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## The role of hypoxia-inducible factors in metabolic diseases

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### Abstract

Hypoxia-inducible factors (HIFs), a family of transcription factors activated by hypoxia, consist of three  $\alpha$ -subunits (HIF1 $\alpha$ , HIF2 $\alpha$  and HIF3 $\alpha$ ) and one  $\beta$ -subunit (HIF1 $\beta$ ), which serves as a heterodimerization partner of the HIF $\alpha$  subunits. HIF $\alpha$  subunits are stabilized from constitutive degradation by hypoxia largely through lowering the activity of the oxygen-dependent prolyl hydroxylases that hydroxylate HIF $\alpha$ , leading to their proteolysis. HIF1 $\alpha$  and HIF2 $\alpha$  are expressed in different tissues and regulate target genes involved in angiogenesis, cell proliferation and inflammation, and their expression is associated with different disease states. HIFs have been widely studied because of their involvement in cancer, and HIF2 $\alpha$ -specific inhibitors are being investigated in clinical trials for the treatment of kidney cancer. Although cancer has been the major focus of research on HIF, evidence has emerged that this pathway has a major role in the control of metabolism and influences metabolic diseases such as obesity, type 2 diabetes mellitus and non-alcoholic fatty liver disease. Notably increased HIF1 $\alpha$  and HIF2 $\alpha$  signalling in adipose tissue and small intestine, respectively, promotes metabolic diseases in diet-induced disease models. Inhibition of HIF1 $\alpha$  and HIF2 $\alpha$  decreases the adverse diet-induced metabolic phenotypes, suggesting that they could be drug targets for the treatment of metabolic diseases.

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Hypoxia-inducible factors (HIFs) are members of the basic helix-loop-helix Per-Arnt-Sim (bHLH-PAS) transcription factor superfamily and consist of a heterodimer of an oxygen-sensitive  $\alpha$ -subunit and a constitutively expressed  $\beta$ -subunit (HIF1 $\beta$ )<sup>1,2</sup>. HIF1 $\beta$  was described as the aryl-hydrocarbon receptor (AHR) nuclear translocator (ARNT) because it was first discovered as a dimerization partner for the AHR<sup>3</sup>. Three oxygen-sensitive HIF $\alpha$  subunits are found in mammals — HIF1 $\alpha$ , HIF2 $\alpha$  and HIF3 $\alpha$  — with HIF1 $\alpha$  and HIF2 $\alpha$  being the most widely studied  $\alpha$ -subunits. The functions of HIF3 $\alpha$  are less well established,

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#### Author contributions

F.J.G. and C.J. researched the data for the article, contributed to discussion of the content, wrote the article and reviewed and/or edited the manuscript before submission. C.X. reviewed and/or edited the manuscript before submission.

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but a number of alternatively spliced variants from *HIF3A* could generate dominant negative inhibitors of HIF1 $\alpha$  and HIF2 $\alpha$ <sup>4</sup>. Whether these splice variants are of biological importance is still unknown.

Under normoxia, HIF $\alpha$  proteins are rapidly hydroxylated by a group of prolyl hydroxylase domain (PHD) enzymes: PHD1, PHD2 and PHD3 (FIG. 1). Once hydroxylated, HIF $\alpha$  is subjected to conjugation with the E3 ubiquitin ligase complex containing the von Hippel-Lindau disease tumour suppressor (VHL) protein, leading to rapid degradation of HIF $\alpha$ <sup>5</sup>. In a second mode of HIF $\alpha$  regulation, hydroxylation of an HIF $\alpha$  asparaginyl residue by factor inhibiting HIF1 (FIH1; also known as HIF1AN) inactivates HIF $\alpha$  transcriptional activity by preventing interaction of the transcriptional co-activator cAMP-response element binding protein (CREB)-binding protein (CBP) and histone acetyltransferase p300 (p300 HAT) with HIF $\alpha$ , thus impeding transcription<sup>6</sup>. PHDs and FIH1 are O<sub>2</sub>-dependent oxygenases. Conversely, during hypoxia, the HIF $\alpha$  subunits are not hydroxylated and are stabilized by the limited oxygen that is a co-substrate for PHDs and FIH1. This effect decreases the rate of HIF $\alpha$  protein hydroxylation by PHDs and FIH1 and leads to protein stabilization and CBP-p300 co-activator complex augmented transcriptional activation, respectively, and increases levels of HIF $\alpha$  and the activation of HIF target gene expression<sup>7-9</sup>.

HIFs interact with upstream binding sites (called HIF regulatory elements) of target genes to activate transcription. No evidence exists of any agonist binding sites on HIF that serve to activate transcription as found with other ligand-dependent transcription factors such as the AHR, another member of the bHLH-PAS superfamily<sup>10,11</sup>. By contrast, HIF1 $\beta$ , which serves as the binding partner for all HIF $\alpha$  subunits and the AHR, and other bHLH-PAS transcription factors such as period (PER) and simple minded (SIM), is constitutively expressed in most tissues<sup>10,12</sup>.

HIF1 $\alpha$  and HIF2 $\alpha$  are differentially expressed in various tissues and cell types; HIF1 $\alpha$  is expressed in many tissues and cells, whereas HIF2 $\alpha$  has a more restricted expression pattern and is found only in vertebrates<sup>13</sup>. The single HIF in invertebrates resembles HIF1 $\alpha$  more closely than HIF2 $\alpha$  and modulates the lifespan in *Caenorhabditis elegans*<sup>14</sup>. HIF1 $\alpha$  has a role in mammalian development and in adult physiology as a regulator of intermediary metabolism, notably the control of glycolysis under low O<sub>2</sub> levels, and an activator of genes involved in the regulation of glucose metabolism<sup>13</sup>. Conversely, HIF2 $\alpha$  is preferentially expressed in endothelial cells of the lung and epithelial cells of the intestine and other tissues<sup>15</sup> and has a number of functions in physiology and disease<sup>16</sup>. HIF1 $\alpha$  and HIF2 $\alpha$  both regulate the expression of *Glut1* (encoding glucose transporter 1 (GLUT1; also known as SLC2A1)), *Vegfa* (encoding vascular endothelial growth factor A (VEGFA)) and many other target genes. Both HIF1 $\alpha$  and HIF2 $\alpha$  bind to the same partner HIF1 $\beta$  and response elements; however, some selectivity in target gene activation might exist between the two HIF $\alpha$ s that depends in part on the chromatin context, which influences gene expression in different cell types<sup>13,17,18</sup>. Both HIFs can be stabilized and activated in cancer cells, where they induce expression of genes such as *VEGF*<sup>19,20</sup>, which promotes angiogenesis in solid tumours, and either directly or indirectly activate genes involved in cell proliferation, epithelial-to-mesenchymal transition, apoptosis and metastasis or tumour invasion<sup>18</sup>. To inhibit angiogenesis and tumour growth, antibodies targeting VEGFA were developed for

use in cancer therapy<sup>21</sup>. Inhibitors of HIF2 $\alpha$  have been developed and are in clinical trials to evaluate their use in treating kidney cancers associated with VHL mutations (where HIF2 $\alpha$  is overexpressed because of the genetic loss of VHL expression)<sup>22–24</sup>.

## Hypoxia and HIF1 $\alpha$ in metabolic diseases

Hypoxia occurs within the expanding adipose tissue of people with obesity and in animal models of obesity<sup>25,26</sup>. This hypoxia is largely due to the increased size of adipocytes, decreased adipose tissue vascularization and increased fatty acid metabolism that consume oxygen<sup>27</sup>. Additionally, during the early stages of advancing obesity caused by a high-fat diet (HFD), adipocyte respiration is uncoupled, resulting in increased oxygen consumption and adipocyte hypoxia<sup>28</sup>. The elevated uncoupling consumes oxygen, in part because saturated fatty acids activate the inner mitochondrial membrane ADP/ATP translocase 2 (ANT2; also known as SLC25A5). In vitro studies using 3T3-L1 adipocytes and human subcutaneous abdominal adipocytes show that insulin resistance is also aggravated by HIF $\alpha$  activation during hypoxia<sup>29</sup>. These studies suggest that HIF1 $\alpha$  and HIF2 $\alpha$  in adipocytes would be stabilized and thus accumulate during hypoxia, resulting in the activation of HIF $\alpha$  target genes.

