Study protocol

Title

The effect of intermittent hyperthermia (sauna) on cardiac and functional capacity, mitochondrial function, and metabolomic profiles in patients with HFpEF

(A pilot study)

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1. GENERAL INFORMATION

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2. BACKGROUND & SIGNIFICANCE

Heart failure (HF) is a major health, social and economic problem worldwide [1]. Incidence and prevalence are increasing [2], in part due to an aging population and rising burden of comorbidities [3]. More than half of the patients with HF present with preserved ejection fraction of the left ventricle (HFpEF) [4]. The main symptoms of these patients are fatigue and exercise intolerance [5]. Advances in medical and device therapies have improved mortality in patients with HFrEF [6-9]. On the other hand, only recently Sodium-Glucose-Cotransporter 2- inhibitors (SGLT2-i) proved in large randomized clinical trials (RCT) in patients with HFpEF and HFmrEF to reduce the combined endpoint consisting of hospitalization rate and cardiovascular mortality compared to placebo. This effect was mainly driven by reduced hospitalization [10,11]. Thus, there is still unmet need to improve symptoms, quality of life, morbidity, and mortality in these groups of patients. As a result, further drugs or interventions are still required.

Although patients with HFpEF have preserved EF there is still severe exercise intolerance, primarily due to reductions in peak cardiac output (CO) and peripheral arterial-venous oxygen difference (A-VO_2 Diff) [12,13], as well as a proposed impairment of skeletal muscle oxidative metabolism [14]. There is a continuously increasing volume of evidence showing that peripheral factors such as skeletal muscle function plays a crucial role in explaining the reduced exercise capacity in patients with HF with reduced ejection fraction (HFrEF) and with HFpEF [15,16]. Some studies showed that exercise intolerance can remain for several months after heart transplantation and achieving improvement of cardiac output [17]. Furthermore, exercise training improves exercise tolerance in patients with HF independent of improving cardiac function [13]. Both examples show that skeletal muscle function may play a central role in explaining the limited exercise capacity in patients with HF [18].

The changes of skeletal muscle occur on various levels. This could include loss of skeletal muscle mass, replacement of muscle with fat tissue and reduced muscle strength [19]. Several alterations on the molecular level accompany the above-mentioned changes and include reduced protein synthesis through blunting of the anabolic insulin-like growth factor 1 (IGF1) signaling pathway [20], and/or increased protein degradation including activation of the ubiquitin-proteasome system (UPS) [21], autophagy [22] and apoptosis [23]. Further metabolic and mitochondrial dysfunction are well described in the cardiac myocytes [24] and to a less extent in skeletal muscle.

Through transcriptional profiling, a set of ~120 response genes, or atrogenes, has previously been identified in atrophying muscles [25]. Dysregulation of atrogenes occurs in muscle atrophy, including genes required for ATP production and transcripts for extracellular matrix proteins. Further, protein quality control mechanisms in the cell are closely controlled by cellular autophagy and dysregulation of autophagy has been linked to abnormal protein turnover and overall cellular dysfunction. Dysregulation of autophagy has been linked to abnormal protein turnover in the failing myocardium and skeletal muscle. Suppression of PGC-1α, a central regulator of metabolism and mitochondrial biogenesis, was found in the setting of muscle atrophy [26]. Induction of PGC-1α in rodents prevents muscle atrophy and transcription of atrogenes [27]. However, less is known in this regard in HFpEF patients.

Reduced muscle mass and function in patients with HF could be triggered additionally by the activation of the sympathetic nervous system, renin-angiotensin-aldosterone system [28], proinflammatory cytokines [29], and transforming growth factor ß family such as myostatin [30]. The resulting imbalance between anabolic and catabolic pathways, in favor of catabolic cascade in patients with HF, is very important in explaining the muscle atrophy, reduced muscle mass and function in these patients. Structurally, changes such as reduced size and number of muscle fibers, a switch from slow muscle fibers MHC I to the fast one's MHC II and reduced capillary density have been described in patients with HF[31].

These changes have been studied mainly either in animal experiments or on patients with HFrEF. But not much is known about patients with HFpEF.

The only proven therapy so far to improve exercise capacity, dyspnea, and QoL in patients with HFpEF is exercise training [32]. However, there are two concerns related to exercise training. The first one is the compliance of patients. Secondly, patients with HF have often reduced mobility due to sarcopenia, cachexia or frailty [16,33]. Therefore, new interventions and innovations need to be investigated.

