REVIEW

Hypoxia-regulated mechanisms in the pathogenesis of obesity and non-alcoholic fatty liver disease

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Abstract The pandemic rise in obesity has resulted in an increased incidence of metabolic complications. Non-alcoholic fatty liver disease is the hepatic manifestation of the metabolic syndrome and has become the most common chronic liver disease in large parts of the world. The adipose tissue expansion and hepatic fat accumulation characteristics of these disorders compromise local oxygen homeostasis. The resultant tissue hypoxia induces adaptive responses to restore oxygenation and tissue metabolism and cell survival. Hypoxia-inducible factors (HIFs) function as master regulators of this hypoxia adaptive response, and are in turn hydroxylated by prolyl hydroxylases (PHDs). PHDs are the main cellular oxygen sensors and regulate HIF proteasomal degradation in an oxygen-dependent manner. HIFs and PHDs are implicated in numerous physiological and pathological conditions. Extensive research using genetic models has revealed that hypoxia signaling is also a key mechanism in adipose tissue dysfunction, leading to adipose tissue fibrosis, inflammation and insulin resistance. Moreover, hypoxia affects liver lipid metabolism and deranges hepatic lipid accumulation. This review summarizes the molecular mechanisms through which the hypoxia adaptive response affects adipocyte and hepatic metabolism, and the therapeutic possibilities of modulating HIFs and PHDs in obesity and fatty liver disease.

Keywords Obesity · Hypoxia ·
Hypoxia inducible factor · Lipid metabolism ·
Oxygen homeostasis · Angiogenesis

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Introduction

Obesity and its associated comorbidities have become one of the defining diseases of the last decades. A recent analysis estimated that the number of overweight or obese individuals has risen to 2.1 billion people worldwide [1]. Consequently, non-alcoholic fatty liver disease (NAFLD) has become a global pandemic and is now the most common cause of chronic liver injury in large parts of the world [2, 3]. NAFLD comprises a spectrum of disease that ranges from hepatocellular steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma. Simple steatosis should no longer be considered a benign condition, as it predisposes the liver to further injury, and is a precursor of and an independent risk factor for type 2 diabetes and cardiovascular disease [2, 4, 5].

Despite intensive research, satisfactory pharmacological treatment for either obesity or NAFLD is currently lacking, urging further research into the pathophysiological mechanisms underlying these disorders.

Complex multicellular organisms were able to evolve as a result of energy production through mitochondrial oxidative phosphorylation, which generates energy much more efficiently than alternative fermentation processes such as anaerobic glycolysis. Oxygen functions as the final electron acceptor in this mitochondrial respiratory chain, and is therefore vital for maintaining normal tissue

homeostasis and metabolism [6, 7]. Accordingly, an evolutionary conserved pathway, termed the hypoxia-induced adaptive response, regulates adaptation to low oxygen tensions at the cellular level [8]. Both hypoxia and this adaptive response have been associated with many diseases, such as anemia, lung and cardiovascular disease, and cancer [6, 9].

Regarding obesity-related diseases, studies in animal models [10, 11] have demonstrated that white adipose tissue (WAT) becomes hypoxic as it expands in obesity. As WAT hypoxia is one of the first pathophysiological changes in obesity, it may underlie the observed link between obesity and low-grade chronic WAT inflammation [12, 13]. In human patients, the evidence for WAT hypoxia is more controversial. Some studies have shown a decrease in WAT oxygen levels as body fat increases [14, 15]. Nevertheless, others have not been able to find a metabolic signature characteristic of hypoxia in adipose tissues of obese subjects [16], whereas one study even found an increase in tissue oxygen levels, despite reduced WAT blood flow [17]. In recent years, there has been considerable interest in brown adipose tissue (BAT) as a potential anti-obesity target due to its capacity to burn excess energy in thermogenesis [18]. Research has indicated that obesity also causes BAT hypoxia, contributing to a loss of thermogenic capacity [19].

Adipose tissue expansion and inflammation cause ectopic lipid accumulation in muscle and liver tissue, leading to insulin resistance and liver steatosis [20, 21]. Because the liver is uniquely vascularized, receiving both oxygenated blood via the hepatic artery and deoxygenated blood via the portal vein, it is a hypoxia-sensitive organ. The blood that flows in the hepatic lobule is directed from the periportal region towards the central vein, creating an oxygen gradient with lower oxygen tensions in the perivenous regions of the liver lobule [22]. While it is known that acute and chronic alcohol exposure causes perivenous hypoxia [23, 24], more recently, a similar pattern was established in mice who were fed a high-fat diet (HFD) leading to NAFLD [25].

This review discusses the pathways involved in hypoxia signaling and the effects of modulating the hypoxia response on the development of adipose tissue dysfunction and NAFLD.

The hypoxia-induced adaptive response pathway

Hypoxia-inducible transcription factors (HIF) are the master regulators of the cellular and tissue response to hypoxic stress [26–28]. They are heterodimers consisting of an oxygen-sensitive HIF-α subunit and a stable, constitutively expressed β-subunit (HIF-1β), also known as

ARNT (aryl hydrocarbon receptor nuclear translocator). Three forms of HIF-α, named HIF-1α, HIF-2α and HIF-3α, have been characterized. HIF-α subunit stability is strictly controlled by cellular oxygen concentrations (Fig. 1). Under normoxic conditions, HIF-α is subject to hydroxylation at specific proline residues by prolyl hydroxylase domain proteins (designated PHD1, PHD2 and PHD3). PHD activity requires oxygen and 2-oxoglutarate as co-substrates and ascorbic acid and iron as co-factors [29]. Hydroxylation allows recognition of HIF-α by the von Hippel Lindau (VHL) tumor suppressor gene, which acts as a substrate for the E3 ubiquitin ligase complex, resulting in polyubiquitination and proteasomal degradation [30]. Under hypoxic conditions, PHD activity is inhibited and HIF-α can translocate to the nucleus where it dimerizes with HIF-1β, thereby forming the active transcription factor HIF.

