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Fundamental roles for hypoxia signalling in adipose tissue metabolism and inflammation in obesity

Zoi Michailidou

Over a decade ago the notion that adipose tissue could become hypoxic during adipose expansion with obesity was proposed. A series of elegant studies in mouse models of obesity have demonstrated that severe adiposity could lead to low oxygen levels within adipose tissue, triggering induction of the hypoxia inducible factor (HIF) signalling cascade, and consequently an upregulation of pro-inflammatory responses that lead to insulin resistance. However, genetic models targeting different components of the HIF pathway have produced conflicting results and revealing that distinct HIF isoforms play tissue-specific roles in metabolic function. This review discusses the consequences and mechanisms of altered oxygenation on adipocyte function and the potential for therapeutic targeting of HIF signalling in obesity and its metabolic complications.

Address
Centre for Cardiovascular Sciences, University of Edinburgh, United Kingdom

Corresponding author: Michailidou, Zoi (v1zmicha@exseed.ed.ac.uk)

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Introduction

Hypoxia, or low oxygenation, is a feature of normal tissues in health and a pathogenic consequence of disease. Hypoxia triggers a plethora of cellular responses to restore oxygenation, normal metabolism and cell survival. The master regulators of this adaptive response are the hypoxia-inducible factors (HIFs) [1]. Exquisite regulation of HIFs is tightly regulated by the cellular oxygen sensors, HIF-prolyl hydroxylases (PHDs) [2]. PHDs hydroxylate HIFs, tagging them for recognition by the Von Hippel Lindau (VHL) ubiquitin ligase system and thus facilitating HIF proteasomal degradation in an oxygen-dependent manner [2]. HIFs and PHDs are implicated in physiological processes and this is dysregulated in pathological conditions [1,2]. Their role in regulating adipocyte function has been demonstrated in transgenic models targeting the HIF signalling system either systemically (whole animal gene knockout) or specifically in adipose tissue.

Adipose tissue expansion in obesity can lead to local hypoxia and induction of HIF-α (3). Activation of the HIF-α-signalling cascade has been implicated in adipocyte dysfunction, increased adipose tissue inflammation and insulin resistance in mouse models of diet-induced obesity (DIO) [3–7]. Tissue oxygen tension is maintained by the balance between oxygen demand and supply. Most studies attributed the adipocyte hypoxia to reduced oxygen supply due to defective vascularization during expansion [3–7]. Recently, however, increased oxygen demand has been implicated as a cause of adipocyte hypoxia [8••]. Thus, during high fat feeding, saturated fatty acids may drive stimulation of adenine nucleotide translocase 2 (ANT2), that in turn increases uncoupled mitochondrial respiration and thus increases oxygen consumption [8••,9•]. In either case, it is evident that the excessive expansion of adipose tissue in obesity disturbs the balance between oxygen supply and demand.

Although the HIF/PHD pathway has been implicated in regulating metabolic pathways in other key metabolic organs such as liver and muscle that are affected by obesity, this review focuses on our current understanding of the hypoxia response principally on adipocyte function, and the therapeutic potential of modulating the PHD/HIF axis in obesity.

The role of HIFs in obesity and related metabolic complications: HIF-1α or HIF-2α?

Activation of adipose-HIF1α could have adverse or beneficial metabolic outcomes. The first study used a transgenic model of adipose overexpression of a constitutively active form of HIF-1α and showed that this led to increased fat mass and adipocyte dysfunction with inflammation, fibrosis and insulin resistance in DIO [6]. This study also showed that HIF-1α activation did not induce the classical Vegfα-vascularisation response [6], which was also confirmed by other studies [10••,11,12•], rather HIF-1α induced a collagen-driven profibrotic response that led to maladaptive adipose tissue remodelling and insulin resistance [6]. Follow-on studies looking at HIF-1α deficiency strategies (using the ap2 [fabp4] promoter driven cre to produce adipose knock-down of 'floxed' gene targets) showed conflicting effects. Zhang *et al.* showed that dominant negative human HIF-1α (also

under the ap2 promoter) led to susceptible to DIO mainly due to loss of thermogenic capacity in brown adipose tissue (BAT) [11]. In contrast a series of studies of adipose HIF-1α deletions showed protection from DIO [8••,13,14•] due to reduced fat mass, adipocyte size and inflammation and increased adipose mitochondrial biogenesis, increased energy expenditure, with improved glucose tolerance and insulin sensitivity. Most of the effects were apparent in white adipose tissue (WAT) with less obvious phenotypes in brown adipose (BAT).