Intermittent hyperthermia such as sauna represents a good alternative to the above-mentioned limitations. Moderate Sauna could be equal to low-and moderate-intensity physical exercise training. Heart rate may increase up to 100/min [34,35]. In one large (2315 proband) prospective population-based cohort-study in middle-aged men from Eastern Finland with a follow-up of 20.7 years, increased frequency and length of sauna sessions were associated with reduced sudden cardiac death, coronary heart disease (CHD), and fatal cardiovascular disease [36]. Some other studies proved improved endothelial function in patients with CHD and HF and shown that sauna is safe in patients with HF and led to improvement of left ventricular ejection fraction and exercise capacity as well as to reduction of natriuretic peptides and arrhythmias [37-39]. A small study on 6 male distance runners who had one sauna session of 30 minutes twice a week for 3 weeks showed 32% improvement in endurance compared to baseline [40]. Furthermore, two 60-minute sessions of whole-body hyperthermia (44°-50° C) and 50% humidity with one week pause showed increased activity of the anabolic cascade in skeletal muscle represented by Akt/mTOR pathway, which is a crucial regulator of skeletal muscle mass [41]. Another small interventional study found that daily locally applied heat treatment to skeletal muscle during immobilization period of 10 days in humans prevented the loss of mitochondrial function, increased heat shock protein (HSP), and reduced skeletal muscle atrophy by 37% compared to a sham treatment group [42].

A further suggested important effect of sauna is enhancing the cognitive and mental health. Whole-body hyperthermia for example via hot water baths in healthy males stimulated a strong increase in serum brain-derived neurotrophic factor (BDNF) levels [43]. BDNF is a protein that activate the growth of new neurons both in the peripheral and central nervous system. BDNF modulates and improves anxiety and depression [44]. A small RCT in 28 patients with mild depression showed, compared to the control group which received bedrest, that patients who were exposed to sauna had reduced symptoms of depression and anxiety and reported improved appetite and reduced somatic complaints [45] (figure 1).

All the potential benefits of sauna seem to be comparable to exercise training with even some more advantages as discussed above. A comprehensive systematic conducted study in patients with HFmrEF and HFpEF to investigate the feasibility of intermittent hyperthermia (sauna) as intervention and the effect of sauna on cardiac function, exercise capacity, skeletal muscle function from bench to bedside, as well as on cognitive capacity, is still missing.

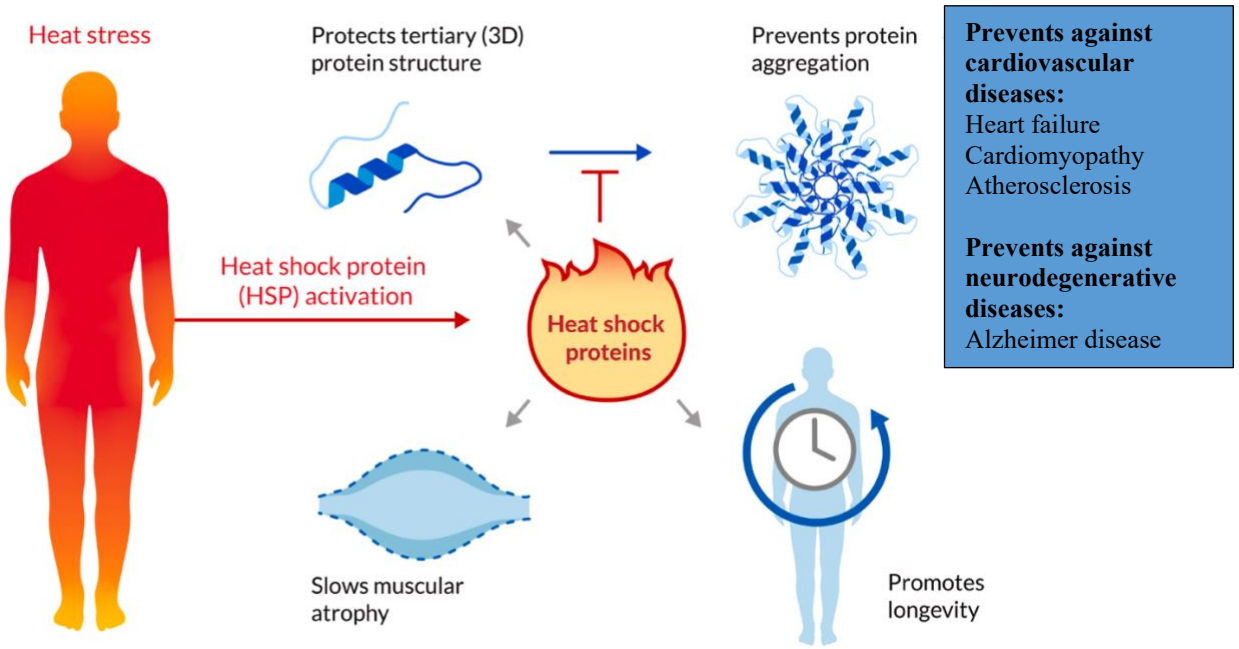

Figure 1: Heat shock proteins (HSPs), which are provoked during sauna, provide protection against cellular stress.
HSPs prevent protein disorder and aggregation by repairing proteins that have been damaged, providing protection against chronic diseases. Increased expression of HSPs also slows muscle atrophy and promotes longevity. (**Modified from Patrick, R. P.; Johnson, T. L. Sauna use as a lifestyle practice to extend healthspan. Exp Gerontol 2021, 154, 111509).**

3. PRELIMINARY STUDIES

The applicant is a heart failure specialist, who has experience in performing and interpreting cardiopulmonary exercise testing (CPET) and in evaluating the structure, function, and metabolism of skeletal muscle in HF patients.