### HIF1 $\alpha$ in adipose tissue

**HIF1 $\alpha$  activation protects against obesity and insulin resistance.**—A number of studies suggest that HIF1 $\alpha$  either promotes or inhibits metabolic diseases (TABLE 1). The first clue that HIF1 $\alpha$  in adipocytes influences obesity and associated metabolic diseases was the observation that mice overexpressing HIF1 $\alpha$  had elevated obesity and insulin resistance associated with increased inflammation and fibrosis<sup>30,31</sup>. However, another group found that mice in which HIF1 $\alpha$  expression is inhibited in adipose tissue, owing to transgenic expression of a dominant negative protein that inhibits HIF1 $\alpha$  signalling, were more obese and insulin resistant after an HFD than wild-type mice after an HFD<sup>32</sup>. These mice also had larger lipid droplets in brown adipose tissue (BAT) that probably resulted from decreased expression of mitochondrial biogenesis-related genes. In addition, another study did not find an effect on mitochondrial biogenesis-related genes in brown adipocytes, as knockdown of HIF1 $\alpha$  expression actually decreased expression of glycolytic enzymes<sup>33</sup>. Another group produced transgenic mice with constitutive expression of both HIF1 $\alpha$  and HIF2 $\alpha$  in adipocytes by tissue-specific knockout of PHD2 (REF.<sup>34</sup>). When these mice were fed an HFD, they were more insulin sensitive with less body weight than the corresponding wild-type mice expressing PHD2 in adipose tissue. The BAT depot in these transgenic mice was also expanded as revealed by increased UCP1 expression<sup>34</sup>. Taken together, these studies suggest that HIF1 $\alpha$  stimulates the thermogenic functions of BAT by controlling mitochondrial biogenesis and glycolysis, implying that activation of HIF1 $\alpha$  in adipose tissue could be of benefit for the treatment of obesity and insulin resistance. Additionally, studies suggesting that HIF1 $\alpha$  protects against obesity and insulin resistance examined mice with forced or transgenic overexpression of HIF1 $\alpha$ ; thus, the data must be carefully interpreted relative to physiological importance in wild-type mice.

**HIF1 $\alpha$  inhibition ameliorates obesity and insulin resistance.**—Although some studies have suggested that HIF1 $\alpha$  activation is beneficial for diet-induced metabolic diseases, other studies have found that HIF1 $\alpha$  in adipose tissue potentiates obesity and insulin resistance instead of alleviating these conditions. It is well established that levels of HIF1 $\alpha$  are elevated in the adipose tissues of obese mice. Two potential mechanisms could account for this increase: hypoxia due to mitochondrial consumption of oxygen and increased insulin signalling. Saturated fatty acids in mouse adipose tissue increased expression of ANT2, resulting in an elevation in adipocyte oxygen consumption via the uncoupling of mitochondrial respiration<sup>28</sup>. This uncoupling leads to cellular hypoxia, which triggers the stabilization of HIF1 $\alpha$  expression. Other studies have shown that insulin also increased HIF1 $\alpha$  protein expression and HIF1 $\alpha$  signalling in adipocytes; however, the mechanism remains unknown<sup>35</sup>. The elevated levels of HIF1 $\alpha$  might potentiate insulin signalling in adipocytes to promote conversion of glucose into fatty acids and triglycerides, resulting in obesity.

To investigate the role of adipose hypoxia and HIF in obesity and insulin resistance, two independent studies characterized mice lacking the expression of HIF1 $\alpha$  and HIF1 $\beta$ <sup>36</sup> and HIF1 $\beta$  alone<sup>37</sup> in adipose tissue. Mouse lines lacking HIF1 $\alpha$  and HIF1 $\beta$  expression exhibited similar metabolic phenotypes, including reduced fat formation, protection from HFD-induced obesity and decreased insulin resistance, suggesting a role for HIF1 $\alpha$  and its dimerization partner HIF1 $\beta$  in the pathogenesis of obesity and insulin resistance<sup>36</sup>. Another group also observed that lack of HIF1 $\alpha$  expression in adipocytes renders mice resistant to HFD-induced obesity, which correlated with increased fatty acid  $\beta$ -oxidation in white adipose tissue<sup>38</sup>. Others found a similar phenotype of decreased insulin resistance when either HIF1 $\alpha$  or both HIF1 $\alpha$  and HIF2 $\alpha$  expression was disrupted in adipose tissue<sup>28</sup>. Furthermore, acriflavine (a molecule that inhibits heterodimerization of HIF1 $\beta$ )<sup>39</sup> reduced insulin resistance in obese mice fed an HFD<sup>40</sup>. Similarly, another selective HIF1 $\alpha$  inhibitor, PX-478, alleviates the HFD-induced glucose intolerance, insulin resistance and obesity that were attributed to inhibition of adipose tissue fibrosis and inflammation<sup>41</sup>. Mice lacking HIF1 $\alpha$  expression in adipose tissue had decreased inflammation, whereas mice with disrupted HIF2 $\alpha$  expression in adipocytes had elevated inflammation and insulin resistance, indicating opposing roles for these two HIF $\alpha$  proteins in adipocytes<sup>28</sup>. However, in adipocytes of obese mice fed an HFD, where HIF1 $\alpha$  is the predominantly expressed isoform, adipocyte-specific HIF1 inhibition protects the mice from metabolic disorders<sup>36</sup>.

**Mechanisms that increase obesity and insulin resistance.**—HIF1 $\alpha$  signalling in adipocytes affects obesity and insulin resistance by several potential mechanisms. In adipose tissue, HIF1 $\alpha$  regulates the gene encoding suppressor of cytokine signalling 3 (SOCS3)<sup>40</sup>. Following the activation of the *Socs3* gene by HIF1 $\alpha$ , SOCS3 inhibits Janus kinase (JAK), which phosphorylates signal transducer and activator of transcription 3 (STAT3) and thus inhibits the expression of adiponectin<sup>42</sup> (FIG. 2). Therefore, when hypoxia occurs during the expansion of adipose tissue, the accumulation of HIF1 $\alpha$  results in decreased adiponectin production from adipocytes and increased insulin resistance<sup>40</sup>. In addition, homocysteine (a sulfur-containing amino acid derived from the metabolism of methionine) treatment triggers HIF1 $\alpha$  activation in adipocytes<sup>43</sup>. Homocysteine markedly induces endoplasmic reticulum

stress, inflammation and subsequent insulin resistance in adipose tissue<sup>44,45</sup>. Adipocyte HIF1 $\alpha$  regulates lysophosphatidylcholine metabolism, as revealed by the identification of a novel HIF1 $\alpha$  target gene encoding phospholipase A2 group 16. Adipocyte-specific HIF1 $\alpha$  knockout abrogated the homocysteine-induced activation of NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3; also known as NALP3) inflammasome (a multiprotein complex that detects pathogenetic stressors and activates inflammatory responses) activation and insulin resistance through the phospholipase A2 group 16-lysophosphatidylcholine pathway. Thus, the adipocyte HIF1 $\alpha$ -lysophosphatidylcholine axis is necessary for homocysteine-induced insulin resistance<sup>43</sup>.

