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11. Inflammation and metabolic dysregulation in diabetic cardiomyopathy

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Abstract. Diabetic cardiomyopathy is characterized by structural and functional alterations in the heart muscle of people with diabetes that finally lead to heart failure. Metabolic disturbances characterized by increased lipid oxidation, intramyocardial triglyceride accumulation and reduced glucose utilization have all been involved in the pathogenesis of diabetic cardiomyopathy. On the other hand, evidences arisen in the recent years point to a potential link between chronic low-grade inflammation in the heart and metabolic dysregulation. Interestingly, the progression of heart failure and cardiac hypertrophy usually entails the activation of pro-inflammatory pathways. Therefore, in this chapter we summarize novel insights into the crosstalk between inflammatory processes and metabolic dysregulation in the failing heart during diabetes.

Introduction

The human heart produces, and immediately hydrolyzes, approx. 30 kg of ATP every day to carry out the mighty job of pumping more than 7,000 L/day

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of blood. Therefore, it is not surprising that any abnormality that deregulates its proper working may become life-threatening. In fact, heart disease is the major cause of death for both men and women in developed countries and, according to the World Health Organization, by 2020 it is expected to be the leading cause of death throughout the world. Heart disease is often grouped with metabolic disorders because it is frequently a consequence of uncorrected type 2 diabetes and obesity-related dyslipidemia. Insulin resistance, which is a hallmark of type 2 diabetes, is a risk factor of heart failure, the leading cause of death in type 2 diabetic patients. Diabetic cardiomyopathy, which refers to structural and functional alterations in the heart muscle of people with diabetes that finally lead to heart failure, is related to disturbances in myocardial energy metabolism. Diabetic cardiomyopathy is only said to exist if heart failure is not accompanied by coronary artery disease or hypertension that may account for the heart muscle disorder.

An increasing body of evidence suggests a potential link between chronic low-grade inflammation and metabolic disorders that are associated with abnormal cytokine production and increased levels of saturated fatty acids, such as insulin resistance, type 2 diabetes and obesity. The progression of heart disease usually entails a local rise in pro-inflammatory cytokines, including interleukin (IL)-6, monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-α (TNF-α) [1]. These molecules exert several autocrine and paracrine effects in cardiac cells via downstream activation of the transcription factor nuclear factor (NF)-κB, which may contribute to states that are associated with myocardial inflammation, for example heart failure and dilated cardiomyopathy. However, the underlying mechanisms linking inflammation, heart failure and dilated cardiomyopathy are complex, since they are coupled to systemic metabolic abnormalities and changes in cardiomyocyte phenotype. In this review we summarize recent insights into the crosstalk between inflammatory processes and metabolic dysregulation in the failing heart during diabetes.

1. Metabolic regulation in the healthy heart

Free fatty acids are the preferred energy substrate in the adult heart, supplying about 70% of total ATP [2]. However, since the human heart requires a constant supply of fuel, other substrates such as glucose (20%) or lactate (10%) may provide additional fuel sources in diverse physiological and nutritional circumstances. Glucose uptake is mostly

regulated by glucose transporters (GLUTs), of which GLUT4 is the most abundant in cardiac cells (Fig. 1) [3]. Once inside the cytosol, glucose is phosphorylated into glucose-6-phosphate, which can either be stored as glycogen or converted into pyruvate, the end product of glycolysis. Then, pyruvate enters the mitochondria where it undergoes oxidative decarboxylation by the pyruvate-dehydrogenase complex (PDC), which catalyses the rate-limiting step of glucose oxidation. Specific PDC kinases (PDKs) are responsible for the phosphorylation-induced inactivation of PDC. As such, PDK4, the most expressed PDK isoform in the heart, decreases glucose oxidation while allowing increased fatty acid β-oxidation. With regard to the fatty acids, they cross the cardiomyocyte cell membrane through passive diffusion or by specific transport proteins, including fatty acid translocase (FAT/CD36), fatty acid binding protein (FABP) and fatty acid transport protein 1 (FATP1). In the cytoplasm, fatty acids are acylated, and either enter the mitochondria by the action of carnitine palmitoyl transferase 1 (CPT-1) or are incorporated into the intracellular lipid pool in the form of triglycerides. In the mitochondrial matrix, fatty acids are oxidized by the β-oxidation pathway to form acetyl-CoA. At this point, pathways for glucose and fatty acid oxidation merge, since acetyl-CoA, which is produced from both pathways, enters the tricarboxylic acid (TCA) cycle. Finally, reduced flavin adenine dinucleotide ($FADH_2$) and nicotinamide adenine dinucleotide (NADH) generated through β-oxidation and the TCA cycle, respectively, carry electrons to the electron transport chain, thus driving ATP synthesis by oxidative phosphorylation.

Under physiological conditions, glucose utilization is reduced in the heart by increased fatty acid oxidation via the Randle cycle. After a high carbohydrate supply, insulin activates the protein kinase B (PKB)/Akt signalling pathway and subsequent translocation of GLUT4 from intracellular vesicles towards the sarcolemma, the cell membrane of cardiac cells. As such, insulin promotes glucose uptake and utilization, thereby reducing myocardial oxygen consumption and increasing cardiac efficiency [4]. Insulin also enhances the uptake of fatty acids, but instead of being oxidized, they are stored in the intracellular pool of lipids. The AMP-activated protein kinase (AMPK) is another important regulator of cardiac energy homeostasis. Conditions leading to energy depletion, for instance the failing heart or increased ATP demand, lead to AMPK activation, which switches off energy-consuming pathways and stimulates ATP-producing pathways such as fatty acid β-oxidation and glycolysis.