HIF belongs to the PAS family of helix–loop–helix transcription factors, and binds DNA at so-called hypoxia responsive elements [31]. HIF regulates the expression of numerous downstream target genes, such as vascular endothelial growth factor (VEGF), erythropoietin and various glucose transporters (GLUTs). In this way, HIF increases oxygen delivery to the tissue, helps adapt the tissue to lower oxygen levels and lowers oxygen consumption. HIF-1α and HIF-2α share structural similarities and coordinate processes such as erythropoiesis, angiogenesis, glucose and lipid metabolism, proliferation, and inflammation [32]. Yet each isoform has non-redundant functions that vary in a tissue-specific way [33, 34]. In addition, the affinity of different PHD isoforms for HIF subunits and their relative abundance in different tissues and cells varies, which allows fine-tuning of a flexible hypoxia adaptive response to varying physiological oxygen tensions [35]. For instance, PHD2 shows greater affinity for HIF-1α, whereas PHD3 and, to a lesser extent, PHD1 predominantly hydroxylate HIF-2α [36].

Hypoxia signaling in adipose tissue

Adipose tissue has the unique property to expand drastically in adulthood, whereas most adult tissues retain a relatively stable size [37]. Although adipogenesis and angiogenesis are tightly interconnected, compensatory WAT angiogenesis is often inadequate to parallel WAT expansion and blood delivery to WAT is diminished [17, 38]. As adipocyte cell expansion also limits oxygen diffusion, oxygen supply can become insufficient, although some inconsistencies exist in the literature regarding WAT oxygen levels in obese humans [14, 16, 17, 39]. Moreover, the increase in free fatty acids in obesity directly increases WAT oxygen consumption through activation of adenine
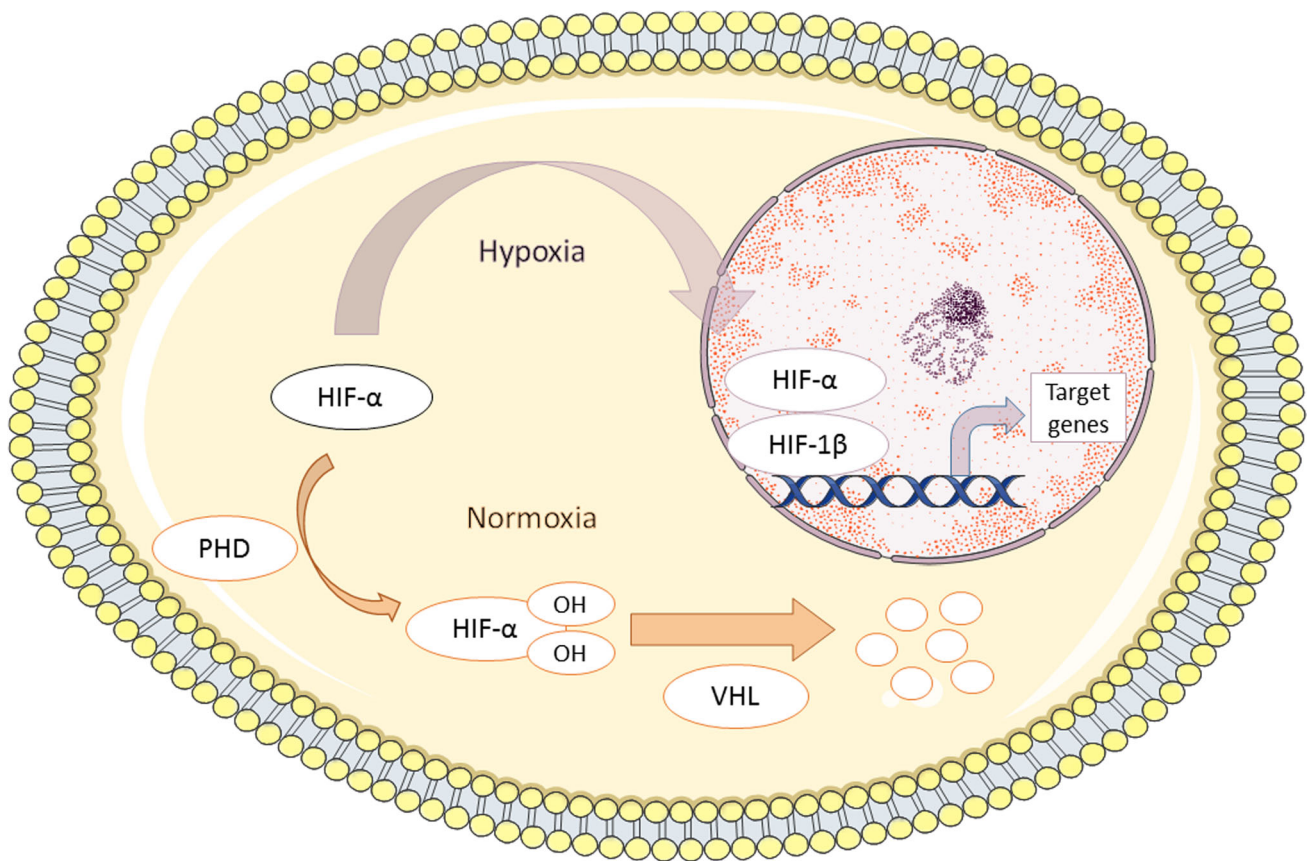
Fig. 1 The Hypoxia adaptive response system. Under normoxic conditions, hypoxia-inducible factor α (HIF-α) subunits are hydroxylated by prolyl hydroxylase (PHD) at specific proline residues (prolines 402 and 564 for HIF-1α and prolines 405 and 531 for HIF-2α). Hydroxylated HIFs are recognized by the von Hippel–Lindau (VHL) protein, leading to ubiquitinilation and proteasomal degradation. PHD activity is inhibited by hypoxia, allowing the nuclear translocation of HIF-α, where it dimerizes with HIF-1β to activate transcription of hypoxia target genes

