie bezpośrednio w tkance sercowej. Ze względu na wysoce inwazyjny charakter biopsji mięśnia sercowego, wykonanie jednak takiej analizy u pacjentów w warunkach mojego badania było technicznie niemożliwe. Z kolei w artykule z ***PLOS ONE*** zabrakło oceny funkcjonalnej mięśnia sercowego za pomocą powszechnie obecnie stosowanej echokardiografii.

Opinie Komisji Bioetycznej i Komisji Etycznej

UCZELNIA ŁAZARSKIEGO

Warszawa, 23 listopada 2022 r.

WNIOSEK NR: 02/KB/2022

UCHWAŁA KOMISJI BIOETYCZNEJ PRZY UCZELNI ŁAZARSKIEGO W WARSZAWIE

nr 02/11/22 z dnia 23/11/2022r.

§ 1

Komisja Bioetyczna przy Uczelni Łazarskiego, działając na podstawie § 4 ust. 9 Regulaminu Komisji Bioetycznej w zw. z art. 29 ust. 3 i 4 pkt 2 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (Dz. U. z 2022 r. poz. 1731)" w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania Komisji Bioetycznych po zapoznaniu się z wnioskiem w wyniku przeprowadzonej dyskusji i głosowania w dn. 23 listopada 2022 r.

postanawia

projekt badawczy „Odkrycie nowych potencjalnych kierunków terapeutycznych w leczeniu niewydolności serca".

zaopiniować pozytywnie

pod warunkiem wykorzystania pobranego materiału biologicznego jedynie w przedstawionym protokole badania.

Komisja Bioetyczna nie wyraża zgody na:

1. Przechowywanie i wykorzystywanie pobranego materiału biologicznego do innych badań naukowych

oraz

2. Przekazywanie pobranego materiału biologicznego innym podmiotom w celu realizowania badań naukowych.

info@lazarski.edu.pl
www.lazarski.pl
tel.: + 48 22 54 35 248
+ 48 22 54 35 216
ul. Świątalowska 43,
02-662 Warszawa
NIP: 527-02-09-936
REGON: 010286164

§ 2

1. W głosowaniu tajnym udział wzięli:
   1) prof. dr hab. n. med. Anna Wilmowska-Pietruszyńska (przewodniczący komisji, lekarz specjalista)
   2) r. pr. Małgorzata Mazur (zastępca przewodniczącego, radca prawny)
   3) dr hab. n. med. Marek Czarkowski, prof. (lekarz specjalista)
   4) dr n. med. Elżbieta Makomaska-Szaroszyk, prof. UŁa (lekarz specjalista)
   5) dr hab. n. med. Marek Stańczyk (lekarz specjalista)
   6) prof. dr. hab. n. med. Ewelina Zawadzka-Bartczak (lekarz specjalista)
   7) dr Wojciech Lewandowski (filozof)
   8) mgr Joanna Stefek (pielęgniarka)

2. Za wyrażeniem:
   1) pozytywnej opinii głosowało 8 członków Komisji,
   2) negatywnej opinii głosowało 0 członków Komisji.

§ 3

Uchwała wchodzi w życie z dniem podjęcia.

.....

prof. dr hab. n. med. Anna Wilmowska-Pietruszyńska
Przewodnicząca Komisji Bioetycznej Uczelni Łazarskiego

info@lazarski.edu.pl
www.lazarski.pl
tel.: +48 22 54 35 340
+48 22 54 35 318
ul. Świeradowska 43,
02-662 Warszawa
NIP: 527-02-09-936
REGON: 010286164

UCZELNIA
ŁAZARSKIEGO

Podpisy:

prof. dr hab. n. med. Anna Wilmowska-Pietruszyńska .....

r. pr. Małgorzata Mazur .....

dr hab. n. med. Marek Czarkowski, prof. .....

dr n. med. Elżbieta Makomaska-Szaroszyk, prof. Uła .....

dr hab. n. med. Marek Stańczyk .....

prof. dr. hab. n. med. Ewelina Zawadzka-Bartczak .....

dr Wojciech Lewandowski .....

mgr Joanna Stefek .....

info@lazarski.edu.pl
www.lazarski.pl
tel.: +48 22 54 35 260
+48 22 54 35 318
ul. Świeradowska 43,
02-662 Warszawa
NIP: 527-02-09-936
REGON: 010286164

do 19/IV

UCHWAŁA NR 24/2011
z dnia 20 września 2011r.