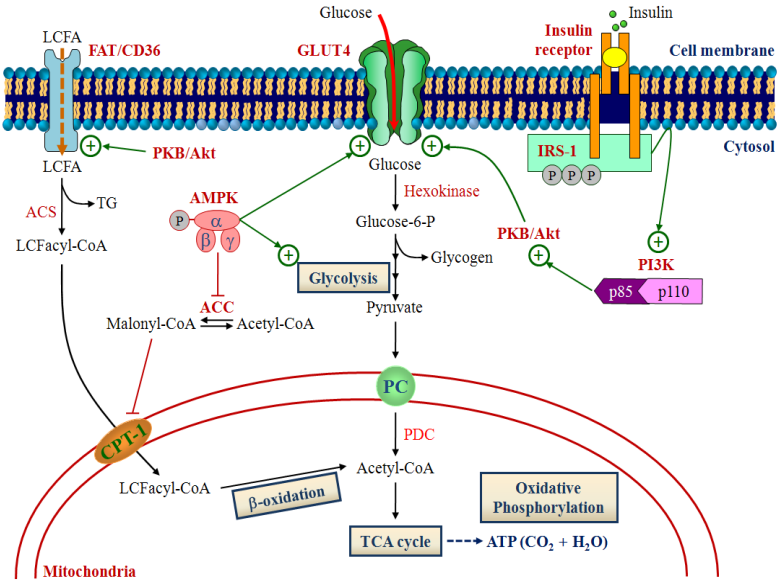

Figure 1. Metabolic regulation in the normal heart. Glucose, after uptake by specific transporters (GLUT4), is phosphorylated and can either be stored as glycogen or converted into pyruvate (glycolysis). In the mitochondria, pyruvate undergoes oxidative decarboxylation by the pyruvate-dehydrogenase complex (PDC). Long-chain fatty acids (LCFA) that enter into the cytoplasm by passive diffusion or specific transporters (FAT/CD36) are acylated by acyl-CoA synthetase (ACS), and either enter the mitochondria (carnitine palmitoyl transferase 1, CPT-1), or are incorporated into triglycerides (TG). In the mitochondrial matrix, fatty acids undergo β-oxidation to form acetyl-CoA. The acetyl-CoA formed during glucose or fatty acid oxidation enters the tricarboxylic acid (TCA) cycle and finally generates ATP through oxidative phosphorylation. Initiation of the insulin receptor substrate 1 (IRS-1) cascade by insulin induces the phosphatidylinositol 3 kinase (PI3K), which activates the protein kinase B (PKB)/Akt. AMPK promotes myocardial GLUT4 activity and stimulates glycolytic enzymes, while it induces CPT-1 activity through acetyl-CoA carboxylase (ACC) phosphorylation-mediated inhibition.

Cardiac metabolism is mostly regulated at the transcriptional level by the peroxisome proliferator-activated receptor (PPAR) transcription factor family, which consists of three isoforms, PPARα (or NR1C1), PPARβ/δ

(NR1C2) and PPARγ (NR1C3). The PPAR family of ligand-activated nuclear receptors require heterodimerization with another nuclear receptor, the 9-*cis*-retinoic acid receptor (RXR or NR2B), in order to be activated. Heterodimerization is then followed by coactivator recruitment and subsequent binding to DNA specific sequences called PPAR-response elements (PPRE), located within the promoter regions of PPAR target genes. Notable among the PPAR target genes with identified PPREs are those coding for lipid and glucose homeostasis. PPARα and PPARβ/δ are the predominant isoforms in the heart, where they share some overlapping functions [5]. Transgenic mice with constitutive overexpression of PPARβ/δ in the heart show increased myocardial glucose utilization, do not accumulate myocardial lipids and display normal cardiac function [6]. Another study has reported that constitutive cardiac overexpression of PPARβ/δ in adult mice results in elevated myocardial oxidative metabolism, while myocardial glycogen content and the activity of the PDC and AMPK are markedly reduced, overall resulting in improved cardiac function [7]. In agreement with this, cardiomyocyte-restricted PPARβ/δ deficiency in mice leads to cardiac pathological development [8]. The best-characterized coactivator of PPARs is the cardiac-enriched PPARγ coactivator-1 (PGC-1)α, which regulates the expression of several genes involved in the electron transport chain, mitochondrial biogenesis, fatty acid β-oxidation and glucose oxidative metabolism, including *PDK4*. *PDK4* gene expression is known to be controlled by a plethora of different transcription factors. Some of them are coactivated by PGC-1α: PPARs, oestrogen-related receptors (ERRs) and the forkhead transcription factor (FOXO1 or FKHR); but others acting in a PGC-1α-independent manner: E2F1, LXR (liver X receptor) or RXR (retinoid X receptor).

