https://doi.org/10.1038/s41575-018-0089-3

Metabolic aspects in NAFLD, NASH and hepatocellularcarcinoma: the intriguing role of PGC-1 coactivators

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Abstract

Alterations of hepatic metabolism are critical to the development of liver disease. The PPAR gamma 1 coactivators (PGC-1) are able to orchestrate, on a transcriptional level, different aspects of liver metabolism, such as mitochondrial oxidative phosphorylation, gluconeogenesis and fatty acid synthesis. As modifications affecting both mitochondrial and lipid metabolism contribute to the initiation and/or progression of liver steatosis, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC), a link between disrupted PGC-1 pathways and onset of these pathological conditions has been postulated. However, despite the large quantity of studies, the scenario is still not completely understood and some issues remain controversial. Here, we discuss the roles of PGC-1s in healthy liver, shedding a light on their contribution to the pathogenesis and future therapy of NASH and eventually HCC.

[H1] Introduction

The liver is a key organ for whole-body energy homeostasis. In addition to its unique specialized functions, such as detoxification of endogenous or xenobiotic compounds and the production of many plasma proteins and bile acids, the liver has a central role in the regulation of energy metabolism by integrating tissue and organ interrelationships. The liver functions as a mediator between dietary and endogenous sources of energy and extrahepatic organs that present high energy demands. In this way, in both physiological and pathological conditions, the liver contributes to the adaptive responses that provide the energy necessary for sustenance of different organ functions. For instance, food deprivation and/or physical activity immediately activate glycogen degradation in liver cells to control blood glucose levels. Moreover, during fasting the liver switches its metabolic programme towards gluconeogenesis, an almost exclusively hepatic pathway that leads to the synthesis of glucose from lactate and other non-carbohydrate compounds; this process is the first source for endogenous glucose production to fulfil the metabolic need of other organs. During prolonged fasting, free fatty acids released to the bloodstream following the activation of lipolysis in the white adipose tissue are rapidly taken up and metabolized by the liver, leading to the production of ketone bodies that can be used as fuel from different organs, the brain especially, thus delaying the degradation of endogenous proteins to provide additional energy [1, 2] .The liver is also essential for cholesterol homeostasis by playing a major part in lipoprotein metabolism and bile production. Furthermore, the nitrogen balance is tightly connected to hepatic function as ammonia, produced by amino acids catabolism, is mostly detoxified trough the liver-specific urea cycle [3].

If the liver is central to energy balance at the level of the body, mitochondria are the main metabolic hubs at the cellular level. Most of the pathways involved in energy metabolism are completely or at least partially compartmentalized within these organelles, or depend on their function. Apart from their unique bioenergetic role in aerobic ATP

production by oxidative phosphorylation, mitochondria are involved in calcium and iron homeostasis, apoptosis and reactive oxygen species (ROS) metabolism [4]. Consequently, it is not surprising that mitochondrial dysfunctions are implicated in several diseases, mainly related to altered metabolism [5].

Alterations of hepatic physiological functions are critical in the development of nonalcoholic fatty liver disease (NAFLD), a disease spectrum ranging from nonalcoholic fatty liver without inflammation to nonalcoholic steatohepatitis (NASH). In turn, NASH can lead to liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) [6-9]. Despite substantial improvements in the clinical management of these diseases, limited understanding of the molecular mechanisms underlying the onset of these conditions has hindered the development of effective therapeutic strategies. Thus, molecular modifications that lead to altered gene expression and subsequently contribute to disease development could be potential targets to improve hepatic functions.

The peroxisome proliferator-activated receptor (PPAR) γ coactivator-1 (PGC-1) family of transcriptional coactivators act as molecular switches in many metabolic pathways. Activated in several organs upon conditions of increased energy demands, in liver the functions of PGC1s are mainly promoted during fasting. PGC-1s are powerful regulators of various metabolic pathways and have substantial involvement in several diseases characterized by energetic misbalance, including cancer.

In this Review, we examine the mechanistic roles of PGC1 coactivators in hepatic tissue. We consider the similarities and differences in the function of these proteins and examine their roles in the development of NAFLD, NASH and HCC.

[H1] Gene expression and coactivators

Control of gene expression at the cellular level is finely regulated through many mechanisms, including the interaction of transcription factors or nuclear receptors with

specific DNA binding sites [10]. Concomitantly to or after binding of the transcription factor to a promoter, a histone acetyltransferase complex is recruited on DNA to modify chromatin, unwind double-stranded DNA and increase the access of RNA polymerase II to specific DNA sites [11]. The biochemical activities required for these processes are usually carried out by coregulatory proteins. These regulatory proteins can bind specific DNA sites, even those distant from the promoter, to switch on (in the case of co-activator proteins) or to shut off (for corepressor proteins) gene expression [10]. Expression of coregulatory proteins can be the primary target of signal transduction pathways; therefore, these proteins can participate in gene expression regulation not only as fundamental units of the transcriptional machinery but also because their expression levels and subsequently their activities can be finely tuned by upstream signalling pathways [12].

[H1] PGC1s

Some of the best characterized examples of coregulatory proteins are the PGC-1 (peroxisome proliferator-activated receptor γ coactivator 1) family, which comprises PGC-1α, PGC-1β and PGC-related coactivator (PRC) [13]. They carry out biological responses by indirectly and/or directly modulating the activity of key enzymes that control metabolic fluxes, which enables the cell to cope with the alterations of energy demands and substrate uptake and utilization. Their versatile actions are achieved by interacting with different transcription factors and nuclear receptors in a tissue-specific manner. In light of the multifaceted actions of mitochondria in cell metabolism, the well-established role of PGC-1s as master regulators of mitochondrial biogenesis adds another level of complexity to the functions of these proteins in liver homeostasis and disease. PGC-1s are able to shape the bioenergetic capacity of the cell by promoting the growth and division of pre-existing mitochondria, thereby increasing mitochondrial mass. The first studies on PGC-1s suggested that these coactivators had low intrinsic transcriptional activity when they are not bound to a

transcription factor [11, 14]. However, further analyses demonstrate that PGC-1s docking to transcription factors and nuclear receptors promote a conformational change in the coactivator that enables its binding to acetyltransferase, absent in PGC-1s, thus boosting its transcriptional activity [11, 14-17].

PGC-1α was the first member of the PGC-1 family to be identified, owing to its functional interaction with the nuclear receptor PPARγ in brown adipose tissue, a mitochondria-rich tissue specialized for thermogenesis [18]. PGC-1β (also known as PERC) and PRC were subsequently identified as structurally and functionally related to PGC-1 [15, 19].The coactivators PGC-1α and PGC-1β have a similar expression pattern, being highly expressed in tissues characterized by elevated aerobic energy requirements and hence by a substantial mitochondrial content, such as heart, type I skeletal muscle fibers and brown adipose tissue [18, 20-22]. PRC, however, is ubiquitously expressed [15]. Whereas the tissue-specific modulation of gene expression regulated by PGC-1α and PGC-1β has been studied in detail, the role of PRC in adult tissues remains comparably unknown. This protein seems to be predominantly involved in the regulation of cell growth with peculiarities of immediate early genes, which are rapidly induced during quiescence-to-cell proliferation transition. *In vitro* studies have demonstrated that induction of PRC expression is particularly high in proliferating cells and expression is rapidly upregulated in response to growth-factor-enriched (epithelial growth factor, fibroblast growth factor and nerve growth factor) serum stimulation in quiescent cells [15, 23, 24]. PRC is mainly involved in the expression of electron transport chain proteins, therefore knocking down PRC in proliferating cells leads to severe mitochondrial dysfunctions [25]. However, as the role of PRC in adult liver remains mostly unknown, in this Review we focus attention on the other two PGC-1s family members, PGC-1α and PGC-1β.

Although the fundamental contribution of PGC-1s in mitochondrial biogenesis is well described, PGC-1α and PGC-1β have different roles in the regulation of energy metabolism

depending on the tissue in which they are expressed and the physiological or pathophysiological context. For instance, in the liver both coactivators can increase mitochondrial biogenesis and oxidative phosphorylation to the same level (Figure 1). However, whereas PGC-1α is mainly implicated in promoting gluconeogenesis and fatty acid β-oxidation, PGC-1β does not drive the generation of glucose, and instead has a major role in upregulating *de novo* lipogenesis and VLDL trafficking. The latter are two processes that, conversely, are not affected by PGC-1α [26]. The differing roles in metabolic processes observed for these coactivators in the liver, with PGC-1β mainly supporting anabolic pathways, is perhaps indicative of their differing contributions to disease onset. Although many studies have been carried out on this topic, controversies about the specific roles of the different members of PGC-1 family remain.

[H1] PGC-1s control liver metabolism

The liver is probably the organ in which the divergent roles of PGC-1α and PGC-1β in regulating antagonistic physiological processes, besides the shared role in promoting mitochondrial biogenesis, is best illustrated [27, 28]. Expression of PGC-1α and PGC-1β is induced during the early postnatal period and in human adult liver following fasting [29, 30]. Conversely, in fed conditions, expression levels of both coactivators are lower in liver than in other tissues that rely on aerobic metabolism for ATP production such as heart, skeletal muscle and brown adipose tissue [18, 31]. Upon transition from the fed to the fasted state, the liver undergoes marked metabolic modifications to facilitate organism adaptation to a period of food shortage. These modifications include activation of mitochondrial metabolism, gluconeogenesis, fatty acid β-oxidation, ketogenesis, haeme biosynthesis and bile acid homeostasis, mostly controlled by PGC-1s [13, 26] (Table 1).