The applicant has previously studied the role of muscle function and the prevalence of muscle wasting (sarcopenia) in Heart Failure patients with preserved Ejection fraction (HFpEF) at the Charité, Humboldt University, Berlin.

In one study, a total of 117 symptomatic outpatients with HFpEF were enrolled prospectively in Germany, England, and Slovenia as part of in a multi-center European study (SICA-HF). Appendicular skeletal muscle (ASM) mass (the sum of muscle mass in both arms and legs) was assessed by dual energy X-ray absorptiometry. Echocardiography, 6-minute walk testing (6-MWT), muscle strength assessment, CPET and quality of life (QoL) evaluation were performed. Muscle wasting was defined as ASM 2 standard deviations below the mean of a healthy reference group aged 18-40 years. Patients were divided into 3 groups according to the E/e' value as group A ≤8, group B 9-14, and group C ≥15. **Muscle wasting was present in 19.7% of all patients**. These patients performed worse in 6-MWT and showed lower absolute peak oxygen consumption. Values of muscle strength/ASM were associated with a better QoL. Logistic regression showed ASM to be independently associated with reduced walked distance in 6-MWT adjusted for NYHA, height, left atrium diameter, ferritin and forced expiratory volume in 1 second (FEV1) [16].

Furthermore, in a further project financed through a grant from the interdisciplinary center for clinical research in the University Hospital Jena, Dr. Bekfani performed a comparison among HFpEF, HFrEF and HC regarding skeletal muscle function, structure and metabolism. We found that HFpEF patients have reduced muscle function, reduced mitochondrial size and elevated levels of atrophy-related genes and protein (FBXO-32, MSTN-2) compared to HFrEF and HC. Perturbations in fatty acid oxidation and glucose metabolism as well as reduced mitochondrial volume density were noted in HFpEF and HFrEF patients compared to HC. These changes were associated with reduced exercise capacity. The results remained unchanged after adjusting for age and gender.

By dividing the cohort into two groups according to the mean value of muscle endurance, we described for the first time the molecular, metabolic and clinical profile of reduced muscle endurance and found that patients with reduced muscle endurance showed reduced gene expression of slow muscle fibers myosin (MYH 7), and elevated levels of atrophy genes and proteins (TGF- ß1, FOXO-1, UBB, TRIM63, FBXO-32), and lysosomal gene (CTSL). Furthermore, the expression of PPARα and GLUT4 were reduced in these patients. This was associated with an elevated inflammatory biomarker level (GDF-15) and reduced values of peak VO_2 and 6MWT. There was neither an association with age nor with physical daily activity estimated in the QoL-questionnaire [46].

The metabolic profile of patients with reduced muscle endurance (RME) among HFpEF and HFrEF patients showed two different metabolic profiles and likely different activation of metabolic pathways. Furthermore, we presented a clear association between metabolic and functional changes occurring in skeletal muscle and the metabolomic profiles found in serum in patients with HF. Additionally, we showed for the first time in HFpEF patients with RME elevated levels of Kyn. Kyn correlated with elevated inflammatory biomarker levels, was an independent predictive factor for RME, and showed very high sensitivity and specificity (AUC 0.83) in detecting RME. One of the most novel findings of this study was showing several indices of mitochondrial dysfunction and perturbations fatty acid oxidation in multiple levels in patients with HFpEF and RME such as a reduction of long-chain and medium-chain ACs, and a decrease of the ratio of medium-/long-chain-ACs. Studies in obese patients with diabetes, and in those on high fat feeding have shown, increased rather than decreased rates of β-oxidation in skeletal muscle [47]. The high rates of fatty acid catabolism in insulin-resistant muscles were attributed principally to ‘‘incomplete’’ fat oxidation, where a large proportion of fatty acids enter the mitochondria but are only partially degraded. [48].
These novel findings indicate the development of an intrinsic skeletal myopathy impairing muscle performance contributing to increased fatigue and frailty in HF.

In a further related publication Bekfani et al. found that outpatients with HFpEF had worse QoL and higher anxiety and depression scores compared with HFrEF and non-HF controls. Depression was associated with reduced QoL and was an independent predictor for reduced coordination capacity in these patients [49].