2. Metabolic remodelling in the development of diabetic cardiomyopathy

The heart has the capacity to adapt to various pathophysiological conditions by adjusting its relative metabolism of carbohydrates and fatty acids. The loss of this metabolic flexibility is associated with pathological cardiac hypertrophy and heart failure. During diabetes, the occurrence of insulin resistance in the myocardium, together with increased rates of systemic lipolysis, means that the heart relies almost exclusively on mitochondrial fatty acid β-oxidation as the sole fuel source [6]. Reduced

myocardial glucose uptake and utilization owing to altered insulin signalling may account for the loss of this capacity to switch between glucose and fatty acids in insulin-resistant and insulin-deficient forms of diabetes. Despite the higher fatty oxidation rate that occurs in the diabetic heart, myocardial lipid accumulation is a feature of this disease. The heart is not a major site of lipid storage, but fatty acids can be stored as triacylglycerols and phospholipids within the cardiomyocyte, particularly when they are abundant, as occurs with diabetes or obesity. If this accumulation persists over time, the heart will begin to accumulate toxic lipid intermediates linked to the development of insulin resistance and lipotoxic cardiomyopathy. In fact, there is a strong correlation between intracardiac lipid accumulation and heart failure in humans [9].

Metabolic dysregulation occurring in the heart of obese and diabetic patients often involves derangements in the activity of both PPARs and PGC-1α. The expression and activity of PPARα and PGC-1α are increased in the early stages of insulin resistance in the heart [10], thus promoting excessive utilization of fatty acids instead of glucose, and driving diabetic cardiomyopathy [6]. In contrast, in overt diabetes the expression and activity of PPARα and PGC-1α are reduced, inducing mitochondrial dysfunction and cardiac hypertrophy [11]. Some debate exists about the role of PGC-1α during diabetes, since it has been reported to be down-regulated in the myocardial tissue of streptozotocin-induced diabetic rats, a fact which is accompanied by a reduction in left ventricular function [12], but also activated in streptozotocin-induced mice [13]. PPARβ/δ also plays an important role in diabetic cardiomyopathy. It has been demonstrated that deletion of the *PPARβ/δ* gene in the heart results in cardiac dysfunction, cardiac hypertrophy and myocardial lipid accumulation [14]. Diabetic cardiomyopathy induced with streptozotocin in rats is associated with ventricular hypertrophy, intramyocardial lipid accumulation and a marked decrease in cardiac PPARβ/δ protein levels [15]. Furthermore, PPARβ/δ protein levels are reduced in neonatal rat cardiomyocytes and rat H9c2 cardiomyoblasts exposed to hyperglycaemia [15].

Transgenic mice with cardiac-specific overexpression of PDK4, and thus with chronic suppression of glucose oxidation, exhibit an insulin-resistant profile characterized by low glucose oxidation rates and high fatty acid catabolism [16]. This shift in the substrate preference constrains metabolic flexibility and predisposes to cardiomyocyte fibrosis and cardiomyopathy. Interestingly, transgenic PDK4 mice are protected against

high-fat diet-induced myocyte lipid accumulation, probably owing to their increased capacity for mitochondrial fatty acid oxidation. This is related to the activation of AMPK and its known target, PGC-1α, and the increased capacity for uncoupled mitochondrial respiration.

3. The role of NF-κB-induced inflammation in diabetic cardiomyopathy

Under various pathological stimuli, the human myocardium secretes a number of pro-inflammatory cytokines and chemokines, which exert several pleiotropic effects in cardiac cells. Sustained increases in their levels may contribute to states that are associated with myocardial inflammation (i.e. heart failure and dilated cardiomyopathy) [17]. Pro-inflammatory cytokine expression is under the control of the ubiquitous and inducible transcription factor NF-κB, which is itself activated in congestive heart failure and cardiac hypertrophy [18]. Several exogenous and endogenous stimuli may induce NF-κB transcriptional activity, notably the pro-inflammatory cytokines themselves, hyperglycaemia, elevated free fatty acid levels in plasma, reactive oxygen species, angiotensin II, endothelin 1, lipoproteins and anoxia.

In mammals, NF-κB consists of five members: p65 (RelA), RelB, c-Rel, NF-κB1 (p50 and its precursor p105) and NF-κB2 (p52 and its precursor p100), which form either homodimers or heterodimers. The most abundant form of the NF-κB family is the p65/p50 heterodimer, which is often used synonymously for NF-κB. In resting cells, NF-κB is present in the cytoplasm as an inactive heterodimer bound to an inhibitor protein subunit, IκB. After stimulation, the canonical or classical pathway of NF-κB signalling involves the activation of the IκB kinase (IKK) complex, which specifically phosphorylates IκB. Phosphorylation of IκB induces its subsequent proteasome-mediated degradation, thus releasing the NF-κB heterodimer, which then translocates to the nucleus and binds to specific promoter sequences on its target genes to begin the transcription machinery. Furthermore, the p50/p65 heterodimer may undergo a series of post-translational modifications including phosphorylation, acetylation and methylation, which allow finely-tuned regulation of transcriptional activity.