[H2] PGC-1s in mitochondrial metabolism

The main and best-described effect of PGC-1s on liver metabolism is the ability of all family members to promote mitochondrial biogenesis, primarily owing to their stimulatory effects on nuclear respiratory factor 1 and 2 (NRF1 and NRF2), estrogen-related receptor α (ERRα) and transcriptional repressor protein Yin Yang 1 (YY1) [21, 32, 33] (Figure 2). The expression of mitochondrial transcription factor A (TFAM), a nuclear-encoded transcription factor crucial for replication, preservation and expression of mitochondrial DNA, is primarily regulated by NRFs, although ERRα and YY1 can also modulate its expression [34]. NRF1 and NRF2 also control the expression of nuclear genes coding for components of the oxidative phosphorylation (OXPHOS) apparatus [35, 36]. The simultaneous upregulation of mitochondrial proteins encoded by mitochondrial DNA and genomic DNA by PGC-1α and PGC-1β increases the enzymatic capacity for fatty-acid β-oxidation, the citric acid cycle and OXPHOS [21, 32, 37-39]. Surprisingly, PGC-1α-null mice display a physiological amount of morphologically normal liver mitochondria despite reduced levels of mRNA encoding mitochondrial proteins and lower oxygen consumption [27]. Compensatory responses and extra-hepatic tissue cross-talk could have a role in explaining this phenotype. For instance, although hepatic PGC-1β expression levels in PGC-1α-null mice have not been published, it is plausible a compensatory mitochondrial production driven by this coactivator, given the high expression of specific PGC-1β target genes observed in the liver of PGC-1α-null mice [28].

Despite the high sequence homology between PGC-1α and PGC-1β, these two coactivators stimulate the mitochondrial biogenesis with distinct metabolic features, as they regulate the expression of overlapping but separate sets of mitochondrial genes. In particular, PGC-1α and PGC-1β promote the induction of specific genes involved in the removal of ROS, with PGC-1β being a more powerful regulator of antioxidant proteins such as manganese superoxide dismutase [40]. Thus, controlling the relative activity of PGC-1α and PGC-1β within the cell can lead to a fine-tuning of mitochondrial functions in response

to specific metabolic needs. For instance, in mice consumption of a diet enriched for saturated fatty acids specifically activates PGC-1β in the liver without affecting PGC-1α expression [41, 42]. Conversely, glucocorticoids decrease PGC-1β expression, but can induce and interact with PGC-1α to promote mitochondrial biogenesis and hepatic gluconeogenesis [43-47].

[H2] PGC-1s in fatty acid β-oxidation

Upon fasting, a shift in fuel usage from glucose to fatty acids, ketone bodies and amino acids in peripheral tissues is required to maintain systemic glucose homeostasis. Fatty acid β-oxidation in particular is the key source of ATP and acetyl-coA for hepatic glucose and ketone body production, respectively[48]. Both PGC-1α and PGC-1β can promote the PPARα-mediated expression of genes involved in hepatic fatty acid oxidation, notably medium-chain acyl-CoA dehydrogenase (MCAD) and carnitine palmitoyltransferase 1A (CPT1a).

Fasting induces hepatic lipid accumulation in PGC-1α-deficient mice, probably as a consequence of downregulated hepatocyte fatty acid oxidation [28, 49]. Intriguingly, the expression of PPARα target genes related to β-oxidation is not impaired in PGC-1α-null mice. However, these mice have a decreased mitochondrial respiration rate, despite displaying an equal amount of morphologically normal mitochondria [28]. This observation could be related to the ability of PGC-1α to increase mitochondrial cristae surface density [40]. Thus, blocking PGC-1α expression would reduce both the number, hence, the functional capacity of the electron transport complexes of the mitochondrial inner membrane cristae, thus resulting in a lower respiratory capacity.

In contrast to PGC-1α-null mice, PGC-1β-null mice do not develop overt hepatic metabolic failure, probably due to the robust compensatory effects exerted by PGC-1α [50]. However, when exposed to high-fat diet for short time, PGC-1β-null mice have increased

liver mass and develop fatty liver, attributable to defective mitochondrial fatty acid oxidation that cannot be rescued by PGC-1α compensation [51].

[H2] PGC1s in gluconeogenesis

Through interaction with forkhead box protein O1 (FOXO1), hepatocyte nuclear factor 4α (HNF4α) and glucocorticoid receptor (GR), PGC-1α also directly activates the transcription of hepatic gluconeogenic genes (such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphate dehydrogenase (G6PD)), whereas PGC-1β does not initiate this pathway [31, 52, 53]. However, the intrinsic transcriptional activity of PGC-1α is upregulated following deacetylation by the NAD^+-dependent sirtuin 1 (SIRT1), the activity of which increases in the liver during fasting [16, 54, 55]. Interestingly, SIRT1 deacetylation of PGC-1α does not affect mitochondrial gene expression, suggesting that variations of metabolic state can post-transcriptionally modify PGC-1α and fine tune coactivator recruitment to the promoters of specific genes [54]. Furthermore, SIRT1 can recruit corepressor NCoR that, in muscle, negatively affects OXPHOS gene expression and antagonizes PGC-1α coactivation of ERRα, thus offering a possible explanation for the selectivity of SIRT1 and PGC-1α towards gluconeogenesis in the liver [56, 57]. Similarly, insulin-induced phosphorylation of PGC-1α on Ser570 and on Ser568-572 negatively affects its interaction with HNF4α, which results in decreased expression of gluconeogenic genes in the liver[58, 59]. *In vitro* and *in vivo* studies, using RNA interference or mice lacking PGC-1α, support the fundamental role of PGC-1α in promoting hepatic gluconeogenesis via cAMP response element-binding protein (CREB) activation [29, 30]. Specifically, when cAMP levels raise, CREB is activated in the nucleus where it directly stimulates expression of gluconeogenic genes by binding to cAMP response elements in their promoters. At the same time, CREB has an indirect effect on the glucose synthesis, through the induction of PGC-1α that binds to HNF4α and regulates hepatic gluconeogenic genes [29, 30]. However, although PGC-1α-deficient mice are

hypoglycaemic, the basal hepatic expression of the master gluconeogenic gene phosphoenolpyruvate carboxykinase (PEPCK) is either normal or increased [27, 28]. Overall, it is still not well known whether the most important role of PGC-1α in hepatic glucose disposal is the direct regulation of the gluconeogenic gene expression, or the secondary modulation of the gluconeogenic flux mediated by fatty acids oxidation.

[H2] PGC-1s in de novo lipogenesis

In contrast to PGC-1α, PGC-1β does not regulate gluconeogenesis. PGC-1β expression in the liver is induced in response to dietary intake of saturated or trans fatty acids, although no effect of MUFAs (monounsaturated fatty acids) or PUFAs (polyunsaturated fatty acids) has been described yet; in the same context, PGC-1α levels do not change [41]. Ectopic PGC-1β overexpression in the liver in rats increased production and secretion of triglycerides via very low-density lipoproteins (VLDL), resulting in hypertriglyceridaemia and hypercholesterolaemia [41]. Conversely, high levels of PGC-1α in the liver are associated with a reduction in triglyceride production and secretion [60].

PGC-1β promotes the expression of genes involved in regulating lipid homeostasis in the liver via direct coactivation of sterol regulatory element-binding protein 1c (SREBP1c) and liver X receptor α (LXRα)[41]. Targets of SREBP1c include the lipogenic genes fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD1). Genes regulated by LXRα include Cyp7a1 and ATP-binding cassette transporter (ABCA1), which encode proteins that promote lipid extraction from the plasma and regulate cholesterol homeostasis [41]. The region of PGC-1β that mediates the interaction with SREBP1c (aminoacids 350–530) is not conserved among PGC-1 family members, thus providing a possible explanation for the isoform selectivity [41]. Notably, in the fed state SREBP1c is induced and directly interacts with HNF4α, thereby competitively interfering and inhibiting PGC-1α recruitment, which causes the suppression of hepatic gluconeogenesis [61]. By blocking gluconeogenesis,

SREBP1c minimizes hepatic glucose output while preserving it for the synthesis of new lipids. In mice, PGC-1β liver-specific overexpression protects the liver from lipid overload and from progression to fibrosis by increasing hepatic VLDL secretion [42].

PGC-1β also interacts with hepatocyte nuclear factor 3β (FOXA2) to regulate fatty acid β-oxidation and VLDL synthesis and secretion from the liver, thereby controlling lipid content [37]. High insulin levels inhibit the PGC-1β binding to a region closed to the Foxa2 C-terminal transactivation domain, leading to a decreased VLDL secretion [37]. Moreover, PGC-1β and its target gene apolipoprotein C3 (ApoC3) are downstream targets of nicotinic acid, a hypotriglyceridaemic drug. Acute or chronic treatment with nicotinic acid suppresses the hepatic expression of PGC-1β and ApoC3 in mice [62]. Overall, these findings suggest that PGC-1β activity could be influenced by nutritional status and/or be the result of hormonal milieu under different physiological conditions. It is therefore possible that available ligands and post-translational modifications engender PGC-1β promoter selectivity, thus culminating in a finely tuned gene expression.

[H2] Summary

PGC-1 target genes are finely regulated in the liver under different energetic states to respond to the metabolic needs of hepatic cells. During fasting, as well as in conditions that mimic starvation and low energy disposal, PGC-1 functions are regulated at both transcriptional and post-transcriptional levels. Interestingly, PGC-1α, but not PGC-1β, can also be stimulated by glucocorticoids. Enhanced PGC-1 activity induces the expression of pathways aimed to improve glucose and energy disposal in the liver to counteract the metabolic stress induced by food shortage. Both PGC-1α and PGC-1β coactivate several transcription factors and nuclear receptors to promote mitochondrial biogenesis, OXPHOS and fatty acid β-oxidation, and compensatory actions in the regulation of these pathways have been observed when one of the two coactivators was experimentally downregulated.