Furthermore, we showed recently that novel cardiac markers such as left atrial strain (LAS) might be capable to demonstrate the link between cardiac and peripheral limitations of exercise capacity [50]. We found in this study in patients with HFpEF and HFrEF that reduced LAS was a sensitive detector for reduced skeletal muscle endurance in these patients with area under the curve AUC (0.8).

Altogether, these data demonstrate that the applicant has ample experience testing exercise function in HF, specifically with cardiopulmonary exercise and skeletal muscle functional testing. Furthermore, the applicant has experience working with muscle tissue and bench science techniques and will be supported by an established group of clinical-translational investigators in the lab of Prof. Braun-Dullaeus.

The next logical step would be to investigate if an intervention such as sauna is safe and if it could reverse these symptoms and manifestations. If this will be proved to be correct, we will plan a larger scale trial.

Our results will likely give a new direction for future research especially in patients with HFpEF This might finally have direct therapeutic implications. We plan as a next step a RCT to confirm our results.

4. AIMS and Hypothesis

The aim of the study is to test the safety and efficacy of intermittent hyperthermia (sauna) in a pilot study on patients with HF. We will compare the results among patients with HFmrEF, and HFpEF regarding cardiac function, exercise capacity, skeletal muscle structure and function, metabolomic changes, cognitive function and quality of life both at baseline and after the intervention with sauna.

Hypothesis: We hypothesize that intermittent hyperthermia (sauna) is a feasible intervention in patients with HF regardless of LVEF. Exploratively we will test the effect of the intervention on filling pressure (E/é) as well as on the level of NT-pro-BNP. Peripherally, skeletal muscle function (including metabolic and mitochondrial function) and structure. Finally, the effects of the intervention on QoL and of cognitive function will be tested.

Questions to be answered through our investigation, and how:

1) **Is hyperthermia intervention (sauna) feasible in patients with HF?**
2) **Does sauna improve exercise capacity? (CPET, 6MWT, skeletal muscle strength and endurance).**
3) **Does sauna improve cardiac function (Echocardiography).**
4) **Understanding the related pathophysiology of the observed results on skeletal muscle and exercise capacity on molecular and mitochondrial level (skeletal muscle biopsies and the following tissue analysis parallel to blood analysis).**
5) **The role of inflammation in the altered muscle structure and function and its possible reversibility through sauna. (Skeletal muscle biopsies: NFkB and NEMO).**
6) **Observing changes in QoL and mental health (depression and anxiety). This will be evaluated by questionnaires as well as by testing the hypothesis that correlates Kynurenine to depression and inversely to exercise (here sauna). (Kyn levels both in skeletal muscle biopsy samples and in serum both at baseline and at the end of the intervention).**

While the main aim of the current study is to answer the first question, we might get some level of evidence to answer the other questions. This would support planning the next and larger study.

5. RESEARCH DESIGN & METHODOLOGY

Study Population: HF patients will be recruited from in the clinic of cardiology and angiology in the University Hospital in Magdeburg. The HF outpatient clinic includes a diverse group of both men and women; therefore, study subjects will not be limited by gender. Altogether, 30 patients, 15 in each group will be recruited prospectively and will enter the final analysis. At least 12 patients per group will be analysed with an expected drop-out-rate of 20%. Stratifications will be performed regarding sex and age to guarantee similar distribution in and among these groups.

1.1.1. Inclusion Criteria

- NYHA class II or III, clinically stable in the previous three months
- Females and males, age ≥50 years
- Left ventricular EF ≥50%, and at least 2 of the following: left atrial volume index (LAVI ≥34ml/$m^{2)}$ or E/e' >9 or interventricular septal thickness >12mm or pulmonary arterial pressure >35mmHg (non-invasive measurement) for HFpEF. HFmrEF will be defined as recommended according to the ESC-HF-guidelines similarly to HFpEF, however with 50 > LVEF ≥ 40.
- Stable on standard HF medication according to the ESC-HF guidelines in the last 4 weeks.

1.1.2. Exclusion Criteria

- Major cardiovascular event or procedure such as myocardial infarction, percutaneous coronary intervention (PCI), aortocoronary bypass operation in the previous 3 months.
- Patients who have an indication for dual platelet inhibition.
- Stroke.
- Patients who have mechanical heart valves.
- Pulomary embolism.
- Deep vein thrombosis.
- Patients with implanted cardiac devices.
- Severe valvular disease
- Individuals with altered or reduced sweat function, such as in autoimmune diseases, spinal cord injuries, Fabry disease.
- Symptomatic hypotension regardless of the measured value and those with BP systolic <110mmHG
- Patients on oral anticoagulation for other reasons than atrial fibrillation
- Uncontrolled diabetes mellitus (HbA1c>8%), severe renal dysfunction (GFR<30ml/min), or pulmonary disease (COPD GOLD ≥3)
- Primary muscle disorder (e.g. muscular dystrophies)
- Neurological disease (e.g. Dementia, Parkinson)
- right ventricular dysfunction
- uncontrolled arrythmias
- Any condition that in the opinion of the investigator could prohibit performance of 6-MWT.

