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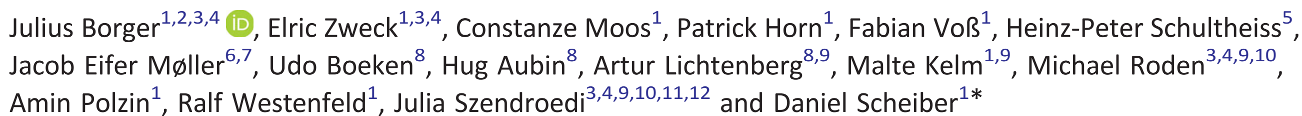
ORIGINAL ARTICLE

Myocardial inflammation is associated with impaired mitochondrial oxidative capacity in ischaemic cardiomyopathy



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Abstract

Aims Myocardial inflammation and impaired mitochondrial oxidative capacity are hallmarks of heart failure (HF) pathophysiology. The extent of myocardial inflammation in patients suffering from ischaemic cardiomyopathy (ICM) or dilated cardiomyopathy (DCM) and its association with mitochondrial energy metabolism are unknown. We aimed at establishing a relevant role of cardiac inflammation in the impairment of mitochondrial energy production in advanced ischaemic and non-ischaemic HF.

Methods We included 81 patients with stage D HF (ICM, n = 44; DCM, n = 37) undergoing left ventricular assist device implantation (n = 59) or heart transplantation (n = 22) and obtained left ventricular tissue samples during open heart surgery. We quantified mitochondrial oxidative capacity, citrate synthase activity (CSA) and fibrosis and lymphocytic infiltration. We considered infiltration of $>$14 $CD3^+$ cells/mm^2 relevant inflammation.

Results Patients with ICM or DCM did not differ regarding age (61.5 ± 5.7 vs. 56.5 ± 12.7 years, P = 0.164), sex (86% vs. 84% male, P = 0.725), type 2 diabetes mellitus (34% vs. 18%, P = 0.126) or chronic kidney disease (8% vs. 11%, P = 0.994). ICM exhibited oxidative capacity reduced by 23% compared to DCM (108.6 ± 41.4 vs. 141.9 ± 59.9 pmol/(s*mg), P = 0.006). Maximum production of reactive oxygen species was not significantly different between ICM and DCM (0.59 ± 0.28 vs. 0.69 ± 0.36 pmol/(s*ml), P = 0.196). Mitochondrial content, detected by CSA, was lower in ICM (359.6 ± 164.1 vs. 503.0 ± 198.5 nmol/min/mg protein, P = 0.002). Notably, relevant inflammation was more common in ICM (27% vs. 6%, P = 0.024), and the absolute number of infiltrating leucocytes correlated with lower oxidative capacity (r = −0.296, P = 0.019). Fibrosis was more prevalent in ICM (20.9 ± 21.2 vs. 7.2 ± 5.6% of area, P = 0.002), but not associated with oxidative capacity (r = −0.13, P = 0.327).

Conclusions More than every fourth ICM patient with advanced HF displays myocardial inflammation in the range of inflammatory cardiomyopathy associated with reduced mitochondrial oxidative capacity. Future studies may evaluate inflammation in ICM at earlier stages in standardised fashion to explore the therapeutic potential of immunosuppression to influence trajectories of HF in ICM.

Keywords Heart failure; Inflammation; Mitochondria; Reactive oxygen species

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Introduction

Heart failure (HF) is a worldwide medical and societal burden with still rising prevalence.[1] The aetiology of HF is variable and complex; however, patients with ischaemic as well as non-ischaemic HF share T-cellular myocardial inflammatory components.[2] Immunosuppression has improved clinical long-term results in non-ischaemic HF (referred to as DCM) with marked myocardial cellular inflammation.[3–5] Although it is known that inflammation also drives ischaemic HF,[2] current diagnostic and therapeutic regimens neither quantify nor target myocardial inflammation in patients with ischaemic cardiomyopathy (ICM). These ICM patients are often excluded from immunosuppressive trials.[3–5] An analysis from the CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcome Study) trial reported promising results concerning the reduction of HF-related hospitalisations by immunosuppression in patients with cardiovascular disease but did not assess myocardial inflammation.[6]

Mitochondrial damage can trigger myocardial inflammation.[7] Impaired mitochondria and altered myocardial bioenergetics have been identified to play a pivotal role in HF.[8,9] A central feature is oxidative phosphorylation (OXPHOS) capacity—closely linked to cardiac function[8,10]—being reduced across various HF aetiologies[11,12] and enhanced production of reactive oxygen species (ROS).[10,13,14] Oxidative stress favours HF by further disturbing mitochondrial energy homeostasis[15] and is itself enhanced by deranged substrate metabolism in the failing heart.[9,16] Both myocardial inflammation and oxidative stress drive cardiac fibrosis, which also accompanies ischaemic as well as non-ischaemic HF and is a predictor for adverse clinical outcomes in HF.[17,18]

While differences in mitochondrial content, morphology and DNA damage between ICM and dilated cardiomyopathy (DCM) have been observed,[19] little is known about quantitative differences in inflammation between these aetiologies.

