Review

Novel links between HIFs, type 2 diabetes, and metabolic syndrome

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Hypoxia inducible factors (HIFs) are master-regulators of cellular responses to hypoxia, and thus are crucial for survival. HIFs also play a role in regulating cellular processes in β-cells, liver, muscle, and adipose tissue, have effects on the regulation of weight, and play a role in type 2 diabetes (T2D). Indeed, in people with T2D the HIF pathway is dyregulated in major metabolic tissues involved in the pathogenesis of diabetes. This review covers the contrasting, complementary and conflicting effects of decreasing and increasing HIFs in various tissues, and shows that a delicate balance exists between HIF levels and optimal metabolic function. We propose that increasing the activity of HIFs might be a potential therapeutic strategy for treating T2D.

HIFs

Oxygen is essential to the survival of all multicellular organisms. It is a prime substrate in the generation of ATP, the major energy source, but in excess, oxygen leads to the formation of reactive oxygen species (ROS) and oxidative cellular damage. Therefore its homeostasis must be finely tuned. Beyond whole-organism oxygen homeostasis, reviewed in [1,2], recent emerging evidence suggests that dysregulation of the HIF pathway has a role in the pathophysiology of a range of diseases including metabolic syndrome, diabetes, cancer, heart disease and pulmonary vascular disease [3,4]. Several recent papers have reported seemingly conflicting findings in tissue-specific HIF activity, and these make this a complex field. Here we review the regulation of HIFs and their roles in the major metabolic tissues involved in the pathogenesis of diabetes and the metabolic syndrome.

The HIF family and their regulation

The HIF proteins coordinate at the transcriptional level the cellular response to oxygen availability [2]. Since their discovery 15 years ago remarkable progress has been made in understanding the structure and function of these proteins and their role in basic cellular physiology. There are now hundreds of known HIF target genes, including genes encoding proteins involved in angiogenesis, apoptosis, cell cycle progression, glucose uptake, glycolysis, and lipid metabolism [3]. As an indispensable component for normal development, deletion of the family members ARNT (also termed HIF-1β, see Glossary), HIF-1α, or HIF-2α in mice is lethal before birth or perinatally, with the exception of one line of HIF-2α null mice with partial survival on a mixed genetic background [1,5–9].

HIFs are heterodimeric transcription factors that belong to the basic helix-loop-helix Per–ARNT–Sim (bHLH-PAS) family. HIF-1 is composed of two parts, namely ARNT (also named HIF-1β) and HIF-1α, both of which play interdependent roles in transcriptional regulation. HIF-2 is composed of ARNT2 (also termed HIF-2β) and HIF-2α, and similarly HIF-3 is made up of ARNT3 (also known as HIF-3β) and HIF-3α. The HIF α subunits are tightly regulated at the protein level [1,10], as depicted in Figure 1a.

HIFs are rapidly hydroxylated, ubiquitinated and destroyed by proteolysis, giving a half life of minutes. Hydroxylation is carried out by prolyl hydroxylases (PHDs) and an asparagine hydroxylase named FIH1 (factor inhibiting HIF). Once hydroxylated, HIF α subunits associate with the von Hippel–Lindau (VHL) protein, which forms part of an E3 ubiquitin ligase complex. This ubiquitinates HIFs and targets them for proteolysis. The hydroxylases require oxygen, iron and 2-oxoglutarate (also known as α-ketoglutarate) as cofactors. Thus, hypoxia, iron depletion, or decreased 2-oxoglutarate inhibit proteolysis and HIF activity increases. Mutations in the human *VHL* gene cause a syndrome of the same name which is associated with an increased risk of vascular tumors [11]. Mutations affecting PHDs or FIH also increase HIF activity in people.

Glossary

Akt/PKB (protein kinase B): a serine/threonine-specific protein kinase that plays roles in a variety of cellular processes including glucose metabolism, cell proliferation and apoptosis.
AMP-activated protein kinase (AMPK): an enzyme expressed in several tissues that plays a pivotal role in cellular energy homeostasis.
Aryl hydrocarbon receptor nuclear translocator (ARNT): a basic helix-loop-helix protein required for the activity of the aryl hydrocarbon receptor (AhR). ARNT also dimerizes with hypoxia-inducible factor 1 (HIF1), and this heterodimer functions as a transcriptional regulator of the adaptive response to hypoxia.
db/db: a mouse model of T2D that lacks the leptin receptor.
Factor inhibiting HIF (FIH): an asparagine hydroxylase that inhibits the function of the HIF α-subunits. It requires iron, oxygen and 2-oxoglutarate for function.
Hypoxia-inducible factors (HIFs): heterodimeric transcription factors, with an oxygen-sensitive α subunit.
Non-alcoholic fatty liver disease (NAFLD): steatosis of the liver not caused by alcohol consumption.
PHDs: prolyl hydroxylase domain enzymes (PHDs) that hydroxylate (and thereby regulate) the HIF transcription factors.
TallyHo: mouse model of T2D with diabetes susceptibility loci on chromosomes 7, 13, 15, and 19.
Von Hippel–Lindau (VHL): a protein that forms part of an E3 ubiquitin ligase complex, and is responsible for ubiquitination and proteosomal degradation of HIFs.