Another mechanism by which adipose HIF1 $\alpha$  could influence metabolic disease is through the modulation of inflammation, as the increased inflammation associated with adipocytes in obese mice contributes to obesity and insulin resistance<sup>46,47</sup>. In macrophages, PHD2 serves as a control for the metabolic shift from anaerobic glycolysis to oxidative phosphorylation; inhibition of PHD2 and the resultant increase in HIF1 $\alpha$  reverse the metabolic phenotype of anaerobic glycolysis<sup>48</sup>. Even at the early stages of diet-induced obesity in mouse models, there is increased inflammation associated with infiltration of adipose tissue by macrophages. M1 macrophages release pro-inflammatory cytokines that can damage tissue and inhibit cell proliferation whereas M2 macrophages release anti-inflammatory cytokines that promote proliferation of nearby cells and tissue repair. M1–M2 polarization is a tight process that interconverts M1 and M2 macrophages by a number of mechanisms that involve the tissues in which the macrophages infiltrate and the associated tissue microenvironments<sup>49</sup>.

HIF1 $\alpha$  can promote classic M1 macrophage activation, regulate phosphofructokinase and modulate the expression of inflammation-related genes<sup>50</sup>. These findings suggest that hypoxia is linked to M1 macrophage polarization and inflammation, thus indicating that HIF1 $\alpha$  expression in macrophages might trigger adverse physiological responses, resulting in not only obesity but also insulin resistance, through the modulation of macrophage metabolic reprogramming and inflammation. However, macrophage-specific *hHif1 $\alpha$* -knockout mice showed no indication that HIF1 $\alpha$  expressed by macrophage in adipose tissue has an important role in the early stages of obesity<sup>51</sup>. However, adipose-specific knockout of HIF1 $\alpha$  results in less adipose tissue macrophage infiltration than wild-type mice, decreased inflammation and amelioration of diet-induced obesity<sup>28,36</sup>.

Although HIF1 $\alpha$  from macrophages does not appear to have a major influence on the early stages of adipose inflammation and obesity, others have found that macrophage-specific *Hif1 $\alpha$* -knockout mice have decreased insulin resistance after 18 weeks of HFD treatment<sup>52</sup>. HIF1 $\alpha$  stimulates glucose uptake by increasing GLUT1 expression and glycolysis (by induction of glycolytic enzymes), which promotes the utilization of glucose in lipid synthesis<sup>53</sup>. HIF1 $\alpha$  also suppresses deacetylation of peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) co-activator 1 $\alpha$  (PGC1 $\alpha$ ) and expression of genes involved in fatty acid  $\beta$ -oxidation in white adipocytes mainly through repression of *Sirt2*, encoding sirtuin 2 (an NAD-dependent deacetylase)<sup>38</sup>. These studies might partially explain the obesity phenotype of transgenic mice overexpressing HIF $\alpha$ <sup>30,31</sup> and the lean phenotype in mice lacking HIF1 $\alpha$  or HIF1 $\beta$  expression in adipose tissue<sup>28,36,37</sup>.

Although obesity is associated with increased HIF1 $\alpha$  expression and activated downstream signalling, in normal-weight mice, levels of basal HIF1 $\alpha$  are almost undetectable. Acute or transient exposure of differentiating adipocytes to hypoxia reprogrammes cells for increased triglyceride accumulation, decreased fatty acid  $\beta$ -oxidation and pyruvate dehydrogenase activity and increased insulin sensitivity as revealed by rapid glucose uptake<sup>54</sup>. AMP-activated protein kinase (AMPK; also known as PRKAA2) was activated along with increased levels of mRNAs encoding GLUT1, PPAR $\gamma$ , PGC1 $\alpha$  and sterol regulatory element-binding protein (SREBP). Repeated exposure to short-term hypoxia further increased glucose uptake in adipocytes. Although this study was performed in cultured adipocytes, the results suggest that acute and chronic hypoxia might have opposing effects on the activation of HIF1 $\alpha$  and HIF2 $\alpha$ .

### HIF1 $\alpha$ in pancreatic $\beta$ -cells

HIF1 $\alpha$  expression in pancreatic  $\beta$ -cells has been linked to metabolic diseases. For instance, in pancreatic  $\beta$ -cells from patients with type 2 diabetes mellitus (T2DM), the level of HIF1 $\beta$  mRNA was reduced by 90% compared with non-diabetic controls<sup>55</sup>. Mechanistic studies have revealed that short interfering RNA-mediated knockdown of HIF1 $\beta$  in mouse  $\beta$ -cell-derived MIN6 cells impairs glucose-stimulated insulin release and changes gene expression patterns, which is similar to what is seen in pancreatic islets from patients with T2DM<sup>55</sup>. Additionally, mice lacking the expression of HIF1 $\beta$  in  $\beta$ -cells had abnormal glucose tolerance, impaired insulin secretion and altered gene expression patterns compared with wild-type mice. However, this study was performed with the HIF $\alpha$  heterodimerization partner HIF1 $\beta$ ; thus, it is not clear which HIF $\alpha$  subunit is responsible for the phenotype, as knockdown of HIF1 $\alpha$ , HIF2 $\alpha$  and even a mechanistically unrelated bHLH-PAS superfamily member AHR in MIN6 cells each independently decreased insulin secretion slightly<sup>55</sup>. Furthermore,  $\beta$ -cell-specific HIF1 $\alpha$  disruption exacerbates  $\beta$ -cell dysfunction and glucose intolerance by downregulating glycolysis and electron-transport-chain-related gene expression, leading to decreased ATP generation<sup>56</sup>. Additionally, HIF1 $\alpha$  is also a protective factor for islet cell transplantation<sup>57</sup>. By contrast, other studies have shown that overexpression of HIF1 $\alpha$  and HIF2 $\alpha$  in conjunction with VHL disruption worsens  $\beta$ -cell function and glucose homeostasis<sup>58,59</sup>. These studies suggest that hypoxia and HIF signalling might have a vital role in the function of pancreatic  $\beta$ -cells.

### HIF1 $\alpha$ in liver

HIF1 $\alpha$  influences liver disease through the regulation of genes involved in glucose and lipid metabolism. Altered expression of these genes could occur under conditions of hypoxia, possibly induced by increased mitochondrial metabolism as in adipocytes. HIF1 $\alpha$  regulates genes encoding GLUT1 (REF.<sup>60</sup>) and 3-phosphoinositide-dependent protein kinase 1 (PDK1; also known as PDK1)<sup>61</sup>, which are involved in glucose transport and fructose production, respectively, and could influence the development and progression of non-alcoholic fatty liver disease (NAFLD)<sup>62</sup>. Metabolic diseases such as obesity, NAFLD, T2DM and atherosclerosis are all linked to altered lipid and glucose metabolism. Early evidence for a role of HIFs in metabolic diseases was provided by hepatocyte-specific disruption of VHL<sup>63</sup>, PHD2 (REF.<sup>64</sup>) or PHD3 (REF.<sup>65</sup>), which triggered the O<sub>2</sub>-independent overexpression of both HIF1 $\alpha$  and HIF2 $\alpha$  and induced hepatic steatosis.



Chronic ethanol administration was shown to activate hepatic HIF1 $\alpha$ , and overexpression of hepatocyte HIF1 $\alpha$  aggravated ethanol-induced hepatic steatosis<sup>66</sup>. Hepatocyte-specific HIF1 $\alpha$  disruption ameliorated chronic ethanol-induced hepatic steatosis and inflammation<sup>66</sup>. By contrast, another group reported that hepatocyte-specific HIF1 $\alpha$  disruption exacerbated hepatic steatosis upon chronic ethanol administration<sup>67</sup>. The same group also found that hepatocyte-specific HIF1 $\alpha$  disruption aggravated high-fat and sucrose-diet-induced glucose intolerance<sup>68</sup>. However, others did not observe metabolic phenotypes in mice with hepatocyte-specific HIF1 $\alpha$  disruption<sup>69</sup>.