Given that myocardial inflammation is a potential therapeutical target for which established therapies exist, as well as the unclear burden of inflammation in ischaemic versus non-ischaemic cardiomyopathy, and the yet unclear relationship of aetiology, inflammation and impaired mitochondrial oxidative capacity, we aimed at establishing a relevant role of inflammation in impaired mitochondrial respiration in advanced HF. We hypothesised that the extent of myocardial cellular inflammation and fibrosis differ between ICM and DCM and that fibrosis and inflammation are associated with mitochondrial oxidative capacity and ROS production in advanced HF in humans.

Methods

Study protocol

One hundred twenty-three patients with advanced HF with reduced ejection fraction were screened for potential enrolment in this prospective, single-centre, observational trial. Out of these patients, 22 did not match the inclusion criteria: Eight had received previous ventricular unloading via LVAD, four patients with DCM had coexisting coronary heart disease, nine had HF-aetiologies other than ICM or DCM, and one had type 1 diabetes mellitus. Out of the remaining 101 patients, 20 yielded no respiration data because of sample weights $<$0.5 mg or $>$1.5 mg, or defective outer mitochondrial membranes, or defective OROBOROS Oxygraph-2k. This study's protocol followed the World Medical Association's Declaration of Helsinki and was approved by the ethics board of the University Hospital of Heinrich Heine University Düsseldorf (study number 5263R, sub-analysis of clinicaltrials.gov registration number NCT03386864). All patients gave written informed consent before inclusion.

Participants

We included 81 patients with advanced HF with reduced ejection fraction due to ICM (n = 44) or DCM (n = 37) at the age of 20–80 years requiring implantation of a left ventricular assist device (LVAD, n = 59) or heart transplantation (HTX, n = 22) between May 2016 and January 2020. Patients with previous LVAD were excluded from the HTX group, since mechanical ventricular unloading may improve mitochondrial efficiency and impact ROS production[20] or reduce mitochondrial DNA damage.[19] Other exclusion criteria included cardiomyopathies other than DCM or ICM or active neoplastic diseases.

LVAD surgery and heart transplantation

During LVAD implantation or orthotopic HTX, we excised a small part of the diseased scar-free left ventricular cardiac apex and immediately transferred it into a cooled buffer solution (BIOPS, containing [in mM]: 2.77 CaK_2EGTA, 7.23 K_2EGTA, 20 imidazole, 20 taurine, 50 4-morpholineethanesulfonic acid, 0.5 dithiothreitol, 6.56 $MgCl_2$•$6H_2O$, 5.77 Na_2ATP, 15 disodium phosphocreatine) and stored on ice. We prepared and immediately performed high-resolution respirometry. Remaining tissue was snap-frozen in liquid nitrogen and stored at −80°C for following measurement of citrate synthase activity (CSA) or histology.

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Histology and immunohistochemistry

We performed immunohistochemistry for characterisation of infiltrates and digital image analyses on shock-frozen tissue. $CD3^+$ lymphocytes were stained with anti-CD3 antibodies (Dako; dilution 1:25), counted and normalised to square millimetre. We considered all samples displaying >14 lymphocytes per mm^2 inflammation positive, the threshold to inflammatory cardiomyopathy.[21] Furthermore, we stained $CD45R0^+$ T-memory cells (Dako, Glostrup, Denmark) as well as $CD68^+$ macrophages, $CD4^+$ T-helper cells and $CD8^+$ cytotoxic T cells in all patients displaying >7 $CD3^+$ lymphocytes/mm^2 and used EnVision peroxidase-conjugated anti-mouse antibody (DakoCytomation) as a secondary enhancing antibody. We visualised immunohistological staining using 3-amino-9-ethylcarbazole (Merck, Darmstadt, Germany) as chromogenic substrate. Slides were counterstained in haematoxylin and mounted with Kaiser's gelatine R (Merck). We quantified immunoreactivity by digital image analysis at 200× magnification. Pathologists were blinded to respiration data and all patient characteristics.

High-resolution respirometry

We performed high-resolution respirometry using previously established protocols for ex vivo measurement of myocardial mitochondrial respiration in two-chambered oxygraphs (OROBOROS Oxygraph-2k, Innsbruck, Austria).[10,22] Intact myocardial tissue from LVAD or HTX operations was carefully dissected into fibres, permeabilised in 50 μg/mL saponin (Sigma-Aldrich Chemie GmbH, Germany) for 30 min, washed in respiration medium (MiRO5, containing [in mM]: 0.5 EGTA, 3 $MgCl_2$•6 H_2O, 60 K-lactobionate, 20 taurine, 10 KH_2PO_4, 20 HEPES, 110 sucrose, 1 g/L bovine serum albumin, essentially fatty acid free in sterile water), dried on filter paper, weighed and transferred to the oxygraph chambers. We performed respirometry in duplicates at 37°C in chambers containing 2.3 mL MiRO5 at oxygen concentrations of 120–450 μM with sample masses of 0.5–1.5 mg. For analysis, we used DatLab 6 software (OROBOROS Instruments Corp., Innsbruck, Austria).