nucleotide translocase 2 (ANT2), a mitochondrial inner-membrane protein normally involved in BAT thermogenesis [40]. ANT2 specifically stimulates the uncoupling of mitochondrial oxidative metabolism, causing energy to be dissipated as heat instead of being coupled to ATP production [41, 42]. The activation of ANT2 suggests that, in obesity, oxygen consumption in WAT is increased in a compensatory attempt to increase energy expenditure.

Importantly, research in animal models has shown that WAT hypoxia and HIF-1α upregulation occur as early as 1–3 days after starting a high-fat diet, before inflammation and insulin resistance develop [40, 43]. This WAT hypoxia exerts a profound influence on tissue homeostasis and dysregulates the expression of many adipokines and pro-inflammatory cytokines [11, 44]. As a consequence, adipose tissue is polarized towards a pro-inflammatory phenotype, further contributing to the attraction of circulating macrophages towards the hypoxic regions [11]. These data support the hypothesis that hypoxia is one of the key features in the development of low grade inflammation and adipose tissue dysfunction [13, 39]. Therefore, the ability to modulate the hypoxia response might hold important therapeutic potential (Table 1).

HIF isoform specificity in adipose tissue

HIF-1α can be artificially stabilized by deleting the HIF-α oxygen-dependent degradation domain, thus conferring resistance to hydroxylation by PHDs [45]. Using this approach, Halberg et al. [43] found a HIF-1α dose-dependent increase in adipocyte cell mass. HIF-1α transgene hemizygotic mice gained more weight and experienced decreased glucose tolerance compared to their wild-type (WT) littermates. This led to WAT fibrosis through upregulation of various collagens and the collagen cross-linking protein lysyl oxidase. Fibrosis preceded infiltration by immune cells, and inhibition of lysyl oxidase resulted in a reversal of glucose intolerance, fibrosis, and inflammation, indicating that HIF-associated fibrosis is a central mechanism of WAT dysfunction [43] (Fig. 2a).

Table 1 Summary of key studies on adipocyte-specific HIF modulation

Gene modulation	Study protocol	Body weight	Glucose tolerance	Adipocyte phenotype	Adipose tissue inflammation	Vasculature	Hepatic metabolism	References
HIF-1α overexpression	aP2-HIF-1α-ODD transgene construct	Increased	Impaired	Increased cell size	Increased	Not affected	Impaired insulin sensitivity, TG increase	Halberg et al. [43]
HIF-1α or HIF-1β deletion	aP2-Cre × HIF-1α$^{f/f}$/HIF-1β$^{f/f}$	Decreased	Improved	Smaller adipocytes	Decreased	ND	Increased insulin sensitivity	Jiang et al. [47]
HIF-1β deletion	aP2-Cre × HIF-1β$^{f/f}$	Decreased	Improved	Smaller adipocytes	Unchanged	Reduced permeability	Unchanged lipid accumulation or insulin sensitivity	Lee et al. [53]
HIF-1α deletion	aP2-Cre × HIF-1α$^{f/f}$	Unchanged	Improved	Decreased cell mass and size	Decreased	Not affected	Decreased steatosis and inflammation	Lee et al. [40]
HIF-2α deletion	aP2-Cre × HIF-2α$^{f/f}$	Increased	Impaired	ND	Increased	ND	ND	
HIF-1α deletion	aP2-Dominant-negative (dn)-HIF-1α construct	Increased	Impaired	'Whitening' of BAT Increased WAT mass	Increased inflammation and fibrosis	Impaired (BAT)	ND	Zhang et al. [69]
HIF-2α deletion	Fabp4-Cre × HIF-2α$^{f/f}$	Increased	Impaired	'Whitening' of BAT Increased WAT mass	Increased inflammation and fibrosis	Impaired	Increased steatosis and TG accumulation Progression to NASH	Garcia-Martin et al. [51]
HIF-1α inhibition	Compound PX-478 Apn-rtTA × TRE-dn-HIF-1α	Reduced	Improved	Decreased cell mass and size	Decreased	ND	Decreased steatosis	Sun et al. [48]
PHD2 inhibition	aP2-Cre × PHD2$^{f/f}$	Reduced	Improved	Smaller adipocytes	Reduced	Mildly increased	ND	Matsuura et al. [60]
PHD2 inhibition	aP2-Cre × PHD2$^{f/f}$	Increased	Unaffected	Larger adipocytes	Unaffected	Increased	Decreased liver TG	Michailidou et al. [62]
PHD2 inhibition	PHD2$^{gt/gt}$ mice FG-4497 administration	Reduced Reduced	Improved Improved	Smaller adipocytes Smaller adipocytes	Decreased Decreased	Not affected ND	Decreased steatosis ND	Rahtu-Korpela et al. [114]