STUDY PROTOCOL: All participants will undergo a standardized series of assessments as shown in table 1. In case of clinical improvement, these benefits are likely to diminish over time if sauna bathing is discontinued. To address this, patients will be invited for a follow-up visit three months after completing the intervention. All investigations will be repeated, except for the skeletal muscle biopsy.

Table 1: Timetable of the study

	Week 0 Visit 1	Week 1 Visit 2	Week 2-12	Week 13 Visit 3	Week 14 Visit 4	Week 30 Visit 5
Informed consent	x					
Echocardiography	x			x		x
Cardiopulmonary exercise test (CPET)	x			x		x
6-MWT		x			x	x
Blood tests		x			x	x
QoL-questionnaire	x			x		x
Accelerometer	x			x		x
isokinetic skeletal muscle measurement	x			x		
Skeletal muscle biopsy		x			x	
Sauna Intervention (twice weekly)			x			

METHODOLOGY

Echocardiography: The diameter, size, and function of all 4 chambers will be measured through transthoracic echocardiography. Valve functions as well as the diastolic function will be evaluated. Strain analysis of the left and right ventricle and of the left atrial will be performed as well.

Cardiopulmonary Exercise Testing: Bicycle exercise testing in association with air-gas-exchange is considered to be a good non-invasive method for estimating functional capacity and will be performed in all individuals by Dr. Bekfani [51]. We routinely perform CPET in patients with HF. The applicant has used the technique in multiple research investigations and in clinical daily work. Subjects will perform a ramp biking exercise on an electronically braked cycle ergometer as previously described [52].

Bioelectrical-Impedance Analysis (BIA): It is a non-invasive measurement of the body composition, i.e. muscle, fat, water. Measurements will be performed when patients are in supine positions with arms

lying along the body sides. 4 electrodes will be connected to hands and arms. Patients who have implanted cardiac devices will be excluded [53].

Muscle Strength and Fatigability: For the specific assessment of skeletal muscle function and fatigue, measures of isolated muscle group strength, endurance, and fatigability will be analyzed in association with overall functional capacity. Isokinetic measurements will be completed on the extremities, using the Biodex System 4 Pro (Biodex Medical Systems, Shirley, NY), as shown in **Figure 2**. Isokinetic data will be collected to measure PKTQ/SM, work to body weight (WK/BW), average power, endurance, fatigability, time to peak torque, acceleration and deceleration time. Muscle strength will be further analyzed through the use of hand grip strength [54], repeated three times on both dominant and non-dominant hands.

Six Minute Walk Test: The six-minute walk test is a commonly used clinical tool, which allows testing of the functional capacity in HF patients in a "real-life" setting. Using standard methodology [55], patients will be asked to walk as fast as possible on a 25-meter course for 6 minutes. The test will be scored in rounded meters walked in 6 minutes.

Questionnaire tools to complement functional assessment measurements: To assess measures of daily activity of patients with HF, we will utilize two questionnaires that assess physical limitation, symptoms and quality of life (QOL). The Kansas City Cardiomyopathy Questionnaire [56] as well as HADS-questionnaire [57]will be used to measure disease-specific health related QOL.

Serum analyses: Serum will be obtained from all individuals at the time of CPET and muscle biopsy, to assess changes in serum biomarkers. Standard phlebotomy technique in sterile fashion will be utilized. Serum samples will be analyzed for several biomarkers (IGF-1, myostatin, insulin, growth hormone) which will complement the skeletal muscle assessments. We will use western blot techniques [58] and commercially available ELISA kits (R&D, USA).

Metabolomic analysis and Lipidomics:

Altogether 188 metabolites (ACs, AAs, biogenic amines, glycerophospholipids, sphingolipids, and sugars) will be quantified in serum using the AbsoluteIDQ™ kit p180 (Biocrates Life Science AG, Innsbruck, Austria). The measurement will be performed after exercise (CPET) both in skeletal muscle and serum, and at rest in serum (Figure 3). Circulating fatty acids and ceramide levels will be analyzed in both the serum and skeletal muscle using LC/MS lipidomics. Total lipids will be isolated using the Forch method. Analysis will be performed in collaboration with the Institute of Clinical Chemistry [47].