It is becoming increasingly apparent that HIF-1α and HIF-2α have divergent roles in physiology and pathology in various diseases [15]. It is also evident that this is the case in adipose function as deletion of adipose HIF-1α reverses the obesity-driven WAT inflammation, reduces body fat mass and protects from insulin resistance, whereas deletion of adipose HIF-2α, or HIF-2α heterozygosity, leads to WAT inflammation, higher WAT mass in DIO and insulin resistance [8••,16,17•]. HIF-2α also seems to be more important in BAT function than HIF-1α, as it is one of the most highly induced genes after cold exposure [18•]. Consistent with an important biological role, loss of HIF-2α signalling impaired thermogenesis by decreasing *Ucp1* and *Vegfa* expression and vascularization in BAT which was rescued by VEGF administration [17•]. Finally, adipocyte deletion of HIF-1α and HIF-2α shifted the phenotype towards that seen with HIF-1α deletion alone [8••]. Notably, HIF-1α and HIF-2α depletion in adipocytes was achieved by using the Fabp4-Cre mice. This approach may lead to recombination (gene deletion) in other cells and tissues, such as macrophages or endothelial cells, therefore adipocyte-specificity for the phenotypes of these models cannot be guaranteed.

Nevertheless, results from a number of studies support adipocyte HIF-2α as the major driver of the key protective metabolic effects during adipose expansion, that is, enhanced vascularization potential. Thus, adipose-HIF-1α modulation (deletion or overexpression models) failed to show an effect of the most well studied HIF-α target gene, *Vegfa*. One possibility could be that targeting primarily adipocyte HIF-1α is not sufficient to induce a vascularization programme. Indeed, other cell types were implicated in this response, for example, in a model of diet-induced obesity, mice lacking myeloid HIF-1α developed a normal vasculature despite reduced whole adipose *Vegfa* levels [19]. The authors concluded that myeloid HIF-1α could differentially regulate *Vegfa* levels in different cell types in adipose tissues (macrophages, endothelial cells and preadipocytes). Macrophage HIF-1α was highlighted as a key driver of pathological adipose tissue expansion. Alternatively, in the vascularization process HIF-α isoforms have distinct roles, HIF-1α regulates mainly endothelial cell proliferation, migration and sprouting, whereas HIF-2α controls vascular morphogenesis [20]. As suggested from the studies above [12•,17•], the isoform specificity might be the key, with adipocyte HIF-2α the dominant isoform to drive neovascularization in adipose tissue.

These studies highlight that HIF-isoforms play distinct, non-redundant roles in adipocyte function. HIF-2α in adipocytes is necessary to protect from adipocyte dysfunction in obesity and this could be due to induction of an adipocyte vascularization phenotype. The clear message is that careful targeting of specific HIF-α isoforms will need to be considered within the context of existing therapeutic regimes where adipose tissue function may impact health and for potential HIF targeting for obesity and diabetes therapeutics.

Is there adipose hypoxia in humans?

Studies in humans have been less conclusive in demonstrating that hypoxia and HIF-α are directly linked to the metabolic dysfunction in obese individuals. There is a consensus that arterial blood oxygenation is lower in obese compared to lean individuals [21,22]. Kabon *et al.* and Pasarica *et al.* reported that in obese the subcutaneous upper arm or abdominal WAT partial oxygen pressure to be significantly lower than lean individuals [23,24•]. However, Goossens *et al.* reported higher adipose tissue oxygen partial pressure in obese compared to lean individuals, accompanied by insulin resistance, impaired adipose tissue vascularization and higher adipose tissue gene expression of inflammatory cell markers [25•]. Hodson *et al.* did not find any evidence of hypoxia metabolic signatures (i.e. increased lactate, increased adipose glucose uptake) in obese individuals thus concluding that it is unlikely that adipose in obesity is hypoxic [26•]. Although there are methodological limitations on all of the above studies, we still need to bridge the gap of knowledge of what is considered the normal adipose partial oxygen pressure, a consensus of what is relative hypoxia in humans and whether is the driver of metabolic changes during adipose expansion.