Outcome values in comparison with WT mice after HFD feeding

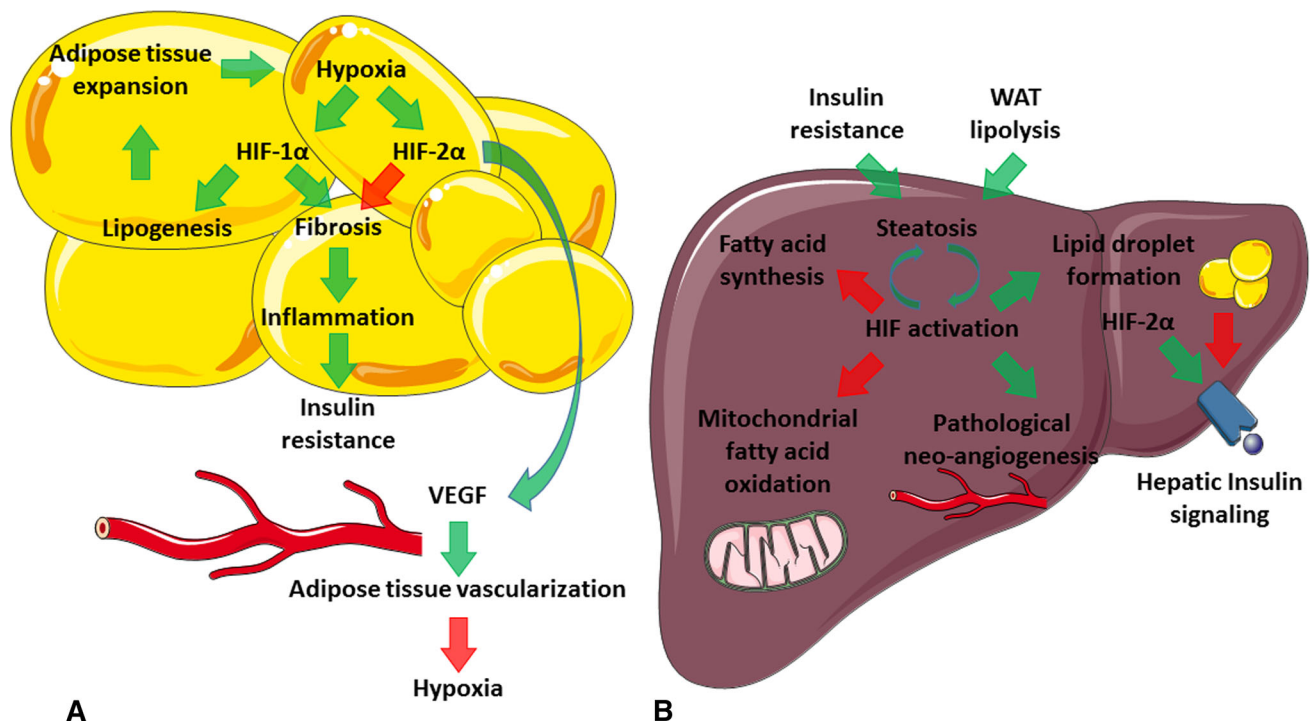*ND* not determined, *TG* triglycerides

Fig. 2 Proposed model of HIF actions in obesity and fatty liver. *Green arrows* activation; *red arrows* inhibition. **a** White adipose tissue (WAT) expansion in obesity causes regional hypoxia, activating the hypoxia adaptive response pathway. HIF-1α increases collagen deposition and crosslinking, causing WAT fibrosis. This in turn underlies the inflammation and insulin resistance characteristic of the metabolic syndrome. HIF-2α has positive effects on WAT, most likely through counteracting the HIF-1α pathway. In addition, HIF-2α also stimulates WAT angiogenesis, improving oxygen delivery. **b** Insulin resistance and WAT lipolysis cause ectopic lipid deposition in the liver. Hepatic steatosis is associated with perivenous hypoxia, again activating HIF signaling. Hypoxia has complicated effects, yet in general lowers oxygen and energy consumption through inhibition of both oxidative metabolism and fatty acid synthesis. Fatty acids are stored as lipid droplets to protect against the toxic effects of free fatty acids. Liver hypoxia is associated with pathological neo-angiogenesis, which further deranges metabolic function. The role of HIF-2α in NAFLD is still under debate, as it also improves hepatic metabolism, in particular hepatic insulin signaling

In addition, a Cre recombinase under the control of the human adipocyte fatty acid-binding protein (aP2) promotor has been used to obtain adipose-tissue specific knock-out (KO) of HIF-1α [46]. This protected against diet-induced glucose intolerance and WAT inflammation [40, 47]. HIF-1α KO increased energy expenditure, in part by increasing physical activity and in part by increasing fatty acid β-oxidation [47], in keeping with the role of HIFs in reducing oxidative metabolism [6]. These results were reproduced by the administration of PX-478, a selective HIF-1α inhibitor. PX-478 treatment reduced HFD-induced weight gain, adipocyte size, and serum leptin levels, suppressed WAT fibrosis and inflammation, and increased glucose tolerance [48]. In agreement with these data, temporally induced overexpression of a defective HIF-1α protein by means of a doxycycline sensitive transgene resulted in the same metabolic phenotype [48].