II Lokalnej Komisji Etycznej ds. Doświadczeń na Zwierzętach w Warszawie, ul. Żwirki i Wigury 61, 02-091 Warszawa.

§1

Na podstawie art.30 ust 1 pkt1 ustawy z dnia 21 stycznia 2005r. o doświadczeniach na zwierzętach (Dz.U.Nr 33, poz. 289) i §14 ust 3 rozporządzenia Ministra Nauki i Informatyzacji z dnia 29 lipca 2005r. w sprawie Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach oraz lokalnych komisji etycznych do spraw doświadczeń na zwierzętach (Dz. U. Nr 153, poz. 1275), po rozpatrzeniu wniosku pt.
„Wpływ pozawałowej niewydolności serca oraz diety wysokotłuszczowej na zmiany ekspresji mRNA i białka receptorów dla apeliny a także na poziom reaktywnych form tlenu (ROS) u szczurów SPRD, poddanych przewlekłemu stresowi oraz u zwierząt kontrolnych" .
z dnia **12. 09. 2011r.** złożonego
przez: **Dr Agnieszkę CUDNOCH-JĘDRZEJEWSKĄ**
z : **Katedry i Zakładu Fizjologii Doświadczalnej i Klinicznej I W L**
ul. Krakowskie Przedmieście 26/28, 00-927 Warszawa

WYRAŻA ZGODĘ
~~**ODMAWIA WYRAŻENIA ZGODY**~~
na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku.

§2

W wyniku rozpatrzenia wniosku, o którym mowa w §1, II Lokalna Komisja Etyczna ds. Doświadczeń na Zwierzętach ustaliła, że:

1.Wniosek należy zaliczyć do kategorii:
Badania naukowe na zwierzętach
~~**Doświadczenia na zwierzętach w dydaktyce**~~
~~**Doświadczenia na tkankach, narządach odzwierzęcych**~~

2.Najwyższy stopień inwazyjności proponowanych procedur i nie przekracza wartości ....4....

3.Doświadczenia będą przeprowadzone na zwierzętach :(gatunek, liczba zwierząt)
Gatunek (liczba zwierząt) :
szczury ....120.....

4.Doświadczenia będą przeprowadzone przez : (nazwisko i imię, nazwa jednostki doświadczalnej)
Imię i nazwisko wnioskodawcy: **Dr Agnieszka CUDNOCH-JĘDRZEJEWSKA**
Nazwa jednostki doświadczalnej: **Katedra i Zakład Fizjologii Doświadczalnej i Klinicznej I W L**
ul. Krakowskie Przedmieście 26/28 , 00-927 Warszawa

§3

Integralną częścią niniejszej uchwały stanowi uzasadnienie i kopia wniosku, o którym mowa w §1.

II LOKALNA KOMISJA ETYCZNA
Ds. Doświadczeń na Zwierzętach
przy Warszawskim Uniwersytecie Medycznym
ul. Żwirki i Wigury 61 02-091 Warszawa
tel. 022 5720-110, 5720-304, fax. 022 5720-169

Przewodniczący II Lokalnej Komisji Etycznej
Prof. dr hab. Bogdan Ciszek

Strona niezadowolona z niniejszej uchwały może wnieść odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 dni od dnia otrzymania uchwały. Odwołanie składa się za pomocą lokalnych komisji etycznych do spraw doświadczeń na zwierzętach (Dz. U Nr 153 poz 1275)

Dr Janina KWIATKOWSKA - Z-ca Przewodniczącego .....

Dr Anna PASSINI - członek .....

Doc. dr hab. Bożena MOSKWA - członek .....

Prof.dr hab. Hanna GRUBEK-JAWORSKA- członek .....

Maciej ONYSZKIEWICZ - członek .....

Lek.wet. Marek WOSZCZYŃSKI - członek .....

Dr Marta WYSOCKA-MINCEWICZ - członek .....

Dr Robert STROSZNAJDER - członek .....

UZASADNIENIE UCHWAŁY NR 24
z dnia 20 września 2011r.

II Lokalnej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w Warszawie, ul. Żwirki i Wigury 61, 02-091 Warszawa.

po rozpatrzeniu wniosku pt.
„Wpływ pozawałowej niewydolności serca oraz diety wysokotłuszczowej na zmiany ekspresji mRNA i białka receptorów dla apeliny a także na poziom reaktywnych form tlenu (ROS) u szczurów SPRD, poddanych przewlekłemu stresowi oraz u zwierząt kontrolnych" .
z dnia **12. 09. 2011r.** złożonego
przez: **Dr Agnieszkę CUDNOCH-JĘDRZEJEWSKĄ**
z : **Katedry i Zakładu Fizjologii Doświadczalnej i Klinicznej I W L ul. Krakowskie Przedmieście 26/28, 00-927 Warszawa**

II LKE uznała, że wniosek spełnia warunki określone ustawą o ochronie zwierząt oraz standardami międzynarodowymi.