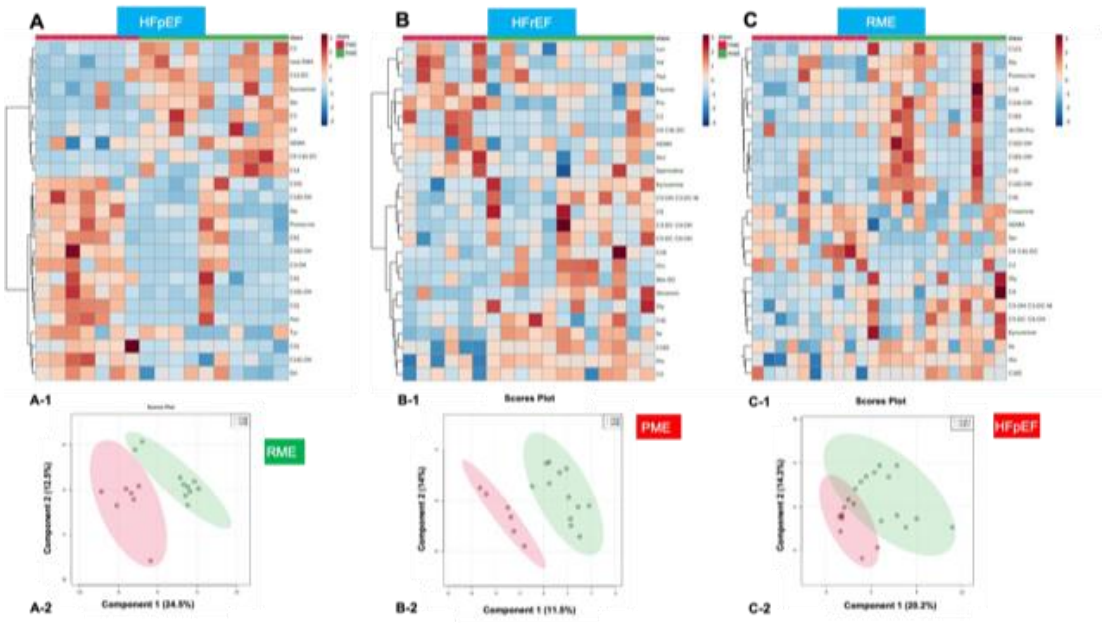

Figure 3: Metabolomic profiles of patients with reduced muscle endurance (RME) vs. preserved muscle endurance (PME) in HFpEF vs. HFrEF suggest different distorted metabolites and likely different mechanisms and metabolic pathways accompanying the RME. Visualization of the metabolomic profile using heat maps to show changes in the top 25 metabolite concentrations, and partial least squares-discriminant analysis (PLS-DA) among HFpEF ((**A**), **A-1** and **A-2**), HFrEF ((**B**), **B-1** and **B-2**), and in patients with RME and HFpEF vs. RME and HFrEF ((**C**), **C-1** and **C-2**), respectively (Bekfani et al. Cells 2022).

Skeletal muscle biopsies: Obtaining muscle tissue from all individuals will allow us to study the effect of sauna on skeletal muscle both structurally and functionally. Skeletal muscle biopsies will be performed by Dr. Bekfani, who has performed several series of biopsies (>50 biopsies) without complications. Aspirin, oral anticoagulation as a therapy for atrial fibrillation will be held for two days (the day prior to the muscle biopsy and the day of the muscle biopsy). Other indications for oral anticoagulation are contraindications in our study. Patients will lie comfortably in the supine position for biopsy of the vastus lateralis muscle in the right leg. Biopsy sites will be prepped with betadine and 2% xylocaine (without epinephrine), with a small stab cut then completed with a blade scalpel. A 5 mm Bergstrom muscle biopsy needle will be used to remove 100-200 mg muscle tissue. After harvesting the first sample, the needle will be rotated 90 degrees and a second sample will be extracted to maximize yield for analysis. The wound site will be closed with steri-strips and a sterile pressure bandage. One fragment of the biopsied muscle will be frozen in liquid nitrogen cooled isopentane for histomorphological and enzymatic analysis. The second fragment will be cut and placed in cryotubes containing RNA preservation solution (RNAlater) and immediately frozen in liquid nitrogen. All samples will be stored at -80°C. Analyses will be performed blinded. The patients will be asked to return for reassessment of the wound site [59].

A systematic review shows a very low incidence of complications (total 0.15%, 22 complications in the course of 13914 muscle biopsies in children and adults from different muscles [M. quadriceps femoris, M. deltoideus, M. biceps humeri) (Tarnopolsky et al. 2011). These were local skin infections (8 cases), arterial bleeding (2 cases), pain symptoms (>3 days, 5 cases), cutaneous nerve injuries (5 cases), and hematomas (2 cases) [60,61].

All of the following analyses will be performed before and after the intervention.

Oxidative Capacity: Fatty acid oxidation will be analyzed in muscle tissue of all subjects by measuring the production of [^{14}C] CO_2 from [$_{14}$C] oleic acid.