Conversely, HIF-2α is a promotor of adipocyte differentiation [49] and plays a protective role in adipose tissue. HFD feeding downregulates WAT HIF-2α levels [47], and HIF-2α haplodeficient mice displayed more glucose and insulin intolerance and showed increased expression of fibrotic collagen genes and pro-inflammatory macrophages in WAT compared to WT littermates [50]. Adipocyte-specific HIF-2α ablation had similar effects [40, 51]. Nevertheless, some ambiguity remains, as another group reported that VHL deletion, resulting in HIF-α accumulation, caused WAT inflammation. This effect was HIF-2α-dependent and resulted in critical hypertrophic cardiomyopathy, independent of weight gain or insulin intolerance [52].

A combined adipocyte-specific deletion of HIF-1α and HIF-2α phenotypically resembled HIF-1α KO mice, indicating that HIF-1α is the dominant isoform in WAT [40]. Correspondingly, deletion of HIF-1β, which impairs both HIF-1α and HIF-2α signaling, had comparable effects to HIF-1α deletion [47, 53].

At the molecular level, hypoxia induces a switch from aerobic to anaerobic metabolism. An increase in anaerobic glycolysis depends on increased glucose transport, which is highly upregulated in WAT by hypoxia [39]. Indeed, glucose uptake and lactate production progressively increase

as oxygen levels drop in adipose tissue, with major shifts in glucose utilization between oxygen tensions of 10 and 3 % O_2 [54]. As one animal study showed that oxygen levels were about 45 mm Hg (6.5 % O_2) in lean mice and 15 mm Hg (2 % O_2) in obese mice [10], these results show that adipocytes are highly sensitive to oxygen. A relatively small expansion of WAT can change oxygen tensions, which in turn can drastically impact WAT gene expression.

Interestingly, WAT HIF-1β ablation decreased glucose uptake through downregulation of the HIF-targets GLUT1 and 4, with a secondary decrease in glucose uptake and lipogenesis [53]. It has been suggested that this mechanism is involved in the reduced fat accumulation in HIF-1α and HIF-1β adipose KO mice. This hypothesis needs to be corroborated, as high-altitude hypoxia has also been known to induce marked weight loss through HIF signaling [55]. Moreover, insulin induces translocation and activation of GLUT4 in adipose tissue, whereas HIF-1β ablation greatly improved insulin sensitivity, so that an increase rather than a decrease in GLUT4 levels was to be expected [53, 56].

Role of PHDs to modulate HIFs in adipose tissue

A relatively novel approach in modifying the hypoxia adaptive response in obesity and metabolic disease has focused on inhibition of PHDs, inspired by promising applications of PHD inhibitors (PHI) in animal models of anemia and ischemic disease [57]. Two recently published clinical trials using the PHI roxadustat for anemia in chronic kidney disease show potential benefit, via upregulation of HIF-dependent genes in iron metabolism and hemoglobin synthesis [58, 59]. Trials investigating PHIs in coronary and peripheral vascular ischemia are underway [57].

Concerning adipose tissue, Matsuura et al. [60] found that adipocyte-specific deletion of PHD2 diminished HFD-induced weight gain. Adipocytes were smaller and glucose and insulin tolerance were likewise improved, secondary to a HIF-induced switch to anaerobic glycolysis. This resulted in inefficient metabolism, comparable to the weight-reducing effects of hypoxia in high altitude and chronic lung disease [55, 61]. However, Michailidou et al. [62] stated different conclusions using an almost identical study protocol. Their mice gained considerable weight but maintained normal glucose tolerance and experienced a remarkable reduction in liver triglyceride content. Reasons for this discrepancy could include an upregulation of PHD3 found only in one of these studies [60]. Indeed, PHD3 upregulation compensates for decreased PHD activity in continued hypoxia to adapt cells to a novel set point [35, 63]. A similar mechanism could account for the observed effect in the study performed by Matsuura et al., as PHD2

deletion artificially pre-adapts tissues to a lower oxygen set point [60].

Modulation of HIFs and PHDs in adipose tissue have thus resulted in discrepant outcomes, which are not easily reconciled. In this context, it is important to note that both HIF-1α and PHDs stand at cross-roads of diverse signaling pathways. PHDs not only sense oxygen but also various metabolites, such as citric acid cycle intermediates and reactive oxygen species [64, 65], whereas HIF-1α expression is, for example, also affected by adipocyte differentiation and insulin levels [66]. Furthermore, PHDs regulate targets other than HIF-1α, such as the key glycolytic enzyme pyruvate kinase M2 and the nuclear factor kappa-light chain-enhancer of activated B cells (NFκB) pathway [65, 67].

Concerning the role of PHDs in hypoxia, the hypoxia autoregulatory feedback mechanism could possibly be exploited by PHD inhibition to create a therapeutic window of HIF-1α and HIF-2α upregulation. A study by the group of Giaccia [68] provided the first proof-of-concept by demonstrating that liver-specific PHD3 deletion improves hepatic insulin signaling, mainly through HIF-2α stabilization. A further rise in HIF-2α (and HIF-1α) levels, obtained by simultaneous PHD1 and/or PHD2 deletions, did not further improve insulin signaling and even caused liver steatosis.

Although PHD inhibition is a promising approach, further research in this area is needed to explore these discordant results.

