review article

Diabetes, Obesity and Metabolism 12 (Suppl. 2): 159–167, 2010.

REVIEW ARTICLE

The hypoxia response pathway and β-cell function

J. Cantley[1,2], S. T. Grey[3,4], P. H. Maxwell[1] & D. J. Withers[1,5]

[1]*Faculty of Medicine, Rayne Institute, University College London, London, UK*
[2]*Diabetes and Obesity Research Program, Garvan Institute of Medical Research, Sydney, Australia*
[3]*Gene Therapy and Autoimmunity Group, Garvan Institute of Medical Research, Sydney, Australia*
[4]*Australian Islet Transplant Consortium*
[5]*Metabolic Signalling Group, MRC Clinical Sciences Centre, Imperial College London, London, UK*

β-cells sense glucose and secrete appropriate amounts of insulin by coupling glucose uptake and glycolysis with quantitative ATP production via mitochondrial oxidative pathways. Therefore, oxidative phosphorylation is essential for normal β-cell function. Multiple cell types adapt to hypoxia by inducing a transcriptional programme coordinated by the transcription factor hypoxia-inducible factor (HIF). HIF activity is regulated by the von Hippel–Lindau (Vhl) protein, which targets the HIFα subunit for proteasomal degradation in the presence of oxygen. Several recent studies have shown that *Vhl* deletion in β-cells results in Hif1α activation, impaired glucose-stimulated insulin secretion (GSIS) and glucose intolerance. This was found to be because of alterations in β-cell gene expression inducing a switch from aerobic glucose metabolism to anaerobic glycolysis, thus disrupting the GSIS triggering pathway. Situations in which islets may become hypoxic are discussed, in particular islet transplantation which has been reported to cause islet hypoxia because of an inadequate blood supply post-transplant. Aside from this principal role for HIF in negatively regulating β-cell glucose sensing, other aspects of hypoxia signalling are discussed including β-cell differentiation, development and vascularization. In conclusion, recent studies clearly show that hypoxia response mechanisms can negatively impact on glucose sensing mechanisms in the β-cell and this has the potential to impair β-cell function in a number of physiological and clinical situations.
Keywords: atmosphere, glucose-stimulated insulin secretion, hypoxia response element, reactive oxygen species

Date submitted 27 March 2010; date of final acceptance 9 June 2010

The Hypoxic Response

Oxygen is critical to support multicellular life and the evolution of complex body plans correlates with a rapid rise in atmospheric oxygen [1]. Oxidative phosphorylation greatly increases the amount of energy that can be released from fuel as oxygen acts as an electron acceptor for the respiratory chain, enabling the redox-dependent coupling of fuel oxidation with quantitative ATP production. Increased dependence on the high energy yield from fuel oxidation has resulted in the evolution of molecular mechanisms to protect against a drop in ambient oxygen tension (hypoxia). Although some specialist cell/tissue types are able to sense oxygen as part of a specific physiological function, such as the carotid body, many cell types are equipped with a basic molecular response mechanism to adapt to hypoxia. Central to this molecular mechanism is the transcription factor hypoxia-inducible factor (HIF) which has been well characterized over the past 17 years and implicated in regulating cellular metabolism in a number of cell types, in particular during tumourigenesis. A role for HIF in the regulation of systemic metabolism and glucose homeostasis has recently been elucidated in tissues such as muscle [2–5], liver [6,7] adipose tissue [8] and the β-cell [9–12], which is the focus of this review. A number of HIF-independent signalling pathways/networks are activated by hypoxia, which include the unfolded protein response [13], AMP-activated protein kinase and the regulation of mammalian target of rapamycin (mTOR) activity [14]. However, this review mainly deals with the hypoxia response pathways involving HIF.

For a human, hypoxia can be defined as a drop in the partial pressure of oxygen from that found in the atmosphere at sea level (21.2 kPa/0.21 ATM/159 mmHg). Two definitions of hypoxia could be used when considering the behaviour of cells in a human or other complex organism. First, hypoxia could be defined as the level at which mitochondrial metabolism (respiration) is compromised. This corresponds to about 1 mmHg within the mitochondrion. Second, hypoxia could be defined as a lower level of oxygen than that normally experienced by a particular type of cell. It is widely recognized that all mammalian cells undergo very large adaptive changes in gene expression in response to alterations in oxygenation in the range 15–50 mmHg, that is, well above that which has any direct effect on mitochondrial respiration. From a physiological standpoint the second definition is much more appropriate and will be used in this review. However, there is some uncertainty about the precise oxygen concentration experienced by cells in normal tissues or under standard culture conditions. What is clear is that some tissues (e.g. the medulla of the kidney and cartilage) have lower average levels of oxygen than others, such as cardiac muscle. It is also clear that within a tissue such as the kidney or liver there