PGC-1α alone coregulates HNF4α, FOXO1 and glucocorticoid receptor expression of gluconeogenic genes. Conversely, only PGC-1β is induced by saturated fatty acid consumption, leading to increased *de novo* lipogenesis via coactivation of LXR and SREBP1c. These opposite activities exerted by PGC-1α and PGC-1β probably prevent futile substrate cycles. At the same time, the upregulation of gluconeogenesis via PGC-1α during fasting and new lipid synthesis via PGC-1β in fed conditions enables fine tuning of glucose disposal by hepatic cells.

[H1] NAFLD, NASH and HCC

NAFLD, the most common chronic liver disease in the Western world, is closely associated with insulin resistance and oxidative stress, which represent a clear manifestation of metabolic syndrome in most cases [63, 64]. NAFLD encompasses a large spectrum of conditions, ranging from simple steatosis to non-alcoholic steatohepatitis, which can progress to liver fibrosis, cirrhosis and end-stage liver diseases [65] (Figure 3). Although cirrhosis is still the main risk factor for HCC development, it is now emerging that HCC can develop in earlier stages of NAFLD, as indicated by the same molecular alterations found in both diseases [8, 9]. The obesity-induced chronic inflammatory response via tumour necrosis factor (TNF) and interlukin-6 (IL-6), two potent activators of pro-oncogenic signalling, has been linked to HCC onset [66]. Moreover, in patients with the metabolic syndrome who do not have liver fibrosis, deregulation of the oncogenic Wnt–β-catenin pathway has a role in the development of HCC, even if it does not represent the main carcinogenic process involved in HCC related to the metabolic syndrome [67].

Different theories have been formulated to explain the development and progression of NAFLD. In the traditional “two-hit” hypothesis, the first insult, hepatic accumulation of lipids, enhances the liver susceptibility to a subsequent second hit characterized by mitochondrial dysfunction, oxidative stress, inflammatory response and fibrinogenesis,

which finally lead to liver injury [68-70]. Although initially considered the most reliable theory explaining the pathogenesis of NAFLD, it is now becoming clear that the “two-hit” hypothesis is too simplistic to unravel the complexity of this disease. Recent findings delineate a “multiple-hit” hypothesis, in which multiple parallel pathogenic events act synergistically, and not in a consequent manner, to promote liver injury. In this view, any single event concurring to NASH development can be considered a potential therapeutic target [71].

In a clear obesity background, dietary and environmental factors could lead to substantial dysfunction and changes in liver, adipose tissue and gut microbiome. Hepatocytes begin to amass fat when they synthesize new lipids through *de novo* lipogenesis pathway, an adaptive response to counteract the generation of toxic lipid metabolites and to balance free fatty acid excess [72, 73]. The accumulation of toxic metabolites, such as saturated fatty acids and by-products of lipid peroxidation (TBARs), promotes a hepatic inflammatory state that is further exacerbated by adipose tissue, intestine and immune system (endotoxins derived from an altered gut permeability and dysbiosis, as well as, the release of Interleukin 6 and TNFα from inflamed adipose tissue), supporting the idea that the hepatic inflammatory microenvironment has a critical role in the development of NAFLD and progression towards HCC [66, 74-78].

Cytokine-mediated hepatocyte injury and death is followed by hepatic progenitor cell population growth which, in an inflammatory environment, induces the fibrogenic response in hepatic stellate cells, thereby promoting progression towards liver fibrosis and NASH [79-81].

Metabolic functions in the liver are connected to one another in an intricate, spatially regulated network. Despite their nearly homogenous histological appearance, hepatocytes show substantial heterogeneity with respect to subcellular, biochemical and physiological functions. Similarly, active metabolic pathways are kept spatially separated in distinct zones

of the liver (a property called “metabolic zonation”) to prevent competition for common substrates and futile cycles, and to enable more efficient detoxification [82-84].

About 50% of expressed liver genes are zonated, and all pathways uniformly increase or decrease in a portal–central manner [85]. For example, fatty acid β-oxidation and gluconeogenesis are increased on the portal side, whereas lipogenesis and triglycerides synthesis are predominant on the central side [85-87]. We have previously illustrated in this Review how PGC-1α and PGC-1β are able to control distinct processes within the liver, given the ability of PGC-1α to increase fatty acid β-oxidation and gluconeogenesis, whereas PGC-1β controls mainly the synthesis of new lipids and triglycerides. This aspect raises the question of whether PGC1 coactivators are subjected to liver zonation. Further studies are needed to address this unexplored issue.

In adult patients, steatosis is mainly localized in the pericentral zone, in a strong association with the presence of inflammatory infiltrates [88]. Whether the changes in lipid zonation trigger or follow NAFLD onset is still debated, but the accumulation of fat in hepatocytes around the central vein facilitates the activation of hepatic stellate cells that deposit collagen, thereby promoting disease progression towards severe forms of hepatic injury, such as cirrhosis [89, 90]. In contrast to the adult disease, paediatric NAFLD is usually closely associated with a dietetic regime enriched with carbohydrates and fructose (as opposed to carbohydrates and fat enrichment in adults), and intestinal dysbiosis with hepatic lipid accumulation localized around the periportal area, suggesting that different molecular processes are implicated in the onset of adult and paediatric NAFLD forms [91]. Once the localization of PGC-1α and PGC-1β has been defined, it would be intriguing to understand whether these coactivators display distinct activity in early life or childhood versus adulthood.

[H1] Benefits and harms of PGC-1s

During NAFLD, compensatory metabolic responses are induced to counteract fat accumulation. One of the most important is the adaptation of mitochondrial functions, the so-called hepatic mitochondria flexibility [92]. In early stages of the disease, mitochondrial respiratory activity increases to oppose hepatic lipid accumulation and NAFLD progression [92]. As a result of increased electron flux through the respiratory complexes, mitochondria produce more ROS, which leads to oxidative damage of biomolecules in the context of low antioxidative capacity [92-94]. In conditions of high substrate supply, this damage could cause the mitochondrial uncoupling and impaired energy output of livers affected by NASH [95].Furthermore, ROS upregulate and activate uncoupling protein 2 (UCP2) as a rescue feedback loop to release the increased redox pressure on the respiratory chain (both in terms of the electrochemical gradient existing across the inner mitochondrial membrane and the amount of reducing substrates that can be more rapidly oxidized through the respiratory chain), thereby causing mitochondrial proton leakage in the livers of patients with NASH [94, 96]. Until now, these mechanisms have been separately investigated in different cohorts of patients with NASH, but no evidence suggests that they are mutually exclusive [92, 94, 95]. Compensatory mitochondrial adaptations observed in subjects with NAFLD are completely lost in patients with NASH; these individuals have low hepatic mitochondrial respiration rates despite higher mitochondrial mass than patients without NASH [97, 98]. In any event, increasing mitochondrial mass while preserving an intact antioxidant defence able to provide protection against ROS accumulation can be considered a suitable option for NAFLD treatment at early stages. In mice, the altered NAD^+ homeostasis observed in NAFLD is rapidly corrected by NAD^+ repletion and a mitohormetic response occurs, which is able improve mitochondrial function, enhance β-oxidation and decrease *de novo* lipogenesis, thereby preventing NAFLD progression into severe hepatic injury or even enabling NASH regression [99, 100]. Following on from these findings, therapies directly targeting PGC-1s as master regulators

of mitochondrial biogenesis and antioxidant pathways should be evaluated as future research perspectives.

[H2] PGC-1s in NAFLD

[H3] Role in steatosis

NAFLD development is characterized by triglyceride accumulation within hepatocytes, mainly owing to an oversupply of fatty acids that exceeds the β-oxidation capacity of the cell. Obesity-related hepatic insulin resistance exacerbates fatty acid oversupply to the liver through the de-repression of hepatic lipogenesis [101]. A substantial proportion (26%) of hepatic fat in patients who are hyperinsulinaemic and obese is derived from *de novo* lipogenesis [102, 103]. However, mouse models suggest that *de novo* lipogenesis exerts a possible protective effect, preventing the progression of liver injury in NAFLD [73, 104]. In response to lipid excess in the liver, increased synthesis of triglycerides (in the form of MUFAs) is a protective mechanism to prevent the accumulation of lipotoxic lipids such as saturated fatty acids, highlighting that quality rather than quantity of fatty acids is essential in NASH progression [105]. PGC-1β is a key regulator of hepatic lipid synthesis, and mice overexpressing this coactivator solely in the liver are protected from steatohepatitis development when administered a high-fat diet [42]. Indeed, in the liver PGC-1β not only induces mitochondrial function and limits ROS production but also coregulates PPARα to counteract fatty acid accumulation by inducing fatty acid β-oxidation [42, 49]. At the same time, PGC-1β coactivates the master regulators of lipogenesis (LXR, SREBP1c and ChREBP), thereby inducing SCD1 and FOXO2, which promote unsaturated fatty acid synthesis and triglyceride excretion, thus conferring another level of protection against NASH [37, 41, 106]. Correspondingly, PGC-1β-null mice fed with high-fat diet display high levels of hepatic steatosis and increased serum triglyceride and cholesterol levels, mainly attributable to defective mitochondrial energy metabolism that the compensatory increase of PGC-1α is

not able to sustain [51]. This phenotype could also relate to low PGC-1β expression in other organs, given the whole body PGC-1β ablation. Supporting this hypothesis, loss of PGC-1β expression in adult pancreatic β-cells in cell culture and mouse model has been linked to a disrupted lipid metabolism; decreased glucose-stimulated insulin secretion, mediated by dysfunctional mitochondrial OXPHOS, is associated with limited PGC-1β functions and could exacerbate the consequences of reduced PGC-1β in other tissues [107].

