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NF-κB-mediated metabolic remodelling in the inflamed heart in acute viral myocarditis

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ABSTRACT

Acute viral myocarditis (VM), characterised by leukocyte infiltration and dysfunction of the heart, is an important cause of sudden cardiac death in young adults. Unfortunately, to date, the pathological mechanisms underlying cardiac failure in VM remain incompletely understood. In the current study, we investigated if acute VM leads to cardiac metabolic rewiring and if this process is driven by local inflammation. Transcriptomic analysis of cardiac biopsies from myocarditis patients and a mouse model of VM revealed prominent reductions in the expression of a multitude of genes involved in mitochondrial oxidative energy metabolism. In mice, this coincided with reductions in high-energy phosphate and NAD levels, as determined by Imaging Mass Spectrometry, as well as marked decreases in the activity, protein abundance and mRNA levels of various enzymes and key regulators of cardiac oxidative metabolism. Indicative of fulminant cardiac inflammation, NF-κB signalling and inflammatory cytokine expression were potently induced in the heart during human and mouse VM. In cultured cardiomyocytes, cytokine-mediated NF-κB activation impaired cardiomyocyte oxidative gene expression, likely by interfering with the PGC-1 (peroxisome proliferator-activated receptor (PPAR)-γ co-activator) signalling network, the key regulatory pathway controlling cardiomyocyte oxidative metabolism. In conclusion, we provide evidence that acute VM is associated with extensive cardiac metabolic remodelling and our data support a mechanism whereby cytokines secreted primarily from infiltrating leukocytes activate NF-κB signalling in cardiomyocytes thereby inhibiting the transcriptional activity of the PGC-1 network and consequently modulating myocardial energy metabolism.

1. Introduction

Viral myocarditis (VM) is an important cause of sudden cardiac death in young, otherwise healthy individuals. The incidence of biopsy-confirmed VM in unexplained heart failure cases in this population is approximately 10% [1]. In a subgroup of these patients, VM elicits an autoimmune response predisposing to dilated cardiomyopathy and heart failure [2]. Despite recent advances, the pathogenesis of VM remains incompletely understood. Current treatment of VM focuses primarily at symptoms of cardiac dysfunction and on targeting the immune response, but fails to be effective [3]. In order to develop new evidence-based treatment modalities, detailed insight into the molecular mechanisms governing VM-induced cardiac dysfunction is of the utmost importance.

It is well-established that cardiac disease is accompanied by perturbations in cardiac energy metabolism that compromise cardiac function and contribute significantly to disease progression [4]. Tissue inflammation has been postulated as a potential driver of this metabolic

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remodelling process [5]. More specifically, activation of inflammatory signalling pathways is thought to interfere with the expression and function of multiple genes controlling cardiac mitochondrial biogenesis and oxidative metabolism, by acting on a transcriptional network involving various nuclear receptors and co-regulatory proteins, in which peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) has been shown to play a central role [6].

It has been demonstrated earlier that in both the acute and chronic phase of Coxackievirus B3 (CVB3)-induced VM in mice, transcript abundance of genes involved in fatty acid β-oxidation and the mitochondrial respiratory chain in the heart was reduced [7,8]. In chronic VM this was associated with profound cardiac dilatation and a significantly reduced cardiac output [8]. These studies, however, do not allow one to discriminate whether these changes are an accurate reflection of the metabolic state of the heart and fail to provide mechanistic insight in the origin of these changes. Whether or not cardiac metabolism is perturbed in patients with acute VM is unknown. Accordingly, in the present study we investigated if the inflammatory response associated with acute VM in men and mice initiates reprogramming of cardiomyocyte energy metabolism.

2. Results

2.1. Transcriptome analysis reveals down-regulation of oxidative metabolic pathways during acute VM

To identify cellular processes that are affected by VM, whole genome transcriptome analysis was performed on cardiac biopsies of a previously well-characterised subgroup of patients with active VM [9] and on cardiac tissue of mice with acute CVB3-induced VM. Presence of VM was verified by determination of viral load and quantification of immune cell infiltration in the heart (Table S1; Fig. S1A, B). Subsequent enrichment analysis revealed that genes involved in processes related to cardiac energy metabolism were by far the most prominently dysregulated during VM in both men and mice (Table S2, S3). Subsequently, heat maps were constructed of all genes implicated in glycolysis, the tricarboxylic acid (TCA) cycle, fatty acid β-oxidation and the electron transport chain (ETC). As depicted in Fig. 1A, the majority of genes involved in fatty acid β-oxidation, the TCA cycle and the ETC was down-regulated during VM in patients compared to controls (Visualisation of transcriptional changes in metabolic pathways in human VM are depicted in Fig. S2–S5). The same response was seen in CVB3-infected mice, especially 7 days post-infection when cardiac inflammation is most fulminant (Fig. 1A) [10]. Significant overlap in differentially-regulated metabolic genes was observed between human and mouse (Fig. 1B). Similar to observations in VM patients, disturbances in cardiac metabolic gene expression were associated with a reduced ejection fraction and decreased fractional shortening in the absence of cardiac dilatation during acute VM in mice (Fig. 1C, Table S1).

2.2. VM-induced metabolic remodelling of the heart

To verify whether the changes in metabolic gene expression observed in our transcriptomic analyses were indicative of cardiac metabolic remodelling, we performed MALDI-IMS analyses of tissue metabolite levels in cryosections of control and CVB3-infected hearts. First, biostatistical analysis of the entire section revealed that the metabolic profile of VM hearts is markedly distinct from control hearts (Fig. 2A). More specifically, MALDI-IMS spectra showed a reduction of ATP, ADP, AMP and nicotinamide adenine dinucleotide (NAD) levels while UDP-*N*-acetylglucosamine (UDP-GlcNAc), phosphatidylinositol (PI) and arachidonic acid (AA) were increased in VM hearts compared to sham-treated animals (Fig. 2B). In addition, MALDI-IMS spectra revealed that cardiolipin, a phospholipid species almost exclusively found in the mitochondrial inner membrane and crucial for mitochondrial function [11], was reduced in VM heart sections compared to controls (Fig. S6A). Upon quantification, levels of ATP, ADP, AMP, total adenine nucleotide (TAN) and NAD content were found to be significantly reduced in VM hearts compared to controls reflecting extensive degradation of high-energy phosphates in VM hearts (Fig. 2C). Also, MALDI-IMS at 30 μm resolution revealed that inflammatory patches within the myocardium are clearly distinguishable from non/sparsely infiltrated areas of myocardium, specifically by presenting higher levels of UDP-GlcNAc and lower levels of PI (Fig. 2D and Fig. S6B).

