



Right ventricular pressure overload alters cardiac lipid composition

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ABSTRACT

Introduction: Right ventricular (RV) failure due to pressure load is an important determinant of clinical outcome in pulmonary hypertension, congenital heart disease and left ventricular failure. The last decades it has become clear that metabolic dysregulation is associated with the development of RV-failure. However, underlying mechanisms remain to be unraveled. Recently, disruption of intracardiac lipid content has been suggested as potential inducer of RV failure. In the present study, we used a rat model of RV-dysfunction and aimed to obtain insight in temporal changes in RV-function, -remodelling and -metabolism and relate this to RV lipid content.

Methods and results: Male Wistar WU rats were subjected to pulmonary artery banding ($n = 25$) or sham surgery ($n = 14$) and cellular, hemodynamic and metabolic assessments took place after 2, 5 and 12 weeks. In this model RV dysfunction and remodelling occurred, including early upregulation of oxidative stress markers. After 12 weeks of pressure load, lipidomics revealed significant decreases of myocardial diglycerides and cardiolipins, driven by (poly-)unsaturated forms. The decrease of cardiolipins was driven by its most abundant form, tetralinoleoylcardiolipin. Mitochondrial capacity for fatty acid oxidation preserved, while the capacity for glucose oxidation increased.

Conclusion: RV dysfunction due to pressure load, is associated with decreased intracardiac unsaturated lipids, especially tetralinoleoylcardiolipin. This was accompanied with preserved mitochondrial capacity regarding fatty acids oxidation, with increased capacity for glucose oxidation, and early activation of oxidative stress. We suggest that early interventions should be directed towards preservation of lipid availability as possible mean in order to prevent RV failure.

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1. Introduction

Right ventricular (RV) failure is a main determinant for mortality and morbidity in patients with pulmonary hypertension [1] and in patients with congenital heart diseases [2]. RV failure due to progressive

pressure load is characterized by diastolic dysfunction and uncoupling of the RV and pulmonary vasculature [3,4]. The last decade, experimental studies have identified that RV dysfunction due to pressure load is associated with RV hypertrophy, fibrosis, and metabolic derangements [4–6]. Unfortunately, the increased knowledge of the cellular signature of RV dysfunction has not yet evolved into a RV specific therapy [7]. Also, the use of therapeutic strategies developed for left heart failure, e.g. ischemic heart diseases or hypertension, has not led to significant clinical improvements in patients with RV disease yet [7–9].

Derangements of RV metabolism associated with RV dysfunction due to pressure load is a recognized feature in various experimental studies [4,10–18] and is confirmed in several studies in patients with pulmonary arterial hypertension (PAH) [5,19,20]. The RV under pressure has been shown to be vulnerable to changes in coronary perfusion pressure [1] and several experimental studies have described a state of so-called capillary rarefaction [4,21,22], both of which may add to the metabolic derangements. The metabolic changes described involve suppression of genes involved in fatty acid metabolism

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[4,10,12–15,18,23,24], as well as deviation away from the glucose oxidation pathway [4,10,11,13,14,18,23]. More recently, studies are focusing on alterations in cardiac lipid content and its potential harmful effect. Up to now only lipotoxicity has been recognized in the pressure loaded RV in a model of bone morphogenetic receptor type 2 (BMPR2)-mutation [12]. However, also myocardial shortage of lipids has been suggested to have negative reflections on cardiac remodelling and function [25]. Together these observations emphasizes the relevance of a deeper understanding of RV dysfunction induced by different types of disease and the therapeutic potential of lipid modulation therapies.

Hereby, it is necessary to expand our knowledge on early and temporal changes in RV metabolic derangements during disease progression and its relation with functional cardiac performance. This will help to understand whether metabolic modulation is a potential therapeutic candidate in RV pressure load as has been suggested in left heart failure [26–30].

Here, we aimed to characterize the alterations in RV lipid content during chronic pressure load and to assess its correlation with RV-function, –remodelling and –metabolism over time.

2. Methods

2.1. Animal experiments

Animal care and experiments were conducted according to the Dutch Animal Experimental Act and conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Animal Experiments Committee of the University of Groningen, in the Netherlands approved the experimental protocol (permit number: AVD105002015134-2).

Male Wistar WU rats (160–180 g) were randomly subjected to pressure load by means of pulmonary artery banding (PAB, $n = 25$) or sham surgery (control, $n = 13$), and were checked daily for clinical signs of RV failure according to ABCDE criteria, as previously described [31]. Animals were terminated at 2 ($n = 5$ vs. 4), 5 ($n = 11$ vs. 4), and 12 weeks ($n = 9$ vs. 5) following surgery.

2.2. Hemodynamic measurements

Echocardiographic assessment of PAB-gradient, LV cardiac output (LV CO), stroke volume (SV) eccentricity index end diastolic (EI ED), eccentricity index end systolic (EI ES), and tricuspid annular plan systolic excursion (TAPSE) was performed at 2, 5 and 12 weeks according to previous described protocol [31]. Invasive pressure measurements performed by heart catheterization including end diastolic pressure (EDP), RV contractility (dP/dt_{max}), RV stiffness (dP/dt_{min}) and cardiac power were performed before termination, whereafter blood and organs were taken out and preserved. For detailed description of hemodynamic measurements, see Supplemental methods.

2.3. Histology

Ventricular remodelling was characterized by cardiomyocyte cross-sectional area (wheat germ agglutinin), fibrosis (Masson Trichrome) and capillary density (Lectin) in the RV free wall, as described previously [4]. Perivascular fibrosis $>200 \mu m$ was excluded for analysis of the section.