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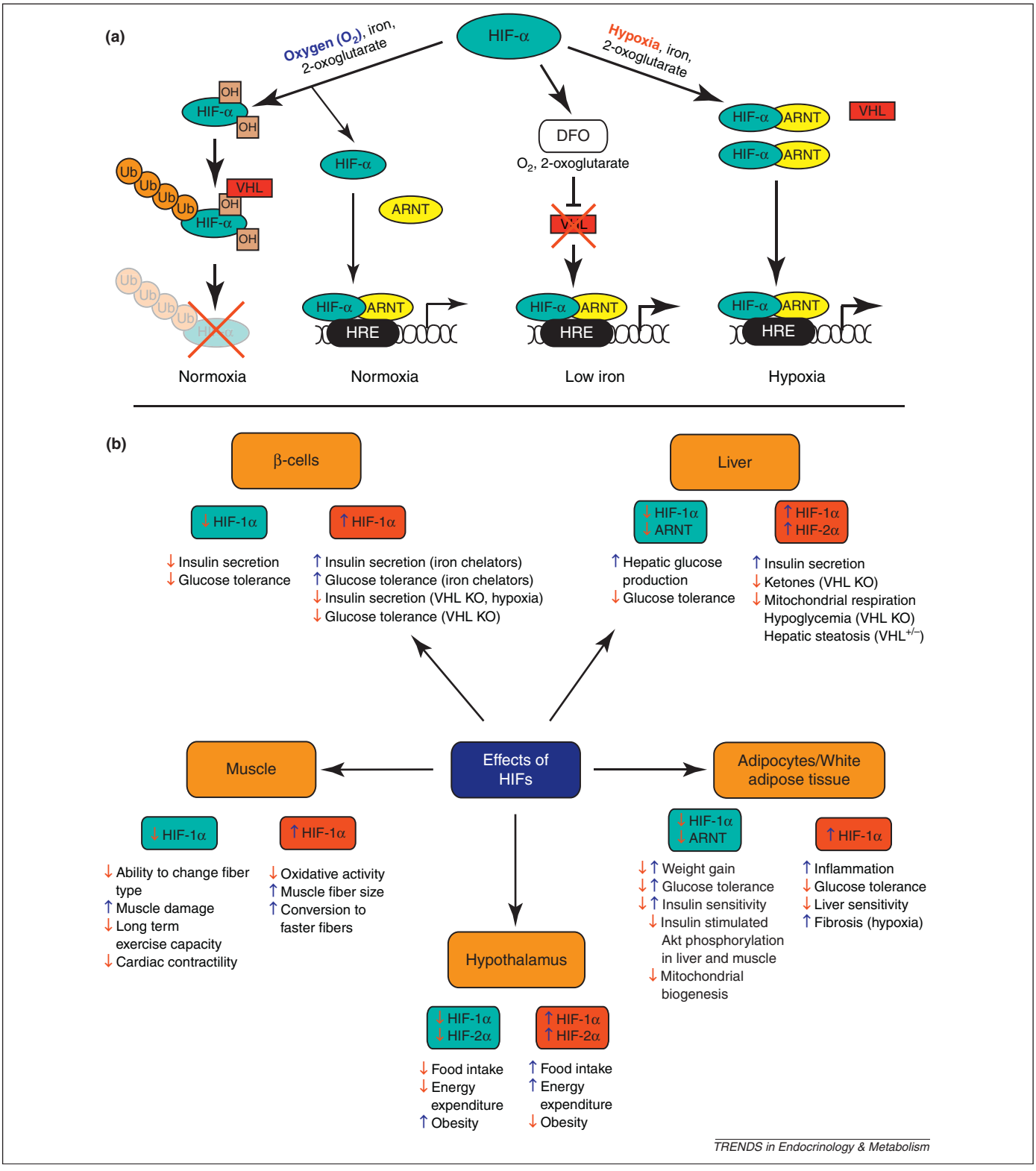

Figure 1. Regulation of HIF protein and the role of HIFs in various tissue types. **(a)** Under normoxic conditions HIF α subunits are hydroxylated, ubiquitinated, and targeted for proteosomal degradation. A small amount escapes degradation and binds to ARNT to regulate target genes. Under hypoxic conditions, HIF α subunits are unable to be hydroxylated by the oxygen-dependent hydroxylases and thus escape degradation. The iron chelator deferoxamine is able to stabilize HIF-1α because the hydroxylases also require iron to function. **(b)** HIFs are present in several tissue types and the effects of reducing or increasing HIFs from previous studies are summarized. Upward arrows indicate an increase, and downward a decrease. Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; HIF, hypoxia inducible factor; VHL, von Hippel–Lindau; KO, knockout.

HIFs, diabetes and metabolic syndrome

Diabetes has become a problem of global proportions. The two major risk factors for T2D are insulin resistance and β-cell dysfunction. Recent research suggests that disturbances in HIF-1 signaling may play a detrimental role at several stages in diabetic pathogenesis, including the innate failure of β-cells to secrete sufficient insulin [12,13], insulin resistance [14,15], adipocyte dysfunction,

and inflammation [16–18]. For example, hyperglycemia interferes with protein stabilization, although the mechanisms are not fully understood [12,13]. Conversely, insulin and other growth factors may stabilize HIF-1α [14].

HIF-1 and β-cell function

β-Cell dysfunction leads to inadequate insulin secretion. In normal β-cells, rising glucose levels lead to its increased uptake and metabolism through glycolysis, the Krebs cycle,