Correspondence to: James Cantley, Diabetes and Obesity Research Program, Garvan Institute of Medical Research, Darlinghurst, Sydney NSW 2034, Australia.
E-mail: j.cantley@garvan.org.au

are large gradients of oxygenation. Changes in oxygenation will occur as part of a normal physiological or developmental process, such as hypoxic gradients forming during embryonic development (physiological hypoxia), or from a pathophysiological process such as tissue ischaemia because of stroke, myocardial infarction or organ transplant (hypoxic stress).

Central to the hypoxic response is the transcription factor HIF which was first identified as binding to the erythropoietin gene enhancer during hypoxia [15], thereby providing a mechanism as to how erythropoeisis is increased during systemic hypoxia. Since this discovery, hundreds of potential HIF target genes have been identified and a large number (>70) have been validated as possessing a functional hypoxia response element (HRE) capable of binding HIF and activating transcription [16]. However, a much larger number of genes in the genome have candidate HREs present in their enhancer region suggesting that many genes have the potential to be regulated by HIF at the level of expression, possibly in a cell-type-specific or conditional manner. It is also clear that HIF influences gene expression indirectly, for example, by increasing the expression of transcriptional activators or repressors, and by interacting with other transcription complexes. The majority of HIF target genes validated to date are involved in diverse processes that adapt the cell/tissue/organism to the hypoxic environment. These principally include genes that adapt cellular metabolism to anaerobic fuel use (such as lactate dehydrogenase), genes that enhance the supply of blood oxygen (such as the pro-angiogenic vascular endothelial growth factor) and genes that act to conserve energy (such as DNA-damage-inducible transcript 4). Some examples of classical HIF target genes are given in figure 1. An additional number of genes modulate the hypoxic response which include elements of the machinery regulating HIF stability and activity, signalling elements mediating cross talk with other pathways and epigenetic modifier genes

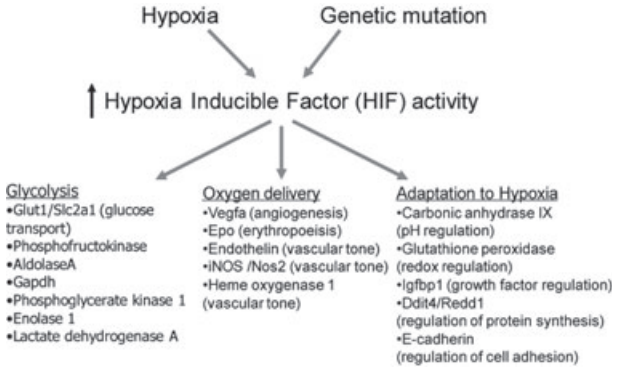

Figure 1. HIF target genes. Examples of HIF target genes involved in increasing oxygen supply and adapting cellular metabolism to anaerobic conditions in various cell types. Over 70 genes, including those in this figure, have been confirmed as HIF targets by DNA binding or reporter assays as previously excellently reviewed [16]. HIF, hypoxia-inducible factor; Glut1, glucose transporter 1; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Vegfa, vascular endothelial growth factor a; Epo, erythropoeitin; iNOS, inducible nitric oxide synthase; IGFBP1, insulin-like growth factor binding protein 1; Ddit4, DNA-damage-inducible transcript 4.