The main cardiac responses to diabetes are oxidative stress, inflammation, endothelial dysfunction, cardiac fibrosis, hypertrophy and

apoptosis, in all of which NF-κB may participate. Cardiac inflammation is an early and notable response to diabetes and is actively involved in the development of heart failure during diabetic cardiomyopathy [19]. Several pro-inflammatory cytokines that have elevated levels in circulation in obese and diabetic patients, such TNF-α and IL-6, are involved in the physiopathological process that relates obesity and insulin resistance in the heart [2].

4. Crosstalk between inflammation and metabolism in the diseased heart

4.1. PPARβ/δ activation blocks lipid-induced inflammation in the heart

In the heart, excess dietary fat may result in myocardial insulin resistance, and is related to a range of direct effects, including inflammation, hypertrophy, fibrosis and contractile dysfunction. PPARs are capable of limiting myocardial inflammation independently of binding to DNA, through a mechanism termed trans-repression. Several mechanisms have been proposed to explain these anti-inflammatory effects, for example the physical interaction between PPARs and NF-κB, thereby resulting in a functional cross-inhibition of their transcriptional activity. In the heart of mice fed a high-fat diet or in human cardiac AC16 cells, treatment with the saturated fatty acid palmitate induced the expression of *TNF-α*, *MCP-1* and *IL-6*, together with the activity of NF-κB (Fig. 2A) [20]. Interestingly, the PPARβ/δ agonist GW501516 abrogated this pro-inflammatory profile. Similarly, NF-κB-dependent inflammation was induced in the heart of PPARβ/δ knockout mice, a fact which is consistent with the anti-inflammatory activity of PPARβ/δ [20]. This latter study reported that PPARβ/δ activation by GW501516 strongly enhanced the physical interaction between the p65 subunit of NF-κB and PPARβ/δ (Fig. 2B), thereby suggesting that this mechanism may also interfere with NF-κB trans-activation capacity in the heart. Furthermore, addition of GSK0660 partially blocked the enhanced interaction between p65 and PPARβ/δ in AC16 cells, thus linking this physical interaction with PPARβ/δ availability in the nucleus and its subsequent activity. Likewise, PPARβ/δ activation with its agonists L-165041 or GW0742 inhibits phenylephrine- and lipopolysaccharide-induced NF-κB activation in cultured neonatal rat cardiomyocytes and H9c2 cells through enhanced physical interaction between p65 and PPARβ/δ [21,22]. Other studies also

demonstrate that PPARβ/δ activation by ligand administration or adenoviral overexpression in cultured cardiac myocytes suppresses the NF-κB signalling pathway and displays potent anti-inflammatory effects [13,23].

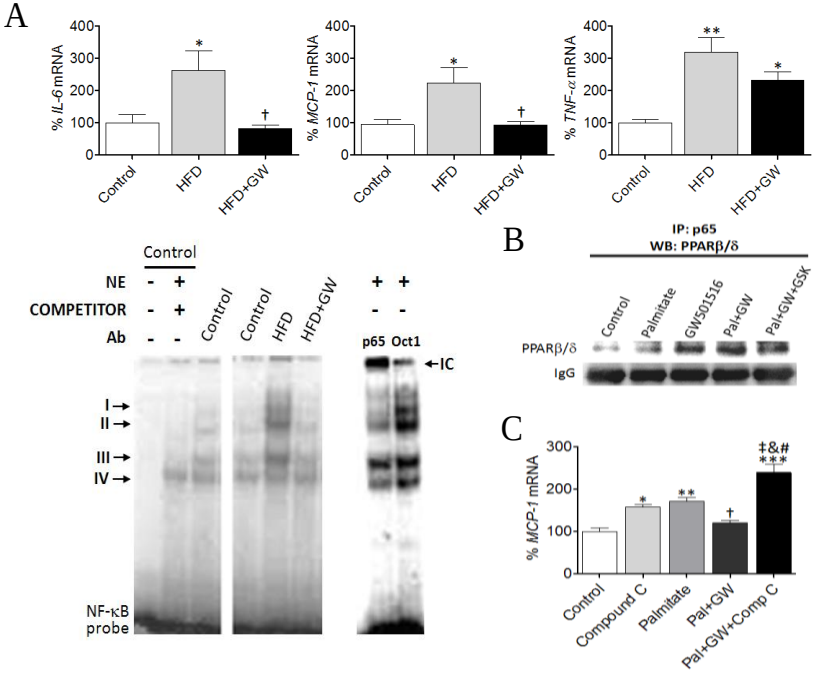

Figure 2. The PPARβ/δ agonist GW501516 (GW) prevents inflammation in the heart of mice fed a high-fat diet (HFD). (A) mRNA levels assessed by real-time RT-PCR (*upper panel*) and electrophoretic mobility shift assay showing NF-κB activity (*lower panel*, Ab, antibody; NE, nuclear extract; IC, immunocomplex). *P<0.05, **P<0.01 vs. Control; †P<0.05 vs. HFD. (B) Co-immunoprecipitation of nuclear protein extracts obtained from AC16 cells treated with palmitate (Pal), GW501516 (GW) and GSK0660 (GSK). (C) *MCP-1* mRNA levels assessed by real-time RT-PCR in AC16 cells treated with palmitate, GW501516 and compound C (Comp C). *P<0.05, **P<0.01 vs. Control; †P<0.05, ‡P<0.01 vs. Palmitate; &P<0.001 vs. Pal+GW.