2.3. VM-induced impairments in mitochondrial metabolic pathways

Next, we performed a more detailed analysis of key constituents and molecular regulators of several mitochondrial processes essentially involved in cellular metabolism. First, to validate our micro-array data, we assessed mRNA transcript levels of several genes involved in cardiac oxidative metabolism by qPCR in CVB3-infected mice. Importantly, we subsequently verified whether alterations in mRNA transcript abundance of selected genes were associated with alterations in protein abundance and enzymatic activity of key constituents of these metabolic pathways.

In line with our array data, which indicated reduced expression of multiple genes involved in the fatty acid β-oxidation pathway, transcript levels of very long-chain acyl-CoA dehydrogenase (ACAD-VL) and β-hydroxyacyl-CoA dehydrogenase (HAD) were significantly reduced in VM hearts compared to controls. Furthermore, enzymatic activity of HAD, the rate-limiting enzyme in fatty acid β-oxidation, was significantly lower in VM hearts compared to controls (Fig. 3A). In addition, VM-induced abnormalities in mitochondrial oxidative phosphorylation (OXPHOS) were apparent as mRNA abundance of sub-units of ETC complex I to III was reduced. This coincided with marked reductions in protein levels of ETC complex I and IV and decreased enzyme activity of cytochrome *c* oxidase (COX; Complex IV) in CVB3-infected mice compared to sham-treated animals (Fig. 3B).

The consistent reduction in the expression and activity of multiple mitochondrial proteins critically involved in oxidative substrate metabolism suggests involvement of key regulatory proteins, like PGC-1 s and PPARs [6]. Indeed, PGC-1α, PGC-1β and PPAR-α, but not PPAR-δ, mRNA levels were significantly reduced in VM hearts (Fig. 3C). In line with its key role in regulating mitochondrial oxidative metabolism, PGC-1α transcript abundance significantly correlated with expression levels of constituents of oxidative phosphorylation and fatty acid β-oxidation in our study (Table S4). In addition to reduced expression levels of PGC-1 and PPAR, protein levels of Tfam and NRF-1, which control mitochondrial biogenesis [6], were significantly decreased in VM further suggesting disturbances in the molecular regulation of mitochondrial oxidative energy metabolism in the heart (Fig. 3D). Collectively, the changes in activity and abundance of key constituents and regulators of mitochondrial metabolic pathways that we observed in VM hearts were associated with an increased phosphorylation status of the cellular energy sensor adenosine monophosphate kinase (AMPK) in VM hearts compared to sham-treated animals pointing to an energy-deprived state of the heart (Fig. 3E).

In addition to disturbances in mitochondrial oxidative metabolic pathways, our transcriptomic analysis also pointed to an increased activity of anaerobic glycolysis in VM hearts (Fig. S5). This was confirmed by increased mRNA expression of glucose transporter-1 (Glut-1), several glycolytic enzymes as well as lactate dehydrogenase-1 (LDH1) and the lactate transporter monocarboxylate transporter-4 (MCT-4) in VM hearts (Fig. S7A). Notably, transcript abundance of hypoxia-inducible factor 1α (HIF-1α), a key regulator of cardiac glycolytic metabolism, and of carbonic anhydrase IX (CA9) and heme oxygenase 1 (HMOX), two well-established HIF-1α target genes, were increased in VM hearts (Fig. S7B). In accordance with its pivotal role as regulator of cardiac glycolytic metabolism, HIF-1α expression also correlated strongly with the expression of glycolytic genes (Table S4).