2.4. Gene expression

Gene expression of markers of cardiomyocyte stress, hypertrophy, fibrosis, metabolic regulators, substrate transporters, inflammation, oxidative stress, and cardiolipin synthesis and remodelling were assessed at mRNA level measured with standard qPCR as described in detail in the Supplemental methods.

2.5. Mitochondrial measurements

Mitochondria were isolated from fresh RV tissue subjected to 12 weeks of pressure load by PAB, as described in the Supplemental methods. Mitochondrial respiration was measured by the oxygen consumption rate with either pyruvate and malate, or palmitoyl CoA and malate as substrate, in the presence of ADP in a stirred, 2-channel high-resolution respirometry (Oroboros, Innsbruck, Austria). The different states, including the ADP-driven state 3 (State 3) as well uncoupled state 3 (State 3u), representing mitochondrial conditions (ADP or respectively ATP-rich environment, and intact respectively absence of membrane gradient), were analyzed as described in the Supplemental methods. Oxygen consumption rate was corrected for protein content. Citrate synthase activity kit (Sigma Aldrich, USA) was used as a marker of mitochondrial density.

2.6. Assessment triglyceride level plasma

Triglyceride (TG) levels in plasma were measured by enzymatic methods using commercial kits according to the manufacturer's instruction (Roche Diagnostics, Mannheim, Germany).

2.7. Assessment of cardiac lipid content

Lipidomics as performed on snap frozen RV tissue subjected to 12 weeks of pressure load by PAB. Sample work up and semi-quantitative analysis of the lipodome was performed as previously described [32,33].

2.8. Bioinformatics and statistical analysis

Quantative data (except the lipidomic data, see below) are expressed as mean \pm standard error of the mean (SEM). GraphPad Prism 5.04 was used for data analysis. Comparisons between control and PAB-groups were tested with unpaired Students *t*-test, whereas comparisons over time (2 versus 5 weeks, 5 versus 12 weeks and 2 versus 12 weeks) within groups (control or PAB) were tested with one-way ANOVA. Bonferroni post hoc correction was used for multiple testing. The *p*-value of <0.05 was considered as statistical significant. Control groups were pooled, since no differences were observed in time. With respect to the measurements of the mitochondrial respiration, control groups were presented individually. Bioinformatics and statistical analyses of the lipidomics data were performed as described before [32]. Totals for the major classes were defined as the summation of the relative abundance of all identified lipids within the same class normalized to the corresponding internal standard. Statistical analysis of the lipid classes were performed using the statistical programming language R (<https://www.r-project.org/>) together with the "MixOmics" package (<https://doi.org/10.1371/journal.pcbi.1005752>). A *q*-value of 0.01 was assumed to be significant. Partial least squares-discriminant analysis (PLS-DA) was used to assess the variable importance in the projection (VIP)-score of individual lipids. Lipids were assumed to be significant if $p < 0.05$, false discovery rate (FDR) < 0.1 and VIP > 1 . Boxplots displays the full range (minimum, first quartile, median, third quartile, and maximum), including statistical outliers.

2.9. Assessment of inflammatory status and oxidative stress

RV gene expression of different inflammatory and oxidative stress markers were assessed at mRNA level by standard qPCR. Macrophage infiltration in the RV was assessed by cluster of differentiation 68 (CD68) staining, as described previously [34]. Advanced oxidation protein products (AOPP) (ab242295, Abcam, Cambridge, United Kingdom) and anti-oxidant capacity assay (ab65329, Abcam, Cambridge, United Kingdom) were performed in RV tissue to the manufacturer's instruction.

Plasma levels of growth differentiation factor 15 (GDF-15) were measured by ELISA (MGD150, R&D, USA). AOPP were assessed in plasma as well (ab242295, Abcam, Cambridge, United Kingdom).