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It has been reported that GW501516 is able to regulate lipid and glucose metabolism in human skeletal muscle by AMPK-dependent, but PPARβ/δ-independent, mechanisms [21]. Since activation of AMPK may block NF-κB signalling pathway through the blockade of IKK activity [22], it might be feasible that GW501516 was blocking lipid-induced inflammatory pathways in cardiac cells through AMPK-dependent mechanisms. An experiment in which the AMPK inhibitor compound C was added before GW501516 and palmitate confirmed the anti-inflammatory role of this kinase in human cardiac AC16 cells (Fig. 2C). These results are relevant, especially taking into account that PPARβ/δ has been postulated as a potential target in the treatment of obesity and the insulin resistance state. Since chronic low-grade inflammation plays a significant role in cardiac hypertrophy and heart failure, and GW501516 has been shown to ameliorate metabolic disturbances in heart caused by high-fat diets, it is tempting to speculate that PPARβ/δ might serve as a therapeutic target to prevent cardiac disease in metabolic disorders.

4.2. The role of the PPAR/PGC-1α/PDK4 axis in the crosstalk between inflammatory processes and metabolism

Cardiac *PGC-1α* expression, along with its target transcription factors PPARs and ERRα, are all reduced in animal models of heart failure [23,13] and in pathological forms of cardiac hypertrophy [24,25], suggesting that this decrease may be responsible for an energetic failure that can eventually lead to cardiac dysfunction. Likewise, PGC-1α knockout mice exhibit lower cardiac power and increased reliance on glucose oxidation [26]. Exposure of human cardiac AC16 cells to TNF-α inhibited the expression of *PGC-1α*, a fact which resulted in a reduction in *PDK4* expression and subsequent increase in the glucose oxidation rate (Fig. 3A) [27]. It is worth mentioning that all these changes were abrogated with parthenolide, thus demonstrating the involvement of the NF-κB pathway [28]. In consonance with this, transgenic mice with constitutive and specific overexpression of TNF-α in the heart, a well-characterized model of cytokine-induced cardiomyopathy, displayed reduced *PGC-1α* and *PDK4* expression in the heart, and this was accompanied an increase in glucose utilization. In a similar way, phenylephrine and LPS down-regulate the expression of *PGC-1α* and *PDK4* in rat neonatal cardiomyocytes, resulting in an increase in glucose utilization and a decrease in fatty acid oxidation, a phenotype which resembles that observed during cardiac hypertrophy [28,29]. Interestingly,

the PPARβ/δ agonist L-165041 prevented the reduction in *PDK4* expression induced by both phenylephrine and LPS [22]. Overall data suggest the hypothesis that the NF-κB-mediated inhibition of PGC-1α accounts for the shift towards increased glycolysis during cardiac hypertrophy and heart failure.

An appealing question arose about the specific molecular mechanisms by which *PGC-1α* was down-regulated after NF-κB activation in cardiac cells. Co-immunoprecipitation studies not only demonstrated that the p65 subunit of NF-κB interacted with PGC-1α in the basal state, but also this

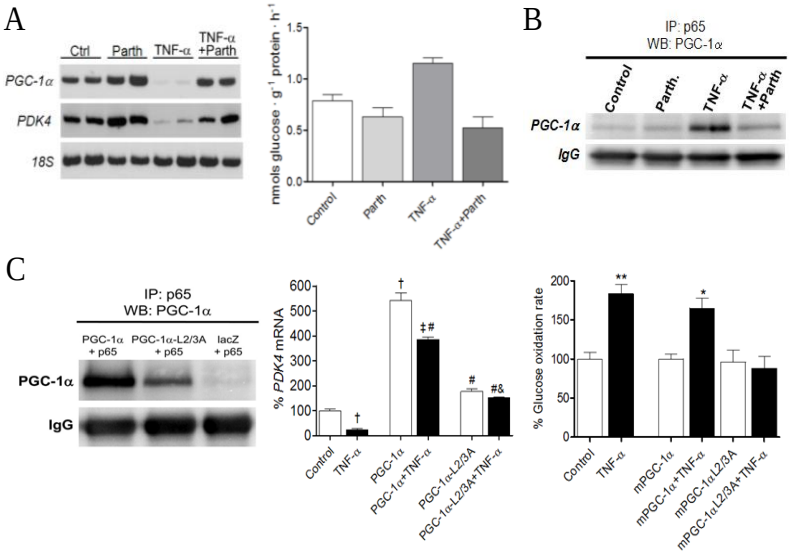

Figure 3. The p65 subunit of NF-κB binds to PGC-1α in cardiac cells. (A) *PGC-1α* and *PDK4* expression determined by RT-PCR and [U-^{14}C]-glucose oxidation rate in AC16 cells treated with TNF-α and the NF-κB inhibitor parthenolide (Parth). (B) Co-immunoprecipitation of nuclear protein extracts obtained from AC16 cells treated with TNF-α and/or parthenolide. (C) Co-immunoprecipitation (*left panel*) of nuclear protein extracts obtained from AC16 cells transfected with PGC-1α, PGC-1αL2/3A or lacZ (Control); analysis of *PDK4* gene expression determined by RT-PCR (*middle panel*, † P<0.01 vs. Control; ‡ P<0.01 vs. PGC-1α; # P<0.01 vs. TNF-α; & P<0.001 vs. PGC-1α+TNF-α); and [U-^{14}C]-glucose oxidation rate expressed as a percentage with respect to control cells (*right panel*, *P < 0.05, **P < 0.01 vs. Control).

binding was increased after stimulation of NF-κB activity, and owing to p65 accumulation in the nucleus (Fig. 3B) [30]. Modulation of the PGC-1α protein levels by means of overexpression or gene silencing demonstrated that the main factor limiting the degree of association between p65 and PGC-1α is the amount of p65 present in the nucleus.