Hypoxia-induced angiogenesis in adipose tissue

One of the central hypoxia-adaptive mechanisms induced by HIFs, in virtually every tissue, is the stimulation of angiogenesis through VEGF upregulation.

Zhang et al. [69] found that HIF-1α inhibition in BAT results in a marked disruption of BAT angiogenesis, and secondarily in impaired thermogenic activity, glucose intolerance and WAT inflammation [69]. It is therefore surprising that neither HIF-1α overexpression nor deletion significantly altered angiogenesis in WAT [40, 43]. Conversely, adipocyte HIF-2α deletion resulted in deficient vascularization in both WAT and BAT [51]. Michailidou et al. further observed a twofold increase in WAT vessel density as a result of adipocyte-specific PHD2 deletion, mediated by HIF2α stabilization [62]. This was accompanied by a 50 % increase in WAT mass, but not with metabolic dysregulation. Mechanistically, a prophylactic vascular remodeling of the adipose tissue was induced by HIF upregulation, allowing a further increase in WAT mass whilst preventing WAT inflammation and ectopic fat deposition, a major cause of insulin resistance development

[62, 70, 71]. Several studies have indeed shown that transgenic overexpression of VEGF prevents and even reverts HFD-induced metabolic dysfunction through increased vascularization [72–74]. Accordingly, WAT contained less hypoxic areas and HIF-1α protein levels were significantly decreased compared to WT controls [73, 74]. VEGF also induced transdifferentiation of white to thermogenic beige adipocytes [18, 72, 75].

In summary, VEGF is a potent inducer of adipose tissue angiogenesis and can pre-adapt WAT to hypoxia [37]. This mechanism is HIF-2α dependent, while HIF-1α intriguingly seems to regulate vascularization in brown but not white adipose tissue (Fig. 2a).

Hypoxia signaling in fatty liver disease

Cross-talk between adipose tissue hypoxia and fatty liver disease

Adipose tissue hypoxia also stimulates basal lipolysis, another key feature of adipose tissue dysfunction. Although a study by Lolmede et al. could not demonstrate an influence of hypoxia on basal lipolysis in vitro [76], other groups subsequently reported an increase in lipolysis in vitro and in vivo in hypoxic conditions [77–79]. Corresponding to the latter studies, lipolysis is inversely correlated with WAT oxygen tensions in human patients [80]. The resultant release of fatty acids into the bloodstream leads to re-esterification and deposition of fatty acids in insulin-sensitive organs such as the liver, causing both insulin resistance and NAFLD [20, 21].

Accordingly, adipocyte HIF modulation affected liver lipid accumulation. Use of the hyperinsulinemic-euglycemic clamp technique to quantify insulin resistance revealed that adipocyte HIF-1α KO leads to decreased gluconeogenesis and thus improved insulin sensitivity [40, 47]. Moreover, both genetic and pharmacological HIF-1α inhibition were able to prevent NAFLD [40, 47, 48] as well as hepatocyte inflammation [40], which is an essential step in the progression to NASH [81], while adipocyte-specific HIF-1α overexpression had the opposite effects [43].

Hypoxia affects hepatic lipid metabolism

Because of the unique vascularization of the liver, there exists an oxygen gradient across the liver lobule, with lower oxygen tensions in the perivenous regions [22]. Research in animal models has shown that liver lipid deposition induces perivenous hypoxia and HIF-1α activation [25].

Important evidence for a pathophysiological role of hypoxia on hepatic lipid metabolism is provided by the now well established independent link between the obstructive sleep apnea syndrome, causing intermittent hypoxia, and NAFLD development and stage [82–84]. In experimental models of high-fat diet-induced obesity, exposure to intermittent hypoxia exacerbated insulin intolerance and hepatic steatosis [85]. Additionally, HIF-1α haplodeficiency limited steatosis development when mice were submitted to intermittent hypoxia, suggesting a role for HIF-1α in sleep apnea-related NAFLD [86].

Hypoxia signaling exerts its effects through a direct regulation of hepatic lipid metabolism (Fig. 2b). Breakdown of fatty acids through mitochondrial β-oxidation consumes large amounts of oxygen and is a major HIF target [87]. Hepatic fatty acid β-oxidation is regulated by the transcriptional control of peroxisome proliferator-activated receptor α (PPARα) over key mitochondrial oxidative enzymes [88]. Gene expression analysis revealed that PPARα and its target genes are indeed downregulated in hepatocyte HIF-2α overexpressing mice [89, 90], whereas HIF-1α KO in hepatocytes leads to an induction of oxidative enzymes [91]. PPARα agonism has beneficial effects in preventing NAFLD, NASH and progression to fibrosis [92]. Unsurprisingly, it has since long been an attractive pharmacological target [93], and promising new PPAR agonists are currently being developed for the treatment of metabolic disorders [94].

The effect of hypoxia signaling on lipogenesis, another oxygen-consuming metabolic process, is more ambiguous. Qu et al. [90] have shown a downregulation of key lipogenic enzymes 2 weeks after genetic induction of HIF-α in hepatocytes, in an attempt to limit energy consumption in hypoxic cells [87]. Nevertheless, 3 days after HIF-α induction a marked upregulation of lipogenic enzymes was observed. Possibly, this upregulation was secondary to a HIF-mediated increased uptake of glucose by hepatocytes [6, 95].