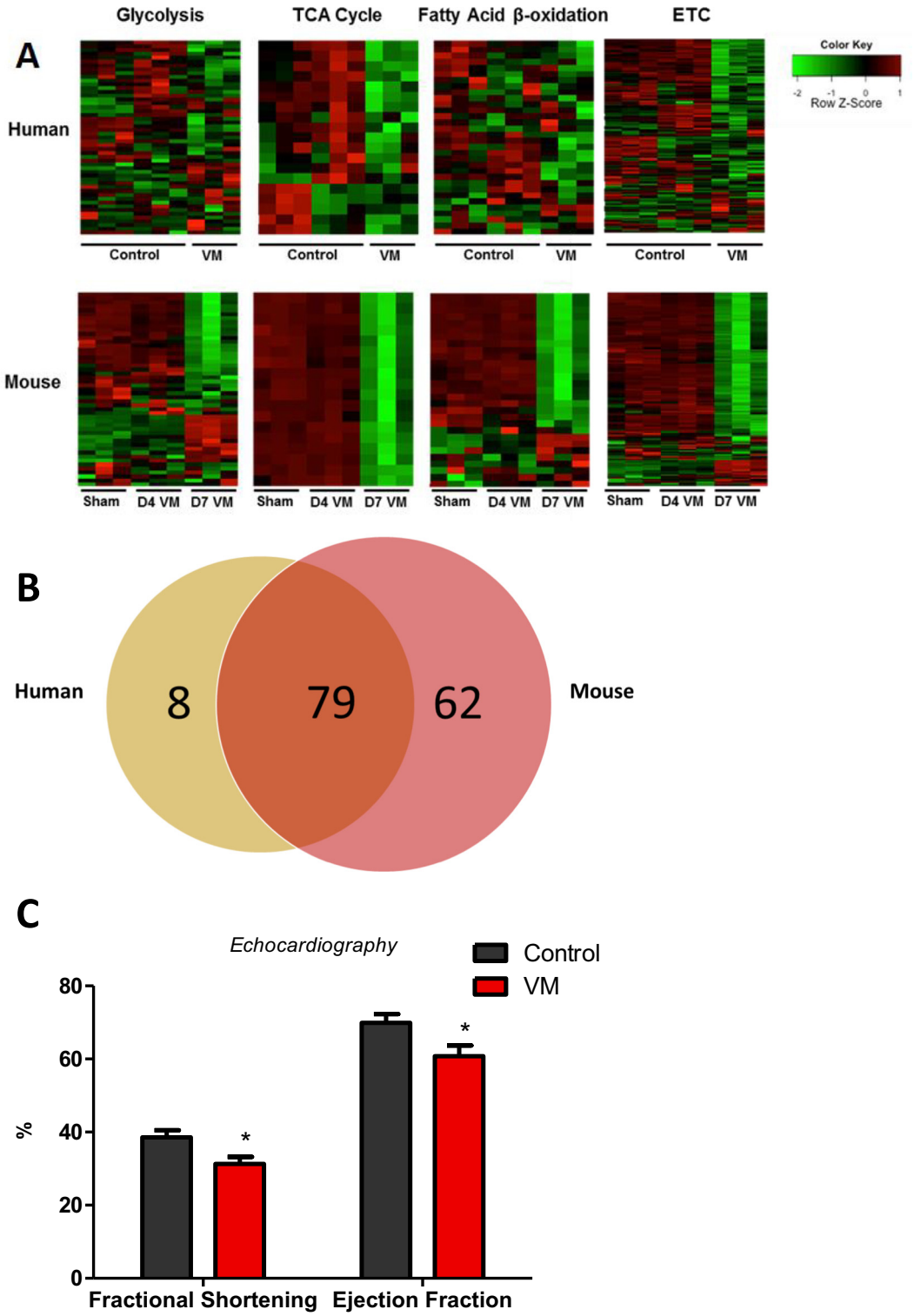

Fig. 1. Transcriptomic analysis reveals down-regulation of oxidative metabolic pathways in the heart during VM. Heat maps of differentially-regulated ($p \leq 0.05$) metabolic genes in right ventricular septal biopsies from patients with viral myocarditis (VM) (n = 3) and controls (n = 6) and in cardiac tissue of mice with CVB3-induced VM, 4 and 7 days post inoculation, and sham-treated animals (n = 3 for all groups) (A). Venn diagram depicting overlap in differentially-regulated metabolic genes in human and mouse VM (B). Echocardiographic assessment of the impact of VM (7 days post-inoculation) on cardiac function in mice (C). * $p \leq 0.05$ compared to control.

2.4. Cardiomyocyte-specific alterations in metabolic gene expression in response to VM

To verify that VM-induced remodelling of metabolic processes that we observed in whole hearts specifically involves changes in the cardiomyocytes, cardiomyocytes were isolated from VM and control hearts and expression levels of metabolic genes were assessed. As expected, CVB3 viral RNA was abundantly present in cardiomyocytes isolated from infected animals and was not detectable in sham-treated animals (Fig. 4A). In line with our *in situ* data, cardiomyocytes isolated

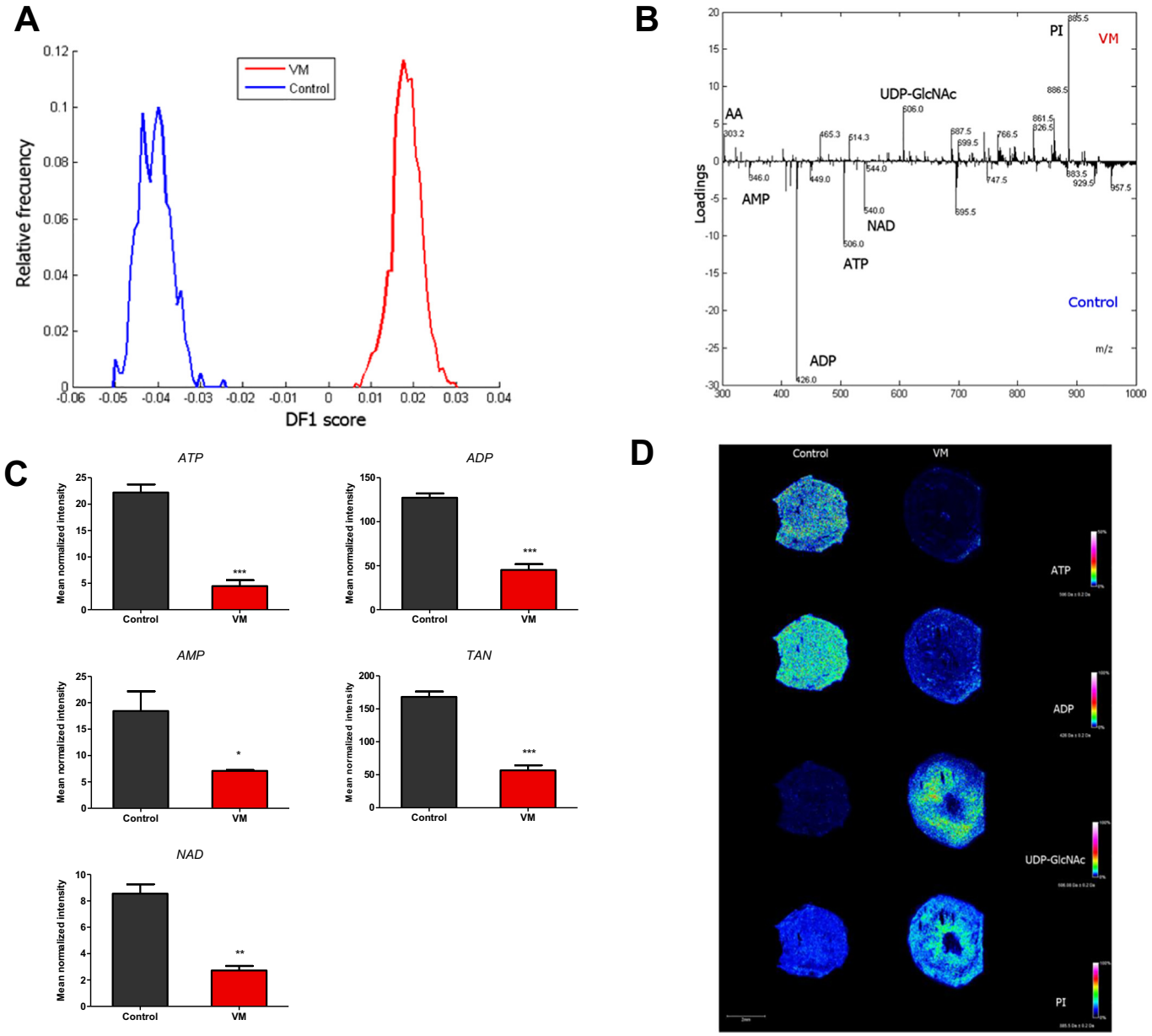

Fig. 2. Metabolic profiles of VM and control heart mice by MALDI-IMS. MALDI-IMS revealed a different molecular profile (negative side of DF1 plot) *vs.* VM heart tissue sections (positive side of the DF1 plot) as assessed by principal components and discriminant analysis (A). The spectrum characteristic of each condition showed a reduction of AMP, ADP, ATP and nicotinamide adenine dinucleotide (NAD) levels while, UDP-*N*-acetylglucosamine (UDP-GlcNAc), phosphatidylinositol (PI) and arachidonic acid (AA) were increased in VM hearts (B). Quantification of high-energy phosphates revealed significant reductions in ATP, ADP, AMP, NAD and total adenine nucleotide levels (TAN) (C). Spatial distribution and abundance of metabolites in VM and control heart mice (D). The intensity scale bar correlates with the abundance of each metabolite. Data were normalised by the total ion count. 3 sections of the heart of VM animals (n = 3) and control animals (n = 3) were analysed. Shown is mean ± SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ compared to control.

from VM animals were characterised by significantly reduced expression levels of genes involved in fatty acid β-oxidation (ACAD-VL) and nuclear- and mitochondrial-encoded genes involved in oxidative phosphorylation (Fig. 4B–D) as well as increased expression of multiple glycolytic genes (Fig. S7C) and of HIF-1α target genes when compared to control (Fig. S7D). Moreover, expression levels of PGC-1β, but not PGC-1α, were significantly decreased in cardiomyocytes isolated from VM hearts compared to control hearts (Fig. 4E).