PGC-1α binds nuclear receptors through three leucine-rich LXXLL motifs (named L1, L2 and L3), which are located within the N-terminus of the coactivator, and it is widely recognized that PGC-1α associates with other co-regulators via these LXXLL motifs [31]. Given that NF-κB also requires the binding to specific LXXLL motifs located within the sequence of specific coactivators to drive gene expression [32], we investigated whether these motifs were responsible of the modulation of PGC-1α activity by p65. On the basis of co-immunoprecipitation studies, and using a mutated form of PGC-1α, we reported that the L2 and L3 motifs play a crucial role in p65 binding (Fig. 3C) [30]. Therefore, in cardiac cells, the increased physical interaction between p65 and the L2/L3 motifs of PGC-1α after NF-κB activation might reduce *PGC-1α* expression, thereby leading to a reduction in *PDK4* expression and the subsequent increase in glucose oxidation observed during the pro-inflammatory state (Fig. 3C) [30].

4.3. PPAR-independent mechanisms in the regulation of PDK4 during inflammation

Other mechanisms might also account for the down-regulation of PGC-1α after NF-κB activation. For instance, NF-κB activation may indirectly stimulate PKB/Akt [30] and, since PGC-1α contains a consensus binding site for PKB/Akt phosphorylation that reduces its stability, it may result in a diminution in its transcriptional activity [33]. PKB/Akt also has the capacity to phosphorylate the FOXO1 transcription factors [34], thereby inducing their ubiquitination-dependent degradation and eventually leading to a decrease in the expression of their target genes (i.e. *PGC-1α*) [35].

We have also recently proposed a novel mechanism by which the inflammatory processes driven by NF-κB can down-regulate *PDK4* through inhibition of the E2F1 transcription factor in a PPAR- and ERRα-independent manner. E2F1 is known for its major role in regulating the G1/S phase transition during cell cycle progress, hence acting as a critical regulator of cell survival and proliferation [36]. However, it has also been demonstrated that it may regulate *PDK4* expression through specific sites

located within the promoter of the gene that encodes for the latter [37]. Protein co-immunoprecipitation analyses revealed that *PDK4* downregulation entailed enhanced physical interaction between the p65 subunit of NF-κB and E2F1 (Fig. 4A) [38]. The association between p65 and E2F1 has already been established in human [39] and murine fibroblasts [40]. Chromatin immunoprecipitation analyses demonstrated that p65 translocation into the nucleus prevented the recruitment of E2F1 to the *PDK4* promoter and its subsequent E2F1-dependent gene transcription in human cardiac cells (Fig. 4B), thus influencing glucose oxidation (Fig. 4C). Interestingly, the NF-κB inhibitor parthenolide prevented the inhibition of E2F1.

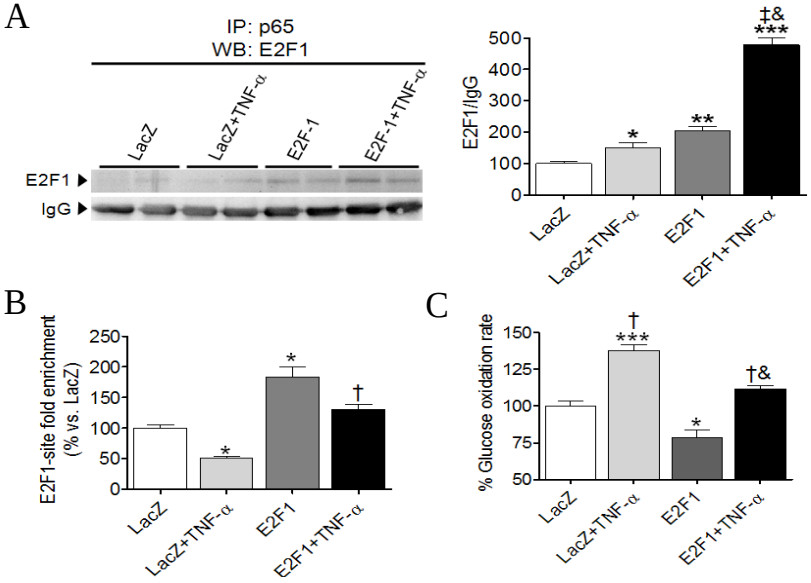

Figure 4. The interplay between NF-κB and E2F1 regulates metabolism in cardiac cells. (A) Co-immunoprecipitation of nuclear protein extracts obtained from AC16 cells treated with or without TNF-α and transfected with LacZ- or E2F1-carrying plasmids. (B) Chromatin immunoprecipitation demonstrating the E2F1-site fold enrichment at the PDK4 promoter and (C) [U-^{14}C]-glucose oxidation rate in AC16 cells overexpressing the human LacZ-control or the E2F1 genes incubated with TNF-α. *P<0.05, **P<0.01, ***P<0.001 vs. LacZ; †P<0.01 vs. E2F1; &P<0.01 vs. LacZ+TNF-α.