High levels of hepatic PGC-1α might also ameliorate NAFLD. PGC-1α overexpression in rat hepatocytes results in reduced levels of hepatic triglyceride *in vitro* and *in vivo*, probably owing to greater mitochondrial mass and function that increases fatty acid β-oxidation [38]. These effects are partially mediated by Lipin1 a phosphatidate phosphatase catalysing the penultimate step in triglycerides synthesis, which when mutated causes hepatic steatosis in a genetic mouse model of lipodystrophy [108]. Lipin1 is induced by PGC-1α and it increases expression of a subset of PGC-1α target genes involved in fatty acid β-oxidation via PPARα, while also suppressing lipogenic genes and reducing circulating lipids [44].

Correspondingly, loss of PGC-1α has been linked to increase susceptibility to NAFLD. PGC-1α heterozygosity in mice liver results in a chronic downregulation of coactivator expression, which causes substantial changes in hepatic metabolism, such as negative regulation of fatty acid β-oxidation and triglyceride production and assembly, and leads to hepatic steatosis as well as hepatic insulin resistance [109]. Moreover, PGC-1α expression is blunted in steatotic liver in a NAFLD mouse model. As a result, the coactivator fails to interact with promoters containing NRF1 and NRF2 responsive elements, which decreases levels of mitochondrial and antioxidant proteins and leads to ROS accumulation [110, 111]. Conversely, the beneficial effect on hepatic steatosis due to downregulation of hyperglycemia and lipids accumulation in the liver of mice treated with PPARδ agonist

(GW0742) partially correlates with improvement of hepatic insulin sensitivity caused by a reduced PGC-1α expression [49, 112].

[H3] Role in NASH progression

PGC-1α seems to protect against NASH progression by limiting immune responses. Ablation of PGC-1α in mice fed with a high-fat diet raises the hepatic level of the proinflammatory cytokine IL-6 compared with wild-type mice [113]. Additionally, the overexpression of PGC-1α increases the levels of Interleukin-1 receptor antagonist (IL-1Rn), attenuating the effects of IL-1β [114].

PGC-1α regulates inflammatory responses by stabilizing the IκB-NF-κB complex in the cytoplasm, thereby preventing NF-κB nuclear translocation and transcription activation, and by promoting an anti-inflammatory environment through balancing the numbers of M1 and M2 macrophages [113, 115, 116]. Macrophages are key members of the immune response that actively contribute to liver homeostasis and respond to tissue damage. Upon environmental signals (e.g. microbial products, damaged tissue and activated lymphocytes), macrophages undergo polarization to M1 or M2 phenotypes, characterized by different cytokine release, cell surface markers and activation of distinct transcriptional profiles [117]. Although this classification does not fully reflect the complex biology of macrophages subsets, M1 macrophages are usually linked to early inflammation in response to hepatic injury and fibrogenesis induction, with a notable generation of mitochondrial ROS. Conversely, M2 macrophages are associated with resolution of inflammation, and display decreased ROS content and enhanced oxidative metabolism [117-119]. Both PGC-1α and PGC-1β have been associated with the anti-inflammatory environment and possibly with M2 macrophage polarization [116]. However, studies regarding PGC-1s and hepatic macrophage polarization are still lacking. Notably, PGC-1β induction in macrophages sustains the

activation of the M2 phenotype and a concomitant inflammatory state reduction both *in vitro* and *in vivo* [120].

In line with these results, genetic polymorphisms that lower hepatic PGC-1α expression or a reduced gene expression *per se* have been correlated to the development of NAFLD in a large cohort of patients [121, 122]. In a population of 781 children between 7 and 18 years old who were obese, almost 59% of patients (23% of the entire population) diagnosed with NAFLD by the presence of specific ultrasonography pattern had the PGC-1α polymorphism rs8192678 versus only 49% of individuals who did not have NAFLD [122]. Interestingly, this polymorphism does not discriminate between those who are obese and those who are not obese (68% and 65% prevalence respectively) [122]. In a population of Japanese adults, in which 115 individuals were diagnosed with NAFLD (65 with NASH and 50 with steatosis) on the basis of liver biopsy tissue histology, the PGC-1α polymorphism rs2290602 was associated with an increased risk of developing NAFLD (odds ratio (OR) NAFLD versus non-NAFLD 2.73) and NASH (OR NASH versus non-NAFLD 3.40; OR NASH versus steatosis 1.57) [121]. However, the effect of these polymorphisms on PGC-1α structure or function remains unclear.

In mouse models, high-fat diet lowers the expression of PGC-1α and, consequently, hepatic respiratory function [59, 109, 111, 123, 124]. Reduced hepatic PGC-1α expression impairs ROS detoxification and results in prominent oxidative injury, thereby exacerbating liver steatosis and inflammation induced by high-fat diet in mice [125]. Specifically, PGC-1α coactivates estrogen receptor α (ERα) in a ligand-dependent and synergistic manner, which promotes expression of antioxidant genes including superoxide dismutase 2 (SOD2) and glutathione peroxidase 1 (GPX1). In addition, treatment with an ERα agonist activates PGC-1α, which through the upregulation of NRF1 and NRF2 further enhances ROS scavenging mechanisms and reduces hepatic oxidative damage [125]. Correspondingly, mice with liver-specific deletion of PGC-1α display hepatic steatosis, mostly caused by an impaired

mitochondrial oxidative capacity [28]. In particular, as PGC-1α is required for oestrogen-dependent response to oxidative stress in liver, it is plausible that decreased levels of PGC-1α concomitant with low oestrogen abundance might contribute to the sex-dependent differences observed in steatosis pathogenesis [125, 126]. Whereas the disease is usually more common in men, the progression of NAFLD is fast in post-menopausal women, with similar or higher disease incidence than men, which suggests a protective role of oestrogens on liver fat accumulation and steatosis [127]. Overall, these studies point to a protective role of both coactivators in the pathogenesis of NAFLD.

However, the role of PGC-1α and PGC-1β in the development of insulin resistance and type 2 diabetes mellitus (T2DM) complicates delineating the function of these proteins in NAFLD. NAFLD is strongly associated with insulin resistance and T2DM, and more than 70% of patients with T2DM develop NAFLD [128]. Although in most cases NAFLD is considered the hepatic manifestation of insulin resistance, in developing countries, a substantial proportion (65%) of patients with NAFLD often do not have insulin resistance, probably due to high carbohydrate consumption that induces dyslipidaemia and fatty liver before insulin resistance develops [129, 130]. We have previously reported in this Review that patients with NAFLD have decreased oxidative, and other studies have suggested that the development of T2DM and insulin resistance is secondary to diminished mitochondrial function [92, 94, 97, 98, 131-133]. Thus, in overt NASH conditions, the downregulation of PGC-1s could positively correlate with diminished mitochondrial function and subsequent decreased insulin sensitivity. Moreover, low PGC-1 levels in the liver lead to a proinflammatory environment that further contributes to NAFLD progression.

Nevertheless, when evaluated in a background of insulin resistance and T2DM, PGC-1s expression display an opposite tendency, with a trend to increase. Indeed, PGC-1α expression is highly induced in the liver in diabetic animal models, where it promotes gluconeogenesis and glucose output and results in fasted hyperglycaemia [29, 30]. Ablation of

PGC1α in mice specifically in the liver increases hepatic insulin sensitivity and reduces fasting blood glucose levels [49], reflecting PGC-1α-mediated regulation of gluconeogenic genes activated by PPARα [44, 49]. Blocking PGC-1β expression ameliorates the fructose-induced hepatic insulin resistance, reducing the process of *de novo* lipogenesis mediated by SREBP1c [134]. Hypomorphic mutation of PGC-1β (loss of exons 3 – 4) also leads to reduced mitochondrial function and hepatic insulin resistance in mice [135]. Patients who are insulin resistant and obese, characterized by enhanced hepatic expression of pseudokinase tribbles homologue 3 (TRIB3) have increased mRNA levels of PGC-1s[136]. Particularly, both PGC-1α and PGC-1β are able to promote TRIB3 transcription via coactivation of PPARα and SREBP1c, respectively [49, 136]. Further studies are required to better clarify how PGC-1s are modulated and, therefore, contribute in all different stages of NASH development.

[H2] PGC-1 in hepatocellular carcinoma

Over the past decade, NAFLD has emerged as a leading cause of end-stage liver disease and HCC. Although HCC was considered the terminal stage of liver disease progression, strong evidence now shows that HCC can develop in NASH in the absence of cirrhosis [137, 138]. The chronic low-grade inflammation in NAFLD, accompanied by obesity and ROS accumulation, might influence and alter hepatic metabolism, enabling HCC development [139]. To support proliferation and escape from apoptosis, hepatic cancer cells rapidly consume energy that is efficiently supplied by altered lipid metabolism, enhanced glycolysis and fatty acid β-oxidation [140]. Altered lipid metabolism also protects cancer cells from oxidative damage. Indeed, the shift from exogenous lipids uptake to endogenous lipids synthesis in cancer cells leads to increased levels of saturated and monounsaturated fatty acids, that potentially protect cancer cells from oxidative damage, inasmuch polyunsaturated acyl chains are more prone to lipid peroxidation [141]. Given the critical role

of PGC-1s in the maintenance of liver homeostasis, unbalance of these coactivators has been assessed in HCC initiation and progression.

[H3] PGC1 and metabolic reprogramming.