2.5. Activation of NF-κB signalling impairs cardiomyocyte oxidative gene expression

Next, we searched for potential triggers and molecular mechanisms underlying the derangements in cardiomyocyte energy metabolism associated with acute VM. Thereto, we first exposed cultured neonatal cardiomyocytes to CVB3. CVB3 infection potently induced expression of genes involved in viral uptake and host response to viral infection, but failed to induce any significant decreases in oxidative metabolic gene expression (Fig. S8A–E), suggesting that viral infection itself does not directly impede on cardiomyocyte metabolic gene expression.

Interestingly, in mice with acute VM, cardiac expression levels of the pro-inflammatory cytokine tumour necrosis factor α (TNF-α) showed a strong significant inverse correlation with expression levels of various genes involved in fatty acid β-oxidation, oxidative phosphorylation and PGC-1 signalling (Fig. 5A–C). As pro-inflammatory cytokines like TNF-α are known activators (as well as target genes) of the

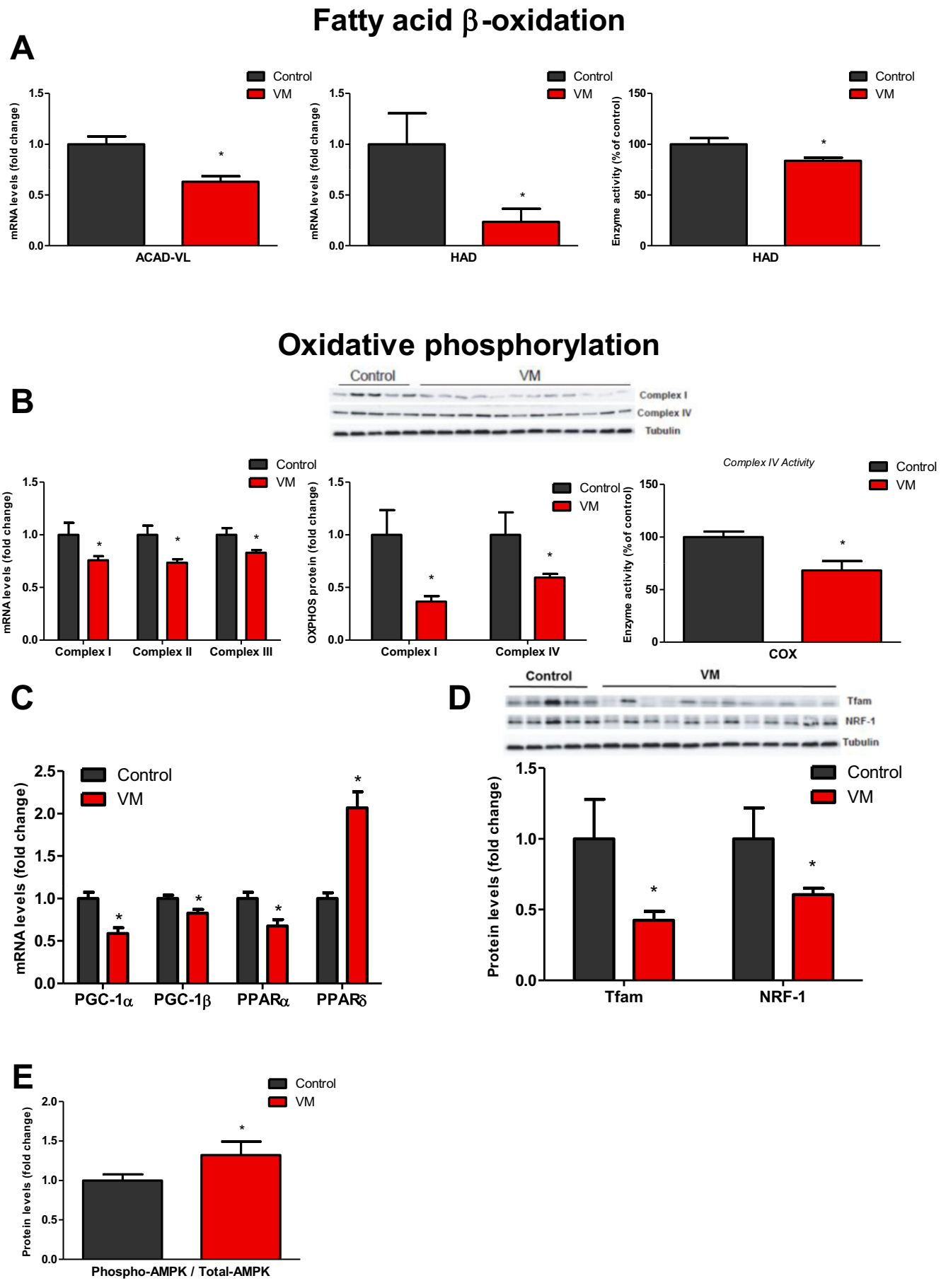

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Fig. 3. Down-regulation of mitochondrial oxidative metabolic pathways and its molecular regulation in the heart during CVB3-induced VM. Intact hearts were obtained from mice with CVB3-induced VM (n = 12) and sham-treated animals (n = 5). The activity of individual metabolic enzymes was determined and corrected for total protein content (A, B). Western blot was performed to determine protein content of proteins of interest (B, D, E) and metabolic gene expression was determined by qPCR (A–C). Western blots were corrected for α-tubulin. * $p \leq 0.05$ compared to control.

inflammatory NF-κB pathway, this may point to a potential role for NF-κB in VM-induced changes in cardiac metabolic gene expression.