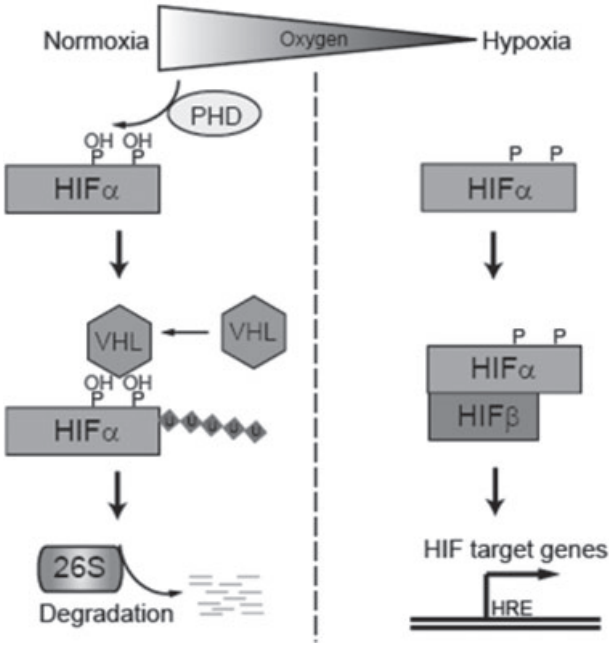

Figure 2. Cellular oxygen sensing. The transcriptional activity of HIF is regulated by proteasomal degradation of HIFα in the presence of oxygen. Prolylhydroxylases (PHD) use molecular oxygen to hydroxylate HIFα, allowing recognition by the von Hippel–Lindau (VHL) protein, polyubiquitination and proteasomal degradation. This is the core mechanism controlling HIFα stability and therefore activation, although there are several other known mechanisms that regulate/modulate the activity of HIF which are discussed in the main article.

and microRNAs capable of modulating the hypoxic response as recently reviewed elsewhere [17].

HIF activity is regulated by direct sensing of molecular oxygen in the cell (figure 2). The active HIF transcription factor dimer is composed of alpha and beta subunits. Aryl hydrocarbon nuclear translocator (ARNT, otherwise known as HIF1β) is not oxygen sensitive *per se* and is constitutively stable in the cell and capable of binding HIFα subunits (as part of the hypoxic response), the aryl hydrocarbon receptor (AHR) or other ARNT/HIFβ subunits [18–20]. HIFα (used here as the collective term for HIF1α/HIF2α/HIF3α gene products) is the oxygen-sensitive component of the transcription factor complex and confers the specificity and selectivity of the hypoxic response. When oxygen is present, two proline residues in the oxygen-dependent degradation domain of HIF1α (Pro402 and Pro564 of human HIF1α) are hydroxylated, using molecular oxygen, by the prolylhydroxylase enzymes (PHD) [21]. These hydroxylated motifs allow capture by the von Hippel–Lindau (VHL) protein that forms the recognition component of an E3 ubiquitin ligase complex, which targets the hydroxylated HIFα for polyubiquitination and proteasomal degradation [22]. This elegant mechanism provides a succinct explanation for the presence of highly vascularized tumours in VHL patients: a mutation in the VHL gene predisposes patients to constitutive HIF activation and tumourigenesis in a classic Knudson two-hit manner [23]. In a normal cell, decreased oxygen tension results in reduced hydroxylation enabling HIFα

to escape VHL capture and proteasomal degradation, enabling stabilized HIFα to dimerise with ARNT/Hif1β and bind the HRE of HIF target genes. An additional level of regulation is achieved by hydroxylation of a C-terminal asparagine residue of HIF1α (Asn803 of human HIF1α) by factor inhibiting HIF (FIH), a hydroxylase which blocks the binding of the CBP/p300 HIF co-factors and blunts the HIF transcriptional response. FIH has a lower K_m for oxygen than PHDs [4,24], therefore allowing a second level of HIF activation with more severe hypoxia.

β-Cell Function and Diabetes

Normal β-cell function is crucial for glucose homeostasis. β-cells sense glucose and secrete appropriate amounts of insulin to stimulate glucose uptake in muscle and fat and inhibit hepatic glucose output. The canonical mechanism whereby β-cells sense glucose and trigger insulin secretion has been well characterized [25] and is summarized as follows. Glucose is taken up into the β-cell via the high K_m/low affinity glucose transporter 2 (non-rate limiting), is phosphorylated by glucokinase (rate limiting) and enters glycolysis to produce pyruvate. Pyruvate enters mitochondrial oxidative pathways, where it drives quantitative ATP production. In turn, an increased ATP/ADP ratio closes ATP-sensitive potassium channels (K_{ATP} channels) resulting in membrane depolarization, opening of voltage-gated calcium channels, calcium influx and insulin vesicle exocytosis. The β-cell is an evolved glucose sensor and as such glucose-derived metabolites are driven towards mitochondrial oxidative phosphorylation and ATP production, in part by suppression of the pentose phosphate pathway [26] and low activity of lactate dehydrogenase and monocarboxylate transporters [27]. The dependence of this glucose sensor role on oxidative phosphorylation therefore means that hypoxia or mechanisms reducing the aerobic capacity of the β-cell would probably have profound effects on glucose-stimulated insulin secretion (GSIS).