We assessed the capacity of the electron-transferring flavoprotein after saturation with malate (2 mM), medium-chain fatty acid octanoylcarnitine (1 mM) and adenosine diphosphate (2.5 mM). Glutamate (10 mM) and succinate (10 mM) supported State 3 respiration, indicating mitochondrial OXPHOS capacity. Cytochrome c (0.01 mM) was added to ensure integrity of the outer mitochondrial membrane: A rise in respiration of no more than 5%–15% is considered an indication of an intact outer mitochondrial membrane.[23] Inhibition of adenosine triphosphate synthase by oligomycin (0.005 mM) yielded mitochondrial leak respiration (State 4o). Uncoupling agent carbonyl cyanide-trifluoromethoxyphenylhydrazone (FCCP) was added by stepwise titration (2 μM) until maximum uncoupled respiration was reached (State u), indicating full electron transfer capacity (ETC). Addition of complex (C) I inhibitor rotenone (0.5 μM) followed by CIII inhibitor antimycin A (5 μM) yielded residual oxygen consumption. Respiratory control rates (RCR = State 3 respiration/State 4o) and leak control rates (LCR = State 4o/State u) served as markers for mitochondrial coupling efficiency.

A second protocol was prepared and conducted in the same way as the first, lacking the addition of octanoylcarnitine, thus providing only direct substrates glutamate and succinate for CI and CII, to assess fatty-acid-independent respiration. Substrates were added in the following order and concentrations (in mM): 2 malate, 10 glutamate, 2.5 adenosine diphosphate, 10 succinate, 0.5 μM rotenone, 0.01 cytochrome c, 0.005 oligomycin, 2 μM carbonyl cyanide-trifluoromethoxyphenylhydrazone (FCCP) titration, and 5 μM antimycin A. Due to limited availability of tissue, this protocol was only assessed in a subgroup of patients (n = 34, n [ICM] = 20, n [DCM] = 14).

Fluoroscopic H_2O_2 measurements

We assessed ROS production via fluoroscopic measurements as described elsewhere.[10,24] In short, tissue was permeabilised and prepared equally to respirometry experiments and transferred into OROBOROS Oxygraph-2k combined with the Fluorescence-Sensor Green of the O2k-Fluo LED2-Module for hydrogen peroxide (OROBOROS Instruments, Austria). Emitted H_2O_2 is captured by the fluorophore Amplex red (10 mM, Thermo Fisher Scientific, USA), producing the red fluorescent compound resorufin (excitation wavelength 563 nm, emission 587 nm) catalysed by horseradish peroxidase (1 U/mL, Sigma-Aldrich Chemie GmbH, Germany). The change in intensity of emitted fluorescence while succinate is added stepwise is directly proportional to H_2O_2 production.[24]

CSA

We measured CSA as a marker for mitochondrial content spectrophotometrically and following previously described protocols.[20,25] In short, CSA was measured in lysed tissue at a wavelength of 412 nm after addition of 10 mM dithionitrobenzoic acid, 30 mM acetyl-CoA and 10 mM oxaloacetic acid (Citrate Synthase Assay Kit, Sigma-Aldrich, St. Louis, USA). We assessed overall protein content using colourimetric bicinchoninic acid assay (Thermo Scientific Applied Biosystems, USA) and normalised CSA values to protein.

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Statistical analyses

All data are shown as means ± SD in text and means ± SEM in pictures unless otherwise indicated. We considered P-values $\leq$ 0.05 statistically significant. We used Prism 9 (Version 9.2.0, GraphPad Software, Inc., USA), and IBM SPSS v27 (IBM Corp. Released 2020. IBM SPSS Statistics for Macintosh, Version 27.0.1. Armonk, NY: IBM Corp) for analyses. Comparisons of groups were performed using unpaired two-tailed Student's t-tests, Mann–Whitney U tests, chi-square tests, Fisher's exact tests or repeated measures two-way ANOVA with Sidak's post hoc tests, respectively, as appropriate. Correlations between normally distributed continuous variables were tested via Pearson's correlation coefficient or Spearman's rank correlation coefficient and shown with a simple linear regression line of best fit.

Results

Patients' characteristics

All 81 patients exhibited advanced HF according to current HF guidelines.[26] Characteristics of patients with ICM or DCM were comparable regarding age, sex, body mass index, co-morbidities, most laboratory results, medication and number of HTX patients. Patients with ICM showed lower mean haemoglobin values as well as higher platelet counts, the latter ranging within physiological boundaries. Both groups showed pathological laboratory results, for example, elevated C-reactive protein, bilirubin and creatinine, which are common findings in HF patients.[26] Importantly, T2DM patients were distributed equally between the groups, and mean glycated haemoglobin A_{1c} was comparable. An overview on demographics is provided in *Table 1*.