Our transcriptome analysis revealed a potent up-regulation of multiple constituents and target genes of the NF-κB pathway in cardiac biopsies of VM patients as well as in cardiac tissue of VM mice (Fig. 5D). To confirm these data, expression levels of various known NF-κB target genes were analysed by qPCR and found to be dramatically induced during CVB3-induced VM (Fig. S9A). Also, cardiac expression levels of key constituents of NF-κB signalling itself were increased (Fig. 5E) and correlated with the degree of cardiac immune cell influx (Table S4). Moreover, immunohistochemical staining for RelA (v-rel avian reticuloendotheliosis viral oncogene homolog A), the main transcriptionally-active subunit of the NF-κB pathway, showed prominent NF-κB activation in cardiomyocytes during CVB3-induced VM (Fig. 5F). This was further confirmed by the increased expression levels of constituents and target genes of the NF-κB pathway in cardiomyocytes isolated from VM hearts (Fig. S9B).

To further explore a potentially direct detrimental impact of TNF-α

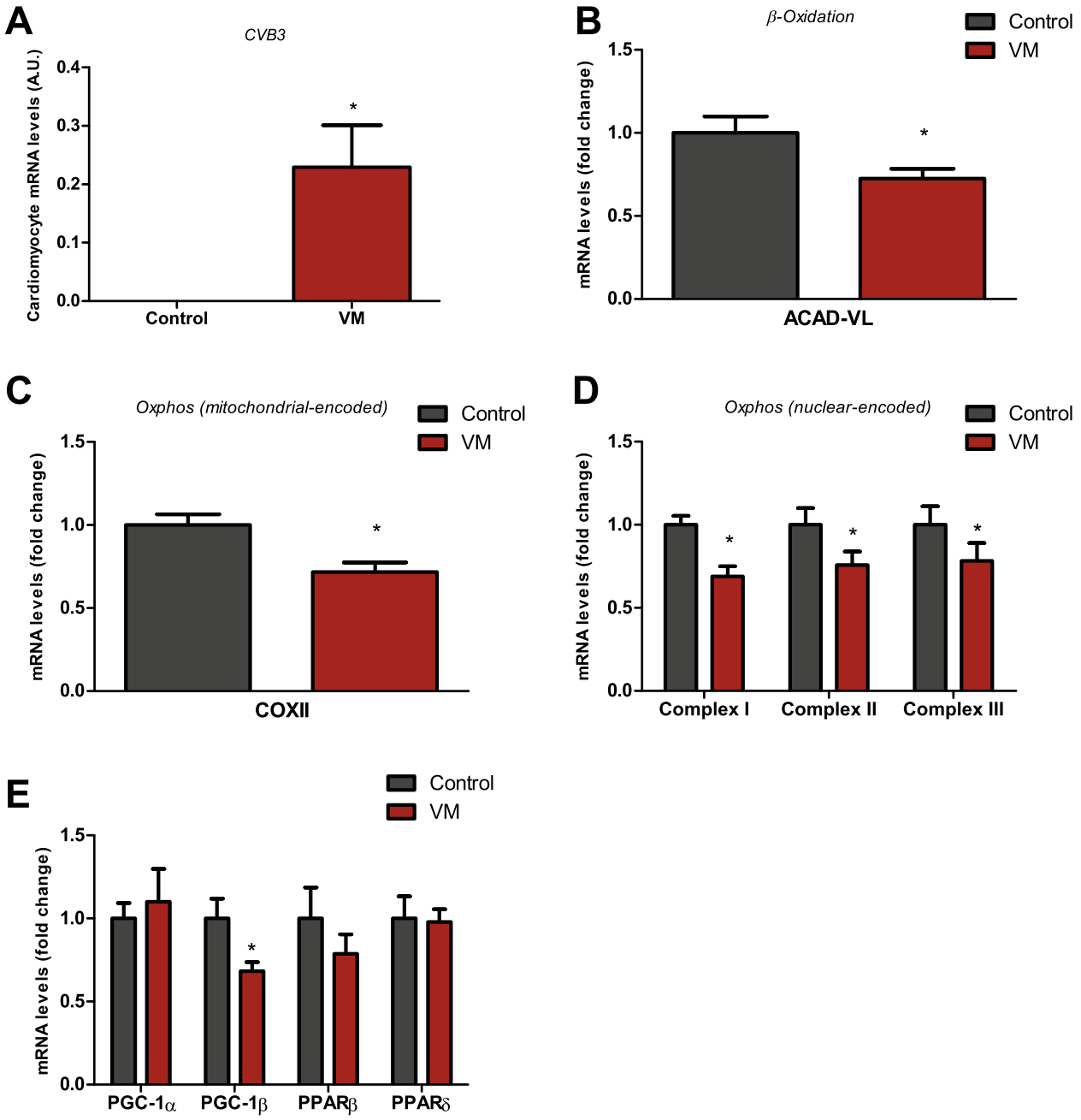

Fig. 4. Cardiomyocyte metabolic gene expression in VM. Cardiomyocytes were isolated from hearts of mice with CVB3-induced VM (n = 8) and sham-treated animals (n = 8) and CVB3 load was determined (A). qPCR analyses revealed down-regulation of genes involved in oxidative phosphorylation (B, C), fatty acid β-oxidation (D) and PGC-1 signalling (E). * $p \leq 0.05$ compared to control.