Metabolic reprogramming is one of the hallmarks of cancer [142, 143]. During tumorigenesis, cells undergo a series of substantial changes in energy metabolism, in which they switch from mitochondrial OXPHOS to glycolysis (a process known as the Warburg effect). Although early studies described the shift to aerobic glycolysis as the result of defective mitochondrial function, new evidence suggests that mitochondrial components rarely harbour inactivating mutations in tumours, including in HCC, with the glycolytic phenotype the result of OXPHOS inhibition by active glycolysis rather than defects in mitochondrial function [144-147]. Thus, after the glycolytic phenotype switch induced by the hypoxic environment in the first stages of cancer development, nutrient shortage caused by high cellular proliferation rates during malignancy restore the more efficient OXPHOS to fulfil increased energy demands [148]. In addition, cancer cells and stromal cells can coevolve in tumorigenesis; in this scenario, stromal cells provide energy metabolites (lactate and pyruvate) derived from aerobic glycolysis for ATP production by OXPHOS in cancer cells (a process known as the reverse Warburg effect) [149]. Thus, mitochondria can sustain cancer cell survival and proliferation not only because they generate ATP by OXPHOS but also because of their involvement in many biochemical pathways, such as glutaminolysis and *de novo* lipogenesis, which lead to precursors for biomass accumulation and calcium homeostasis and apoptosis [150].

Studies on the role of PGC1s in hepatocellular carcinoma depict an incoherent scenario in which these coactivators can both sustain or interfere with tumorigenesis. Genetic deletions, amplifications or mutations in PGC-1s are rarely detected in cancer, in contrast to classical tumour suppressors or oncogenes [151]. However, cancer cells seem to

take advantage of PGC-1 functions through dynamic modulation of their expression, primarily by metabolic adaptation to energy variation through activation of AMPK (5' AMP-activated protein kinase), which functions as an intracellular sensor of AMP levels and therefore cellular energy state, and epigenetic changes [151, 152] (Figure 4).

During hepatocarcinogenesis, negative regulation of the gluconeogenesis pathway might enable cells to upregulate glycolysis, thereby favouring their survival in the hypoxic environment that characterizes early stages of tumorigenesis before angiogenesis has been initiated [153]. The hepatic expression of PGC-1α and its target genes involved in gluconeogenesis is markedly reduced in a mouse model of HCC (induced by choline-deficient diet) and in primary human HCC, which leads to suppression of gluconeogenesis and a concomitant increase in glycolysis. These changes prevent the conversion of glucose 6-phosphate to glucose, necessary for its release into the blood, resulting in decreased circulating glucose levels in tumour-bearing mice and cancer progression [154]. Downregulated PGC-1α expression in human hepatoma cell lines *in vitro* promotes dedifferentiation of hepatocytes via functional impairment of HNF4α [155]. HNF4α is key transcription factor regulating hepatocyte differentiation, as it has a critical role in the specification of cellular phenotype during mammalian liver development and controls the expression of genes involved in glucose, cholesterol and fatty acid disposal [156, 157]. A key early event in HCC is the phenotypic dedifferentiation of tumour cells that leads to the loss of hepatocyte-specific functions (such as xenobiotic detoxification) [158]. Thus, given that the role of HNF4α in regulating hepatocyte differentiation and gluconeogenesis requires PGC-1α coactivation, it can be hypothesized that altered glucose metabolism could be related to hepatic dedifferentiation in HCC.

Several studies in mouse models and cells *in vitro* have described the action of tumour suppressor p53, via sirtuin 6 (SIRT6) activation, in arresting cellular proliferation by lowering gluconeogenesis. SIRT6 is an NAD^+-dependent deacetylase that activates

acetyltransferase GNC5, which contributes to PGC-1α acetylation, therefore suppressing gluconeogenic gene expression. FOXO1, a key pro-gluconeogenic transcription factor, is also directly deacetylated by SIRT6, which leads to its exclusion from the nucleus and inhibition of transcriptional activity [159-161]. In hepatoma cell lines PGC-1α binds to p53 and enhances the p53-mediated transactivation of genes relating to growth arrest (such as p21 and GADD45) and metabolic genes (such as TIGAR and SCO2), that increase glucose utilization [162]. When cells are subjected to glucose starvation, both p53 and PGC-1α are induced in an AMPK-dependent manner [163-165]. Specifically, under glucose-limited conditions, in liver and cancer cells the AMPK–p38–PGC-1α axis confers a growth advantage to cancer cells by promoting oxidative metabolism via increased mitochondria biogenesis and OXPHOS [166, 167]. The PGC-1α–p53 interaction is modulated by the extent of food deprivation [162], suggesting that the coactivator might have different roles on tumour development in different environmental and particularly metabolic conditions.

Metabolic reprogramming in cancer cells is also necessary to provide molecules for rapid cellular proliferation; for instance, fatty acids are required for new membrane formation, but they can also be utilized in signalling pathways or catabolized for energy production [168]. PGC1α supports hepatocarcinogenesis via coordinated regulation of mitochondrial and fatty acid metabolism. Knockdown of PGC-1α or inhibition of fatty acid synthesis in an HCC mouse model blunts the pro-growth effects of PGC-1α and ultimately protects against liver cancer development [169]

Another important feature of cancer cells is their ability to escape cell death. Since large amount of ROS mediate cell death, to actively support cancer cell survival, increased expression of PGC-1β limits the amount of ROS through the induction of ROS scavenger genes, including SOD2, TXN2 and PRDX5. In gain-of-function and loss-of-function PGC-1β mouse models, this coactivator confers metabolic advantages to promote cancer cell proliferation by upregulating lipid synthesis, TCA cycle and OXPHOS activity and ROS

scavenging [170]. Accordingly, during hypoxia, a common feature of tumour microenvironment at advanced stage, increased expression of hypoxia-inducible factor 1 (HIF-1) suppresses PGC-1β, thereby inhibiting expression of fatty acid β-oxidation genes (such as MCAD and LCAD), which facilitates lipid anabolism and tumour progression [171]. The opposite function on tumour fatty acids β-oxidation exerted by the two coactivators could appear as a paradox. If PGC-β fosters tumour growth highly supporting *de novo* lipogenesis while blocking fatty acids catabolism in order to prevent futile cycles and optimize all the supplies, PGC-1α promotes HCC by sustaining fatty acids catabolism to provide new substrates for oxidative metabolism that confer advantage to cancer cells. Although the concomitant modulation of PGC-1α and PGC-1β in tumour has been never investigated, it is possible that different metabolic conditions, as glucose deprivation, can affect PGC-1s activity and downstream metabolism. The synthesis of new lipids is also increased by hypoxic stress in several cancer cell lines, included HCC cells lines [172, 173]. Thus, it is plausible that expression of PGC-1s is induced in stressful liver tumour microenvironments to increase cancer cells proliferation. One can also speculate on whether different stimuli can explain the controversial role of PGC-1s in cancer: various oncogenes and metabolic modifications, such as fasting and hypoxia, could supply diverse signals upstream of PGC-1 activity that might explain the dynamic roles of these coactivators in cancer development.

The enhanced mitochondrial biogenesis and respiration induced by PGC-1α is crucial in promoting metastasis [174]. In liver cancer cell lines and in mouse models of HCC metastasis, SIRT1 activates PGC-1α, which promotes mitochondrial biogenesis and ATP production via OXPHOS and consequently drives cancer dissemination [175]. The PGC-1α-mediated promotion of mitochondrial OXPHOS could be an important signature of invading cells. While they are moving to more oxygenated areas of the tumour, cancer cells can shift to more efficient aerobic energy production to bolster their migrating phenotype. As discussed earlier, understanding the zonated expression of PGC1s in relation to oxygen and

substrate gradients in the liver might be helpful to shed light on their role on HCC progression. The alteration of metabolic programmes is a fundamental feature of malignant cells and is therefore an attractive target for the development of novel therapeutic approaches [176]. Once the molecular mechanisms by which PGC-1s are involved in cancer are better understood, therapeutic approaches to treat HCC and prevent metastasis formation based on modulation of PGC1s could be explored.

[H2] Therapeutic targeting of PGC1s

The PGC-1s are deeply involved in liver homeostasis, and deregulation of their expression results in metabolic changes that can lead to NAFLD and its sequelae, cirrhosis and HCC. Targeting PGC-1s might represent an appealing strategy in HCC, as these coactivators are able to regulate a number of metabolic pathways. However, targeting PGC-1s is not straightforward. In contrast to nuclear receptors that are considered druggable targets owing to the presence of highly specific ligand-binding domains, coactivators are hard to target, because of the lacks of these domains, their large and flexible structures, as well as, their nuclear localization[177]. However, modulating the transcriptional and post-transcriptional activators of PGC-1s to increase their expression or to activate the existing pool of endogenous PGC-1s, respectively, is a viable therapeutic approach to treat NAFLD.

In liver, energy deprivation, such as induced by fasting, is one of the principal mechanisms through which PGC-1 expression could be regulated. Additionally, the PPARδ agonist GW0742 lowers PGC-1α expression in rats and cell culture and decreases hepatic palmitic acid accumulation in diabetic rats [112]. Since now, these ones are the only approaches that directly modify PGC-1s expression, without interfere with post-translational modulation of both coactivators.