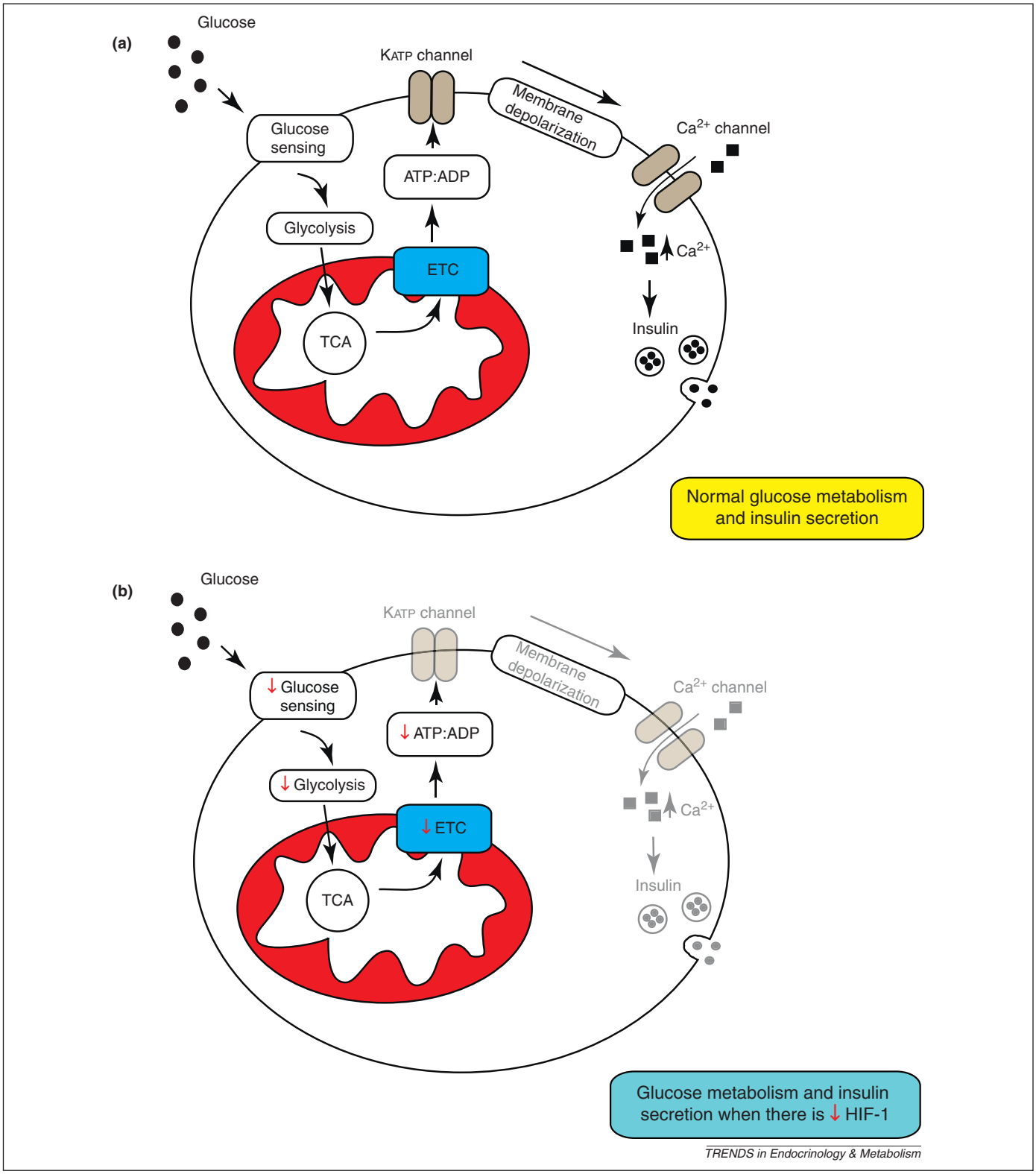

Figure 2. Hypoxia inducible factor (HIF) and pathways in β-cell dysfunction in diabetes. **(a)** Under normal conditions, glucose is taken up and broken down by glycolysis to provide substrates for the TCA cycle and the electron-transport chain. An increase in the ATP:ADP ratio closes the ATP-dependent potassium channels, leading to membrane depolarization, opening of the voltage-gated calcium channels and increase in intracellular calcium. This subsequently results in insulin exocytosis. **(b)** Decreased HIF-1 leads to decreases in genes associated with glucose sensing, glycolysis, and the electron-transport chain, ultimately leading to a decrease in ATP generation. A decrease in insulin secretion is a direct result of a decrease in the ATP:ADP ratio.

and then in the mitochondrial electron-transport chain (Figure 2a). Metabolism of glucose leads to generation of ATP from ADP. The ATP-sensitive potassium channels close, leading to membrane depolarization and insulin release. Thus, impaired glucose uptake can impair glucose-stimulated insulin release (GSIS).

Islets isolated from people with T2D have a 90% decrease in ARNT expression (mRNA) compared to glucose-tolerant donors [12]. Mice with β-cell-specific knockout of ARNT (β-ARNT) had reduced glucose tolerance and impaired *in vivo* and *in vitro* GSIS, and similar changes in gene expression to those seen in T2D islets, including decreased HNF4α, insulin receptor, aldolase, phosphofructokinase and others [12]. Cellular studies examining ARNT knockdown in three different β-cell culture models (MIN6, primary islets and 832/13) show consistent results, with significantly impaired insulin secretion, reduced glycolytic enzyme expression, and dysregulation of metabolic pathways [12,19]. These findings suggest that ARNT plays an important role in β-cell function. Another recent cellular study has identified carbohydrate-responsive element-binding protein (ChREBP) as a negative regulator of ARNT and has suggested that ChREBP-mediated repression of the HIF complex might contribute to glucotoxicity-induced β-cell dysfunction [20].

HIF-1α, the heterodimeric partner of ARNT, plays an important role in insulin secretion, because insulin secretion is impaired in mice with β-cell-specific knockout of HIF-1α and in MIN6 cells with siRNA knockdown of HIF-1α [21]. As with β-ARNT knockout mice, these mice had decreased expression of glycolytic genes including glucokinase and phosphofructokinase, key components of the β-cell glucose-sensing machinery [21] (Figure 2b).

Consistent with impaired glycolytic gene expression, β-HIF-1α null mice had severely reduced glucose-stimulated ATP generation and therefore impaired insulin release (Figure 2b). This provides a mechanism by which a transcription factor can regulate insulin secretion. Further linking these components is the observation that the uptake and metabolism of glucose by the electron-transport chain in a β-cell model was shown to deplete oxygen. Oxygen depletion induces HIF-1α and increased glycolysis [22].

VHL is required for HIF proteolysis, and thus deletion or inactivating mutations increase HIF protein. Interestingly, mice with β-cell deletion of VHL have markedly impaired insulin secretion and glucose intolerance [23–26]. Therefore, both depletion of ARNT or HIF-1α and excess of HIF-1α and HIF-2α (with are both increased with VHL deletion) impair β-cell function, suggesting an 'inverse-U' relation.

Because hydroxylation and concomitant degradation of HIFs requires iron, iron chelators induce modest increases in HIF protein levels. Use of iron chelators resulted in improved insulin secretion and normalized ARNT mRNA and downstream gene expression in islets from people with T2D [21]. The β-cell benefits of iron chelation are HIF-1α-dependent because iron chelators did not affect β-cell function in mice lacking HIF-1α in β-cells. Collectively, these data suggest that iron chelators might have therapeutic potential. Consistent with the inverse U-relation, deletion of FIH, which causes relatively modest increases in HIFs, also results in improved glucose tolerance in mice challenged with high-fat diet (HFD) [27].

The various effects in HIF-1 dyregulation of β-cells are shown in Figure 1, and the effects on β-cell glucose-sensing are shown in Figure 2b. Smaller increases such as those seen with iron chelation or FIH deletion are beneficial for β-cell function and survival [28–30]. Mice on a HFD with the addition of an iron chelator (deferasirox) have better glucose tolerance than mice on a HFD alone. Beneficial effects on β-cell function were not limited to mice because human islets treated with the iron chelator deferoxamine had improved glucose-stimulated insulin secretion and *ARNT* mRNA expression was restored [28]. Deferoxamine improves islet survival following the hypoxic insult of islet transplantation [29]. Mice were cured of diabetes with a lower number of transplanted islets when islets were pretreated with deferoxamine. Deferoxamine-treated islets had increased anti-apoptotic gene expression and decreased apoptosis [29].

The available evidence suggests that HIF-1α activity operates on a spectrum, with the two extremities of too little and too much HIF-1α causing detrimental effects on β-cell function. In this context it is interesting that whereas homozygous β-cell deletion of VHL is very clearly deleterious for β-cell function, heterozygous mutations in people with VHL syndrome do not appear to be associated with diabetes. In fact, there may be a decrease in diabetes risk after partial pancreatectomy in people with VHL syndrome (discussed in [28]). Our belief is that there is an optimal point for HIF-1α for β-cell function, with 'normal' probably being below that level of HIF-1α. Then, if HIF-1α is modestly increased, such as with iron chelation, β-cell function will improve.

Consistent with that concept, human islets from people with T2D have decreased expression of both HIF-1α and ARNT, and increasing HIF-1α with iron chelation improved human islet function and gene expression [28]. Iron chelators are currently approved for human use for treatment of iron overload and it will be of great interest to determine the effects in human diabetic patients in clinical trials.

HIFs and liver

In the liver, the highest oxygen concentrations are in the periportal regions and the lowest in the centrilobular area. Periportal hepatocytes specialize in oxidative metabolism and the synthesis of bile. Centrilobular cells have lower oxygen tension, higher HIF protein levels, take up more glucose, synthesize glutamine, and metabolize xenobiotics – functions related to the activity of ARNT and HIFs. Xenobiotic metabolism is regulated by ARNT plus Aryl hydrocarbon receptor (AhR) [31,32]. The liver is sensitive to hypoxia and to reperfusion injury: apneic episodes in people who have obstructive sleep apnea can induce acute elevation of liver enzymes [33,34].