Zgoda obejmuje okres jak we wniosku czyli 2 lata.
Okres ważności zgody na prowadzenie doświadczeń na zwierzętach liczy się od daty rozpoczęcia badań, co nie może nastąpić nie później niż w ciągu jednego roku od momentu wydania zgody.

Przewodniczący
II Lokalnej Komisji Etycznej

Prof. dr hab. Bogdan Ciszek

Pieczęć II LKE

II LOKALNA KOMISJA ETYCZNA
Ds. Doświadczeń na Zwierzętach
przy Warszawskim Uniwersytecie Medycznym
ul. Żwirki i Wigury 61, 02-091 Warszawa
tel. 022 5720-110, 5720-304, fax. 022 5720-169
pok. 304

Oświadczenia współautorów publikacji

Publikacja 1:

„Evaluation of Nitrosative/Oxidative Stress and Inflammation in Heart Failure with Preserved and Reduced Ejection Fraction"

Lp.	Autor	Rodzaj wkładu w powstanie publikacji	Procentowy wkład (%)	Podpis
1	Karol Momot	• Stworzenie projektu • Opracowanie tekstu artykułu • Uzyskanie finansowania	60	
2	Kamil Krauz	• Opracowanie tekstu artykułu • Stworzenie rycin i tabel	5	
3	Katarzyna Czarzasta	• Opracowanie metodologii • Przeprowadzanie oznaczeń laboratoryjnych	5	
4	Maciej Zarębiński	• Nadzór merytoryczny • Opracowanie tekstu artykułu	5	
5	Liana Puchalska	• Opracowanie metodologii • Analiza statystyczna	5	
6	Małgorzata Wojciechowska	• Opracowanie metodologii • Nadzór merytoryczny	20	

Publikacja 2:

„Endoplasmic Reticulum Stress and Expression of Nitric Oxide Synthases in Heart Failure with Preserved and with Reduced Ejection Fraction – Pilot Study"

Lp.	Autor	Rodzaj wkładu w powstanie publikacji	Procentowy wkład (%)	Podpis
1	Karol Momot	• Stworzenie projektu • Opracowanie tekstu artykułu • Uzyskanie finansowania	55	
2	Małgorzata Wojciechowska	• Opracowanie tekstu artykułu • Stworzenie projektu	20	
3	Kamil Krauz	• Opracowanie tekstu artykułu • Stworzenie rycin i tabel	5	
4	Katarzyna Czarzasta	• Opracowanie tekstu artykułu • Przeprowadzanie oznaczeń laboratoryjnych	5	
5	Liana Puchalska	• Opracowanie metodologii • Analiza statystyczna • Opracowanie tekstu artykułu	5	
6	Maciej Zarębiński	• Stworzenie projektu • Opracowanie tekstu artykułu	5	
7	Agnieszka Cudnoch-Jędrzejewska	• Nadzór merytoryczny	5	

Publikacja 3:

„Post-myocardial infarction heart failure and long-term high-fat diet: cardiac endoplasmic reticulum stress and unfolded protein response in Sprague Dawley rat model"

Lp.	Autor	Rodzaj wkładu w powstanie publikacji	Procentowy wkład (%)	Podpis
1	Karol Momot	• Opracowanie tekstu artykułu • Analiza statystyczna • Uzyskanie finansowania	50	
2	Kamil Krauz	• Opracowanie tekstu artykułu • Stworzenie rycin i tabel	5	
3	Katarzyna Czarzasta	• Opracowanie tekstu artykułu • Opracowanie metodologii	5	
4	Jakub Tomaszewski	• Opracowanie tekstu artykułu	5	
5	Jakub Dobruch	• Przeprowadzanie doświadczeń	5	
6	Tymoteusz Żera	• Przeprowadzanie doświadczeń	5	
7	Maciej Zarębiński	• Opracowanie tekstu artykułu	5	
8	Agnieszka Cudnoch-Jędrzejewska	• Nadzór merytoryczny	5	
9	Małgorzata Wojciechowska	• Nadzór merytoryczny • Opracowanie tekstu artykułu	15	

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