Several drugs have been linked to post-transcriptional modifications of PGC-1α and PGC-1β that alter coactivator activity [152, 178] . AMPK activation by AICAR (an analog of AMP)

or metformin (a widely used drug for treatment of T2DM) in hepatic cells downregulates gluconeogenesis, in a mechanism depending directly and indirectly on PGC-1α functions. Activated AMPK phosphorylates and inactivates PGC-1α but also mediates the degradation of TORC2 (transducer of regulated CREB protein 2), thereby promoting the dissociation of the transcriptional CREB–CBP–TORC2 complex that, in turns, blocks PGC-1α transcription and decreases expression of gluconeogenic genes (including G6Pase and PEPCK), and improves liver insulin sensitivity [179-182]. Moreover, AMPK can increase the expression of the small heterodimer partner (SHP), which leads to FOXO1-mediated repression of PGC-1α, thus lowering the formation of glucose [183, 184]. As SHP is also a downstream effector of bile acids signalling, therapeutic targeting of bile acid receptor FXR (Farnesoid X Receptor) might be useful in decreasing gluconeogenesis via PGC-1α–HNF4α [184]. Although no evidence suggests that AMPK regulates PGC-1β in the liver, the effect of metformin on downregulation of SREBP1c and *de novo* lipogenesis genes allows to speculate an AMPK-mediated PGC-1β deregulated activity [185]. Although in mice metformin improves hepatic steatosis and inflammation, the heterogeneity of clinical studies prevents us from stating a beneficial effect also for human steatosis [186, 187]. Moreover, if targeting AMPK-PGC-1α axis by metformin treatment might ameliorate insulin sensitivity, on the other side the PGC-1α deregulation could promote a diminished mitochondrial function and a proinflammatory environment that further exacerbates NAFLD.

PGC-1s action can also be negatively regulated by flavonoids such as forskolin via SIRT6-mediated GNC5 activation [17, 161]. GNC5 is an acetyltransferase activated by caloric excess and high levels of acetyl-CoA, which acts synergistically with SRC3 (also known as nuclear receptor coactivator 3) to acetylate PGC-1s and lower expression of genes involved in fatty acid β-oxidation and gluconeogenesis [188, 189]. Mice lacking ablation are protected against high-fat diet induced obesity and have improved insulin sensitivity owing to reduced hepatic lipid accumulation and glucose production [188].

Resveratrol can activate both AMPK and SIRT1, which act in concert to stimulate fatty acid β-oxidation and mitochondrial functions through both PGC-1α and PGC-1β activation [178, 189, 190]. However, SIRT1-null animals treated with resveratrol do not display its beneficial effects, suggesting that SIRT1 is required for the AMPK-mediated effects of the drug. Indeed, whereas only small doses of resveratrol are necessary to stimulate SIRT1, higher doses of the compound are necessary to activate AMPK [191]. SIRT1 activators mimic caloric restriction and decrease PGC-1s acetylation in mouse models [190, 192]. Although effects of resveratrol on NAFLD are inconclusive, clinical studies demonstrate that this drug is beneficial for patients with neurological disorders, cardiovascular diseases, diabetes, and some types of cancer [193].

Finally, a study published in 2017 reported that the small synthetic molecule SR18292 suppresses gluconeogenic gene expression in primary hepatocytes and diet-induced obesity mice. The drug reduced glucose production, improved hepatic insulin sensitivity and glucose homeostasis and ameliorated T2DM. Mechanistically, this molecule increases the PGC-1α–GNC5 interaction, which augments PGC-1α acetylation and decreases the coactivation of HNF4α [194]. Despite this molecule has not been yet explored in human trials, it represents the only direct manipulation of PGC-1α so far reported. This molecule is able to bypass the coactivator “undruggability”, by interfering with protein-protein interaction. Future studies are recommended in order to find specific coactivator interactions, therefore modulating specific metabolic programme.

[H1] Conclusions

PGC-1α and PGC-1β have a key role in the transcriptional regulation of liver metabolism. Although their abilities to orchestrate different hepatic physiological processes have been almost discerned, their specific contributions to liver disease remain unclear. While more studies are needed to clarify the function of PRC, both PGC-1α and PGC-1β

have important roles in modulating initiation and progression of NAFLD and HCC in mouse models of these diseases, thus opening new putative avenues for the therapy of these disorders. Nevertheless, future studies are needed to unravel the functions of PGC-1 coactivators in the complex landscape of human liver disease.

Key points

- PGC-1s have a key role in liver metabolism and contribute to energy homeostasis
- PGC-1α and PGC-1β exert divergent functions on liver metabolism and regulate different pathways. Although the hepatic expression of both PGC-1α and PGC-1β negatively correlates with NAFLD severity, HCC development is inhibited by PGC-1α and promoted by PGC-1β.
- Although direct coactivator targeting is problematic, pharmacological modulation of transcriptional and post-transcriptional activators of PGC-1 is an appealing therapeutic avenue.

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Acknowledgements

Moschetta A. is funded by Italian Association for Cancer Research (AIRC, IG 18987) NR-NET FP7 Marie Curie ITN, Italian Ministry of Health (Young Researchers Grant GR-2010-2314703).

Author contributions

Competing interests statement

The authors declare no competing interests.

Referee accreditation trial placeholder

Figure 1 | *Physiological roles of PGC-1α and PGC-1β in the liver.* PGC-1α and PGC-1β regulate overlapping and distinct hepatic genes that contribute to liver metabolism homeostasis. Besides the ability of both PGC-1α and PGC-1β to regulate oxidative metabolism and promote mitochondrial biogenesis, antioxidant responses and fatty acid β-oxidation, each coactivator is able to activate distinct opposite pathways under physiological conditions. Whereas PGC-1α promotes gluconeogenesis during fasting, saturated fatty acids-enriched diets consumption induce PGC-1β involvement in *de novo* lipogenesis pathways as well as synthesis and export of VLDL particles.

Figure 2. Common pathways regulated by PGC-1α and PGC-1β in the liver.

PGC-1s are finely regulated in the liver in response to changes in cellular energy. During fasting, AMPK activation enables PGC-1 coactivation of different transcription factors to maintain mitochondrial metabolic homeostasis, via the upregulation of genes involved in mitochondrial biogenesis, oxidative phosphorylation and fatty acid β-oxidation. In caloric excess conditions, the acetyltransferase GNC5 shut-off PGC-1s activity. To express genes involved in mitochondrial biogenesis, YY1 requires PGC-1s coactivation. NRF1, NRF2 and ERRα not only contribute to mitochondrial biogenesis, but also promote the expression of antioxidant genes that protect the cell from ROS accumulation. ERRα is also involved in the regulation of fatty acid β-oxidation, together with PPARα, the main nuclear receptor regulating this pathway. (PGC-1: peroxisome proliferator-activated receptor γ coactivator 1; AMPK, 5' AMP-activated protein kinase; YY1, yin yang 1; NRF, nuclear respiratory factor; ERRα, estrogen-related receptor alpha; PPARα, peroxisome proliferator-activated receptor).

Figure 3. Principal metabolic alterations characterizing NAFLD and HCC.

In healthy liver mitochondrial metabolism provides for most of the energy requirement by oxidative phosphorylation (OXPHOS), and reactive oxygen species (ROS) levels are kept low. Once the liver starts to accumulate lipids NAFLD can rapidly develop. In the early stages of NAFLD, mitochondrial respiratory functions are increased to mitigate disease progression, yet in NASH mitochondrial adaptations are completely lost, and a low respiration rate, despite increased mitochondrial mass, is observed. Although HCC mostly develops in cirrhotic livers, in many cases liver tumours can form in noncirrhotic pathological states, including in NAFLD. In HCC, a switch from mitochondrial OXPHOS to glycolysis is observed. Mitochondria in liver tumours are still able to efficiently generate ATP by OXPHOS, and are involved in many biosynthetic pathways that lead to biomass accumulation and provide fundamental metabolites for cancer growth and progression.

Alongside the primary metabolic shift from OXPHOS to glycolysis, at later disease stages the liver becomes more reliant on *de novo* lipogenesis and glycolysis than on fatty acid β-oxidation and gluconeogenesis. Thus, increasing mitochondrial mass while preserving intact antioxidant defences could be a treatment target for early NAFLD stages. By contrast, during late phases, alterations of the metabolic programme would be a better target for the development of novel therapeutic strategies. In this context, PGC-1s, as master regulators of mitochondrial biogenesis and various liver metabolic processes including gluconeogenesis and fatty acid oxidation, could be suitable targets of new therapies. ATP: adenosine triphosphate, ROS: reactive oxygen species, NAFLD: nonalcoholic fatty liver disease, NASH: nonalcoholic steatohepatitis, HCC: hepatocellular carcinoma, PGC-1: peroxisome proliferator-activated receptor γ coactivator 1.

Figure 4. PGC-1α and PGC-1β in hepatocellular carcinoma.

a | Studies on the role of PGC-1α and PGC-1β indicate that these coactivators can sustain or interfere with liver tumour development. On the basis of findings from cell lines and transgenic and orthotopic mouse models, PGC-1α can promote HCC development by sustaining mitochondrial biogenesis, fatty acid β-oxidation and, in contrast to its physiological functions, *de novo* lipogenesis. However, other studies demonstrate the ability of PGC-1α to block tumour progression, mainly due to the downregulation of gluconeogenesis. The PGC-1β scenario is clearer: the coactivator fosters tumour growth by blocking fatty acid β-oxidation while supporting *de novo* lipogenesis and antioxidant responses. The opposite role of PGC-1α and PGC-1β in modulating fatty acids β-oxidation is probably related to the different metabolic conditions affecting PGC-1s activity and downstream metabolism.

b | The central pathway involved in the regulation of PGC-1s actions in liver tumours. Upon glucose deprivation in HCC, AMPK is activated and modulates the regulation of PGC-1s in

concert with SIRT1. AMPK can positively regulate p53, which in turn interacts with PGC-1α and induces expression of cell-cycle arrest and metabolic genes. At the same time, p53 induces SIRT6 activity, which inhibits gluconeogenesis by deacetylating PGC-1α and interfering in the PGC1-α–FOXO1 interaction. AMPK can also promote PGC-1β activity, which leads to increase lipogenesis. In hypoxic conditions, such as in rapidly growing tumours, HIF-1 suppresses PGC-1β expression and its target genes involved in fatty acid β-oxidation. (HCC: hepatocellular carcinoma, PGC-1: Peroxisome Proliferator-Activated Receptor γ Coactivator 1; AMPK, 5' AMP-activated protein kinase; SIRT, Sirtuin; FOXO1, Forkhead box protein O1; HIF-1, Hypoxia-inducible factors; GADD45, Growth Arrest and DNA Damage 45; TIGAR, P53-induced glycolysis and apoptosis regulator; SCO, cytochrome c oxidase assembly protein).