In addition, digoxin has been shown to protect mice from liver inflammation and cellular damage caused by non-alcoholic steatohepatitis (NASH)<sup>70</sup>. This protective effect is because digoxin inhibits the interaction between pyruvate kinase PKM and histones and downregulates HIF1 $\alpha$  signalling. Thus, the possibility exists that the effect of digoxin on NASH could be the result of HIF1 $\alpha$  inhibition as other studies have reported that hepatic HIF1 $\alpha$  activation promotes inflammation<sup>66</sup> and further suggests that downregulation or inhibition of HIF1 $\alpha$  in the liver could be a therapeutic strategy for the treatment of metabolic diseases.

Liver disease, such as NASH, is accompanied by increased fibrosis. The HIF1 $\alpha$  inhibitor 3-(5-hydroxymethyl-2-furyl)-1-benzylindazole (YC-1) ameliorated liver fibrosis in part by downregulating SOCS1 and SOCS3, which resulted in the inhibition of nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation and STAT3 phosphorylation<sup>71</sup>. This pathway is similar to that uncovered in adipose tissue that results in adiponectin expression<sup>40</sup>.

HIF1 $\alpha$  is also involved in the control of cholesterol synthesis in the liver. HIF1 $\alpha$  directly activates insulin-induced gene 2 protein (INSIG2), which is located in the endoplasmic reticulum membrane and subsequently inhibits the rate-limiting cholesterol synthesis enzyme 3-hydroxy-3-methylglutaryl-coA reductase (HMGCR)<sup>72</sup>. This observation indicates a potential beneficial role for hepatic HIF1 $\alpha$  activation, which triggers the degradation of HMGCR under conditions of lipid overload.

## HIF2 $\alpha$ in metabolic disease

### HIF2 $\alpha$ in the liver

Although the role of HIF2 $\alpha$  in cancer has been extensively studied, its function in metabolism and metabolic disease has received more attention in recent years. Several studies have shown that HIF2 $\alpha$  either promotes or inhibits metabolic disease (TABLE 1). Furthermore, a study published in 2017 revealed that HIF2 $\alpha$  has a role in the control of glucose and fatty acid metabolism in the liver, as summarized in FIG. 3 (as discussed in this review<sup>73</sup>). The first study to report a more definitive connection between HIF2 $\alpha$  and lipid metabolism showed that *Vhl* and *Hif1 $\alpha$* -double-knockout mice, which have constitutively stabilized HIF2 $\alpha$ , and not HIF1 $\alpha$ , exhibit severe hepatic steatosis with decreased fatty acid  $\beta$ -oxidation<sup>74</sup>. However, administration of the PHD inhibitor FG-4497 decreased serum levels of cholesterol and de novo lipid synthesis and protected mice from hepatic steatosis and atherosclerosis<sup>75</sup>. In addition, increased hepatic hypoxia and HIF2 $\alpha$  (but not HIF1 $\alpha$ ) expression, which was assessed using temporal VHL disruption with a cre-*ER*<sup>T2</sup> system,

caused hepatic steatosis by regulating hepatic fatty acid uptake, synthesis and catabolism<sup>76</sup>. Acute activation of HIF2 $\alpha$  in the liver upregulated the expression of genes involved in fatty acid synthesis, including fatty acid synthase (FASN), which is controlled by SREBP1C and fatty acid uptake (via CD36); the latter is a plasma membrane transporter responsible for the import of fatty acids into cells. HIF2 $\alpha$  activation is also correlated with downregulation of PPAR $\alpha$  and enzymes encoded by its target gene, including peroxisomal acyl-coA oxidase 1 (ACOX1), which is involved in fatty acid  $\beta$ -oxidation<sup>76</sup>. The mechanism by which HIF2 $\alpha$  controls SREBP1C and PPAR $\alpha$  in the liver has not been determined. However, in hepatocytes, HIF2 $\alpha$  represses PPAR $\alpha$  and exacerbates acetaminophen-induced hepatotoxicity<sup>77,78</sup>. Furthermore, HIF2 $\alpha$  directly regulates angiotensin-related protein 3 (ANGPTL3)<sup>76</sup>, an endogenous lipoprotein lipase inhibitor and an important mediator of lipid homeostasis<sup>79</sup>. Additionally, HIF2 $\alpha$  activation increases liver inflammation and fibrosis, although the mechanism is still unclear<sup>76</sup>.

Clinical biopsy samples from patients with NAFLD showed an overexpression of HIF2 $\alpha$ ; a mouse model of NASH (with NASH induced by feeding mice a diet deficient in methionine and choline) further supported this result<sup>80</sup>. Disruption of HIF2 $\alpha$  expression ameliorated liver fibrosis and inflammation via downregulation of hepatocyte production of histidine-rich glycoprotein, which potentiates M1 macrophage migration and polarization leading to increased hepatic inflammation<sup>81</sup>, suggesting a potentially harmful outcome from overexpression of HIF2 $\alpha$  in the liver.

Most studies investigating the relationship between HIF and NAFLD have focused on evaluating the effects of HIF1 $\alpha$  and HIF2 $\alpha$  in the liver. By contrast, activation of HIF2 $\alpha$  in the liver ameliorates hyperglycaemia through an insulin-dependent pathway with increased levels of insulin receptor substrate 2 (IRS2) or through the insulin-independent pathway through repression of glucagon action<sup>16,73,82–84</sup>. Evidence that HIF2 $\alpha$  regulates lipid and glucose metabolism was further revealed in hepatic *Phd3* (also known as *Egln3*)-null mice<sup>83</sup>. Acute disruption of *Phd3* stabilized HIF2 $\alpha$  expression and further upregulated *Irs2* expression, which increased insulin-stimulated AKT activation and forkhead box protein O1 (FOXO1)-dependent suppression of gluconeogenesis. Physiological liver hypoxia and VEGF inhibition through vascular regression are two stimuli that can activate the HIF2 $\alpha$ -IRS2 pathway to modulate glucose metabolism (FIG. 3). Furthermore, hepatic *Phd3*-null mice exhibited decreased  $\beta$ -oxidation and increased insulin sensitivity. In contrast to *Phd1* and *Phd2*-knockout mice, hepatic *Phd3*-null mice specifically stabilized HIF2 $\alpha$ , which was not associated with increased hepatic toxicity<sup>83</sup>. Additionally, HIF2 $\alpha$  attenuates postprandial glucagon signalling through the extracellular-signal-regulated kinase (ERK; also known as MAPK)-dependent increase in phosphodiesterase-mediated hydrolysis of intracellular cAMP, resulting in decreased protein kinase A (PKA)-mediated activation of CREB<sup>82</sup>. This decreased activation leads to the suppression of the gluconeogenic target genes encoding the enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6 phosphatase (G6Pase).

These studies imply that pharmacological inhibition of hepatic HIF2 $\alpha$  might not be a suitable target for NAFLD therapy owing to the risk of increased hepatic glucose production and T2DM. However, transcription factor signalling pathways in the intestine are also



involved in the development of metabolic disease, including NAFLD<sup>85–88</sup>. These studies demonstrate the complexity of the role of HIF2 $\alpha$  in obesity, insulin resistance, NAFLD and other metabolic diseases involving dysfunction of glucose and lipid metabolism.