Table 1 Patients' characteristics

All patients	ICM (n = 43)	DCM (n = 37)	P
Demographic and clinical data			
HTX (%)	20	37	0.139
Male (%)	86	84	0.725
BMI (kg/m^2)	27.8 ± 4.9	26.2 ± 5.5	0.188
Age (years)	61.5 ± 5.7	56.5 ± 12.7	0.164
LVEF < 30% (%)	90	91	0.868
LVEF 30%–40% (%)	10	9	0.868
Comorbidities			
Arterial hypertension (%)	50	32	0.099
Chronic kidney disease > stage III (%)	8	11	>0.999
Atrial fibrillation (%)	31	24	0.175
T2DM (%)	34	18	0.126
Medication			
Insulin (%)	22	11	0.228
PPI (%)	84	92	0.479
Diuretics (%)	84	81	0.719
Beta blockers (%)	70	61	0.465
ACEI (%)	24	42	0.140
MR-antagonists (%)	62	42	0.112
PDE-5 inhibitors (%)	70	60	0.360
Statins (%)	81	61	0.080
ASS (%)	78	72	0.595
Phenprocoumon (%)	70	46	0.080
Laboratory results			
Blood glucose at start of surgery (mg/dL)	144 ± 56	132 ± 28	0.265
HbA$_{1c}$ (%)	5.89 ± 1.17	5.9 ± 0.73	0.994
Haemoglobin (g/dL)	10.8 ± 1.8	12.1 ± 2.4	**0.008**
White blood cells (×1000/μL)	8.5 ± 3.1	8.6 ± 4.9	0.449
Platelets (×1000/μL)	220.7 ± 114.9	175.5 ± 66.8	**0.032**
Creatinine (mg/dL)	1.35 ± 0.49	1.72 ± 1.77	0.439
C-reactive protein (mg/dL)	4.3 ± 6.7	2.9 ± 5.2	0.298
LDH (U/L)	381 ± 292	450 ± 383	0.638
GOT (U/L)	88 ± 216	139 ± 387	0.661
GPT (U/L)	93 ± 192	190 ± 497	0.411
Bilirubin (mg/dL)	1.62 ± 1.71	1.89 ± 2.64	0.572
hs-TropT	477 ± 1558	637 ± 1890	0.912

Characteristics of patients with ischemic or non-ischemic heart failure. Comorbidities, medication and laboratory results are presented as mean ± SD. Statistics were performed as Student's t-tests or Mann–Whitney U tests, as appropriate.
ACEI, angiotensin converting enzyme inhibitor; ASS, acetylsalicylic acid; BMI, body mass index; DCM, dilated cardiomyopathy; HbA1c, glycated haemoglobin A1c; hs-TropT, high-sensitive troponin T; HTX, heart transplantation; ICM, ischemic cardiomyopathy; LDH, lactate dehydrogenase; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; MR, mineralocorticoid receptor; PDE, phosphodiesterase; PPI, proton pump inhibitor; T2DM, type 2 diabetes mellitus.

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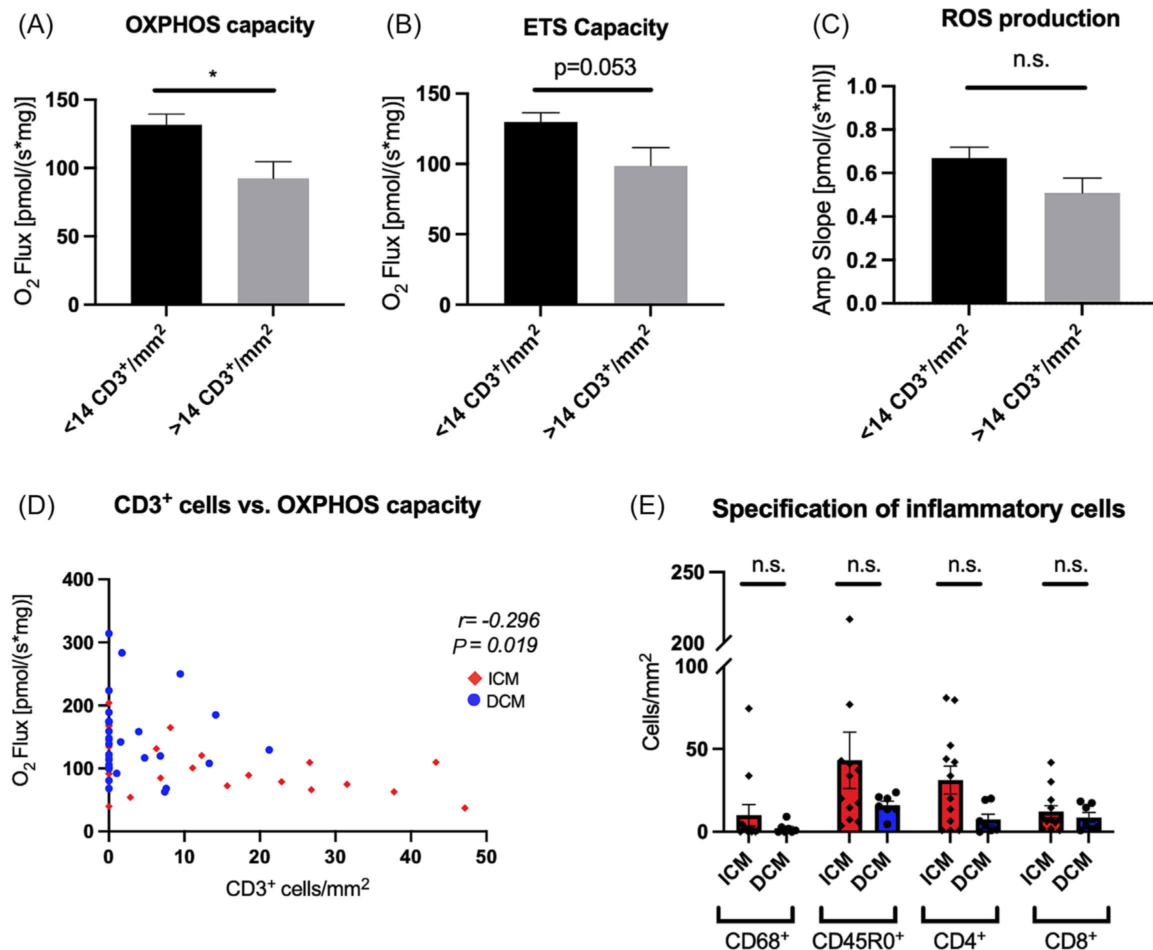
Figure 1 Myocardial inflammation was more common in patients with ICM compared to DCM. Patients with inflammation differed in some parameters of myocardial function. (A–C) Patients with relevant myocardial inflammation showed lower OXPHOS capacity than those without inflammation. The difference in ETS capacity did not reach statistical significance. ROS production did not differ. (D) T-lymphocytic infiltration was associated with lower OXPHOS. (E) No differences in specification for CD4$^+$ T-helper cells, CD8$^+$ cytotoxic lymphocytes, CD45R0$^+$ T-memory cells or CD68$^+$ macrophages were found between patients with ICM or DCM and more than 7 CD3$^+$ lymphocytes per mm^2. (A) Chi square test. (A–C) Unpaired *t*-tests. (D) Spearman correlation. (E) Mann–Whitney *U* tests. *n* (ICM) = 33, *n* (DCM) = 32, *n* (inflammation) = 11, *n* (no inflammation) = 51. CD, cluster of differentiation; DCM, dilated cardiomyopathy; ETS, electron transfer system; ICM, ischaemic cardiomyopathy; ROS, reactive oxygen species.