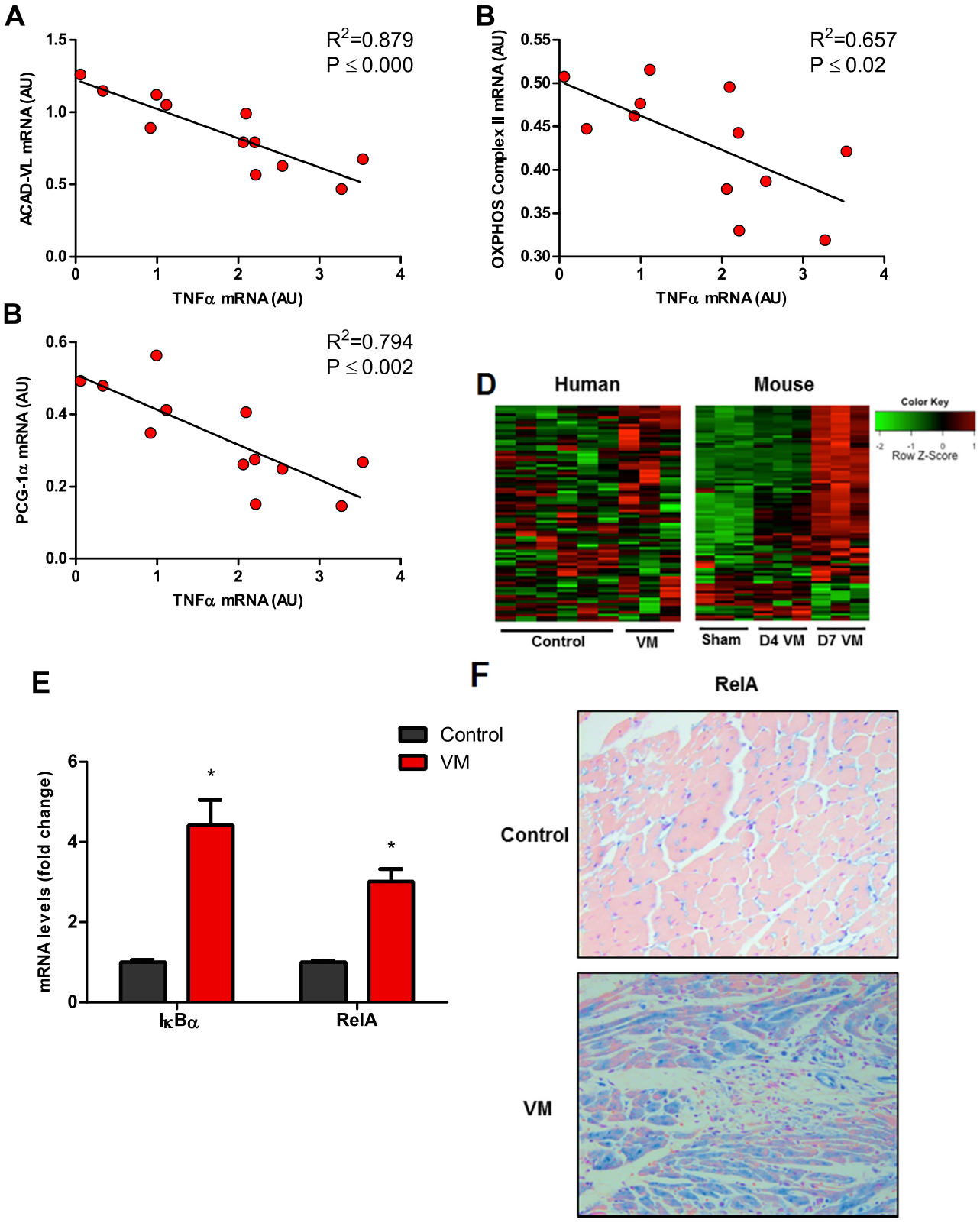

Fig. 5. Activation of NF-κB signalling in cardiomyocytes during VM. Correlations between cardiac TNF-α expression and expression of constituents of fatty acid β-oxidation (A), oxidative phosphorylation (B) and PGC-1 signalling (C) were explored in mice with CVB3-induced VM (n = 12). Heat map analysis revealed activation of NF-κB signalling in right ventricular septal biopsies from patients with viral myocarditis (VM) (n = 3) compared to controls (n = 6) as well as in cardiac tissue of mice with CVB3-induced VM (n = 3) compared to sham-treated animals (n = 3) especially 7 days post-inoculation (D). Gene expression of NF-κB signalling constituents was induced in cardiac tissue of mice with CVB3-induced VM (n = 12) compared to sham-treated animals (n = 5) (E). Immuno-histochemical staining of RelA on sections from whole hearts from sham-treated mice (n = 5) and mice with CVB3-induced VM (n = 9) demonstrated activation of NF-κB in cardiomyocytes (F). *p ≤ 0.05 compared to control.