Another aspect of HIF2 $\alpha$  stabilization and activation is modulation of lipid metabolism in liver and adipose tissue. Peroxisomes, which carry out fatty acid  $\beta$ -oxidation of long-chain and very-long-chain fatty acids, are dependent on oxygen, thus indicating a potential role for oxygen-sensing HIFs in the control of metabolism by this organelle. In *Vhl-Hif1a*-null mice that constitutively express HIF2 $\alpha$ , liver HIF2 $\alpha$  activation leads to a decrease in peroxisomes expressing the gene encoding next to BRCA1 gene 1 protein (NBR1) through pexophagy, the selective autophagy of peroxisomes<sup>89</sup>. The mechanism and functional importance of induced pexophagy in liver and other tissues requires further investigation<sup>90</sup>. Perhaps under low oxygen, peroxisome numbers are reduced, leading to lower oxygen consumption and accumulation of very-long-chain fatty acids because of increased HIF2 $\alpha$  signalling.

Upon global *Pdh2* gene disruption, which stabilizes both HIF1 $\alpha$  and HIF2 $\alpha$ , mice fed either normal chow or an HFD display less adipose tissue and less adipose inflammation<sup>91</sup>. In adipose tissue, loss of HIF2 $\alpha$  signalling exacerbates adipose dysfunction and impairs thermogenesis, coinciding with decreased UCP1 expression (a marker for brown adipocytes)<sup>92</sup>. Administration of VEGFA reversed the obese phenotype and adipose inflammation in the absence of HIF2 $\alpha$ . Overexpression of VEGFA, a gene target of the HIF2 $\alpha$  in adipose, in mice fed an HFD to induce obesity triggers adipose browning, improved metabolism and reduced adipose inflammation<sup>93</sup>.

### HIF2 $\alpha$ in macrophages

Intermittent fasting improves metabolism and obesity by inducing VEGF overexpression in adipose tissue, which coincides with the activation of anti-inflammatory M2 macrophages in adipose tissue<sup>94</sup>. The anti-inflammatory M2 macrophages increased the browning of white adipose tissue<sup>95</sup>. A VEGF-M2 axis was suggested to promote adipose browning<sup>94</sup>, indicating that upstream HIF induction of VEGF might influence adipose browning and thermogenesis. These data suggest that HIF1 $\alpha$  and HIF2 $\alpha$  have opposing effects in adipose tissues (as discussed previously) and knockout or chemical inhibition of HIF1 $\alpha$  in adipose tissue reduces inflammation and obesity<sup>36–38</sup>. Determining the role of adipose hypoxia and HIF1 $\alpha$  in this process and rectifying the differences in other studies showing that hypoxia and increased levels of HIF1 $\alpha$  in adipose tissues exacerbate obesity require additional experimentation.

HIF2 $\alpha$  could also influence the M1–M2 macrophage transition and cooperate with HIF1 $\alpha$  via controlling the balance of inducible nitric oxide synthase (iNOS; also known as NOS2) and arginase 1 (ARG1)<sup>96</sup>. Owing to the tight association between metabolism and inflammation<sup>47</sup>, compared with inflammation-prone HIF1 $\alpha$ , HIF2 $\alpha$  exerts more anti-inflammatory activity that inhibits macrophage activation by inhibiting mitochondrial reactive oxygen species (ROS)<sup>97</sup>. Furthermore, HIF1 $\alpha$  and HIF2 $\alpha$  influence ROS production via modulation of different targets<sup>98</sup>. HIF1 $\alpha$  upregulates cytochrome NADPH oxidase 2 (NOX2; also known as CYBB), which increases ROS production<sup>99</sup>; HIF2 $\alpha$  upregulates mitochondrial superoxide dismutase (SOD2) and then suppresses ROS

production<sup>100</sup>. Taken together, HIF1 $\alpha$  and HIF2 $\alpha$  have different roles in macrophage polarization, ROS production and inflammation.

### Iron transport and metabolic diseases

HIF2 $\alpha$  has a major role in the control of iron transport in the intestine<sup>101–103</sup>. The incidence of iron deficiency is increased in children and adults with obesity, suggesting that intestinal HIF2 $\alpha$  signalling, through its control of iron metabolism, might influence obesity<sup>102–106</sup>. As an explanation for the findings of these epidemiological studies, HIF2 $\alpha$  expression could be suppressed in the intestine, leading to iron deficiency, which causes or potentiates obesity. Alternatively, iron-deficiency-induced activation of intestinal HIF2 $\alpha$  could affect obesity, which is dependent on the modulation of another pathway. Expression of HIF2 $\alpha$  and its target genes encoding divalent metal transporter 1 (DMT1; also known as NRAMP2) and duodenal cytochrome *b* (DCYTB; also known as CYBRD1) are elevated in the ileum of people with obesity compared with individuals who are not obese<sup>88</sup>. The mRNA levels of *Dmt1* and *Dcytb1* are positively correlated with BMI and levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are markers of liver damage associated with NAFLD. Although this finding suggests that HIF2 $\alpha$  is activated in the intestine in obesity and influences obesity-associated phenotypes, the question arises whether hypoxia is the cause or the result of obesity.

### HIF2 $\alpha$ in intestine and metabolic disease

To determine the mechanism by which hypoxia in the intestine affects metabolic disease, a diet-induced obesity mouse model was used with genetically manipulated mice and pharmacological inhibition to explore the role of HIF $\alpha$  in metabolic disease. Transgenic mice expressing an HIF1 $\alpha$  oxygen-dependent degradation domain linked to a luciferase reporter<sup>107</sup> were fed an HFD to induce obesity and showed increased hypoxia<sup>88</sup>. To understand the role of HIF1 $\alpha$  and HIF2 $\alpha$  in the intestine in metabolic disease and identify the precise mechanism responsible, metabolomic profiling of mice with intestine-specific knockout of HIF1 $\alpha$  and HIF2 $\alpha$  or activation of both HIF $\alpha$  subunits by intestine-specific disruption of *Vhl* was undertaken. As summarized in FIG. 4, HIF2 $\alpha$ , but not HIF1 $\alpha$ , signalling in the intestine was activated during obesity. Intestine-specific HIF2 $\alpha$  ablation substantially ameliorated HFD-induced hepatic steatosis. HIF2 $\alpha$  expression and signalling were directly correlated with obesity in humans<sup>88</sup>, thus indicating the potential for the translation of the mouse studies to metabolic disease in humans.

However, there is the question of whether this pathway can be pharmacologically targeted. A family of ligands were developed that inhibit HIF2 $\alpha$  heterodimerization with HIF1 $\beta$ , resulting in loss of DNA-binding activity and HIF signalling<sup>108</sup>. Notably, a specific HIF2 $\alpha$  inhibitor, PT2385 ((*S*)-3-((2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1H-inden-4-yl)oxy)-5-fluorobenzonitrile), is in clinical trials for the treatment of renal cancer<sup>22–24</sup>. Oral administration of PT2385 to obese mice selectively inhibits HIF2 $\alpha$  in the intestine and prevents the development of metabolic disorders in mice fed an HFD<sup>88</sup>. Importantly, PT2385 administration to obese mice markedly decreases all adverse phenotypes associated with obesity, including insulin resistance and NAFLD. This work suggests that HIF2 $\alpha$  in the intestine is a target for the treatment of metabolic disease.

*Neu3*, encoding sialidase 3 (NEU3), a key enzyme in the ceramide salvage pathway that produces ceramide<sup>109</sup>, is an HIF2 $\alpha$  target gene in the intestine. Two inhibitors of NEU3, *N*-acetyl-2,3-didehydro-*N*-acetyl-neuraminic acid (DANA) and the flavonoid naringin, reduced the metabolic abnormalities associated with HFD treatment. Although having low systemic bioavailability, these compounds effectively inhibited NEU3 in the intestine and reduced the severity of HFD-induced metabolic disorders<sup>88</sup>. PT2385 administration to mice shifted the metabolism of bile acids via hepatic cholesterol 7 $\alpha$ -monooxygenase (CYP7A1), the key enzyme in bile acid biosynthesis<sup>110</sup>. Thus, this selective HIF2 $\alpha$  antagonist shows great potential in the treatment of metabolic disease.