Inflammation, but not fibrosis, was associated with oxidative capacity

More patients with ICM than DCM revealed lymphocytic infiltration in the range of inflammatory cardiomyopathy with more than 14 CD3-positive cells per mm^2 (27% vs. 6%, P = 0.024). These patients exhibited lower OXPHOS capacity than patients with inflammation below this threshold (*Figure 1A*). The difference in electron transfer capacity did not reach statistical significance (*Figure 1B*); neither did ROS production differ between patients with or without relevant lymphocytic infiltration (*Figure 1C*). In the overall group of patients, CD3 count related to OXPHOS capacity (r = −0.296, P = 0.019; *Figure 1D*). However, stratification for HF aetiology omitted status of inflammation as a predictor for OXPHOS capacity in a linear regression model containing aetiology, inflammation and OXPHOS capacity (beta [aetiology] = −0.258, P [aetiology] = 0.045, beta [inflammation] = −0.203, P [inflammation] = 0.113).

We found a trend towards denser infiltration by CD4-positive cells in patients with ICM and >7 CD3-positive cells/mm^2 compared to DCM (31.3 ± 29.1 vs. 7.4 ± 8.7 cells/mm^2, P = 0.095). No difference was found between patients with ICM or DCM with respect to infiltration with CD8-positive lymphocytes, CD68-positive macrophages or CD45R0-positive T-memory cells, respectively (*Figure 1E*).

Density of lymphocytic infiltration with CD4-positive lymphocytes did not significantly correlate with OXPHOS (r = −0.298, P = 0.230) or ETS capacity (r = −0.266, P = 0.286), respectively. Additionally, CD4-positive infiltration

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did not significantly relate to ROS production (r = −0.413, P = 0.126). Infiltration with CD8-positive lymphocytes showed no correlation with OXPHOS (r = −0.302, P = 0.223) or ETS capacity (r = −0.46, P = 0.358), either. Notably, a non-significant trend was observed, indicating higher oxidative stress in myocardium with greater CD8-positive lymphocytic infiltration (r = 0.46, P = 0.078).

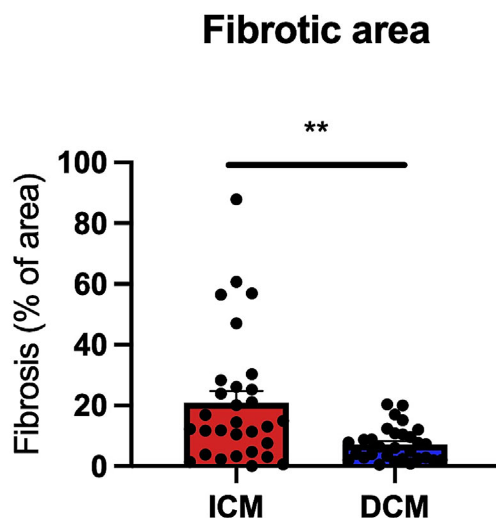
Relative fibrotic area was larger in ICM than in DCM (20.9 ± 21.2 vs. 7.2 ± 5.6%, P = 0.001; *Figure 2*), but the extent of myocardial fibrosis did not relate to OXPHOS capacity (overall: r = −0.13, P = 0.327), electron transfer capacity (r = −0.22, P = 0.086) or ROS production (r = −0.17, P = 0.234), respectively.