Based on these findings, we envision a model for the regulation of *PDK4* expression and cardiac cell metabolism by NF-κB and E2F1, in which NF-κB might serve as a molecular switch that regulates E2F1-dependent *PDK4* gene transcription. As inappropriate PDK4 activity would have catastrophic consequences in high-metabolic-rate organs, the basal repression of E2F1-dependent *PDK4* expression by NF-κB might be crucial for normal cardiac function. Since E2F1 plays an important role in cardiac myocyte growth and is also involved in metabolism regulation through *PDK4* modulation, targeting this transcription factor could provide us with an effective therapy for treating detrimental left ventricular hypertrophy leading to heart failure.

There are several other pathways that may account for the interplay between inflammation and metabolic responses in the heart. For instance, the heart of obese mice fed a high-fat diet display a reduction in glucose metabolism, GLUT and AMPK activity, which is accompanied by macrophage infiltration and enhanced inflammation [41]. A similar profile is observed after IL-6 administration in mice, whereby this cytokine suppresses the glucose metabolism and induces insulin resistance owing to AMPK activity and IRS-1 inhibition [42]. In accordance with this, AMPK activation diminishes NF-κB signalling, attenuates cardiac hypertrophy and improves cardiac function in rats subjected to trans-aortic constriction or in rat neonatal cardiomyocytes induced with angiotensin II [42].

Enhanced saturated free fatty acid (e.g. palmitate) levels up-regulate the lipid intermediates ceramides and diacylglycerol (DAG), the latter being a powerful activator of protein kinase C (PKC)θ. Likewise, hyperglycaemia activates the glycolytic pathway and increases the production of the PKC/DAG pathway [43]. PKC activation may provoke insulin resistance by means of IRS-1 inhibition and inflammation through NF-κB induction [44]. On the other hand, ceramides are lipotoxic molecules that modulate cellular energy metabolism and are enhanced in several models of lipotoxic cardiomyopathy. They are necessary and sufficient intermediates linking saturated fats to the inhibition of insulin signalling and enhanced inflammation [45]. In transgenic mice prone to dilated cardiomyopathy owing to ceramide accumulation in the heart, there is an up-regulation of fatty acid oxidation while glucose oxidation is down-regulated [46]. This has been related to the ceramide-dependent increase in the expression of genes such as *PDK4*, and the fatty acid transporters *FAT/CD36*, *FATP1 or ACS* [46].

Finally, recent studies have revealed an important link between sirtuin 1 (SIRT1), energy metabolism and inflammation [46]. The main reason for this link is perhaps that besides its ability to inhibit NF-κB activity, SIRT1 may associate with and deacetylate PGC-1α, leading to enhanced transcriptional activity of the latter. A paradigmatic example of this is given by the overexpression of SIRT1 in transgenic mice fed a high-fat diet, which show lower lipid-induced inflammation along with better glucose tolerance [47]. This study reported that the beneficial effects of SIRT1 were due both to the induction of the antioxidant proteins via stimulation of PGC-1α, and lower activation of pro-inflammatory cytokines, owing to NF-κB activity down-modulation. Similarly, SIRT1 inhibition in primary myotubes down-regulates PGC-1α and its target mitochondrial transcriptional regulators, ERRα and mtTFA, along with the expression levels of mitochondrial and fatty acid utilization genes [48]. On the other hand, both the activation of SIRT1 with resveratrol and overexpression of SIRT1 reduced phenylephrine-induced hypertrophy and the down-regulation of fatty acid oxidation genes in neonatal rat cardiac myocyte [49]. According to a study by Planavila *et al*. [50], SIRT1 overexpression led to enhanced PPARα binding to the p65 subunit of NF-κB and subsequent p65-deacetylation, thus blocking NF-κB activity. Consistent with this, isoproterenol-induced cardiac hypertrophy, metabolic dysregulation and inflammation were prevented by resveratrol in wild-type mice, but not in PPARα-null mice. Moreover, SIRT1 overexpression led to deacetylation of PGC-1α [50] and phosphorylation-induced AMPK activation [50]. In fact, besides the PPAR/PGC-1α pathway, SIRT1 modulates the activity of a number of proteins involved in cardiac metabolic homeostasis. For instance, it has been reported an important role of SIRT1 in regulating the ERR transcriptional pathway during the progression of heart failure, thus promoting mitochondrial dysfunction [51].