These studies established a novel HIF2 $\alpha$ -NEU3-ceramide pathway that promotes the development of metabolic disease. The different metabolic phenotypes found in wild-type and intestine-specific *Hif2a*-knockout mice were positively correlated with serum levels of ceramide. High serum levels of ceramides were associated with increased risk in all adverse metabolic phenotypes, including obesity, insulin resistance and NAFLD<sup>88</sup>. The role of ceramides in metabolic disease has been established in a number of earlier studies<sup>111–114</sup>. The induction and activation of *Neu3* by HIF2 $\alpha$  in small intestinal epithelial cells resulted in hypoxia, which increases serum levels of ceramides in HFD-fed intestine-specific *Hif2a*-knockout mice, or when wild-type mice treated with PT2385 or the NEU3 inhibitors (DANA and naringin) had decreased serum levels of ceramides by ~30%<sup>88</sup>. Restoration of ceramides to intestine-specific *Hif2a*-knockout mice fed an HFD, by injection of C16:0 ceramide, reversed the improved metabolic abnormalities<sup>88</sup>. No gastrointestinal toxicities (such as diarrhoea or inflammation) were found, as revealed by similar faecal levels of neutrophil gelatinase-associated lipocalin (LCN2) in the drug-treated mice versus controls or in mice genetically deficient in intestinal HIF2 $\alpha$  signalling.

Additionally, HIF2 $\alpha$  is also involved in the control of iron absorption<sup>103</sup>. However, mice lacking expression of HIF2 $\alpha$  in the intestine show no signs of anaemia, suggesting that targeting HIF2 $\alpha$  in the gut is a safe and effective treatment for metabolic disease. Other mechanisms underlie the control of ceramide synthesis in the intestine, such as the farnesoid X receptor (FXR; also known as NR1H4), which is highly expressed in the liver and intestine and controls bile acid synthesis and transport and the enterohepatic circulation of bile acids<sup>87,115</sup>. FXR in the intestinal epithelial cells activates the gene encoding sphingomyelin phosphodiesterase 3 (SMPD3) that catalyses the hydrolysis of sphingomyelin to form ceramide and phosphocholine<sup>116,117</sup>. FXR is constitutively activated by bile acids in the ileum, leading to increased serum levels of ceramides through the induction of *Smpd3*; inhibition of intestinal FXR decreases the incidence of HFD-induced metabolic diseases<sup>86,87,117,118</sup>. Thus, both intestinal HIF2 $\alpha$  and FXR contribute to diet-induced obesity and related disorders, and both can be targeted for the treatment of metabolic disease.

## HIF1 $\beta$ in metabolic diseases

Conditional knockout of HIF1 $\beta$  in mice was produced<sup>119</sup> and used to determine the role of HIF signalling in metabolic disease. However, results from mice in which HIF1 $\beta$  is disrupted in various tissues can be difficult to interpret as HIF1 $\beta$  is the obligate heterodimeric partner of HIF1 $\alpha$ , HIF2 $\alpha$ , AHR and other members of the bHLH-PAS

superfamily<sup>10</sup>. In comparison with hepatic-specific *Hif1a*-null and *Hif1b*-null mice, hepatic-specific *Hif1b*-null mice have increased fasting plasma levels of glucose, glucose tolerance and postprandial triglycerides, which are associated with increased expression of G6Pase, carbohydrate-responsive element-binding protein (ChREBP), FASN and acyl-CoA desaturase (SCD)<sup>69,120</sup>. *Hif1a*-null and *Hif2a*-null mice showed the same metabolic parameters (such as fasting plasma levels of glucose and glucose tolerance) as wild-type mice, thus indicating that HIF1 $\beta$ , the obligate partner of HIF1 $\alpha$ , has an impact on metabolism, possibly through its interactions with another bHLH-PAS transcription factor<sup>69</sup>. For example, expression of fibroblast growth factor 21 (FGF21), which contributes to energy homeostasis during fasting, is repressed by activation of AHR, another partner of HIF1 $\beta$ <sup>121</sup>. These studies indicate that HIF1 $\beta$  has individual functions in metabolic disorders and that understanding the relationship of HIF1 $\alpha$  and HIF2 $\alpha$  with their heterodimeric partner molecule HIF1 $\beta$  requires additional studies.

## HIF inhibitors and therapeutic effects

As HIF has critical functions in cancer, inhibitors of HIF were developed and exhibited therapeutic potential<sup>122</sup>. These inhibitors show a great potential in cancer treatment, but owing to the potential for important roles of HIFs in metabolic disorders, they might have broader therapeutic effects. Although some small molecules were reported to be an inhibitor of HIF, other inhibitors function indirectly. For example, acriflavine, PX-478, 3-(2-(4-adamantan-1-yl-phenoxy)-acetyl-amino)-4-hydroxybenzoic acid methyl ester (LW6), 3,4-dimethoxy-*N*-((2,2-dimethyl-2H-chromen-6-yl) methyl)-*N*-phenylbenzenesulfonamide (KCN1), YC-1 and PT2385 are the most widely used experimental inhibitors. Acriflavine was demonstrated to protect from HFD-induced obesity and insulin resistance dependent on the adipose HIF1 $\alpha$ -SOCS3-STAT3-adiponectin pathway<sup>40</sup>. PX-478 treatment selectively inhibits adipose HIF1 $\alpha$ , leading to improvement in metabolic dysfunctions, partially through reduced adipose fibrosis<sup>41</sup>. LW6 is an adamantyl-based derivative, and this compound indirectly inhibits HIF1 $\alpha$  via the mitochondrial malate dehydrogenase (MDH2) protein<sup>123,124</sup>. LW6 administration can decrease activated human T cell proliferation without affecting cell survival by inhibiting the tricarboxylic acid cycle<sup>125</sup>. KCN1 is a direct inhibitor of HIF1 $\alpha$  by downregulating HIF1 $\alpha$  target gene expression, and it could be used to treat metabolic disorders<sup>126,127</sup>. YC-1 is an HIF1 $\alpha$  inhibitor that is widely used in experimental studies. In lung cancer cells, YC-1 inhibited the HIF1 $\alpha$ -induced reprogramming of glucose metabolism from mitochondrial oxidative phosphorylation to anaerobic glycolysis and lactic acid fermentation<sup>128</sup>. YC-1 can also modulate lipolysis, but in a cell-type-specific manner. In RAW 264.7 macrophage cells, YC-1 increased lipid droplet and oxidized LDL foam cell formation through cGMP-dependent protein kinase<sup>129</sup>, whereas in adipocytes, YC-1 induced lipolysis<sup>130</sup>. Another study revealed that YC-1 is a non-competitive inhibitor of p-glycoprotein (multidrug resistance protein 1 (MDR1; also known as ABCB1)) also act via the cGMP-dependent protein kinase ERK<sup>131</sup>. These studies suggest the potential for a broader therapeutic use for the HIF inhibitor YC-1.

PT2385 is an HIF2 $\alpha$  inhibitor effective in treating renal cell carcinoma<sup>23</sup>. Furthermore, PT2385 administration decreases intestinal and serum levels of ceramides, resulting in metabolic improvements, a finding consistent with studies in the intestinal *Hif2a*-knockout

mice<sup>88</sup>. As the effects of either activating or inhibiting HIF1 $\alpha$  and HIF2 $\alpha$  in different tissues can affect metabolic diseases to different degrees, more studies are needed to focus on tissue-specific targeting of the two HIF $\alpha$  proteins to achieve favourable metabolic end points.