Liver dysfunction is a key component of T2D and is both affected by and contributes to the condition [35–38]. Cardinal features include increased and inappropriate hepatic glucose production (HGP) [39,40] and reduced hepatic insulin sensitivity [41]. Non-alcoholic fatty liver diseases (NAFLD) are common in T2D and further exacerbate metabolic dysfunction [42–48].

Perturbations of the HIF proteins have been demonstrated to play a role in these processes in animal models. It has been shown that feeding mice with a high-fat/sucrose diet that causes fatty liver also causes upregulation of hepatic HIF-1α [49] via an unknown mechanism. However, HFD is a metabolic stress with well-established effects on glucose and energy homeostasis. When mice with a hepatocyte-specific HIF-1α deletion were fed this diet, they exhibited more severe impairment of glucose tolerance and peripheral insulin-resistance than control littermates [49].

Consistent with the decrease in ARNT mRNA in T2D islets, ARNT mRNA and protein are also reduced in human T2D livers [50]. Short-term hepatic ablation of ARNT in mice using adenovirus–*cre* injection increased HGP and impaired glucose tolerance [50]. Conversely, deletion of hepatocyte VHL substantially increased hepatic HIF-1α and HIF-2α proteins and caused life-threatening hypoglycemia and reduced ketones [51]. This phenotype was rescued by hepatic ARNT inactivation [51]. Hepatocyte VHL-null mice developed fatty liver, as did $VHL^{+/-}$ (heterozygous) mice [52]. Further studies reported that this effect is HIF-2α-dependent [53]. It is interesting that VHL knockout in β-cells results in impaired glucose tolerance whereas its specific knockout in the liver leads to hypoglycemia. The data from HIF-1α, ARNT and VHL deletion suggest that in the liver there is also an inverse-U relation, and both deletion and excess of HIF activity are deleterious. Similarly to in β-cells, this suggests that balanced HIF activity is crucial for optimal metabolic health.

As discussed in the β-cell section, the use of iron chelation in the form of deferoxamine may be beneficial to hepatic insulin sensitivity. In HepG2 hepatoma cells, deferoxamine upregulated the glucose transporter Glut1 and the insulin receptor. The latter was ARNT-dependent [54]. Rats treated with deferoxamine displayed improved insulin sensitivity with the upregulation of hepatic insulin receptor and Akt/PKB.

HIFs and muscle

Skeletal muscle is faced with the challenge of matching energy production with demand under widely-ranging circumstances, from resting to intense exercise. The ability of muscle to switch rapidly between aerobic and anaerobic energy production is an important characteristic [55,56]. HIF-1 plays a role in this dynamic process by regulating glycolytic and oxidative pathways of energy production, mitochondrial respiration and muscle fiber composition [55,57]. Its role is suggested by three observations: expression is higher in fast-twitch muscles that rely on glycolysis [58] and is upregulated during bursts of activity [57] and in chronic hypoxia [59].

Mice with muscle-specific HIF-1α deletion are initially able to exercise for longer due to reduced lactate accumulation [60]. Lactate accumulation causes muscle pain, hence decreased lactate associates with improved exercise tolerance. However, the mice lack the ability to change fiber-type between fast- and slow-twitch types, and have a decreased capillary:fiber ratio, making the muscle less able to adapt [55]. They develop muscle damage and fibrosis, eventually resulting in reduced exercise capacity [60]. Conversely, muscle HIF-1α overexpression in rats increased fiber size and conversion to faster fibers [57].

In humans, a HIF-1α polymorphism (Pro582Ser) is more commonly found in elite athletes versus controls, suggesting that it may improve physical performance and muscle function [61]. However, the mechanism is unknown. In a subset of skaters who had muscle biopsies, this polymorphism was associated with a predominance of fast-twitch fibers. Increased fiber size and fast-twitch fibers would improve strength and performance in tasks requiring speed, and therefore some have suggested that muscle HIF may one day constitute a form of doping [62]. The effects of iron chelators would be very interesting to examine in the context of human athletic performance and muscle function.

We have found two studies examining the direct role of muscle HIF-1 in glucose homeostasis. In the first study HIF-1α upregulated GLUT4 mRNA after 10 min of electrically-induced contraction of isolated soleus muscle. Thus HIF-1α might facilitate glucose transport following contraction [63]. Another study showed that insulin-dependent upregulation of glucose transporters was dependent on the HIF-1α/ARNT transcriptional complex [64]. Interestingly, basal and insulin-induced expression of Glut1, Glut3, aldolase, phosphoglycerate kinase, and VEGF were reduced in ARNT-defective cells.

The characteristic features of skeletal muscle in subjects with insulin resistance, namely reduced oxidative and increased glycolytic activities and altered fiber composition [65], may imply a potential regulatory role for HIF-1. However, direct assessment of glucose homeostasis in animal models will be needed to test this possibility.

HIFs, fat and inflammation

Since the discovery of leptin it has been recognized that adipose tissue represents a dynamic organ that produces adipokines and has roles in energy balance, glucose metabolism, inflammation and immunity [66,67]. Obesity is the strongest acquired risk factor for T2D: it gives a 10-fold higher risk in men and 30-fold higher risk in women [68,69].

Three recent reviews have discussed the various links between obesity and insulin resistance [67,70,71]. In insulin-resistant obesity there is adipose tissue hypoxia, and this appears to drive an inflammatory response [72,73]. In cultured adipocytes and preadipocytes, HIF-1α expression is upregulated by exposure to hypoxia or cobalt(II) chloride (a heavy metal poison which inhibits mitochondrial function and increases HIFs), and exposure increased levels of the inflammatory mediators IL-6 and monocyte migration inhibitory factor, and raised leptin and reduced adiponectin levels [74]. In obese mice, regional fat hypoxia colocalized with macrophage and T cell infiltration [75,76].

In obese humans, reduced oxygen pressures within abdominal fat associate with greater macrophage infiltration [77]. Collectively, these observations suggest that (i) adipose tissue hypoxia is deleterious and (ii) that defects in the adipocyte response to hypoxia may contribute to the pathogenesis of insulin resistance and diabetes. This concept was also suggested by a study in which gene expression analysis of cultured adipocytes taken from diabetic

db/*db* and *TallyHo* mice demonstrated that these cells were unable to respond efficiently to hypoxia [78].

Compelling evidence that HIF-1 plays a crucial role in adipocyte function emerges from genetically engineered models. In mice overexpressing constitutively active HIF-1α in white adipose tissue (WAT), inflammation, glucose intolerance and decreased liver sensitivity were observed both on normal chow and on a HFD [16]. Also, as seen in β-cells, constitutively active, unregulatable HIF is deleterious [28].