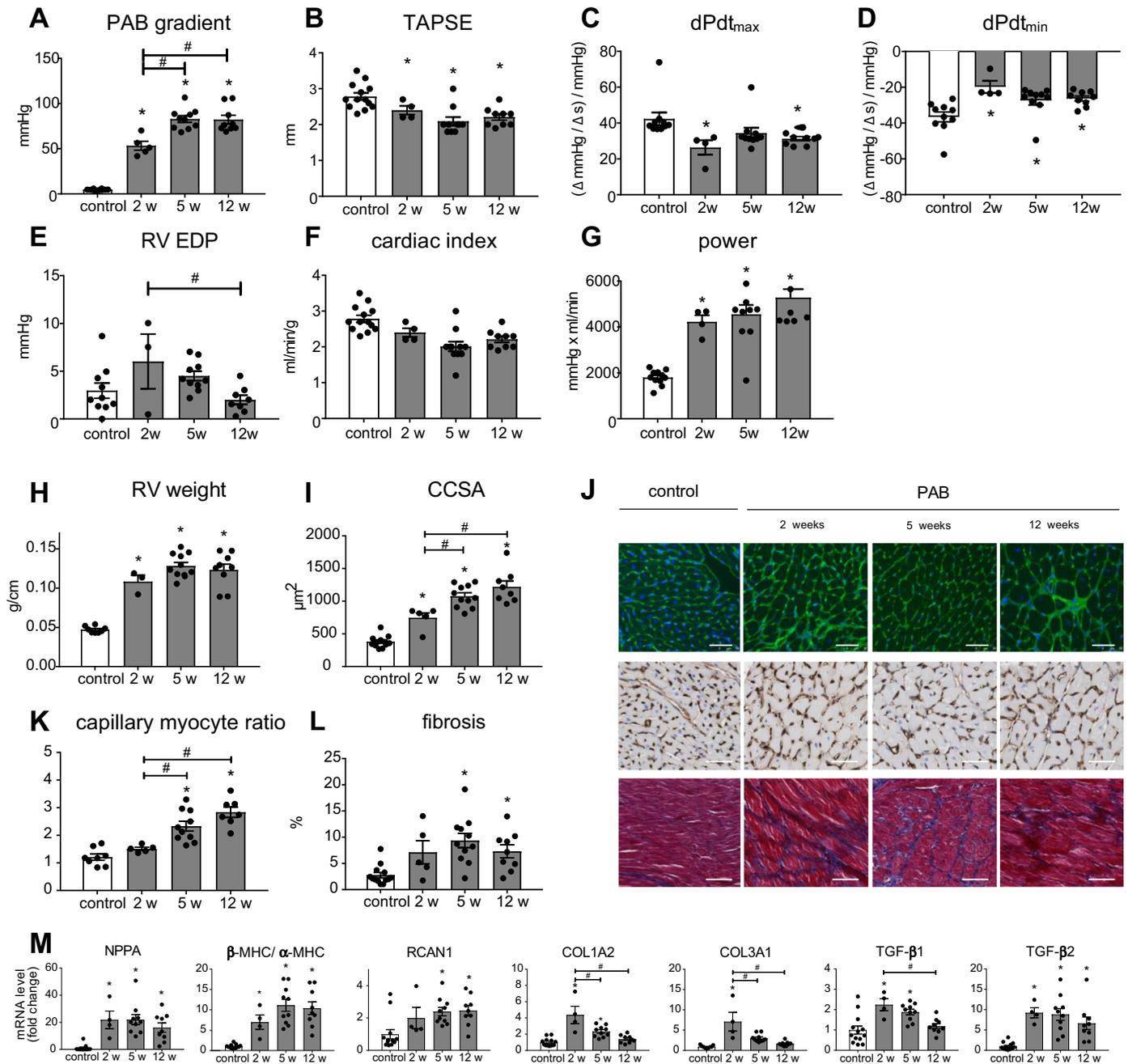
3. Results

3.1. RV pressure load induced RV dysfunction

PAB induced a pressure load of the RV that increased over time (Fig. 1A) and echocardiographic markers of relevant pressure load were present (Suppl. Table 1). In PAB-rats, TAPSE (Fig. 1B), RV dP/dt_{max} (Fig. 1C) and RV dP/dt_{min} (Fig. 1D) were reduced. RV end diastolic pressure (Fig. 1E) tended to increase at 2 weeks after PAB, but gradually decreased again over time. Cardiac index was maintained over 12 weeks in rats with PAB (Fig. 1F) and in line with that, these rats did not develop clinical signs of RV failure using the ABCDE-criteria [8]. Finally, RV workload (Suppl. Table 1) and power (Fig. 1G) increased significantly in the PAB-groups, without changes over time.

3.2. Pressure load induced RV remodelling

PAB induced hypertrophy after 2 weeks, expressed by RV weight normalized for tibia length (Fig. 1H). RV cardiomyocyte cross sectional area (Fig. 1I,J) and capillary myocyte ratio (Fig. 1K) increased in PAB-rats compared to control and over time (5 and 12 weeks, vs. 2 weeks after PAB). The capillary density, irrespectively of the number of cardiomyocytes, decreased at all time points compared to control (21, 20 and 23, vs. 31.05 vessels per square millimeter) (Fig. 1J). RV fibrosis increased significantly compared to control only after 5 weeks of PAB (Fig. 1J,L), whereas gene expression of the fibrotic markers collagen subunits 1A2 (COL1A2) and 3A1 (COL3A1) and transforming growth factor $\beta 1$ (TGF $\beta 1$) and $\beta 2$ (TGF $\beta 2$) were all increased already 2 weeks after



n = 3-13 in each group

Fig. 1. Hemodynamic and molecular changes over time in RV pressure load. PAB-gradient measured by Doppler echocardiography (A). Right ventricular systolic and diastolic function expressed by TAPSE (B) and dP/dt Max corrected for RV_{peak} pressure (C), and dP/dt Min corrected for RV_{peak} pressure (D) and RV EDP (E). Cardiac index (cardiac output corrected for bodyweight) (F). Power (RV_{peak} pressure multiplied by stroke volume) (G). RV weight correct for bodyweight (H), RV cross sectional area (I), RV capillary myocyte ratio (K) and RV fibrosis (L). Representative images of histological analyses: wheat germ agglutinin, lectin and masson-trichrome staining respectively (ruler is 50 μm) (J). mRNA expression of genes involved in cardiomyocyte stress, hypertrophy, fetal gene program, and fibrosis (M). Data presented as mean ± SEM. * = $p < 0.05$ compared to control, # = $p < 0.05$ compared to indicated time point. ● = individual animal, white = control group, grey = pulmonary artery banding groups. PAB = pulmonary artery banding. 2w, 5w and 12w = 2, 5 and 12 weeks after PAB. TAPSE = tricuspid annular plane systolic excursion, dPdt = delta pressure delta time, EDP = end diastolic pressure. CCSA = cross cardiomyocyte sectional area. NPPA = natriuretic pro-peptide type A, RCAN1 = regulator of calcineurin 1, MHC = myocyte heavy chain, COL1A2 = collagen subunit 1A2, COL3A1 = collagen subunit 3A1, TGF-β = transforming growth factor β.

PAB and gradually decreased at 5 and 12 weeks (Fig. 1M). Gene expression of both natriuretic pro-peptide A (NPPA), as marker of myocardial stress, and the ratio of myosin heavy chain isoforms β and α (Suppl. Table 2), as marker of the switch to fetal gene programme, were increased at all time points (Fig. 1M). Finally, gene expression of regulator of calcineurin 1 (RCAN1) involved in activation of hypertrophy was increased at 5 and 12 weeks (Fig. 1M).