5. Conclusion

Free fatty acids are the preferred energy substrate in the adult heart, although other substrates such as glucose or lactate may provide additional fuel sources in diverse circumstances. At the transcriptional level, cardiac metabolism is mostly regulated by the PPAR/PGC-1α/PDK4 axis. The PGC-1α co-activates PPARα, PPARβ/δ and ERRα to regulate the

expression of genes involved in the electron transport chain, mitochondrial biogenesis, fatty acid β-oxidation and glucose oxidative metabolism. One of its key target genes, *PDK4*, may promote insulin resistance through the inhibition of efficient glucose utilization. During diabetes, and owing to the insulin resistance and the dysregulation in the activity of both PPARs and PGC-1α, the myocardium relies almost exclusively on mitochondrial fatty acid β-oxidation as the main fuel source. As a consequence, one of the hallmarks of diabetic cardiomyopathy is myocardial lipid accumulation, which is related to the development of lipotoxic cardiomyopathy. During diabetic cardiomyopathy, the excess of fatty acid oxidation in mitochondria induces the formation of reactive oxygen species and the accumulation of lipid intermediates, promoting NF-κB activation and, as a result, contributing to cardiac inflammation and subsequent development of heart failure.

In this review, we report that PPAR activation is capable of limiting myocardial inflammation by means of trans-repression through several mechanisms, including the physical interaction between PPARs and pro-inflammatory NF-κB. Notably, PPARα and PPARβ/δ activation might prevent metabolic disturbances occurring during diabetic cardiomyopathy, while inhibiting inflammatory processes in the heart. This is relevant, especially taking into account that PPARs have been postulated as potential targets in the treatment of obesity and the insulin resistance state. On the other hand, several studies point to PGC-1α as a potential contributor to cardiac dysfunction and heart failure in metabolic disorders with an inflammatory background. Here, we have described several well-known and new mechanisms by which PGC-1α activity is modulated after NF-κB induction, for instance the sequestration of PGC-1α protein by p65, the PKB/Akt-mediated phosphorylation of PGC-1α, or FOXO inhibition (Fig. 5). PGC-1α down-regulation often results in diminished *PDK4* expression, a crucial step in determining the rates of glucose and fatty acid oxidation in the heart. However, other mechanisms may account for PDK4 down-regulation, including the inhibition of E2F1.

In summary, an increasing body of evidence suggests a potential link between chronic inflammation and metabolic disturbances in the heart. Gaining more insight into the mechanisms by which cardiac inflammatory processes may deregulate metabolic homeostasis in the heart may assist in devising new therapeutic strategies to restore cardiac function during insulin resistance and diabetes.

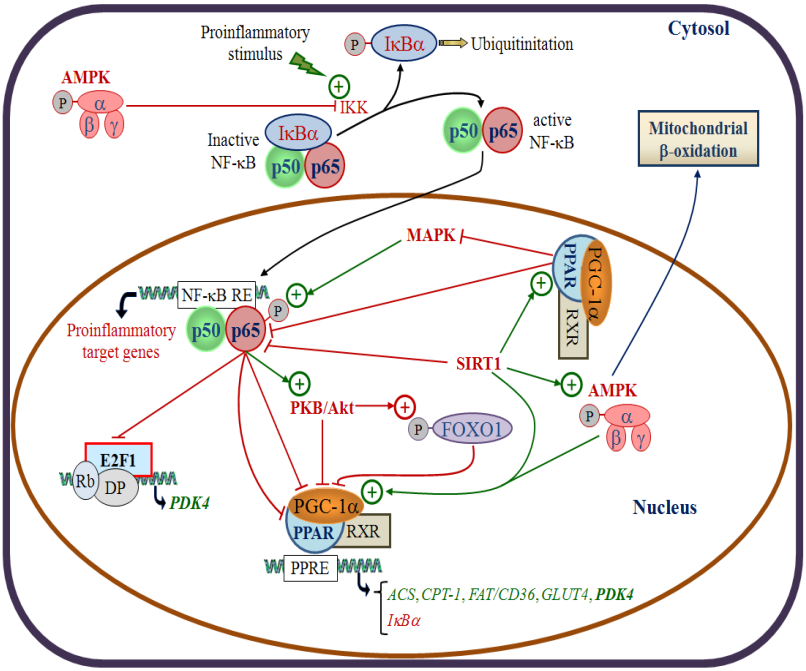

Figure 5. Crosstalk between inflammation and metabolism in the diseased heart. PPAR activation is capable of limiting myocardial NF-κB activity through several mechanisms, such as its physical interaction with the p65 subunit of NF-κB, or the inhibition of MAPK phosphorylation. PPARα is also capable of inhibiting NF-κB activity by inducing *IκBα* expression. After NF-κB activation, the increased physical interaction between p65 and PGC-1α reduces the transcriptional activity of the latter, thus diminishing its target gene expression (*ACS*, *CPT-1*, *FAT/CD36*, *GLUT4*, and *PDK4*). NF-κB may also down-regulate PDK4 through inhibition of the E2F1 transcription factor. SIRT1 down-regulates NF-κB activity through p65 deacetylation and enhances the activities of PGC-1α and AMPK. AMPK also diminishes NF-κB signalling due to IKK inhibition and the subsequent lowering of IkBα degradation. NF-κB activation may also indirectly stimulate PKB/Akt, which catalyzes the phosphorylation-mediated inhibition of PGC-1α, and also has the capacity to phosphorylate FOXO1, thereby inducing its degradation and finally leading to a decrease in the expression of *PGC-1α*.

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