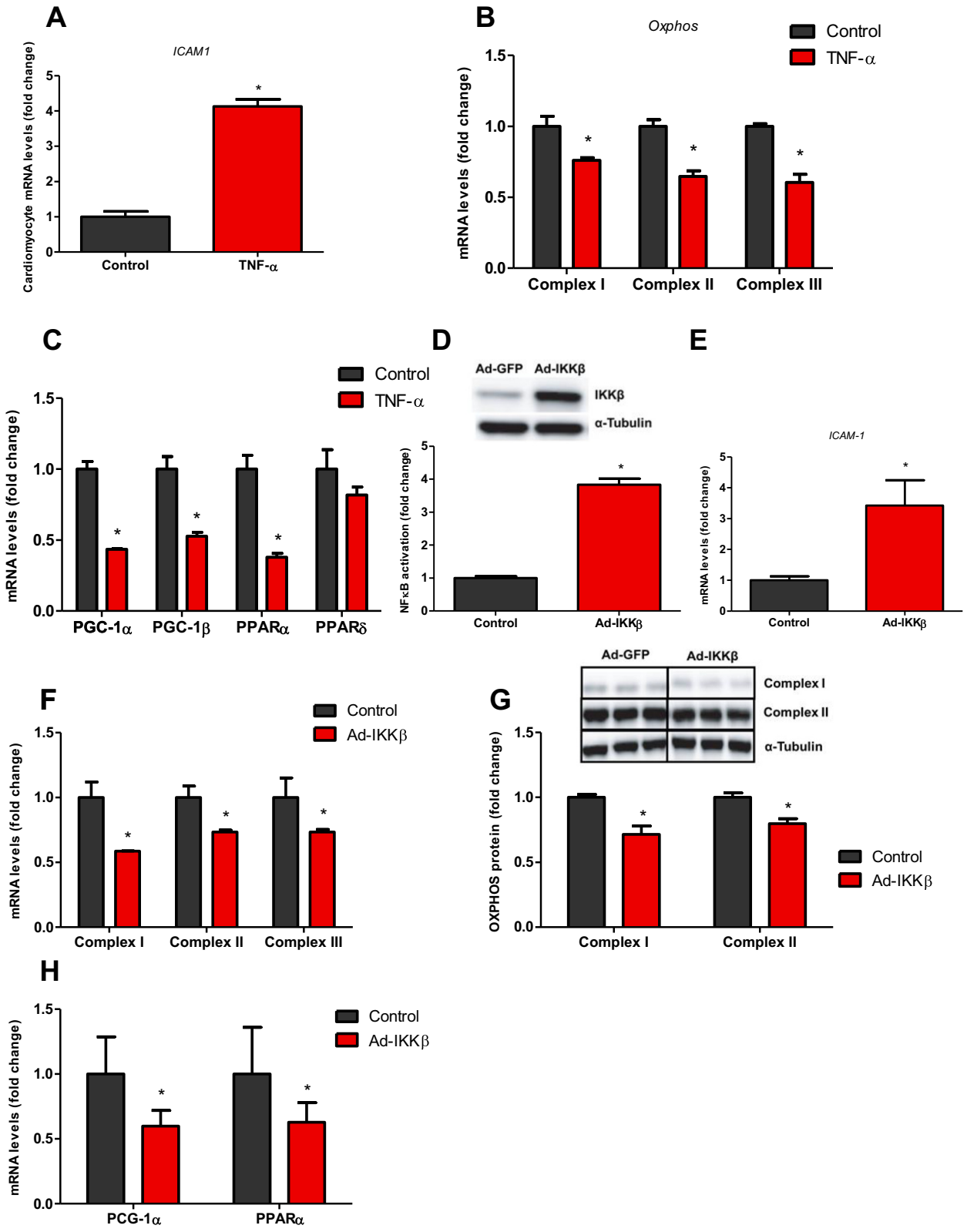

Fig. 6. Activation of NF-κB signalling impairs cardiomyocyte oxidative gene expression. HL-1 cardiomyocytes were stimulated with TNF-α (10 ng/ml) for 48 h and expression levels of ICAM-1(A), OXPHOS sub-units (B) and PGC-1/PPAR (C) were determined. HL-1 cardiomyocytes were subjected to adenoviral over-expression of IKKβ or an adenoviral construct expressing GFP as a control for 48 h. IKKβ protein content, NF-κB transcriptional activity (D), ICAM-1 mRNA (E), OXPHOS mRNA (F) and OXPHOS protein content (G) as well as PGC-1/PPAR mRNA expression was investigated (H). *$p \leq 0.05$ compared to control.

and subsequent activation of the NF-κB pathway on the regulation of cardiomyocyte oxidative metabolism, we stimulated cultured cardiomyocytes with TNF-α. As expected, TNF-α induced inflammatory gene expression (Fig. 6A). In addition, TNF-α significantly reduced abundance of subunits of oxidative phosphorylation complexes I, II and III and of several constituents of the PGC-1 signalling network (Fig. 6B, C). Moreover, TNF-α decreased transcriptional activity of NRF-1 (84.4% ± 2.8 *vs* 100.0% ± 5.1; $P \leq 0.05$) and reduced Tfam promoter transactivation (86.6% ± 4.8 *vs* 100.0% ± 4.7; $P \leq 0.05$), as assessed by transient transfection reporter experiments.

To examine whether NF-κB activation as such (in absence of inflammatory cytokines) yielded similar results, we activated NF-κB signalling in cultured cardiomyocytes by adenoviral over-expression of IKKβ, one of the key kinases involved in activation of the NF-κB pathway. IKKβ over-expression increased NF-κB transcriptional activity and mRNA levels of Intra-Cellular Adhesion Molecule-1 (ICAM-1), a well-known NF-κB target gene (Fig. 6D, E). In addition, mRNA as well as protein content of oxidative phosphorylation complexes decreased in response to IKKβ-induced NF-κB activation (Fig. 6F, G). Also, IKKβ over-expression was sufficient to decrease PGC-1α, PGC-1β and PPAR-α transcript abundance similar to what was observed in response to TNF-α (Fig. 6H). IKKβ over-expression induced similar changes in oxidative metabolic gene expression in cultured rat neonatal cardiomyocytes (Fig. S10A–C).

Collectively, these data suggest that (cytokine-induced) activation of the NF-κB signalling pathway impairs cardiomyocyte oxidative metabolic gene expression, presumably through interference with the PGC-1 signalling circuitry.

3. Discussion

This is the first study showing that acute VM, in both patients and in mice, is associated with extensive remodelling of metabolic pathways in the heart. Our findings indicate that metabolic changes observed at the level of the entire transcriptome are reflected by parallel changes at the protein, enzyme activity and metabolite level. The dysregulation of mitochondrial oxidative metabolism, likely resulting from inflammation-induced activation of NF-κB and subsequent inhibition of the PGC-1 nuclear receptor network, culminates in an energy-starved status of the heart, the extent of which likely impacts cardiac function.

We show that active VM in humans, as defined by virus levels and abundance of inflammatory cells in the heart, was associated with profound changes in cardiac metabolic gene expression, basically affecting multiple aspects of mitochondrial oxidative metabolism. These transcriptomic changes were even more prominent in CVB3-infected mice. The extent of the transcriptomic changes in acute VM, in terms of the number of genes involved and the magnitude of change appears to be far more extensive than in heart failure [4]. Only 2 studies previously investigated parameters of mitochondrial metabolism in a mouse model of CVB3-induced VM. Xu et al. showed that cardiac mRNA levels of genes involved in fatty acid metabolism and mitochondrial oxidative phosphorylation were reduced [7]. However, these authors used a model of persistent, instead of acute, VM (> 100 days post-infection) characterised by marked hypertrophic, fibrotic remodelling and cardiac dilatation. In contrast, Ebermann et al. [8] reported reduced activity of respiratory chain complexes 8 days post CVB3-infection, i. e. in the acute phase of VM. The latter observation is in line with our findings as we show decreased mRNA transcript abundance and protein levels of several OXPHOS complexes and reduced activity of complex IV as well as reductions in cardiolipin, a mitochondrial phospholipid species required for the activity of the electron transport chain [11], in acute VM. In our study these changes were paralleled by reduced activity and transcript levels of the rate-limiting enzyme of the fatty acid β-oxidation (HAD) highlighting that, besides abnormalities in respiratory chain activity, concurrent impairments in fatty acid β-oxidation are evident in acute VM.

Derangements in cardiac substrate- and energy metabolism, resulting in an 'energy-starved' heart, have been postulated to play an important causal role in the development of heart failure [4]. Reductions in cardiac ATP and total adenine nucleotide levels in particular, as we observed in our study, are considered hallmarks of disease progression in chronic heart failure [12]. We show that in acute VM the changes observed at the level of the entire transcriptome were paralleled by reductions in abundance and/or activity of key constituents of cardiac oxidative metabolism and an increased phosphorylation status of the cellular energy sensor AMPK [4]. Importantly, acute VM was associated with severe myocardial energy deprivation as evidenced by a marked reduction in ATP levels and exhaustion of the total adenine nucleotide pool in VM hearts as assessed by MALDI-IMS *in situ*.

In contrast to the earlier studies reporting changes at the whole heart level, our *in situ* metabolic imaging data as well as the changes in metabolic gene expression observed in cardiomyocytes isolated from VM hearts, provide evidence that metabolic remodelling observed in the heart actually takes place in cardiomyocytes. Hence, the observed changes at the tissue level do not simply reflect the contribution of infiltrating immune cells which highly rely on glycolysis for energy generation and anabolic processes [13]. The glycolytic dependency of macrophages was reflected by the high levels of UDP-*N*-acetylglucosamine in the inflammatory infiltrates. UDP-*N*-acetylglucosamine is an intermediate in the hexosamine biosynthetic pathway and was recently shown to be increased in M2-polarized macrophages in particular [14]. This metabolite was subsequently used as a metabolic marker for inflammatory infiltrates in the heart. In this regard, the increased expression of several glycolytic genes, most of which are down-stream targets of HIF signalling, in cardiomyocytes isolated from VM hearts also supports a cardiomyocyte-specific metabolic remodelling process away from oxidative metabolism towards a greater reliance on (anaerobic) glycolysis.