3.3. 12 weeks of RV pressure load induced a discrete shift towards carbohydrate metabolism

Next we assessed mitochondrial respiratory capacity and expression of metabolic regulatory and transporter genes. In rats with PAB, the mitochondrial respiratory capacity using palmitoyl CoA as substrate was not significantly decreased, in both State 3 (Suppl. Fig. 1A) and State

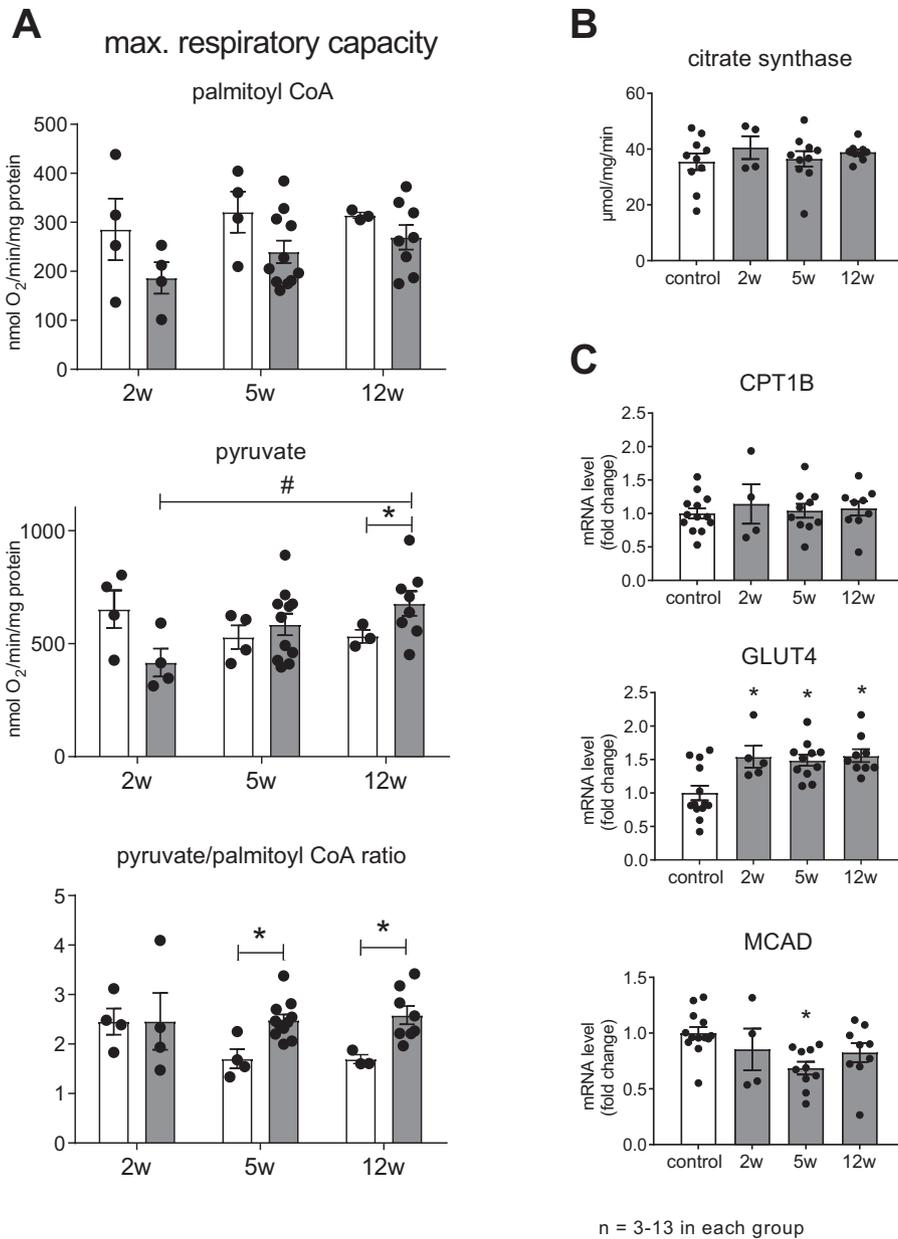


Fig. 2. Right ventricular fatty acid and glucose metabolism during pressure load over time. Maximal mitochondrial respiratory capacity (state 3 uncoupled) measured by oxygen consumption rates in isolated mitochondria from RV tissue in the presence of palmitoyl CoA or pyruvate, and the ratio of these two (A). Citrate synthase as marker of mitochondrial content (B). mRNA level of regulatory genes in metabolic transport and regulation (C). Data presented as mean \pm SEM. * = $p < 0.05$ compared to control, # = $p < 0.05$ compared to indicated time point. ● = individual animal, white = control group, grey = pulmonary artery banding groups, 2w, 5w and 12w = 2, 5, and 12 weeks. O₂ = oxygen. MCAD = medium-chain acyl CoA, PPAR α = peroxisome proliferator-activated receptor α , PGC1 α = PPAR gamma coactivator gene 1 α , CPT1B = carnitine palmitoyltransferase isoform 1B, GLUT4 = glucose transporter 4.

3u (Fig. 2A). The respiratory capacity using pyruvate as substrate was unchanged for State 3 (Suppl. Fig. 1B). However, State 3u, representing the maximal respiratory capacity, did increase after 12 weeks of PAB as compared with control (Fig. 2A). The ratio of both State 3 at 5 weeks (Suppl. Fig. 1C), and State 3u at 5 and 12 weeks (Fig. 2A) shifted in favour of the use of carbohydrates over fatty acids. To test whether these changes correlated with changed mitochondrial content, citrate synthase was assessed, which was not different (Fig. 2B). The expression of carnitine palmitoyltransferase 1b (CPT1B), fatty acid transporter on the mitochondrial membrane, did not change in rats with PAB as compared with control, whereas expression of glucose transporter 4 (GLUT4) was increased at all time points (Fig. 2C). At this stage of disease, metabolic regulators peroxisome proliferator-activated receptor α (PPAR α) and peroxisome proliferator-activated receptor gamma

coactivator 1- α (PGC1 α) remained unchanged (Suppl. Table 2). Medium chain acyl CoA dehydrogenase (MCAD) mRNA levels decreased at 5 weeks (Fig. 2C).