Lower mitochondrial oxidative capacity and mitochondrial content in ICM

Patients with ICM had lower mitochondrial oxidative capacity and electron transfer capacity than patients with DCM (*Figure 3A*). CI-linked respiration was 22% lower, while CI + II-linked respiration (OXPHOS capacity) was 23% lower in ICM versus DCM (108.6 ± 41.4 vs. 141.9 ± 59.9 pmol/(s*mg), P = 0.006). Inhibition of ATP synthase by oligomycin showed 18% lower leak respiration and uncoupled respiration indicating that full electron transfer system capacity was 20% lower in ICM than in DCM patients. Inhibition of CI led to 31% lower respiration in ICM compared to DCM. No difference was found in total myocardial protein content between the groups. However, CSA normalised to protein content in the myocardium as a marker of mitochondrial content was lower in patients with ICM (359.6 ± 164.1 vs. 503.0 ± 198.5 nmol/min/mg protein, P = 0.002; *Figure 3*). We normalised all respiration values to CSA. Here, we found no difference between the groups on any stage of the experiment (*Figure 3D*).

The second protocol only provided glutamate and succinate as direct substrates to the respiratory chain, omitting fatty acids. Here, CI-linked respiration in the ICM group was 32% lower, OXPHOS capacity was 43% lower, and CII-linked respiration was 42% lower, compared to DCM. Leak respiration was reduced by 44% in ICM, and ETS capacity of CI alone was 39% lower in ICM. Residual oxygen consumption (ROX) was also lower in ICM; however, correction for ROX did not alter these results (*Figure 3E*).

Figure 2 ICM myocardium showed larger fibrotic areas. Myocardial samples from ICM patients had larger fibrotic areas as determined by Azan staining with quantification. n (ICM) = 30, n (DCM) = 30. Mann–Whitney U test. DCM, dilated cardiomyopathy; ICM, ischaemic cardiomyopathy.



Comparable oxidative stress in ICM and DCM

We found no difference between patients with ICM or DCM in the production of reactive oxygen species at maximum stimulation (5 mmol/L succinate) (0.59 ± 0.28 vs. 0.69 ± 0.36 pmol/(s*ml), P = 0.196), nor at any lower stage of stimulation. Repeated measures two-way ANOVA revealed no interaction of aetiology and stimulation (P = 0.922) (*Figure 3F*). Normalisation to CSA as a marker of mitochondrial content did not alter these results.

Comparable mitochondrial coupling efficiency indicators

Respiratory control ratios as a marker of mitochondrial coupling efficiency were similar in patients with ischaemic and non-ischaemic HF (2.06 ± 0.70 vs. 2.16 ± 0.59 [*arbitrary unit*], P = 0.517), as were leak control ratios, mirroring comparable mitochondrial uncoupling (0.51 ± 0.12 vs. 0.49 ± 0.11 [*arbitrary unit*], P = 0.412). The contribution of fatty acids to mitochondrial respiration (capacity of the electron-transferring flavoprotein divided by maximum OXPHOS capacity) was found comparable between ICM and DCM patients (0.58 ± 0.13 vs. 0.55 ± 0.11 [*arbitrary unit*], P = 0.274).

Discussion

In this study, we report disturbed mitochondrial bioenergetics accompanying inflammatory cell infiltration in advanced ICM and DCM. The main findings are as follows: (1) Myocardial inflammation is more common in patients with ischaemic than non-ischaemic HF. (2) HF patients with inflammation in the range of inflammatory cardiomyopathy have impaired mitochondrial function. (3) We detected reduced mitochondrial respiration but similar mitochondrial efficiency in ICM and DCM.