Moreover, the lipid binding protein adipocyte differentiation-related protein (ADRP) was upregulated in hepatocyte-specific HIF overexpressing mice [89, 96]. ADRP strongly promotes lipid droplet formation in various tissues [97, 98]. Formation of lipid droplets may represent a protective mechanism against the direct toxic effects of free fatty acids, which cause oxidative stress and inflammation [81], although this initiates a vicious circle of lipid deposition and hypoxia.

Extrapolation from experimental data to the role of hypoxia in human liver metabolism is not straightforward. The intricate regulation of hepatic lipid metabolism by HIFs is made even more complex by zonation of metabolism and oxygen supply in the liver. Perivenous hepatocytes, prone to hypoxia, are more engaged in lipogenesis, whereas the well-oxygenated periportal hepatocytes are more involved in β-oxidation [99]. Hence,

whereas overexpression HIF-α isoforms in mouse models inhibits fatty acid oxidation, perivenous hypoxia would not affect periportal β-oxidation in human NAFLD [87]. In patients with NAFLD, β-oxidation is indeed typically increased instead of reduced [100]. And although hypoxia inhibits lipogenesis, lipogenic genes are upregulated in human NAFLD and play a central role in hepatic fat accumulation [101, 102]. Therefore, zonated modulation of the hypoxia response in fatty liver disease could provide interesting insights.

Hepatocyte-specific HIF modulation in fatty liver disease

To modulate the hypoxia response system in vivo, hepatocyte-selective ablation and overexpression models of HIFs have been used. These were obtained either by injection of adenoviral particles or by hepatocyte-specific Cre-mediated recombination, in most cases by use of the albumin-Cre mouse strain [103] (Table 2).

The first studies showing the significance of HIF modulation in hepatic steatosis used conditional VHL deletions, which prevented proteasomal degradation of hydroxylated HIFs. This resulted in increased vascularization, cavernous liver hemangiomas and severe hepatic steatosis [104]. This phenotype was copied by simultaneous HIF-1α and HIF-2α overexpression using degradation-resistant HIF variants (HIF-1dPA and HIF-2dPA) [105]. Furthermore, HIF-1α activation resulted in moderate steatosis, whereas HIF-2α overexpression greatly increased liver microvascular density with only minimal lipid deposition [105].

By inducing simultaneous deletions in VHL and HIF-1α and/or HIF-2α, to reverse the overexpression of specific HIFs after VHL deletion, a dominant role for HIF-2α in fatty liver disease was asserted, as deletion of HIF-2α, but not of HIF-1α, prevented the severe steatosis associated with VHL deletion [89]. Qu et al. [90] have confirmed these data and showed that temporal disruption of VHL causes severe steatohepatitis with progression to fibrosis. Again, deletion of HIF-2α but not HIF-1α rescued this phenotype.

Conversely, several papers have convincingly shown pathophysiological effects of HIF-1α in fatty liver disease. Using degradation-resistant HIF-1dPA mice, Nath et al. [96] demonstrated that hepatocyte HIF-1α overexpression causes hepatomegaly and liver triglyceride accumulation, which were further exacerbated by liquid ethanol administration, whereas hepatocyte-specific HIF-1α KO provided protection against steatosis. Comparable results were obtained in models of diet-induced obesity, as antisense nucleotide treatment, resulting in both hepatocyte and adipocyte HIF downregulation, protected against adverse metabolic effects of HFD feeding [106]. However,

Nishiyama et al. [91] postulated the opposite hypothesis of HIF-1α as an adaptive mechanism, as they found that hepatocyte HIF-1α deletion aggravated lipid deposition in an alcoholic model of fatty liver disease. The same group also reported the development of insulin resistance when these mice were fed a HFD [107]. These discrepant results are difficult to reconcile, as both groups [91, 96] have used identical genetic approaches. However, different control animals were used by the two groups, which may have biased the outcome. It has also been suggested that differences in the intestinal flora between the two research group animal facilities and between the different mice strains could have influenced steatosis progression, as it is well known that the gut microbiome contributes to the development of NAFLD [96, 108–110]. Research in related fields, such as liver fibrosis, nonetheless suggests that HIF-1α is a mediator of liver disease progression rather than a protective regulator [111].

In addition to HIF1α modulation, hepatocytic deletion of HIF-1β, targeting all HIF signaling pathways, has resulted in increased gluconeogenesis and mild whole-body insulin resistance, yet slightly lowers hepatic triglyceride content [112]. Consistently, another group reported a decrease in steatosis in hepatocyte HIF-1β null mice when subjected to binge ethanol models [113].

Taken together, these studies indicate that both HIF-1α and HIF-2α promote hepatic lipid accumulation. Which HIF-α isoform plays the dominant role, however, remains to be elucidated. The difference in the genetic mouse models used may explain some of the discrepancies. For instance, indications for a pathological role of HIF-2α in WAT [52] and fatty liver disease [89, 90] have solely been obtained in mice with tissue-specific VHL deletions by showing that additional HIF-2α KO reversed these effects. Further research with other genetic approaches to HIF-2α could help to clarify this discussion.