3.4. RV pressure load induced changes in intra-cardiac lipid content

The RV lipid content was determined at the 12 weeks time point by semi-quantitative measurements of lipids such as TG, DG, Cer, cardiolipin (CL), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylglycerol (PG) and phosphatidic acid (PA) (Suppl. Table 3–4). These were also assessed per major class (e.g. all TG), per cluster (e.g. TG within cluster 62) and as individual lipid species (e.g. TG(42:4), representing the lipid (number of carbon atoms: number of double bonds)). PAB induced several changes

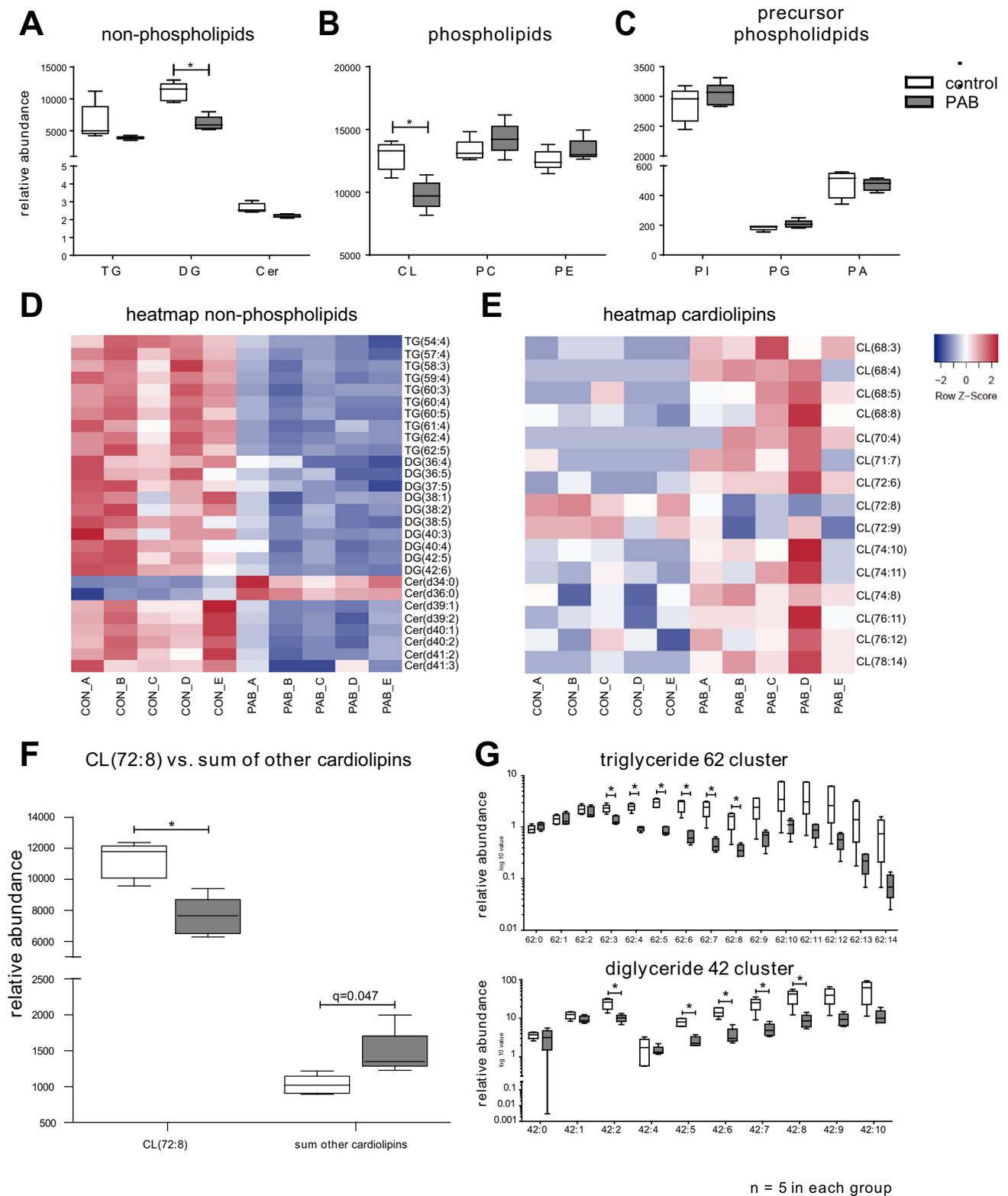


Fig. 3. Right ventricular lipid content. Class totals of non-phospholipids (A), phospholipids (B), and precursor phospholipids (C). Heatmap of individual significantly changed non-phospholipids selected on top ten VIP-score within class (D). Heatmap of individual cardiolipins VIP > 1 (E). CL(72:8) versus sum of other cardiolipins (F). Representative examples of progressive decrease of poly-unsaturated fatty acids within lipid clusters of TG and DG (G). TG = triglyceride, DG = diglyceride, Cer = ceramide, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PG = phosphatidylglycerol, PA = phosphatidic acid, CL = cardiolipin.

in RV lipid content (Fig. 3). In the major class analyses, RV content of DG, one of the non-phospholipids, was decreased 12 weeks after PAB as compared with RVs from control rats (Fig. 3A). TG and Cer showed a negative trend (Fig. 3A). Less uniform were the changes in lipid content

for the phospholipids (Fig. 3B). RV cardiolipin content was decreased at 12 weeks after PAB compared to controls, whereas PC and PE were not decreased. RV content of precursor phospholipids PI, PG and PA did not show any differences between the PAB and control groups (Fig. 3C).