Table

Table 1. Key pathways regulated by PGC-1α and PGC-1β in the liver

Process	Coactivator	Transcription factor	Target genes
Mitochondrial biogenesis and oxidative phosphorylation	PGC-1α and PGC-1β	NRF1 NRF2 ERRα YY1	*TFAM* *ATP synthase* *Cytochrome C* *COX IV*
Fatty acid oxidation	PGC-1α and PGC-1β	PPARα ERRα FOXA2	*TRB-3* *CPT-1a* *PDK4* *MCAD* *Apoc3*
Gluconeogenesis	PGC-1α	FOXO1 HNF4α GR CREB	*PEPCK* *G6Pase*
***De novo* lipogenesis**	PGC-1β	SREBP1c	*FASN* *ACLY* *ACC* *SCD1* *DGAT1*
VLDL synthesis and excretion	PGC-1β	LXRα	*CYP7a1* *ABCA11*

Abbreviations: NRF, Nuclear Respiratory Factor; ERRα, Estrogen Related Receptor α; YY1, Yin Yang 1; PPARα, peroxisome proliferator-activated receptor α; FOX, forkhead box protein; HNF4α, Hepatocyte Nuclear Factor 4α; GR, Glucocorticoid receptor; CREB, cAMP response element-binding protein; SREBP1c, Sterol regulatory element-binding transcription factor 1; LXR, Liver X Receptor; TRB-3, Tribbles-Related Protein 3; CPT-1a, Carnitine Palmitoyltransferase I; PDK4, Pyruvate Dehydrogenase Kinase 4; MCAD, Medium-Chain Acyl-CoA Dehydrogenase; G6Pase, Glucose 6-Phosphatase; FASN, Fatty

acids Synthase; ACLY, ATP Citrate Lyase; ACC, Acetyl-CoA carboxylase; SCD1, Stearoyl-CoA desaturase 1; DGAT1, Diacylglycerol O-Acyltransferase 1; ABCA1, ATP-binding cassette transporter A1.

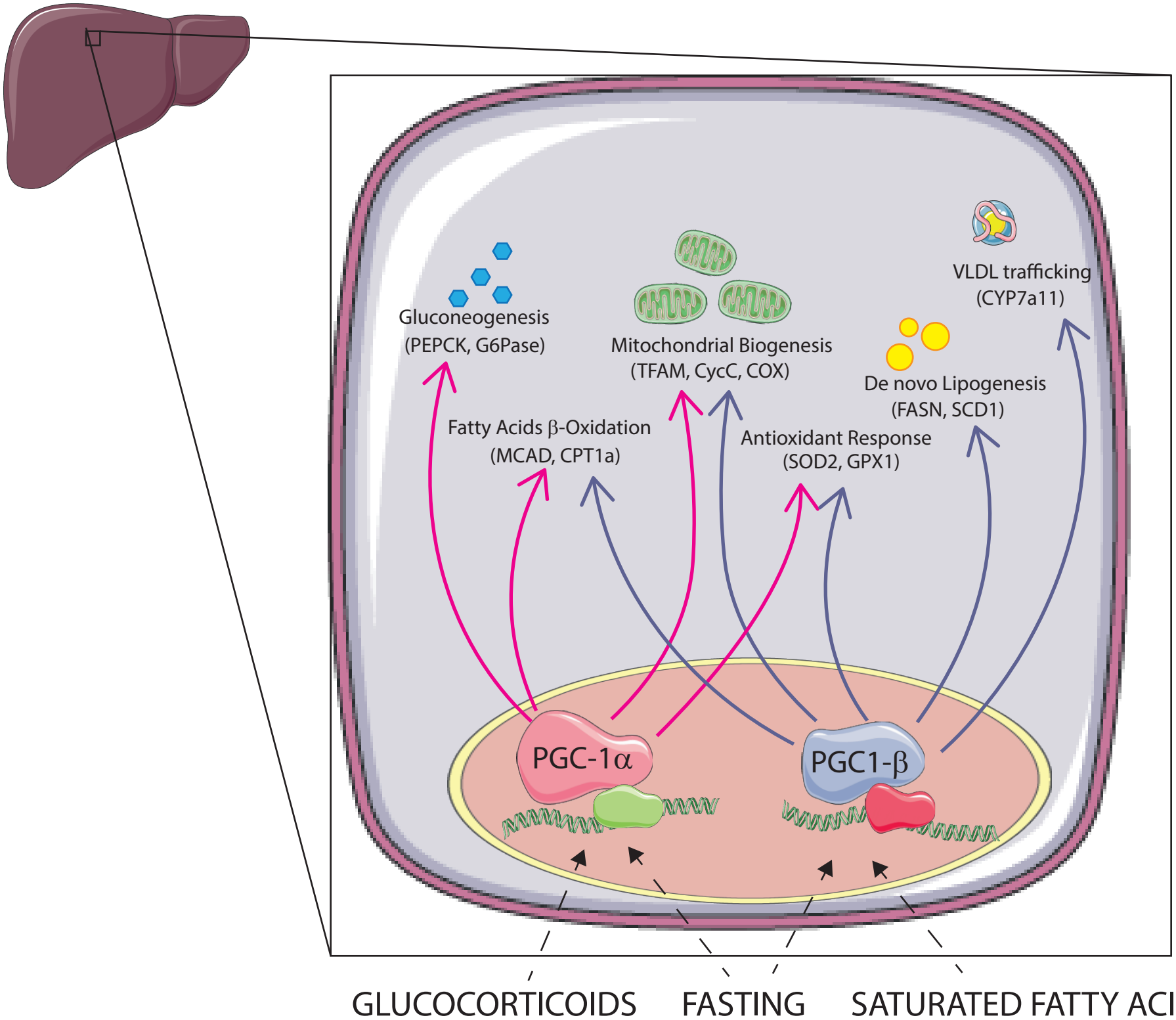
Gluconeogenesis
(PEPCK, G6Pase)
Mitochondrial Biogenesis
(TFAM, CycC, COX)
VLDL trafficking
(CYP7a11)
De novo Lipogenesis
(FASN, SCD1)
Fatty Acids β-Oxidation
(MCAD, CPT1a)
Antioxidant Response
(SOD2, GPX1)
PGC-1α
PGC1-β
GLUCOCORTICOIDS
FASTING
SATURATED FATTY ACIDS

Figure 1

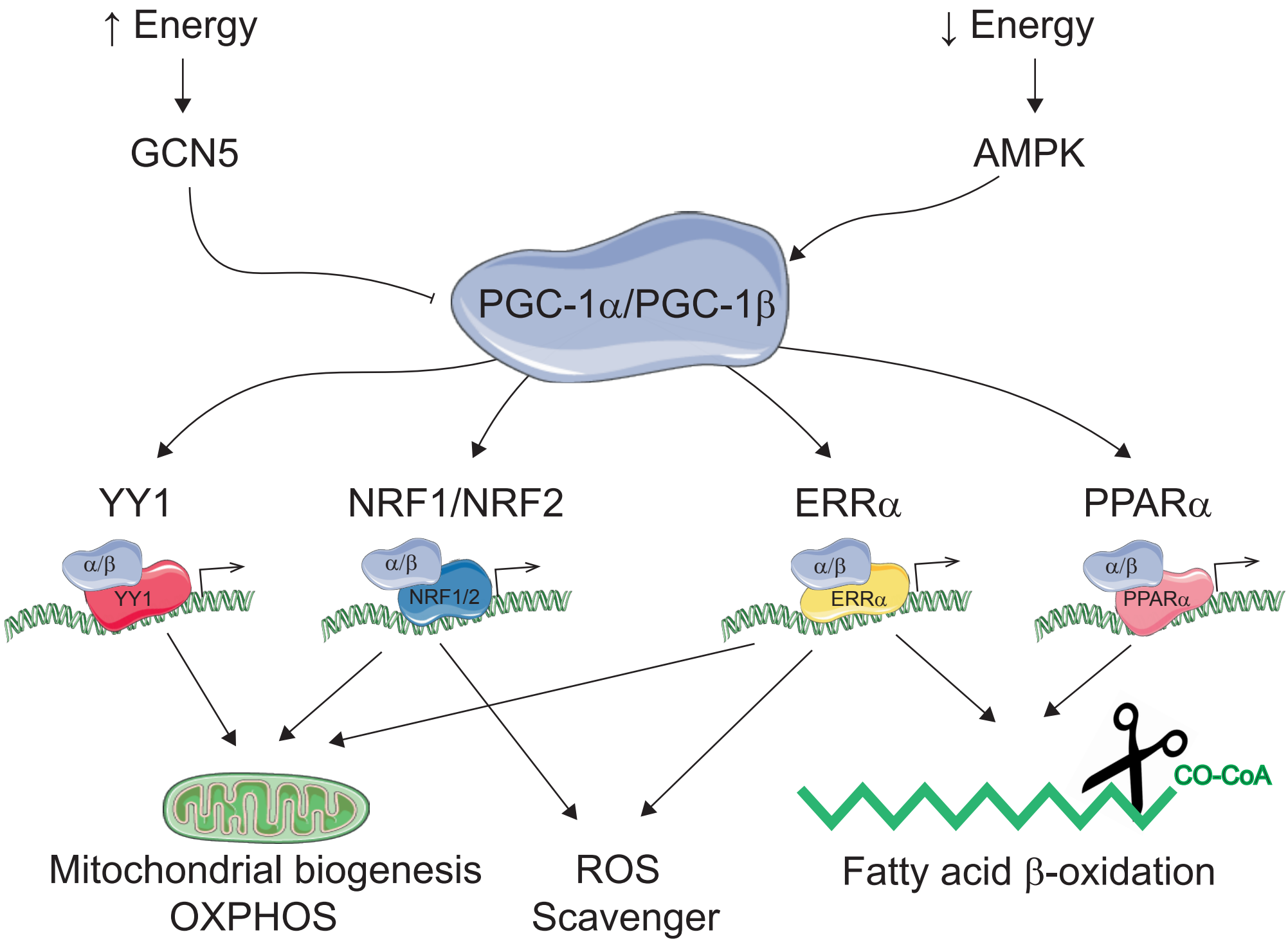
↑ Energy
↓ Energy
GCN5
AMPK
PGC-1α/PGC-1β
YY1
NRF1/NRF2
ERRα
PPARα
α/β
YY1
α/β
NRF1/2
α/β
ERRα
α/β
PPARα
CO-CoA
Mitochondrial biogenesis
OXPHOS
ROS
Scavenger
Fatty acid β-oxidation