Studies examining adipocyte-specific HIF-1α and adipocyte-specific ARNT knockout mice report the protection of these mice from the consequences of HFD. Specifically, these mice were resistant to weight gain, and had substantially better glucose tolerance and insulin sensitivity [17,18]. Increased insulin-stimulated Akt phosphorylation was seen in WAT, liver and muscle in adipocyte-specific HIF-1α knockout mice [17], suggesting crosstalk between adipose tissue and other sites. These mice also displayed central effects with an increase in core temperature and energy expenditure [17,18]. Adipocyte-specific ARNT knockout mice were lean, had smaller adipocytes, and were protected from age-related effects on glucose homeostasis [79]. In these studies, adipocyte specific ARNT and HIF-1α mice were created using the *aP2 (Fabp4)–cre* strain. The presence of *aP2–cre* causes selective deletion of a 'floxed' gene in cells expressing aP2 (adipocyte protein 2/fatty acid binding protein 4) – which include macrophages and potentially some cells in the central nervous system (CNS) [80]. Deletion in macrophages may contribute to the fascinating metabolic phenotype because macrophage accumulation and activity are linked to obesity [81].

By contrast, another mouse model of adipocyte-specific HIF-1α inhibition resulted in entirely different effects [82]. In response to HFD, these mice gained more weight, had worse glucose tolerance and insulin sensitivity, and impaired mitochondrial biogenesis. The reason for the opposite responses is unclear. In these mice a dominant negative mutant of human HIF-1α lacking the DNA-binding domain was overexpressed in adipocytes, and there was also substantially increased expression in brown adipose tissue (BAT). In contrast to 'normal' adipose tissue, the primary function of BAT is not to store energy, but to consume energy in thermogenesis. Thus, some of the effects reported in this paper may relate to preferentially decreased HIF-1α function in BAT. Moreover, *aP2–cre* also deletes in BAT, and therefore in the genetic deletion models discussed above there would also have been HIF-1α deletion in BAT.

Deletion of VHL from adipocytes, also using *aP2–cre* mice, caused brain hemorrhages and lethality, reflecting *aP2–cre* expression in the embryonic CNS [80]. This raises the additional possibility that some of the beneficial effects seen in *aP2–cre* ARNT and *aP2–Hif1a* mice may relate to CNS effects.

The findings from another mouse model further complicate the interpretation. Mice lacking FIH have greater HIF activity in fat and are protected from the effects of HFD, with decreased weight gain, decreased fat accumulation, and improved insulin sensitivity [30]. Many of the effects in the FIH null mice were recapitulated by selective deletion in the CNS [30].

An intriguing finding of these papers is that peripheral knockout of HIF-1α can alter central control of metabolism. Changes in the metabolic rate, as assessed by indirect calorimetry (that measures heat production based on

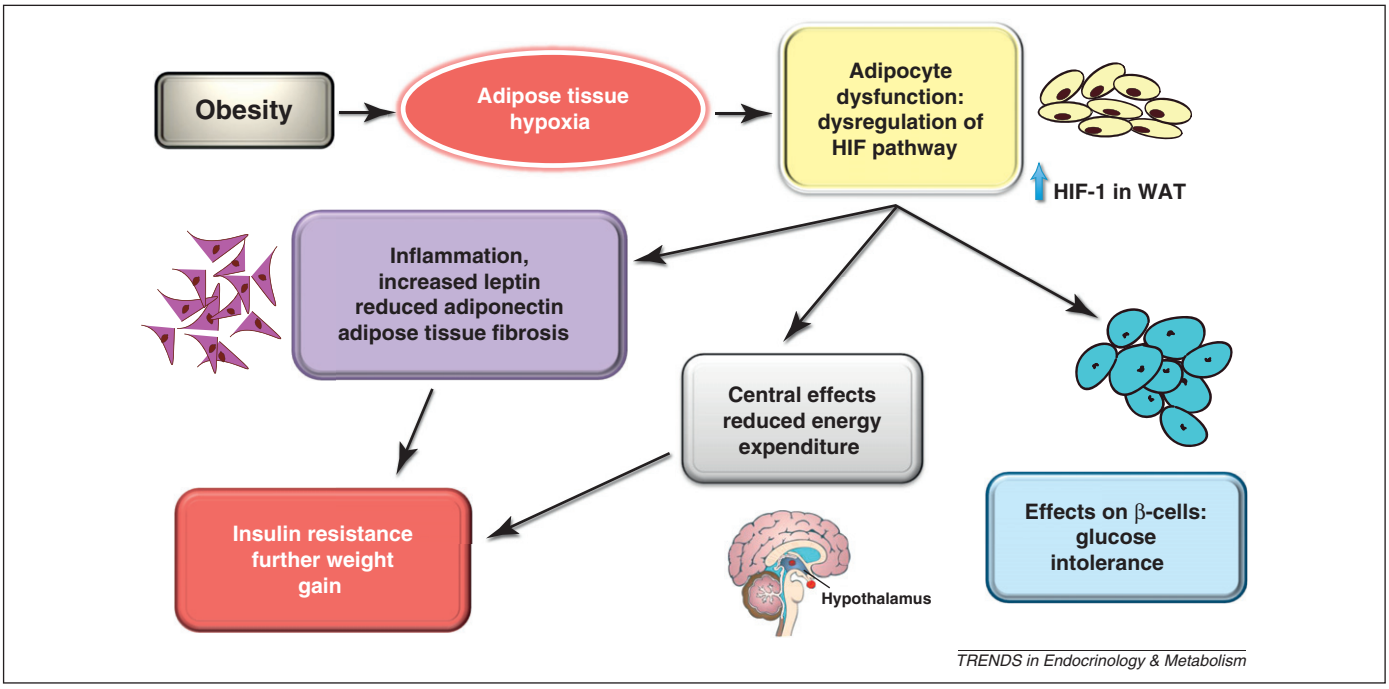

Figure 3. Roles of HIF-1α in adipocyte dysfunction and impaired glucose homeostasis. In obese individuals, hypoxia of adipose tissue leads to a chain of local events set off by dyregulation of the HIF-1 pathway in adipocytes, including inflammation and macrophage infiltration. More distant effects include disturbances in β-cell function and the central control of metabolism, all of which ultimately lead to glucose intolerance, insulin resistance, and further weight gain. The main evidence for this model comes from transgenic mouse studies; whether this takes place in humans under typical pathophysiological conditions is not yet clear. Abbreviations: HIF, hypoxia inducible factor; WAT, white adipose tissue.

oxygen consumption), and changes in core body temperature in adipocyte-specific models, support this possibility [20,21,81]. A recent study focusing on the hypothalamus examined this issue [83]. Neurons in the mediobasal hypothalamus express HIFs, particularly HIF-2α, and this protein was upregulated in response to glucose, suggesting that HIF-2α regulates hypothalamic glucose-sensing. HIF activation upregulated expression of pro-opiomelanocortin, an anorexigenic neuropeptide that is essential in the control of body weight. Conditional loss-of-function of HIF signaling in pro-opiomelanocortin hypothalamic neurons exacerbated dietary obesity. Delivery of HIF-2α/ARNT or HIF-1α/ARNT via lentiviral coexpression in the mediobasal hypothalamus protected these mice from the effects of a HFD. Favorable effects of both HIF-1α and -2α were attributed to reduced *ad libitum* food intake [83].