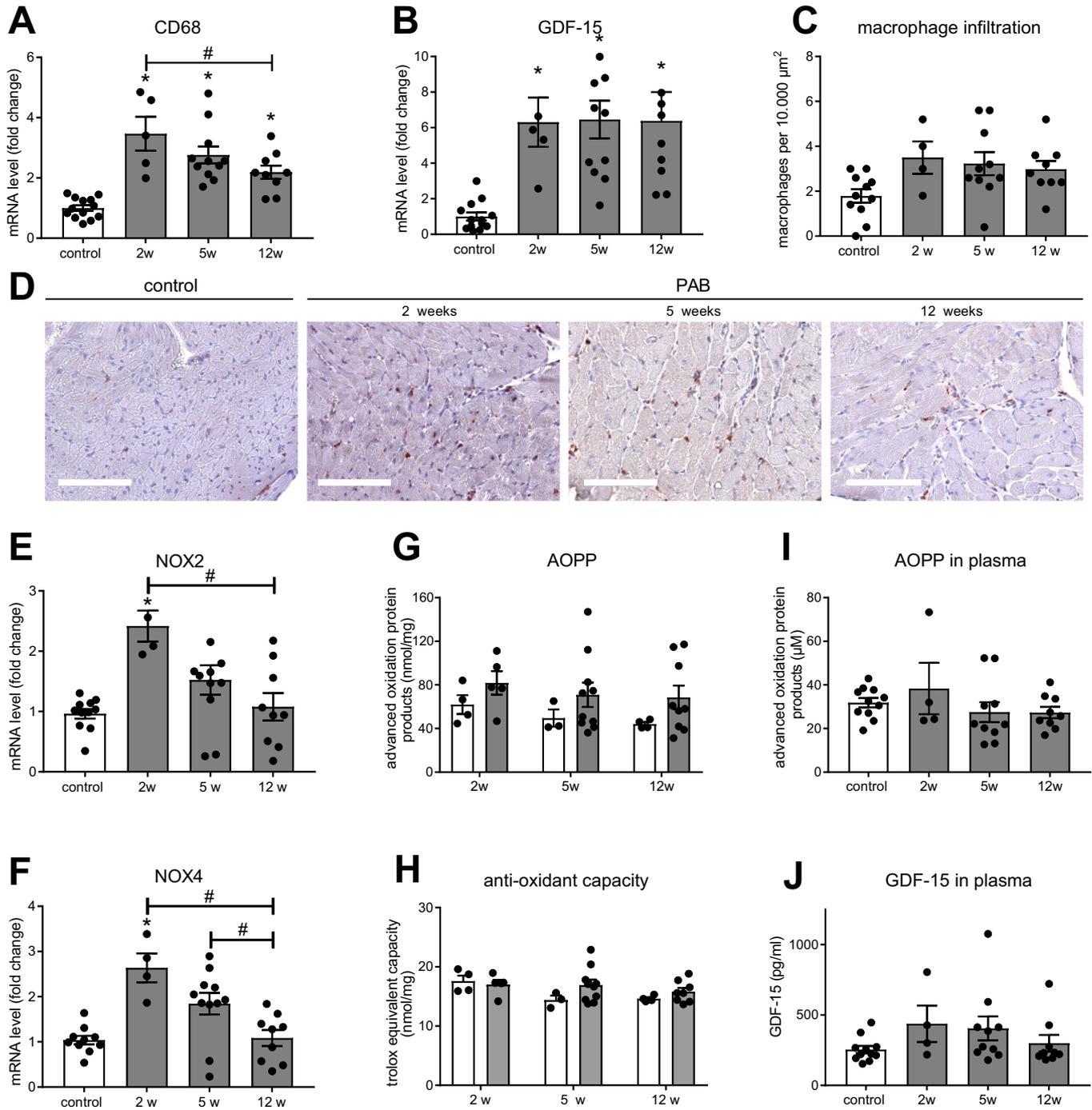


Fig. 4. Inflammation and oxidative stress over time in RV pressure load. mRNA expression of macrophage marker CD68 (A), and GDF-15 (B) in RV tissue. Macrophage infiltration measured by CD68-staining (C), with representative images of histological analysis (ruler is 100 μm) (D). mRNA expression of NOX2 and NOX4 as activators of oxidative stress (E–F). AOPP as marker of actual oxidative stress (G) and anti-oxidant capacity assay (H) in RV tissue. Levels of AOPP and GDF-15 in blood-plasma (I–J). Data presented as mean \pm SEM. * = $p < 0.05$ compared to control, # = $p < 0.05$ compared to indicated time point. ● = individual animal, white = control group, grey = pulmonary artery banding groups. PAB = pulmonary artery banding. 2w, 5w and 12w = 2, 5 and 12 weeks after PAB. CD68 = cluster differentiation 68, GDF-15 = growth differentiation factor 15, NOX = NAPDH oxidase, AOPP = advanced oxidation protein products.

Zooming in at the individual level, the heat map of significantly changed non-phospholipids showed a uniform decrease with exception of Cer(d34:0) and Cer(d36:0) (Fig. 3D). The heat map of individual cardiolipins showed lower levels of CL(72:8) and CL(72:9) (Fig. 3E). CL(72:8), tetralinoleoylcardiolipin, dominated the decrease of cardiolipins, whereas the sum of other, less abundant, cardiolipin species appeared to be increased (Fig. 3F). Within TG and DG species, PAB induced a shift from (poly-)unsaturated fatty acids (PUFA's) to more saturated fatty acids (see data supplement for additional

boxplots and complete heat maps), e.g. TG 62 cluster and DG 42 (Fig. 3G). Plasma total TG levels were unchanged (control vs. PAB after 12 weeks: 1.7 vs. 1.6 mmol/L).