## Conclusion

Since the discovery in 1992 (REF.<sup>1</sup>) that a transcription factor controls the cellular adaptation to low levels of oxygen, there have been many studies showing the unique mechanism by which HIF $\alpha$  proteins are stabilized by the oxygen-dependent PHDs and FIH1 enzymes<sup>132</sup>. The major function of HIF that has received the most attention is its role in the control of angiogenesis during mammalian development and in the growth of tumours, largely by the induction of the gene encoding VEGF. However, evidence has emerged that modulation of HIF1 $\alpha$  and HIF2 $\alpha$  signalling could be of potential benefit for metabolic diseases, which is beyond their known roles in cancer treatment.

These new functions for HIF were discovered primarily through the analysis of *Hif1 $\alpha$* , *Hif2 $\alpha$*  and *Hif1 $\beta$* -conditional-knockout mice and through limited pharmacological studies in which HIFs were chemically inhibited. Notably, targeted inhibition of HIF1 $\alpha$  in adipose tissue and HIF2 $\alpha$  in intestine restored to normal many adverse phenotypes of metabolic disease found caused by feeding mice the high-fat Western diet (including obesity, T2DM and NAFLD). In addition, the direct inhibition of the HIF2 $\alpha$  target gene encoding NEU3, involved in ceramide production, could also be explored as a potential therapeutic target. Because HIF2 $\alpha$  expression and signalling and NEU3 are conserved between mice and humans, it is probable that the studies in mice would translate to humans. Indeed, intestinal HIF2 $\alpha$  expression and activity is associated with human obesity<sup>88</sup>, and increased levels of ceramides, which were recently called the ‘new cholesterol’<sup>114</sup>, are correlated with metabolic diseases in humans and promote obesity, T2DM and NAFLD in mice<sup>87,113</sup>.

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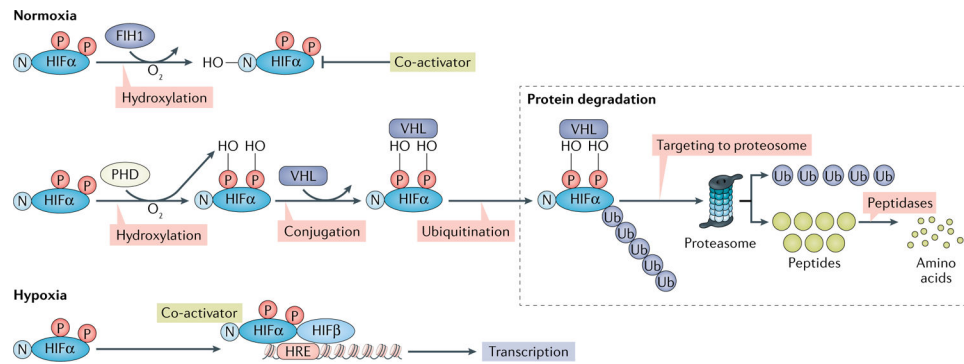
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### Key points

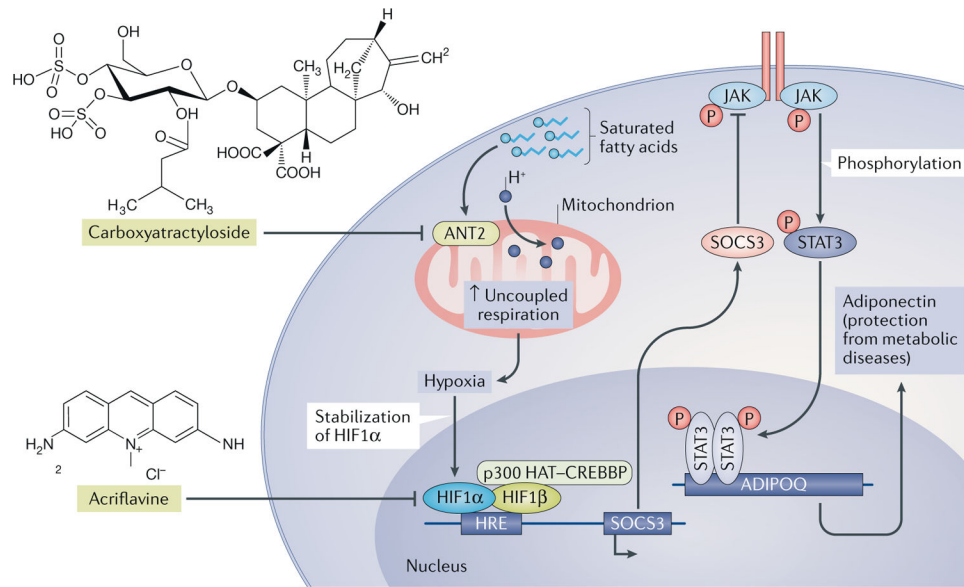
- Obesity triggers hypoxia in adipose tissue and the small intestine, which stabilizes and activates hypoxia-inducible factor (HIF)1 $\alpha$  and HIF2 $\alpha$  signalling, resulting in adverse metabolic effects, including insulin resistance and non-alcoholic fatty liver disease.
- Induction of HIF1 $\alpha$  in adipocytes, through a suppressor of cytokine signalling 3 (SOCS3)-signal transducer and activator of transcription 3 (STAT3) axis, leads to the upregulation of inflammation and downregulation of adiponectin expression, resulting in insulin resistance.
- Activation of HIF2 $\alpha$  in the small intestine increases expression of sialidase 3, resulting in an elevation of small intestinal and serum levels of ceramides that in turn potentiate obesity-associated metabolic diseases.
- Genetic or chemical inhibition of HIF1 $\alpha$  and HIF2 $\alpha$  signalling in adipose tissue and the small intestine ameliorates obesity-associated metabolic diseases, indicating that they could be targeted for treatment of metabolic disorders.





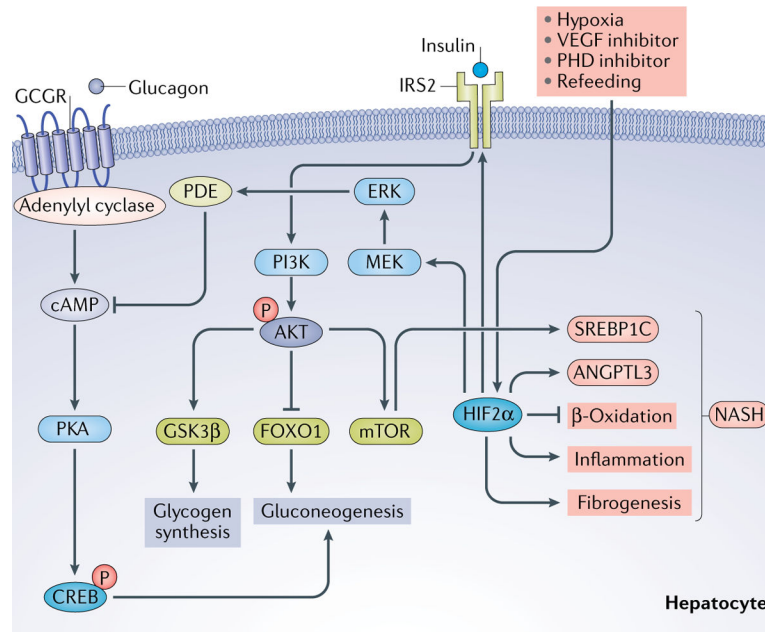
**Fig. 1 | Hypoxia-inducible factor- $\alpha$  proteins are hydroxylated under normoxic conditions by prolyl hydroxylase domain enzymes.**

The hydroxylated hypoxia-inducible factor- $\alpha$  (HIF $\alpha$ ) is then conjugated by the von Hippel-Lindau disease tumour suppressor (VHL) protein, leading to rapid degradation by the proteasome. HIF $\alpha$  can also be hydroxylated at an asparaginyl residue by the factor inhibiting HIF1 (FIH1) enzyme, which inactivates HIF $\alpha$  transcriptional activity by preventing it from interacting with its transcriptional co-activators. Both prolyl hydroxylase domains (PHDs) and FIH1 are O<sub>2</sub>-dependent oxygenases that are active under normoxia. Under conditions of hypoxia, HIF $\alpha$  subunits are not hydroxylated and the protein is stabilized, leading to the accumulation of HIF $\alpha$  proteins and activation of HIF target gene expression. HIF $\alpha$  requires the dimerization partner HIF1 $\beta$  to activate the transcription of HIF target genes. HRE, HIF regulating element; N, nitrate; -OH, hydroxylation; P, phosphate; Ub, ubiquitin.