How do we incorporate these findings into a unifying explanation of the role of HIFs in adipocyte function, energy regulation, and glucose homeostasis? First, the local upregulation of HIF-1α in adipocytes in obese individuals with hypoxic WAT is associated with inflammation and peripheral insulin-resistance. This is corroborated by similar findings from a mouse model which overexpresses HIF-1α in adipocytes. On the other hand, deletion of HIF-1α in adipocytes appears beneficial, conferring protection from disturbances of glucose homeostasis and energy regulation following a HFD. These concepts are depicted in Figure 3. Excess HIF-1α, induced by hypoxia, is deleterious, and deficit appears to be beneficial. However, expression of a dominant negative form of HIF-1α adipocytes is deleterious [82]. Second, whole-body FIH deletion, which modestly increases HIF, is beneficial. Together, this suggests that in adipocytes the relation is more complex than the apparent inverse-U-shape in other tissues. It remains unclear why the relation in adipose tissue is different.

HIF and diabetes complications

The complications posed by diabetes lead to significant morbidity and mortality and a substantial financial burden to the community. Hypoxia is a key feature of these complications whether they occur at a macrovascular (i.e., coronary artery, peripheral and cerebral vascular diseases) or microvascular level (i.e., neuropathy, retinopathy, nephropathy).

Mechanisms by which hyperglycemia results in the degradation of HIF-1α have been described [84], possibly linking the increase in myocardial infarct size amongst rats exposed to hyperglycemia with the reported reduction in HIF-1α [85]. More directly, overexpression of HIF-1α leads to improvement of myocardial circulation and inhibition of cardiac hypertrophy and fibrosis following myocardial injury in diabetic mice [86]. These effects probably take place at the basic level of endothelial function and thus oxygen and nutrient supply, as suggested by the upregulation of VEGF by HIF-1α in these mice.

Biopsies taken from the foot ulcers of human subjects with diabetes displayed significantly lower HIF-1α levels than biopsies taken from venous ulcers, suggesting a role for hyperglycemia rather than hypoxia alone in diabetic peripheral vascular disease [84]. Young *db*/*db* (obese) mice with large wounds display reduced HIF-1α production, and genetic upregulation of HIF-1α in these mice accelerated wound-healing and angiogenesis [87]. In another study, the topical application of deferoxamine improved healing of skin wounds in *db*/*db* mice and systemic administration improved surgical skin-flap survival in streptozocin-induced diabetic mice [88]. Further research is needed to clarify the role of HIF-1 and its potential as a therapeutic target in the management of these important conditions.

Box 1. Outstanding questions

- How does a local imbalance in HIF-1 levels facilitate crosstalk between remote organ systems? For example, adipocyte-specific knockout mice display increased body temperature and energy expenditure, usually under the control of the hypothalamus.
- A better understanding of the complexities of HIF-1 regulation may help to explain some of the discordant findings of various mouse models with unregulated, increased HIF-1 activity. Also, by contrast, when there is whole-body unregulated HIF-1, as in FIH mice, why is this beneficial?
- What are the effects on function of various metabolic tissues in people with VHL syndrome: is their rate of diabetes altered? Do they have an increased or decreased risk of fatty liver?
- What are the effects of mutations in the equivalents of FIH and PHDs in humans? Again, do these people have a decreased risk of diabetes? Are they protected from obesity as are the FIH mice?
- How can we apply this evidence-base to the treatment of T2D and metabolic syndrome?

Concluding remarks

Collectively, the literature indicates important roles for the HIFs in many tissues, with tissue crosstalk. Several transgenic animal studies have reported effects of organ-specific ARNT and HIF knockouts, raising the suggestion that HIF-1-mediated crosstalk plays a role in the pathogenesis of diabetes. Indeed, T2D is a disorder of seemingly disparate phenomena, including defective β-cell function, impaired insulin signaling within muscle, and increased HGP. Each of these individual features can be induced or repressed by manipulating ARNT and/or HIFs. Given the known alterations in human tissues we speculate that ARNT and HIFs may play a central role in these processes. It is interesting to speculate whether HIF-related perturbations in glucose homeostasis point to an underlying problem in hypoxia-sensing or are hypoxia-independent. There are also a number of outstanding questions posed by this body of evidence (Box 1). In adipose tissue the hypoxia model plausibly links together HIF-related maladaptive responses to tissue hypoxia, with subsequent inflammation and peripheral insulin resistance. Likewise, vascular complications in diabetes are characterized by tissue hypoxia, with plausible evidence for a contributory role in the dyregulation in the HIF-1 pathway. For the β-cell and liver the distinction is less clear. It is likely that hypoxia-independent factors play a role in HIF dyregulation in β-cells.

Interpreting these findings in a clinical context is challenging. Tissue-specific manipulation of the HIF pathway is clearly difficult to translate to the whole human organism. However, overall, in most tissues, especially fat, hypoxia is obviously deleterious for function. For β-cell function, modest hypoxia, similar to the exposure *in vivo* (5% oxygen), is not deleterious, but function is clearly impaired at lower oxygen levels. Interestingly, in muscle,

intermittent hypoxia induced by exercise is beneficial, and induces helpful adaptive responses, but chronic hypoxia is deleterious.

Despite its limitations, this body of preclinical data supports a vital role for HIF-1 in the regulation in mice of glucose homeostasis, adipocyte function and the central regulatory control of metabolism. In humans, significant alterations in the expression of HIF-1α and/or ARNT in the islets and liver cells of those with diabetes suggests that HIF-1 pathway may also play similar roles in glucose homeostasis to those demonstrated in mice. Together, the body of animal research and the human data suggest that modest stimulation of the HIF pathway may present a viable therapeutic strategy in the management of T2D and the metabolic syndrome.