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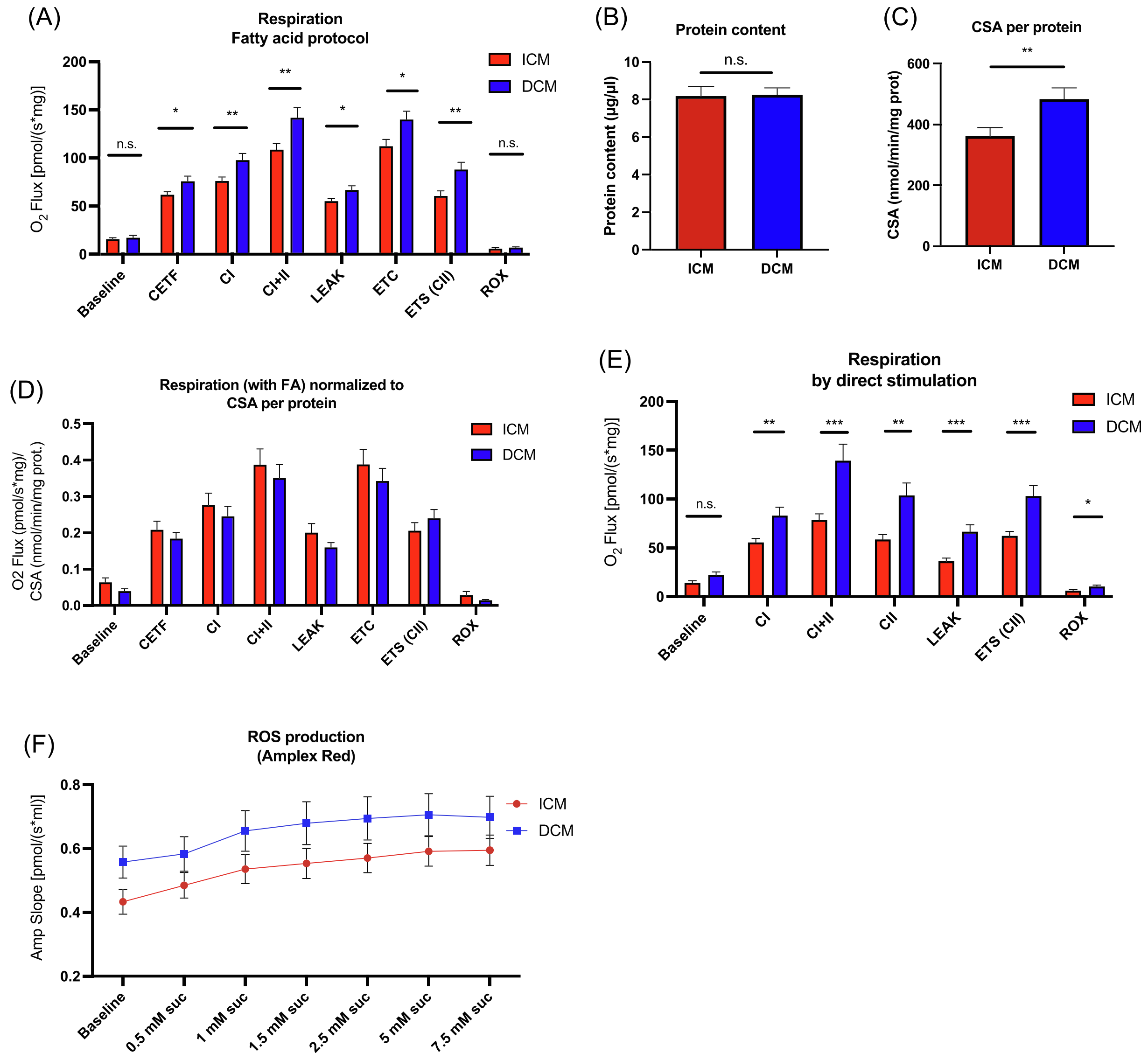
Figure 3 Lower mitochondrial respiration and mitochondrial content, comparable ROS production in patients with ICM or DCM. (A) OXPHOS capacity as well as electron transfer capacity on fatty acids were significantly reduced in patients with ICM compared to DCM. *n* (ICM) = 40 vs. *n* (DCM) = 34. (B) Protein content measured via bicinchoninic acid assay was comparable between the groups. *n* (ICM) = 35 vs. *n* (DCM) = 32. (C) Citrate synthase activity was significantly reduced in ICM patients compared to DCM. *n* (ICM) = 35 vs. *n* (DCM) = 32. (D) No significant difference in mitochondrial respiration after normalisation to citrate synthase activity (CSA) as a marker of mitochondrial content. *n* (ICM) = 35 vs. *n* (DCM) = 30. (E) OXPHOS capacity as well as electron transfer capacity were significantly reduced in patients with ICM compared to DCM. *n* (ICM) = 20 vs. *n* (DCM) = 13, respectively. (F) No significant difference in ROS production in ICM compared to DCM. *n* (ICM) = 37 vs. *n* (DCM) = 30. (A–D) Unpaired *t*-tests and (F) two-way ANOVA with Sidak's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Am, antimycin A; CETF, capacity of the electron-transferring flavoprotein; CI/CII, Complex I/II; CSA, citrate synthase activity; DCM, dilated cardiomyopathy; ETC, electron transfer capacity; ETS, electron transfer system; ICM, ischaemic cardiomyopathy; LEAK = leak respiration (state 4_O); ROS, reactive oxygen species; ROX, residual oxygen consumption; suc, succinate.



Inflammation, but not fibrosis, associates with impaired mitochondrial respiration

In this study, we found that myocardial inflammation and fibrosis were more prevalent in patients with ICM than DCM and that inflammation was associated with impaired mitochondrial oxidative capacity.