Electron microscopy: We will analyze mitochondria and lipid droplet accumulation in the skeletal muscle using electron microscopy. In brief, after glutaraldehyde fixation and resin embedding, the tissue is micro-sectioned and imaged by scanning electron microscopy under computer-guidance for analysis and image preparation (Figure 3).

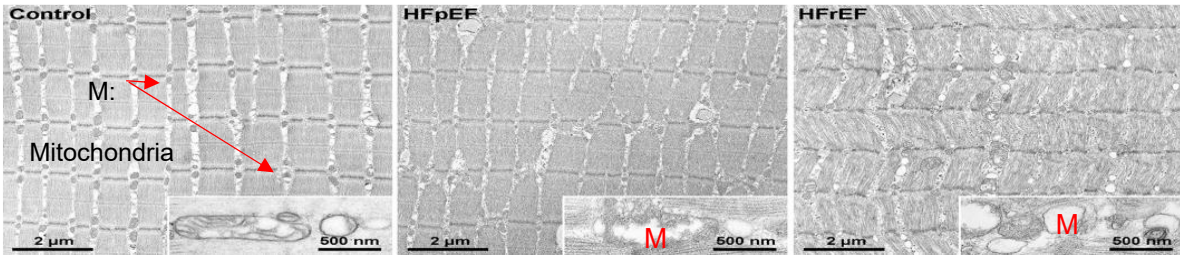

Figure 3: Transmission electron microscopic images of mitochondria in skeletal muscle biopsies in patients with HFpEF, HFrEF and controls (Bekfani et al. Circulation HF 2020).

mRNA Expression: We will analyze mRNA expression of genes related to muscle atrophy in the muscle biopsy specimens from HF patients and controls. Further, we will analyze gene sequences that code for metabolic marker genes related to muscle atrophy in the muscle biopsy specimens from HF patients and controls. As discussed above, these genes belong to a specific set of atrophy genes, so called atrogenes, which were previously characterized to be upregulated in several models of skeletal muscle atrophy [25]. We specifically choose to analyze the expression of these genes since they have been associated with muscle atrophy in several animal models and together seem to form a genetic fingerprint of muscle atrophy. We will particularly focus on atrogin-1 and MuRF-1, but all the following gene sets will also be analyzed:

- Protein degeneration atrogenes: atrogin-1/MAFbx, MuRF1, Polyubiquitin, E3α-II, proteasome subunits, cathepsin L.
- Growth factors: IGF-1, IGFBP5, FoxO1, TGFα, myostatin, follistatin.
- Markers of cellular metabolism. mCPT1, the rate-limiting mitochondrial fatty acid-transferring enzyme; CPT2, MCAD, a representative fatty acid β-oxidation enzyme; PPARα, a key transcriptional regulator of fatty acid genes; PGC-1α, UCP3; GLUT1 and 4; and PDK-4.

Proteomics: To further assess molecular changes of the muscle proteome, we will also analyze changes in protein levels of the above-mentioned genes. Total muscle proteins will be obtained using extraction buffer containing sodium fluorate, protease and phosphatase inhibitors without detergent and centrifugation. Samples will be stored at -80°C until the analysis. Protein lysates will be used for the analysis of changes in proteasome content and activity. Also, changes in the expression of specific genes (e.g. myosin heavy chain) will be investigated on the protein level using western blot techniques as previously described [58] **(Figure 4). Further, we will perform a post-translational modification analyses and global proteome analysis of the entire skeletal muscle proteome.**

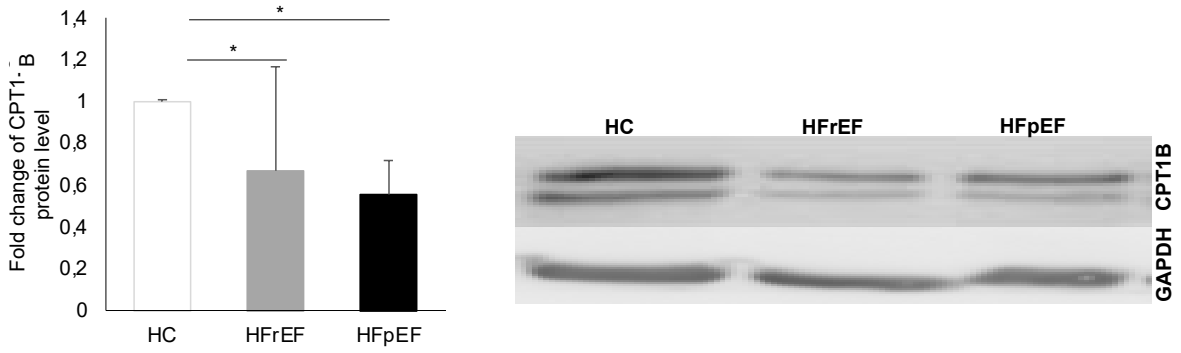

Figure 4: CPT1B-protein level in patients with HFpEF, HFrEF and in HC measured by western blot. ∗: p<0.05.