Since cardiolipin content was decreased, we further investigated enzymes of cardiolipin synthesis and remodelling at the mRNA level. Cytidinediphosphate-diaclyglycerol synthase (CDS), an enzyme involved in cardiolipin synthesis did not change after PAB. Also, cardiolipin remodelling enzymes phospholipase A₂ (PLA₂) and tafazzin (TAZ) were unaffected at the mRNA level (Suppl. Table 2).

3.5. RV pressure load effects on inflammation and oxidative stress

To assess the effects of pressure load on inflammation and oxidative stress in the RV, we analyzed recruitment of cytokines (interleukines) and macrophages (CD68), activation of oxidative stress (NADPH oxidases), oxidation protein products and anti-oxidative markers in RV tissue. Cardiac gene expression of inflammatory markers CD68 (Fig. 4A) and GDF-15 (Fig. 4B) increased after 2 weeks. IL-6 was increased at two weeks only, albeit with a large interindividual variation (Suppl. Table 2). Expression of other cytokines (IL-1 β (with fold changes in control versus PAB of 1:0.94, 0.33:0.47 and 0.55:0.38 at two, five, and twelve weeks respectively) and IL-33 (Suppl. Table 2) did not change in response to pressure load. To assess whether the up-regulated gene expression of CD68 resulted in increased macrophage infiltration, CD68 staining was performed. CD68 staining revealed positive trend of increased infiltration of macrophages in the pressure loaded RV at all time points, yet the increase was not statistically significant (Fig. 4C,D). RV pressure load did induce a transient increase in cardiac expression of NADPH oxidases 2 and 4 (Fig. 4 E,F resp.), both of which are known to induce oxidative stress. Actual measurement of oxidative stress, by using advanced oxidation protein products (AOPP) assay, showed a positive trend at all time points compared to controls, however, statistical significance was not met (Fig. 4G). No decreases in anti-oxidative capacity were observed, possibly due to the relatively low levels of oxidative stress (Fig. 4H). Expression of superoxide dismutase was unaffected (Suppl. Table 2).

Levels of AOPP and GDF-15 in blood plasma, showed no differences when compared to controls at all time points (Fig. 4I,J resp.).

4. Discussion

With this study in chronic experimental RV pressure load, we aimed to characterize the alterations in RV lipid content during chronic pressure load and to assess its correlation with RV-function, -remodelling and -metabolism over time. The main finding of this study is that chronic pressure load of the RV induces a decrease of myocardial lipid content, that is associated with the development of RV dysfunction. The decrease of intracardiac lipids was mostly expressed in the lipid major classes diglycerides and cardiolipins, driven by (poly)unsaturated forms. This included tetralinoleoyl-cardiolipin, the most abundant form of cardiolipin. The decrease in fatty acids was not accompanied by an impairment of mitochondrial fatty acid oxidation, whereas the mitochondrial respiratory capacity for glucose oxidation increased. RV pressure overload induced early expression of inflammatory and oxidative stress markers, that gradually faded again in the following weeks. This pattern corresponds to the pattern of the expression of pro-fibrotic genes, that preceded the occurrence of fibrosis in the RV.

Decrease of cardiolipin levels, predominantly tetralinoleoyl-cardiolipin, has been described in different forms of heart failure, including pediatric heart failure [35–39]. Cardiolipin in the tetralinoleoyl-form (noted as CL18:2₄, L₄ or more generally CL(72:8)) is the most abundant cardiolipin in the mitochondrial membrane of most tissues and is essential for optimal mitochondrial energy production [39–41]. Defects in cardiolipin content affect complexes I, II, III and IV of the electron transport chain [42–46], leading to reduced oxidative capacity and increased production of reactive oxygen species [45–48]. Nevertheless, we did not find evidence of impaired mitochondrial function and one may speculate that the reductions in RV cardiolipin content precede a decrease in respiratory capacity due to a dysfunctional mitochondrial inner membrane leading to progressive oxidative stress.

In addition to a decreased cardiolipin content, the reduction of (P) UFA's also affected other lipid major classes. Since in this study mitochondria were not affected in number and their respiratory capacity for fatty acids, these reductions may be caused by oxidative stress or by reduced levels of common precursor lipids due to decreased uptake

of long-chain fatty acids (LCFA). PUFA's are known to be vulnerable to oxidative stress because of their hydrogen atoms close to multiple double bounds, which are easily taken by hydroxyl radicals. The current study did show initial increases of inducers of oxidative stress, which faded over time. The pattern was also recognized at the level of actual oxidative stress and inflammation, however, these results did not reach statistical significance. We speculate that PUFA's serve as primary preventive response and enables preservation of anti-oxidant capacity in the pressure overloaded RV. Another explanation may be inadequate uptake of essential lipids in the stressed RV. Diminished levels of CD36, a prominent LCFA transport protein in contracting cardiomyocytes [49], have previously been observed in LV hypertrophy and heart failure [50]. In addition, in the LV, adequate lipid turnover mediated by TG-pools has been shown to protect the heart against ceramides, known as inducer of mitochondrial dysfunction and apoptosis, making sufficient lipid availability even more relevant. All this together suggests that limited availability of PUFA's, including cardiolipin, precedes deterioration of RV homeostasis and function.