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References

1 Semenza, G.L. (2004) Hydroxylation of HIF-1: oxygen sensing at the molecular level. *Physiology* 19, 176–182
2 Rocha, S. (2007) Gene regulation under low oxygen: holding your breath for transcription. *Trends Biochem. Sci.* 32, 389–397
3 Semenza, G.L. (2001) Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. *Trends Mol. Med.* 7, 345–350
4 Semenza, G.L. (2000) HIF-1 and human disease: one highly involved factor. *Genes Dev.* 14, 1983–1991
5 Kopp, R. *et al.* (2011) HIF signaling and overall gene expression changes during hypoxia and prolonged exercise differ considerably. *Physiol. Genomics* 43, 506–516
6 Zhang, Y. *et al.* (2009) The HIF-1 hypoxia-inducible factor modulates lifespan in *C. elegans*. *PLoS ONE* 4, pe6348
7 Maltepe, E. *et al.* (1997) Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* 386, 403–407
8 Scortegagna, M. *et al.* (2003) Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1−/− mice. *Nat. Genet.* 35, 331–340
9 Scortegagno, M. *et al.* (2003) The HIF family member EPAS1/HIF-2alpha is required for normal hematopoiesis in mice. *Blood* 102, 1634–1640
10 Zagorska, A. and Dulak, J. (2004) HIF-1: the knowns and unknowns of hypoxia sensing. *Acta Biochim. Pol.* 51, 563–585
11 Hammel, P.R. *et al.* (2000) Pancreatic involvement in von Hippel-Lindau disease. *Gastroenterology* 119, 1087–1095
12 Gunton, J.E. *et al.* (2005) Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. *Cell* 122, 337–349
13 Cheng, K. *et al.* (2010) Hypoxia-inducible factor-1alpha regulates beta cell function in mouse and human islets. *J. Clin. Invest.* 120, 2171–2183
14 Ochiai, D. *et al.* (2011) Disruption of HIF-1alpha in hepatocytes impairs glucose metabolism in diet-induced obesity mice. *Biochem. Biophys. Res. Commun.* 415, 445–449
15 Yu, J. *et al.* (2011) Conditioned medium from hypoxia-treated adipocytes renders muscle cells insulin resistant. *Eur. J. Cell Biol.* 90, 1000–1015
16 Halberg, N. *et al.* (2009) Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. *Mol. Cell. Biol.* 29, 4467–4483
17 Jiang, C. *et al.* (2011) Disruption of hypoxia-inducible factor 1 in adipocytes improves insulin sensitivity and decreases adiposity in high-fat diet-fed mice. *Diabetes* 60, 2484–2495
18 Krishnan, J. *et al.* (2012) Dietary obesity-associated Hif1alpha activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD^+ system. *Genes Dev.* 26, 259–270
19 Pillai, R. *et al.* (2011) Aryl hydrocarbon receptor nuclear translocator/hypoxia-inducible factor-1{beta} plays a critical role in maintaining glucose-stimulated anaplerosis and insulin release from pancreatic {beta}-cells. *J. Biol. Chem.* 286, 1014–1024
20 Noordeen, N.A. *et al.* (2010) Carbohydrate-responsive element-binding protein (ChREBP) is a negative regulator of ARNT/HIF-1beta gene expression in pancreatic islet beta-cells. *Diabetes* 59, 153–160
21 Cheng, K. *et al.* (2010) Hypoxia-inducible factor-1alpha regulates beta cell function in mouse and human islets. *J. Clin. Invest.* 120, 2171–2183
22 Bensellam, M. *et al.* (2012) Glucose-induced o(2) consumption activates hypoxia inducible factors 1 and 2 in rat insulin-secreting pancreatic Beta-cells. *PLoS ONE* 7, pe29807
23 Cantley, J. *et al.* (2009) Deletion of the von Hippel-Lindau gene in pancreatic beta cells impairs glucose homeostasis in mice. *J. Clin. Invest.* 119, 125–135
24 Choi, D. *et al.* (2011) Vhl is required for normal pancreatic beta cell function and the maintenance of beta cell mass with age in mice. *Lab. Invest.* 91, 527–538
25 Puri, S. *et al.* (2009) A role for von Hippel-Lindau protein in pancreatic beta-cell function. *Diabetes* 58, 433–441
26 Zehetner, J. *et al.* (2008) pVHL is a regulator of glucose metabolism and insulin secretion in pancreatic {beta} cells. *Genes Dev.* 22, 3135–3146
27 Zhang, N. *et al.* (2010) The asparaginyl hydroxylase factor inhibiting HIF-1alpha is an essential regulator of metabolism. *Cell Metab.* 11, 364–378
28 Cheng, K. *et al.* (2010) Hypoxia-Inducible Factor-1α Regulates β-Cell Function in Mouse and Human Islets. *J. Clin. Invest.* 120, 2171–2183
29 Stokes, R.A. *et al.* Hypoxia-inducible factor 1α (HIF-1α) potentiates β-cell survival after islet transplantation of human and mouse islets. Cell Transplantation, 2012. (in press)
30 Zhang, N. *et al.* (2010) The asparaginyl hydroxylase factor inhibiting HIF-1alpha is an essential regulator of metabolism. *Cell Metab.* 11, 364–378
31 Jungermann, K. and Kietzmann, T. (2000) Oxygen: modulator of metabolic zonation and disease of the liver. *Hepatology* 31, 255–260
32 Nath, B. and Szabo, G. (2012) Hypoxia and hypoxia inducible factors: Diverse roles in liver diseases. *Hepatology* 55, 622–633
33 Mishra, P. *et al.* (2008) Apnoeic-hypopnoeic episodes during obstructive sleep apnoea are associated with histological nonalcoholic steatohepatitis. *Liver Int.* 28, 1080–1086
34 Jouet, P. *et al.* (2007) Relationship between obstructive sleep apnea and liver abnormalities in morbidly obese patients: a prospective study. *Obes. Surg.* 17, 478–485
35 Rivera, C.A. (2008) Risk factors and mechanisms of non-alcoholic steatohepatitis. *Pathophysiology* 15, 109–114
36 Musso, G. *et al.* (2010) Non-alcoholic fatty liver disease from pathogenesis to management: an update. *Obes. Rev.* 11, 430–445
37 Brown, M.S. and Goldstein, J.L. (2008) Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab.* 7, 95–96
38 Basu, R. *et al.* (2005) Obesity and type 2 diabetes impair insulin-induced suppression of glycogenolysis as well as gluconeogenesis. *Diabetes* 54, 1942–1948
39 Rizza, R.A. (2010) Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. *Diabetes* 59, 2697–2707
40 Basu, R. *et al.* (2004) Both fasting glucose production and disappearance are abnormal in people with "mild" and "severe" type 2 diabetes. *Am. J. Physiol. Endocrinol. Metab.* 287, E55–E62
41 Basu, R. *et al.* (2004) Insulin dose-response curves for stimulation of splanchnic glucose uptake and suppression of endogenous glucose production differ in nondiabetic humans and are abnormal in people with type 2 diabetes. *Diabetes* 53, 2042–2050
42 Sparks, J.D. and Sparks, C.E. (1994) Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion. *Biochim. Biophys. Acta* 1215, 9–32
43 Taghibiglou, C. *et al.* (2002) Hepatic very low density lipoprotein-ApoB overproduction is associated with attenuated hepatic insulin signaling and overexpression of protein-tyrosine phosphatase 1B in a fructose-fed hamster model of insulin resistance. *J. Biol. Chem.* 277, 793–803
44 Lewis, G.F. *et al.* (1993) Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes* 42, 833–842