The relevance of HIF signalling could be considered in individuals with obstructive sleep apnea (OSA) which is highly associated with obesity and has been linked to impaired insulin sensitivity and higher risk of diabetes [27]. OSA is associated with repetitive episodes of hypoxia/reoxygenation (CIH) and has been associated with increased production of reactive oxygen species, fibrosis, and immune cell infiltration [28]. Although OSA is a disorder manifested by repeated and transitory events of extremely variable oxygen desaturation and blood CO_2 retention, it is clear that severe hypoxia (~5% O_2 inspired) per se decreases insulin sensitivity, whereas moderate hypoxia (13–15% O_2) improves insulin sensitivity [29]. Interestingly, CIH did not further increase serum proinflammatory cytokines or adipose tissue ER stress and hypoxia mRNA markers when compared with BMI-matched obesity without CIH [30]. Individuals with OSA and different degrees of obesity or adiposity

(normal, obese and morbidly obese) could provide the key information on molecular hypoxia signatures.

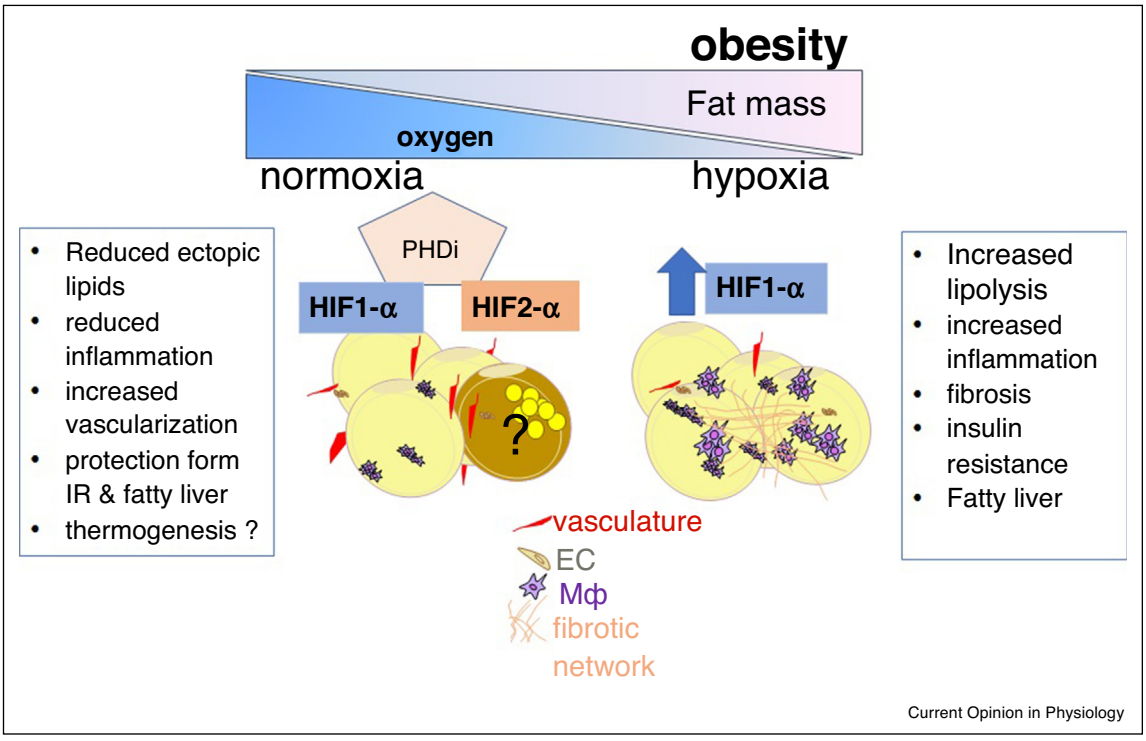
Crucially, there is a need to refine methodology of measuring tissue oxygen availability and a consensus in defining adipose hypoxia in order to define in humans whether the HIF-pathway is switched on after a certain degree of adipose mass expansion, whether hypoxia develops more in certain adipose depots (subcutaneous or visceral), whether is driven by a specific HIF-α isoform and if this is an adaptive or a maladaptive response.

Harnessing the HIF response by inhibiting the oxygen sensors. Programming for metabolic flexibility?