Autophagy: To assess catabolic mechanisms in the skeletal muscle tissue, we will measure LC3I and LC3II using standard western blot techniques and LC3II staining on tissue immunohistology specimens.

Intermittent hyperthermia sessions (sauna):

All participants will be accompanied by medical doctors for two sessions weekly, each between 8-15 minutes. The intervention will be done in the NEMO-sauna in Magdeburg. The duration of the intervention will be 10 weeks. We will start the first 2 weeks with 8 minutes. Week 3-4: 10 minutes, week 5-6: 12 minutes, week 7-10: 12-15 minutes. Sessions will be done in groups each consisting of 5 patients. The temperature of the sauna will be 60°C. After each session a cold shower will follow and then rest for 20 minutes. Blood pressure, heart frequency, O2-Saturation will be measured prior to each session as well as directly after the shower and at the end of the rest.

6. PROJECTED PROJECT TIMELINE & FUTURE PLANS

We will be able to begin our study in July 2023. We have previous experience with an established HF population. We expect to reach full recruitment by the end of 2023. Data will be analyzed immediately with focus on presenting initial results in the annual meeting of the German Society of Cardiology and the European society of Cardiology 2024. We expect to have a full analysis for manuscript preparations by the end of 2024.

7. PARTICIPATING DEPARTMENT

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8. INFORMATION & INFORMED CONSENT

Subjects will sign an informed consent before further inclusion criteria are met.

9. STATISTICS

There is not enough prior data to calculate the sample size. Our aim is to test primarily the safety of intermittent hyperthermia and the feasibility of the intervention on patients with HF. We still do think based on our previous research and in spite of the small sample volume, that we would find some significant differences in the secondary endpoints comparing baseline data to post intervention.

All data and statistics will be reported as mean ± standard deviation (n ± SD) for continuous normally distributed data or as median and interquartile range [25–75%] for variables that were not normally distributed, respectively. Categorical data will be summarized by percentages. The Statistical Package for Social Sciences software (SPSS 26, IBM, Armonk, USA) will be used for statistical analysis. The chi-squared test will be used to look for trend for categorical variables and Kruskal Wallis test will be applied for not normally distributed data, respectively. Analysis of variance (ANOVA), Pearson's or Spearman simple regression will be used as appropriate. A two-tailed p-value <0.05 indicates statistical significance.

10. ETHICAL ASPECTS OF PROPOSAL

Participation is entirely voluntary and will in no way effect their care. There is no direct benefit to the patients except a strong contribution to science.

All efforts will be made to minimize risk to the subject. The cardiopulmonary test and Biodex muscle function testing will occur in the outpatient clinical area. Emergency equipment and medical personnel will be in the immediate vicinity. Risks associated with skeletal muscle sample collection are very small. Blood samples will be taken during the outpatient visits at low risk. Adverse events will be reviewed on a biweekly basis by a cardiologist otherwise not affiliated with this proposal. Serious adverse events will be reported to the Institutional Review Board within 24 hours. All patient information is kept absolutely confidential.

10.1. DECLARATION OF HELSINKI & GOOD CLINICAL PRACTICE

The Study fulfills all principles of the Declaration of Helsinki and GCP.

11. FUNDING

This study will be funded through the internal budget of Prof. Braun-Dullaeus in the Department of Cardiology and Angiology.

12. FINAL REPORT & PUBLIATIONS

Data will be analyzed immediately with focus on presenting initial results in the annual meeting of the German Society of Cardiology and European Society of Cardiology. We expect to have a full analysis for manuscript preparations by the end of 2024. A copy of the final report / publications will be submitted to the ethic commission.

13. INSURANCE

In this clinical study, all study participants are covered by the liability insurance of the University Hospital Magdeburg A.ö.R.

14. DATA PROTECTION

During the study, medical findings and personal information will be collected from you and written down in your personal study file and/or stored electronically in our department for Cardiology and Angiology. In accordance with the currently valid data protection regulations (EU Data Protection Regulation/DS-GVO, State Data Protection of Saxony-Anhalt), the study data will additionally be stored in pseudonymized form, evaluated and, if necessary, passed on to other authorized persons. "Pseudonymized" means that no details of clear names or complete birth data are used, but only a number and/or letter code and your personal data are only known to authorized persons in the clinic. The data are secured against unauthorized access from outside.

All data collected during the clinical trial shall be published in anonymous form.
Records and documents relevant to the study (e.g. informed consent forms) will be kept in the University Hospital Magdeburg, Department of Cardiology and Angiology for at least 15 years.
Patient records and other original data must be kept for the longest possible period provided by Magdeburg University Hospital A.ö.R. (10 years) and secured against unauthorized access.

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