Immunosuppression has improved long-term clinical results in non-ischaemic HF patients with marked myocardial inflammation,[3–5] whereas patients with ischaemic HF are usually not screened for myocardial inflammation. An analysis from the CANTOS trial reported a trend towards fewer HF-related hospitalisations in patients with cardiovascular disease.[6] The said study only screened for systemic inflamma-

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tion by assessment of C-reactive protein that we found comparable in ICM and DCM despite differing myocardial cellular inflammation. In this study, we observed an increased myocardial infiltration with $CD3^{+}$ lymphocytic cells in ICM compared to DCM patients. Increased myocardial inflammation was associated with reduced oxidative capacity in all patients, a finding that has recently been observed in patients after HTX with or without acute rejection.[27] This raises the question whether selection of HF patients by assessment of myocardial inflammation may have had further improved the clinical benefits of immunosuppression in CANTOS. Further studies will have to show whether myocardial inflammation accompanied by lower mitochondrial respiration in ICM is mirrored in clinical endpoints. Subsequently, clinical studies may evaluate immunosuppressive therapy in ICM patients with increased myocardial inflammation.

Oxidative stress by ROS is increased pathologically in HF[15] and promotes inflammation, which again provokes further ROS production, creating a vicious cycle.[7] Although consequently one might suspect elevated oxidative stress in patients with relevant myocardial inflammation compared to those without, this was not the case in this study. Albeit cardiac fibrosis is considered as detrimental for cardiac function and favours ROS production in HF,[17,18] we did not find an association of fibrotic area with mitochondrial oxidative capacity. However, patients with ICM showed larger portions of fibrotic myocardial area paralleled by reduced OXPHOS capacity compared to DCM. A possible explanation for both findings might be that all our patients suffered from terminal HF, and differences that were blunted by overlaying effects may have been observed in less fatal states of the disease. This question could of course not be answered lacking a proper control group.

Reduced mitochondrial function in ICM compared to DCM

We observed diminished mitochondrial respiration in left ventricular myocardium from ICM patients, which applied to OXPHOS capacity, electron transfer capacity and leak respiration, but not oxidative stress. This is in line with previous results by Park *et al*.,[28] but in part different from the results of Sharov *et al*., who recognised a trend towards higher OXPHOS capacity in patients with ICM than DCM.[11] However, the distribution of patients with T2DM remained unclear but might have affected the results of Sharov *et al*.[29]

The relative contribution of fatty acids to OXPHOS capacity was not significantly distinguishable between patients with ICM or DCM. This indicates that for none of the two groups, addition of fatty acids was especially beneficial for respiration.

After normalisation to CSA, results for respiration and ROS did not differ significantly between aetiology groups. Also, flux control ratios as an indicator of intrinsic mitochondrial function independent of mitochondrial content were comparable between the groups. Taken together, these data suggest reduced mitochondrial content in ICM patients while the efficiency of the respiratory chain is preserved. This is in line with the findings of Ahuja *et al*., who reported diminished mitochondrial content and smaller mitochondria in human end-stage HF myocardium from ICM patients compared to DCM, while in DCM abnormal mitochondrial proliferation with higher rates of mitochondrial DNA damage was observed.[19] Although reducing oxidative stress in the failing myocardium is considered a therapeutical concept,[15] and a trend towards a potential difference in the efficacy of a ROS-targeting therapy between patients with DCM or other HF aetiology were found in a study,[30] we could not identify a group of patients that may particularly benefit from this approach. Nevertheless, further studies concerning oxidative stress in DCM myocardium are required to find reliable evidence in this regard. In summary, we were able to identify differences in mitochondrial respiratory chain activity and signs of differing mitochondrial content between patients with ICM or DCM, although mitochondrial efficiency was comparable. Future studies addressing mitochondrial function in HF should consider aetiology as a potential determinant of OXPHOS capacity.

Good overall comparability of aetiology-groups

All patients enrolled in this study suffered from terminal HF. Polymedication and multiple comorbidities are common in HF patient cohorts and were observed among the analysed cohort as well. Equal distribution of patients with T2DM deserves special attention as insulin resistance influences cardiac substrate utilisation and mitochondrial energy metabolism.[29,31,32]

Limitations

Since analyses were performed in human myocardium; some limitations apply to our study. The presented results could not be compared to tissue specimens from healthy human hearts. Disease or comorbidity-associated effects on myocardial mitochondrial function cannot be ruled out. Not all analyses could be performed in all patients, as tissue availability was limited. We only assessed T-lymphocytic infiltration, which may overlook features of humoral inflammatory activity. Haemodynamic parameters such as cardiac output or intracardial pressures were unavailable in many patients, preventing analyses of the relationship between cardiac function and mitochondrial respiration. Furthermore, it should be mentioned that DCM, as a heterogeneous disease, may have resulted from various underlying causes,[33] possibly influencing mitochondrial metabolism.

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Conclusions

Our data confirm higher prevalence of myocardial inflammation in patients with ischaemic than non-ischaemic heart failure. Patients with inflammation showed reduced mitochondrial oxidative capacity. As immunosuppression has shown to be beneficial in non-ischaemic HF with relevant inflammation improving HF-related endpoints, future studies may link myocardial inflammation to worsened clinical outcome in patients with ICM and pave the way for standardised detection of myocardial inflammation in ICM.

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Conflict of interest

The authors declare no conflict of interest in connection with the submitted article; Ralf Westenfeld has accepted a position as Medical Director at Abiomed, Inc., Danvers, MA, USA, after the completion of this manuscript.

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