Lessons from genetic models of PHD deletion

One way of pre-empting and fine tuning the HIF response is by targeting the key HIF regulators, HIF-prolyl hydroxylases, that would permit stabilization of both HIF-1α and HIF-2α. Mouse models of either global or adipocyte-specific deletion of *Phd2* (most abundant adipose isoform) showed beneficial metabolic outcomes. Recently, genetic deficiency of *Phd2* (*hypomorphic for Phd2*) in mice was shown to reduce body weight, white adipose tissue mass and subsequently WAT inflammation [31•]. These mice were protected from the development of age or diet-induced insulin resistance and had lower serum cholesterol levels and improved HDL/LDL ratios [31•]. Further experiments also revealed that Phd2 deficiency protected against steatohepatitis and atherosclerosis [32]. The beneficial metabolic phenotype with reduced body weight, white fat mass, inflammation and protection from insulin resistance was confirmed in another study of adipose-specific deletion of Phd2 [33•]. Furthermore, specifically deleting *Phd2* in adipocytes blunted lipolysis by mechanistically suppressing the phosphorylation of hormone sensitive lipase, therefore reducing ectopic lipid deposition in liver or skeletal muscle [12•]. Supporting evidence towards beneficial metabolic outcomes also came from deletion of the *Phd1* and *Phd3* isoforms. *Phd1* deficiency ($Phd1^{-/-}$) improved glucose tolerance in DIO in mice, normalized hypercholesterolemia and reduced circulating inflammatory cells when crossed to LDLRKO mice [34]. Acute loss of *Phd3*($Phd3^{fl/fl}$ injected with adenoviral Cre) in DIO decreased fasting blood glucose by 30% and fasting serum insulin by 50% compared to the diabetic controls [35••]. Mechanistically, the loss of *Phd3* specifically stabilized HIF-2α, which increased Irs2 transcription

Figure 1



The balance of the HIF-α response in adipocytes determines metabolic outcomes.
Adipose expansion in obesity leads to adipose hypoxia and HIF-1a activation. This drives pro-inflammatory, fibrotic response but fails to induce vascularization thus leading to ectopic lipid accumulation and insulin resistance (right). In contrast, HIF-2α is essential in maintaining vascularization (left) during adipose expansion, facilitates the thermogenic response during cold exposure and has a beneficial metabolic outcome. HIF-prolyl hydroxylase deficiency or inhibition (PHDi), and thus stabilization of both isoforms is permissive towards a favourable metabolic profile (left).

and insulin-stimulated Akt activation thus ameliorating the diabetic phenotype induced by DIO [35••]. Finally, deletion of another HIF regulator, asparaginyl hydroxylase (factor inhibiting HIF, or FIH), allowed HIF-α transactivation capacity [36]. *Fih*$^{-/-}$ mice exhibited reduced body weight, elevated metabolic rate, and improved glucose and lipid homeostasis and were resistant to high-fat-diet-induced weight gain and hepatic steatosis [37•]. Taken together these data suggest that PHDs and FIH are essential regulators of metabolism. Pharmacological targeting of these proteins may provide advantages during or after a hypoxic insult.

Lessons from preclinical models

Is there a scope of activating the HIF signalling pathway to treat obesity and metabolic complications?

HIF-prolyl hydroxylase inhibitors (PHDi) that target PHD1-2-3 are in phase II or III clinical trials for the treatment of renal anaemia. Apart from their role in increasing haemoglobin levels and correcting anaemia, preclinical studies have shown encouraging data that PHD inhibitors (i.e. daprodustat and roxadustat) could lower serum cholesterol levels [38,39], possibly implicating them in novel pathways of regulating lipid homeostasis. In mouse preclinical models of diet-induced obesity or LDL-receptor deficient mice (LDLRKO), the HIF-prolyl hydroxylase inhibitor, FG-4497 (FibroGen Inc), reduced body weight, obesity-related adipose inflammation, insulin resistance and serum cholesterol levels [32]. Additionally, in the LDLRKO mice FG-4497 reduced the atherosclerosis [32]. Careful consideration should be given when designing strategies to activate the HIF pathway, for example to modulate whole body metabolism, as HIF activation targets a plethora of pathways and has been linked to cancer. However, taken together the beneficial effects of the HIF-prolyl hydroxylase inhibition on body weight regulation, glucose and lipid lowering, there is a strong case that this should be explored further as a potential therapeutic avenue to obesity-related complications such as diabetes and fatty liver (Figure 1). Further experiments to identify if targeting of a specific PHD isoform will shift towards the apparently metabolically beneficial effects of HIF-2α activation might provide a way of minimizing complex responses from non-specificity.

Conclusions

In summary, the HIF transcriptional response is protective during states of cellular hypoxia. Harnessing the hypoxic response could have beneficial effects on metabolism and this is an increasingly important concept to be explored in the therapeutic settings of obesity-related metabolic dysfunction. Targeting the HIF/ hydroxylase pathway could offer therapeutic opportunities in for example regulating blood lipid levels. Further preclinical and experimental medicine studies are required to assess safe and effective treatment for human diseases associated with obesity such as dyslipeadimia and type 2 diabetes.

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Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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