Our *in vivo* and *in vitro* data collectively show that the VM-induced aberrations in cardiomyocyte mitochondrial oxidative metabolism are associated with activation of the inflammatory NF-κB pathway. Indeed, during VM we observed that expression of TNF-α in the heart correlated strongly and inversely with abundance of key constituents and molecular regulators of mitochondrial oxidative metabolism, suggesting that inflammatory cytokines, such as TNF-α, may act as potential drivers of cardiac metabolic remodelling during VM. The observation that stimulation of cultured cardiomyocytes with TNF-α, but not exposure to CVB3 virus *per se*, impaired oxidative metabolic gene expression further strengthens this notion. As expression of TNF-α mRNA correlated strongly with the degree of cardiac immune cell influx, infiltrating immune cells likely are the main source of inflammatory cytokines in the heart during VM. However, cardiomyocytes themselves may well contribute to the ensuing inflammatory response in the heart as expression levels of various inflammatory cytokines were increased in cardiomyocytes isolated from hearts of animals with VM. These observations are in line with other *in vivo* studies demonstrating that cardiac-specific over-expression of TNF-α impairs mitochondrial oxidative metabolism [15]. Indeed, TNF-α as well as other members of the TNF superfamily, like TRAIL, have been implicated in cardiomyocyte metabolic remodelling and the development of cardiac dysfunction [16].

Earlier we and others showed that RelA (p65), the main transcriptionally-active sub-unit of the inflammatory NF-κB signalling pathway, interferes with the molecular regulation of cellular oxidative metabolism by impeding on the abundance and activity of key molecules in the PGC/PPAR pathway in skeletal muscle and heart [5] [17–19]. This is in line with our findings as we show that activation of NF-κB signalling during VM was associated with decreases in protein levels of Tfam and NRF-1 as well as reduced transcript abundance of PGC-1 molecules. Interestingly, mRNA abundance of PPAR-δ, a key regulator of mitochondrial fatty acid metabolism, was increased suggesting a compensatory cellular response to activate mitochondrial

oxidative metabolism in response to cardiac energy depletion, a phenomenon also observed in experimental models of hypertrophic heart disease and heart failure [6]. In our study, alterations in mitochondrial metabolic pathways and its molecular regulation by the PGC-1/PPAR network were observed within a few days after viral infection, suggesting that perturbations in cardiac energy metabolism in VM do not arise secondary to cardiac structural remodelling but likely contribute to impairment of cardiac function during disease progression.

Illustrative of its significance, blockade of the NF-κB pathway improved cardiac function and survival during TNF-α-induced cardiomyopathy. *Vice versa*, activation of NF-κB signalling, by cardiac-specific transgenic over-expression of IKKβ, was sufficient to induce foetal reprogramming of cardiomyocytes, cardiomyopathy and heart failure [20,21]. Together with our findings, this suggests that NF-κB activation likely constitutes an important etiological factor involved in the adverse cardiac metabolic remodelling observed during acute VM. Given the changes in expression and activity levels of down-stream targets of PGC-1 signalling, this most likely occurs as a consequence of down-regulation of this signalling network. Interestingly, this appears relevant for other forms of cardiac disease as NF-κB activation has also been shown in other experimental models of heart failure associated with cardiac metabolic remodelling [22].

In addition to abnormalities in key parameters and regulators of cardiac oxidative metabolism, we show that NF-κB activation during CVB3-induced VM is also associated with increased expression of glycolytic genes and elevated levels of HIF-1α and HIF-1α target genes, suggesting compensatory activation of a glycolytic gene program [23]. This is in line with current understanding of these pathways as interactions between the inflammatory NF-κB pathway and the hypoxia-sensitive HIF-1α pathway have been described [24–26]. Accordingly, besides its well established role in regulating immune and inflammatory responses, the NF-κB pathway is increasingly being recognized as a central regulator of energy homeostasis *via* direct interaction with cellular networks that govern glycolysis and mitochondrial respiration [27].

Given the derangements in key constituents of metabolic pathways that we observed in mice and humans suffering from active VM and the notion that correcting cardiac metabolism may offer relief to the diseased heart [28,29], the current findings imply that restoring cardiac energy metabolism may help to improve cardiac function in VM, either directly by stimulating the activity of upstream regulators of cardiac metabolism, such as PGC-1, or indirectly *via* the targeted inhibition of the NF-κB pathway. In this regard, inhibition of NF-κB signalling has already been shown to improve outcome in experimental models of myocarditis by attenuating inflammatory responses in the heart and by inhibiting viral replication in lymphoid cells [30,31]. It remains to be established, however, if this is through its anti-inflammatory and/or metabolic effects.

In conclusion, collectively our findings support the concept that cytokines, including TNF-α, released from inflammatory cells infiltrating the infected myocardium during VM, activate NF-κB within the cardiomyocyte. This leads to a reduction in oxidative metabolism and reduced ATP production in the affected myocardium. It is well feasible that these disturbances in energy generating capacity significantly contribute to the observed depression of cardiac contractile function during acute VM.

4. Methods

Detailed information regarding patient characteristics, animal studies, *in vitro* studies and material and methods is available in the supplementary material online. The acquisition of human samples and clinical parameters has been described previously [9]. In short, all human material was obtained during routine clinical sampling and available for research purposes in accordance with the Declaration of Helsinki and the ethical committee at Maastricht University. Written consent was obtained from all subjects. All mice were bred and maintained in an open animal facility (University of Leuven, Belgium) and experiments were performed according to the guidelines for the care and use of laboratory animals approved by the institution animal committee of the University of Leuven (ECD 2432013 or 067/2008). Mice were sacrificed and the heart and other organs were excised and stored in liquid nitrogen for analysis including, transcriptome analysis, Matrix-Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI-IMS), qPCR, Western blotting, measurements of enzyme activities and histology.

Transparency document

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Author contributions

AR, WD and MB designed the experiments. WD, AR, AP and PC performed the experiments. BC, RH and SE were involved in MALDI-MS analyses while MK, CT and KV were involved in QPCR, Western blot, enzyme activity assays, immunohistochemistry and luciferase experiments. SG performed pathway analyses and SH, AP and WH were involved in transcriptome analyses on human and mouse VM material. PC performed Echocardiography. WD, AR and MB wrote the manuscript.

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Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2018.04.022.

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