Role of PHD inhibition as a treatment strategy in fatty liver disease

Parallel to PHD inhibition in obesity, it also represents an attractive target in fatty liver disease. Rahtu-Korpela et al. [114] have used hypomorphic PHD2$^{gt/gt}$ mice, which express decreased amounts of PHD2 and show differing levels of HIF-1α and HIF-2α stabilization across various tissues [115]. Whereas 1-year-old control littermates spontaneously developed insulin resistance and hepatic steatosis, PHD2$^{gt/gt}$ mice were protected against metabolic dysregulation through an upregulation of insulin receptor substrate 2 (Irs2) [114]. These mice retained improved metabolic parameters after a HFD. As only hepatic HIF-2α was stabilized, these results are concordant with a study by Wei et al. [116]. They found that VEGF inhibition and the

Table 2 Summary of key studies on hepatocyte-specific HIF modulation

Gene modulation	Study protocol	Phenotype	Lipogenic genes	β-Oxidation	References
HIF-1α and/or HIF-2α overexpression	Alb-Cre × VHL$^{f/f}$, HIF-1dPA and/or HIF-2dPA	Severe steatosis in Alb-Cre; VHL$^{f/f}$ and Alb-Cre; HIF1dPA/HIF2dPA mice	ND	ND	Kim et al. [105]
		Moderate steatosis in Alb-Cre; HIF1dPA mice			
HIF-1α and/or HIF-2α overexpression	Alb-Cre × VHL$^{f/f}$ ± HIF-1α$^{f/f}$ and/or HIF-2α$^{f/f}$	Severe steatosis in mice overexpressing HIF-2α (±HIF-1α)	Downregulation	Suppression	Rankin et al. [89]
		HIF-1α overexpression alone does not cause steatosis			
Temporally induced HIF-1α and/or HIF-2α overexpression	SA-Cre-ER^{T2} × VHL$^{f/f}$ ± HIF-1α$^{f/f}$ and/or HIF-2α$^{f/f}$	Severe steatosis in mice overexpressing HIF-2α (±HIF-1α)	Upregulated after 3 days, downregulated after 14 days	Suppression	Qu et al. [90]
		HIF-1α overexpression does not cause steatosis			
HIF-1α deletion	Alb-Cre × HIF-1α$^{f/f}$ + Lieber-DeCarli diet	Exacerbated steatosis	Upregulated	Induction	Nishiyama et al. [91]
		Increased serum TG			
PHD inhibition	Dimethyloxalylglycine	Decreased steatosis and liver TG	Downregulated	ND	
HIF-1α overexpression	Alb-Cre × HIF-1dPA + alcoholic diet	Increased steatosis and liver TG, increased serum ALT	ND	Suppression	Nath et al. [96]
HIF-1α deletion	Alb-Cre × HIF-1α$^{f/f}$ + alcoholic diet	No increase in ALT, protected against steatosis	ND	ND	
HIF-1β deletion	Adenoviral Cre injection in HIF-1β$^{f/f}$ mice ± high-fat diet	Increased gluconeogenesis	Upregulated	Not affected	Wang et al. [112]
		Mild insulin resistance			
		Decreased liver TG content			

ND not determined, *ALT* alanine aminotransferase, *TG* triglycerides

concomitant impaired angiogenesis caused local hypoxia and specifically stabilized HIF-2α, which in turn improved insulin signaling through Irs2 induction. Therefore, these studies postulate a beneficial effect of moderate HIF-2α upregulation in fatty liver disease through PHD inhibition, contrary to the studies that used VHL deletion to stabilize HIFs discussed in the previous paragraph.

Remarkably, pharmacological PHD inhibition with the compound FG-4497 led to highly similar results in the models of metabolic dysregulation, suggesting that treatment could not only prevent but also reverse metabolic dysfunction [114]. In accord with this study, Nishiyama et al. [91] demonstrated a marked improvement in alcoholic fatty liver disease upon treatment with the PHI dimethyloxalylglycine. Although isoform-specific PHD inhibitors are not yet available, these could represent a new approach in the treatment of obesity and its associated metabolic disorders.

Hypoxia-induced angiogenesis in fatty liver disease

In chronic liver disease, especially hepatocellular carcinoma [28, 117], hypoxia-induced angiogenesis is an attractive therapeutic target, yet its role in NAFLD is not extensively researched. We have shown that increased angiogenesis and VEGF upregulation are hallmarks of NAFLD progression, whereas treatment with anti-VEGF receptor 2 antibodies prevents disease progression [118]. Wei et al. [116] have additionally demonstrated that VEGF inhibition improves hepatic insulin signaling through induction of hypoxia and stabilization of HIF-2α.

In general, HIF-2α seems to play a major role in blood vessel normalization and homeostasis and can impact tissue function and metabolism in various organs [34, 119]. Thus, the HIF/PHD pathways could represent an attractive therapeutic strategy to remodel the hypoxia-induced angiogenic response in obesity and NAFLD.

Conclusion and future perspectives

The prevention and treatment of obesity and the associated non-alcoholic fatty liver disease has become one of the major priorities in public health care. Unfortunately, sustained weight loss is difficult to achieve for many patients, and no pharmacological treatment can satisfactorily remedy these conditions. Therefore, a greater understanding of the underlying pathological mechanisms is required. Hypoxia signaling pathways represent an attractive target in obesity and NAFLD. Although HIF modulation in adipose tissue and fatty liver has yielded conflicting results,

prolyl hydroxylase inhibition is a promising approach as studies have consistently yielded beneficial effects.

It is of significance that pharmacological HIF-1α and PHD inhibition were able to prevent or reverse the metabolic dysregulation associated with obesity, thus proving the therapeutic potential of tweaking the hypoxia response. As there are still many gaps in our understanding of hypoxia in metabolic disorders, further research is warranted to determine the physiological functions of each HIF isoform, and to explore their cell-specific role in adipose and hepatic tissue.

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