In the LV, upregulation of NADPH oxidase is known to induce fibrosis by the expression of TGF β 1 [51,52], which is accompanied with diastolic dysfunction [51]. A similar pattern is observed in the current model of RV pressure overload. NADPH oxidase is recognized as activator of oxidative stress [53,54]. In the current study we show upregulation of NADPH oxidase 2 and 4, without significant upregulation of actual oxidative stress. Although this might be due to lack of sufficient statistical power, this might also imply that in the state of compensated RV dysfunction, activation of oxidative stress is mild and might be balanced by protective mechanisms other than anti-oxidants and proteins. How exactly upregulation of NADPH oxidase is triggered, is yet still unknown. In the diabetic mice heart, growing evidence suggest that NADPH oxidase is stimulated by hyperglycaemia [55,56], whereas in hypertensive rats NADPH oxidase seems to be indirectly stimulated by systemic and local effects of angiotensin II [52,57]. In pressure overload ventricles, both left [57,58] and right, the exact mechanism still needs to be unraveled. In this respect, it is of specific interest that the current study shows early upregulation of GLUT4, which might contribute to higher levels of glucose in cardiomyocytes. In diabetic disease, hyperglycaemia leads to fibrosis by inflammation [59]. These observations suggest that similar mechanisms, including oxidative stress, inflammation and pro-fibrotic activity, may be involved in the early adaptation of the RV to increased pressure load and preceding RV failure. Further research should clarify the initial cause of NADPH oxidase activation in the pressure loaded RV.

Levels of oxidative stress and inflammation, measured by AOPP assay and GDF-15 ELISA, appeared to be not increased in blood plasma. This is in line with the relatively low activation of oxidative stress and inflammation in RV tissue, but also with the fact plasma pools are rarely influenced by dynamic changes in cardiac expression only [60]. Furthermore, blood derived biomarkers, other than cardiac specific markers, predominantly reflect systemic effects of heart failure [60], while the animals in the current study developed RV dysfunction, but no clinical overt heart failure.

Our findings are opposed to those in experimental PH due to BMPR2-deficiency, where intracardiac accumulation of fatty acid intermediaries has been associated to progressive RV dysfunction [12]. The results of the present study suggest a difference between chronic pressure load in the presence or absence of PH, or more specifically involvement of the BMPR2-mutation. The ambivalent character of these findings are in line with different changes in metabolic capacity reported in the different models of RV pressure load [13,61–64], and need to be considered in developing therapeutic strategies in RV dysfunction due to different types of disease. The present study suggests that therapies aiming at maintenance of mitochondrial integrity via restoration of cardiolipin content may be more appropriate than targeting fatty acid oxidation itself. Recent studies showed that preservation of cardiolipin by diet or therapeutics led to preservation of normal

mitochondrial function and preservation of left ventricular function. Dietary measures, such as high-linoleate safflower oil, were able to preserve tetralinoleoylcardiolipin content and mitochondrial function, and improved left ventricular function in spontaneously hypertensive heart failure rats [65,66]. Resveratrol is known to improve fatty acid oxidation, to reduce ROS production, to be cardiac protective and to improve survival in experimental models of diabetic cardiomyopathy, myocardial infarction induced tachycardia, exercise training and high-fat diet induced cardiac myopathy [67–70], and has been described as therapeutic option for up regulation of cardiolipin content [71]. In Barth syndrome, a cardiomyopathy due to disruption of the TAZ gene leading to reduced mature cardiolipin levels, substitution of cardiolipin itself via nanoparticles is currently being tested as a new therapeutic strategy. Preservation of substrates availability is a potential candidate for prevention or early interception in the development of RV dysfunction, whereas therapeutics inhibiting oxidative stress and stimulating antioxidants are likely to be more relevant in established heart failure than in early adaptation.

In the current study we did not test the effect of metabolic modulation, used in previous studies in RV-failure. This study aimed at identification of changes in metabolic regulation over time, early in the process of RV adaptation towards RV dysfunction, preceding clinically overt RV failure. The results of this comprehensive study do challenge the widely assumed concept that altered metabolism in RV failure represents “an engine out of fuel”. Rather we speculate that early activation of oxidative stress affects intracardiac lipid content and thereby might contribute to acceleration of oxidative stress in the progression towards RV failure. Indeed, now we have identified early lipid alteration, including cardiolipins, in the development towards RV failure, the mechanism by which increased pressure load leads to this metabolic changes warrants further exploration. The results of this study reveal that both functional (Fig. 1A–G) and histopathological (Fig. 1H–M) changes of the pressure loaded RV precede significant changes in oxidative capacity (Fig. 2). Furthermore, there are no indications that the decrease of specific lipids was preceded by increased fatty acid metabolism. In addition, the initial increase in markers of oxidative stress preceded progressive functional deterioration. Based upon these findings we suggest to design intervention strategies based upon restoration of intracardiac fatty acid pool. To derive insights in metabolic changes, we studied several components of metabolism. By adding functional measurements of mitochondrial respiratory capacity using Oroboros, we attempted to create a better picture of the metabolic capacity in the pressure loaded RV. These data showed that the immediate increase in cardiac power (Fig. 1G), was associated with a slow increase in metabolic capacity of carbohydrates only. Combining these results with the altered lipid profile, indicates a role for preserving intracardiac lipid status in the initial response to pressure overload, rather than a change in fatty acid metabolic capacity itself.

5. Conclusion

In this study we showed that RV dysfunction, preceding RV failure due to chronic pressure load, is associated with decreased intracardiac unsaturated lipids, especially in the most abundant form of cardiolipin. These changes were accompanied by preserved mitochondrial capacity for fatty acid oxidation, with an increased mitochondrial capacity for glucose oxidation, and early expression of oxidative stress markers. We suggest that early interventions to prevent RV failure may be directed towards preservation of intracardiac lipid composition.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijcard.2019.04.004>.

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Conflict of interests

None.

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