45 Malmstrom, R. *et al.* (1997) Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia* 40, 454–462
46 Bourgeois, C.S. *et al.* (1995) VLDL output by hepatocytes from obese Zucker rats is resistant to the inhibitory effect of insulin. *Am. J. Physiol.* 269 (2 Pt 1), E208–E215
47 Biddinger, S.B. *et al.* (2008) Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metab.* 7, 125–134
48 Ginsberg, H.N. (2000) Insulin resistance and cardiovascular disease. *J. Clin. Invest.* 106, 453–458
49 Ochiai, D. *et al.* (2011) Disruption of HIF-1alpha in hepatocytes impairs glucose metabolism in diet-induced obesity mice. *Biochem. Biophys. Res. Commun.* 415, 445–449
50 Wang, X.L. *et al.* (2009) Ablation of ARNT/HIF1beta in liver alters gluconeogenesis, lipogenic gene expression, and serum ketones. *Cell Metab.* 9, 428–439
51 Kucejova, B. *et al.* (2011) Uncoupling hypoxia signaling from oxygen sensing in the liver results in hypoketotic hypoglycemic death. *Oncogene* 30, 2147–2160
52 Haase, V.H. *et al.* (2001) Vascular tumors in livers with targeted inactivation of the von Hippel-Lindau tumor suppressor. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1583–1588
53 Rankin, E.B. *et al.* (2009) Hypoxia-inducible factor 2 regulates hepatic lipid metabolism. *Mol. Cell. Biol.* 29, 4527–4538
54 Dongiovanni, P. *et al.* (2008) Iron depletion by deferoxamine up-regulates glucose uptake and insulin signaling in hepatoma cells and in rat liver. *Am. J. Pathol.* 172, 738–747
55 Mason, S.D. *et al.* (2007) HIF-1alpha in endurance training: suppression of oxidative metabolism. *Am. J. Physiol.* 293, R2059–R2069
56 Bhambhani, Y.N. (2004) Muscle oxygenation trends during dynamic exercise measured by near infrared spectroscopy. *Can. J. Appl. Physiol.* 29, 504–523
57 Lunde, I.G. *et al.* (2011) Hypoxia inducible factor 1 links fast-patterned muscle activity and fast muscle phenotype in rats. *J. Physiol.* 589 (Pt 6), 1443–1454
58 Pisani, D.F. and Dechesne, C.A. (2005) Skeletal muscle HIF-1alpha expression is dependent on muscle fiber type. *J. Gen. Physiol.* 126, 173–178
59 Hoppeler, H. and Vogt, M. (2001) Muscle tissue adaptations to hypoxia. *J. Exp. Biol.* 204 (Pt 18), 3133–3139
60 Mason, S.D. *et al.* (2004) Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. *PLoS Biol.* 2, pe288
61 Ahmetov, I.I. *et al.* (2008) Effect of HIF1A gene polymorphism on human muscle performance. *Bull. Exp. Biol. Med.* 146, 351–353
62 Lippi, G. and Guidi, G.C. (2004) Gene manipulation and improvement of athletic performances: new strategies in blood doping. *Br. J. Sports Med.* 38, p641
63 Lima, G.A. *et al.* (2009) Contractile activity per se induces transcriptional activation of SLC2A4 gene in soleus muscle: involvement of MEF2D, HIF-1a, and TRalpha transcriptional factors. *Am. J. Physiol. Endocrinol. Metab.* 296, E132–E138
64 Zelzer, E. *et al.* (1998) Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT. *EMBO J.* 17, 5085–5094
65 He, J. *et al.* (2001) Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. *Diabetes* 50, 817–823
66 Trayhurn, P. and Beattie, J.H. (2001) Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *Proc. Nutr. Soc.* 60, 329–339
67 Sell, H. *et al.* (2006) The adipocyte-myocyte axis in insulin resistance. *Trends Endocrinol. Metab.* 17, 416–422
68 Colditz, G.A. *et al.* (1995) Weight gain as a risk factor for clinical diabetes mellitus in women. *Ann. Intern. Med.* 122, 481–486
69 Chan, J.M. *et al.* (1994) Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 17, 961–969
70 Unger, R.H. and Scherer, P.E. (2010) Gluttony, sloth and the metabolic syndrome: a roadmap to lipotoxicity. *Trends Endocrinol. Metab.* 21, 345–352
71 Samocha-Bonet, D. *et al.* (2012) Insulin-sensitive obesity in humans - a 'favorable fat' phenotype? *Trends Endocrinol. Metab.* 23, 116–124
72 Trayhurn, P. and Wood, I.S. (2004) Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br. J. Nutr.* 92, 347–355
73 Wood, I.S. *et al.* (2009) Cellular hypoxia and adipose tissue dysfunction in obesity. *Proc. Nutr. Soc.* 68, 370–377
74 Wang, B. *et al.* (2007) Dysregulation of the expression and secretion of inflammation-related adipokines by hypoxia in human adipocytes. *Pflugers Arch.* 455, 479–492
75 Ye, J. *et al.* (2007) Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *Am. J. Physiol. Endocrinol. Metab.* 293, E1118–E1128
76 Rausch, M.E. *et al.* (2008) Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int. J. Obes.* 32, 451–463
77 Pasarica, M. *et al.* (2009) Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. *Diabetes* 58, 718–725
78 Hong, S.J. *et al.* (2011) Impaired response of mature adipocytes of diabetic mice to hypoxia. *Exp. Cell Res.* 317, 2299–2307
79 Lee, K.Y. *et al.* (2011) The differential role of Hif1beta/Arnt and the hypoxic response in adipose function, fibrosis, and inflammation. *Cell Metab.* 14, 491–503
80 Zhang, J. *et al.* (2012) Hypoxia-inducible factor 1 activation from adipose protein 2-cre mediated knockout of von hippel-lindau gene leads to embryonic lethality. *Clin. Exp. Pharmacol. Physiol.* 39, 145–150
81 Weisberg, S.P. *et al.* (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808
82 Zhang, X. *et al.* (2010) Adipose tissue-specific inhibition of hypoxia-inducible factor 1{alpha} induces obesity and glucose intolerance by impeding energy expenditure in mice. *J. Biol. Chem.* 285, 32869–32877
83 Zhang, H. *et al.* (2011) Hypoxia-inducible factor directs POMC gene to mediate hypothalamic glucose sensing and energy balance regulation. *PLoS Biol.* 9, pe1001112
84 Catrina, S.B. *et al.* (2004) Hyperglycemia regulates hypoxia-inducible factor-1alpha protein stability and function. *Diabetes* 53, 3226–3232
85 Marfella, R. *et al.* (2002) Myocardial infarction in diabetic rats: role of hyperglycaemia on infarct size and early expression of hypoxia-inducible factor 1. *Diabetologia* 45, 1172–1181
86 Xue, W. *et al.* (2010) Cardiac-specific overexpression of HIF-1{alpha} prevents deterioration of glycolytic pathway and cardiac remodeling in streptozotocin-induced diabetic mice. *Am. J. Pathol.* 177, 97–105
87 Mace, K.A. *et al.* (2007) Sustained expression of Hif-1alpha in the diabetic environment promotes angiogenesis and cutaneous wound repair. *Wound Repair Regen.* 15, 636–645
88 Thangarajah, H. *et al.* (2010) HIF-1alpha dysfunction in diabetes. *Cell Cycle* 9, 75–79