Figure 2

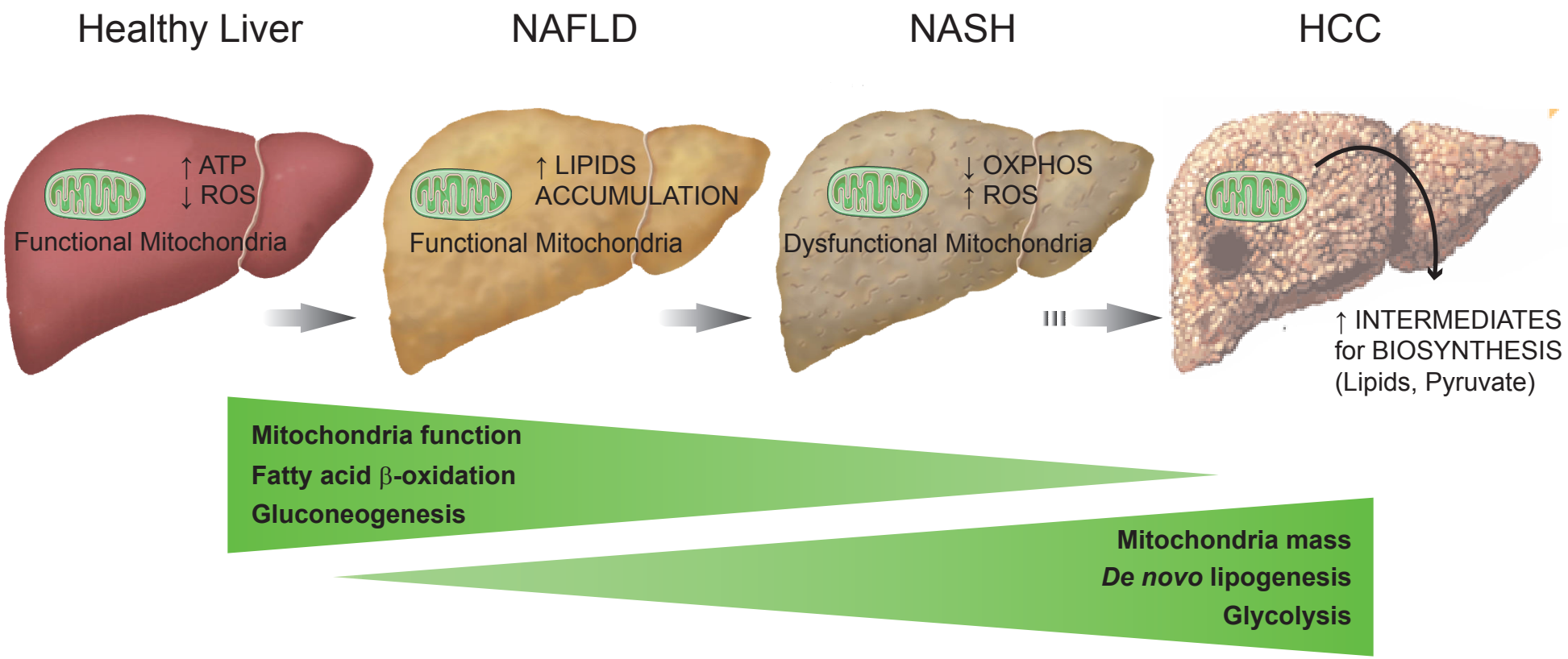
Healthy Liver
NAFLD
NASH
HCC
↑ ATP
↓ ROS
Functional Mitochondria
↑ LIPIDS
ACCUMULATION
Functional Mitochondria
↓ OXPHOS
↑ ROS
Dysfunctional Mitochondria
↑ INTERMEDIATES
for BIOSYNTHESIS
(Lipids, Pyruvate)
Mitochondria function
Fatty acid β-oxidation
Gluconeogenesis
Mitochondria mass
De novo lipogenesis
Glycolysis

Figure 3

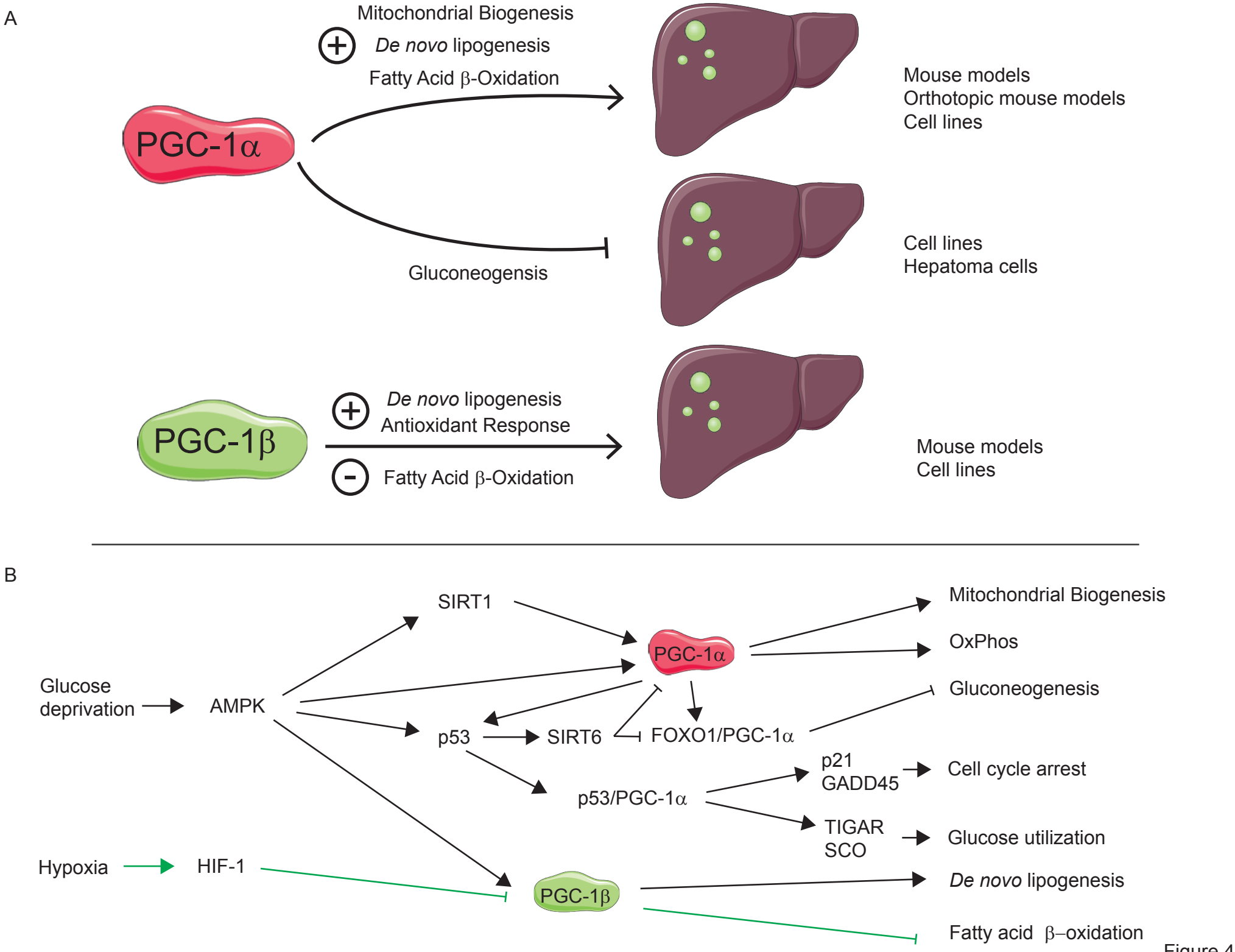
A
Mitochondrial Biogenesis
De novo lipogenesis
Fatty Acid β-Oxidation
PGC-1α
Mouse models
Orthotopic mouse models
Cell lines
Gluconeogensis
Cell lines
Hepatoma cells
PGC-1β
De novo lipogenesis
Antioxidant Response
Fatty Acid β-Oxidation
Mouse models
Cell lines
B
SIRT1
Mitochondrial Biogenesis
PGC-1α
OxPhos
Glucose deprivation
AMPK
Gluconeogenesis
p53
SIRT6
FOXO1/PGC-1α
p21
GADD45
Cell cycle arrest
p53/PGC-1α
TIGAR
SCO
Glucose utilization
Hypoxia
HIF-1
PGC-1β
De novo lipogenesis
Fatty acid β–oxidation

Figure 4