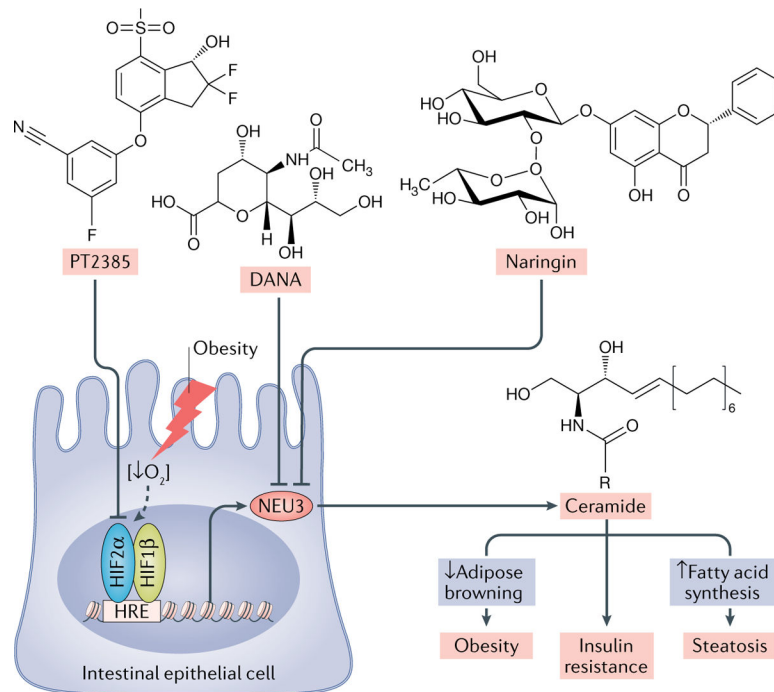
**Fig. 2 | Hypoxia-inducible factor 1α in adipose tissue.**

Adipose tissues become hypoxic because of saturated fatty acids binding to ADP/ATP translocase 2 (ANT2) in mitochondria, which increases uncoupled respiration. This uncoupling causes the stabilization of hypoxia-inducible factor 1α (HIF1α). Inhibition of ANT2 by carboxyatractyloside lowers saturated fatty acid-induced hypoxia. HIF1α induces expression of the suppressor of cytokine signalling 3 (SOCS3) and, by activating Janus kinase (JAK), SOCS3 phosphorylates and activates signal transducer and activator of transcription 3 (STAT3), which inhibits the expression of adiponectin (encoded by *ADIPOQ*). Acriflavine inhibits the dimerization of HIF1α and HIF1β, resulting in non-transcriptional activation of target genes. H<sup>+</sup>, proton; HRE, HIF regulating element; P, phosphate; p300 HAT-CREBBP, histone acetyltransferase p300-cAMP-response element binding protein (CREB)-binding protein.



**Fig. 3 | Hypoxia-inducible factor 2 $\alpha$  in liver glucose metabolism.**

Liver glucose metabolism and transport are activated by insulin signalling through insulin receptor substrate 2 (IRS2), which activates phosphoinositide 3-kinase (PI3K) to phosphorylate AKT. This phosphorylation results in the activation of glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), stimulating glycogen synthesis and mechanistic target of rapamycin (mTOR), which activates fatty acid synthesis through sterol regulatory element-binding protein 1C (SREBP1C) and inhibits forkhead box protein O1 (FOXO1), which controls gluconeogenesis. Hypoxia-inducible factor 2 $\alpha$  (HIF2 $\alpha$ ) levels can be increased in the liver by hypoxia, vascular endothelial growth factor (VEGF) inhibition, prolyl hydroxylase domain (PHD) inhibition and refeeding. Glucagon exerts the opposite effects on glucose than insulin by binding to glucagon-like protein receptor 1 (GCGR), which increases cAMP through adenylyl cyclase. An increase in cAMP leads to the activation of protein kinase A (PKA) and phosphorylation of cAMP-responsive element-binding protein (CREB), which controls hepatic gluconeogenesis. Chronic activation of HIF2 $\alpha$  also leads to increased inflammation and fibrosis and decreased fatty acid  $\beta$ -oxidation, which suggest that chronic activation would have detrimental consequences to liver physiology, such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH). ANGPTL3, angiopoietin-related protein 3; ERK, extracellular-signal-regulated kinase; MEK, mitogen-activated protein kinase kinase (also known as MAP2K); P, phosphate; PDE, phosphodiesterase.



**Fig. 4 | Hypoxia-inducible factor 2 $\alpha$  in metabolic disease.**

Under conditions of obesity, the small intestine becomes hypoxic, leading to the accumulation of hypoxia-inducible factor 2 $\alpha$  (HIF2 $\alpha$ ) in epithelial cells. HIF2 $\alpha$  activates the gene encoding sialidase 3 (NEU3), which hydrolyses gangliosides (located in the plasma membrane) to form ceramides. Increased levels of ceramides cause obesity as a result of decreased adipose browning, increased steatosis owing to upregulation of fatty acid synthesis and increased insulin resistance. Inhibition of HIF2 $\alpha$  by PT2385 or inhibition of NEU3 by *N*-acetyl-2,3-didehydro-*N*-acetyl-neuraminic acid (DANA) or naringin decreases serum levels of ceramides, reduces obesity and fatty liver and increases insulin sensitivity. HRE, HIF regulating element.

Table 1 |

Summary of the effects of HIF1 $\alpha$ , HIF2 $\alpha$ , and HIF1 $\beta$  on metabolic disease

HIF	Tissue	HIF signalling	Phenotype	Refs
HIF1 $\alpha$	Adipose	Activation	Increased obesity and insulin resistance	30
HIF1 $\alpha$	Adipose	Inhibition with dominant negative HIF1 $\alpha$	Increased obesity and insulin resistance	32
HIF1 $\alpha$	Adipose	Inhibition	Decreased obesity and insulin resistance	31,36,38,40,41
HIF1 $\alpha$	Adipose	Inhibition	Decreased insulin resistance and unchanged obesity	28
HIF1 $\alpha$	Macrophage	Inhibition	No phenotype	51
HIF1 $\alpha$	Pancreatic $\beta$ -cell	Inhibition	Increased $\beta$ -cell dysfunction and glucose intolerance	55
HIF1 $\alpha$	Pancreatic $\beta$ -cell	Activation	Increased $\beta$ -cell dysfunction and glucose intolerance	58,59
HIF2 $\alpha$	Liver	Activation	Increased hepatic steatosis and fibrosis	63,65,74,76
HIF2 $\alpha$	Liver	Activation	Decreased glucose intolerance, gluconeogenesis and glucagon response	82
HIF2 $\alpha$	Liver	Inhibition	Decreased non-alcoholic steatohepatitis	80
HIF2 $\alpha$	Intestine	Inhibition	Decreased obesity, insulin resistance and hepatic steatosis	88
HIF2 $\alpha$	Adipose	Inhibition	Slightly increased insulin resistance	28
HIF1 $\beta$	Pancreatic $\beta$ -cell	Inhibition	Increased $\beta$ -cell dysfunction and glucose intolerance	55
HIF1 $\beta$	Liver	Inhibition	Increased glucose intolerance	69

HIF, hypoxia-inducible factor.