Role of Hif1α in β-Cell Function

Three recently published studies from independent laboratories, including ours, investigated the effect of HIF activation on β-cell function [9–11]. These studies utilized cre-loxP systems to conditionally delete the *Vhl* gene in the β-cells or pancreas of mice: notably, each study used different lines of cre-transgenic mice to achieve gene deletion, thereby increasing our confidence in the results. *Vhl* deletion resulted in the activation of Hif1α but not Hif2α in β-cells as shown by nuclear staining. This caused glucose intolerance because of postprandial hypoinsulinaemia [9–11] and was completely reversed by additional deletion of floxed *Hif1α* [9,11]. Interestingly, deletion of *Hif1α* alone in β-cells did not alter glucose homeostasis, arguing that this factor is not required for β-cell function under normal conditions [9,11]. We found that *in vitro* GSIS was not altered from β-cell *Hif1α*KO islets; however, we did observe a slight increase in basal insulin secretion suggesting a restraining role of Hif1α on basal β-cell metabolism [9]. *Vhl* deletion/Hif1α activation in the β-cell severely blunted islet GSIS and this was accompanied by impaired NAD(P)H/$FADH_2$, ATP, membrane depolarization and calcium levels in response to glucose [9,11] (figure 3). Lactate efflux was significantly elevated despite normal oxygen levels [10,11]. Mitochondrial substrates were found to elicit normal calcium and insulin secretory responses, arguing for a mechanistic defect upstream of mitochondrial metabolism [9].

Analysis of gene expression showed that *Vhl* null/Hif1α active β-cells had altered the expression of genes involved in glucose uptake, glycolysis and pyruvate utilization. Glut2 expression was decreased and Glut1 upregulated, which altered the uptake rate of glucose into the cell [9,10]. Glucokinase was slightly downregulated, possibly limiting the rate of glucose utilization further. Perhaps most importantly, a strong induction of pyruvate dehydrogenase kinase 1 (*Pdk1*), lactate dehydrogenase A (*Ldha*) and monocarboxylate transporter 4 (*Mct4*) was detected, which probably act in concert to direct pyruvate away from mitochondrial oxidation and instead into lactate (figure 3). These genes are normally barely detectable in β-cells [27] as they oppose the coupling of GSIS: Pdk1 inhibits pyruvate dehydrogenase, the rate limiting step of pyruvate entry into oxidative pathways; Ldha converts pyruvate to lactate; Mct4 will enable the export of lactate from the β-cells and into the circulation thus supporting glycolytic flux. Forced overexpression of Ldha alone in the β-cell has been shown to impair GSIS [29], whereas overexpression of PDK3 does not [30]. Although pyruvate dehydrogenase activity was not measured in *Vhl* null/Hif1α active β-cells, the combination of Pdk1 with Ldha expression may have an additive effect. These studies clearly show that activation of Hif1α in the β-cell causes a switch in metabolism from aerobic to anaerobic glycolysis, despite normal oxygen tension. This effect is akin to the Warburg effect in cancer, where adequately oxygenated tumours preferentially utilize anaerobic glycolytic pathways rather than aerobic respiration, which is thought to be due in part to HIF activation [31].

The three papers cited above clearly show that HIF1α activation impairs β-cell function by switching glucose metabolism from aerobic oxidative phosphorylation to anaerobic glycolysis. In contrast, a previous study has assessed the effect of deleting the Hif1α binding partner Arnt in the β-cell [12]. These mice were reported to have sexually dimorphic minor glucose intolerance and impaired GSIS. It is not clear from this study which mechanism is responsible for the impaired GSIS; however, as Arnt is constitutively stable (i.e. not regulated by oxygen *per se*) and able to bind all three Hif1α subunits, the Ahr and other Arnt proteins, it may potentially impact on a number of pathways. Accepting our results describing Hif1α activation and the results of Gunton et al. describing deletion of Arnt/Hif1β, this would imply that the effects of *Arnt* deletion on β-cell function may be because of a Hif1α-independent pathway. The authors also report that siRNA knock-down of Hif1α or Hif2α impairs GSIS in mouse insulinoma cells (Min6). However, data recently published by us and others [9–11] clearly show that Hif1α protein is not stabilized in the β-cells of normal wild-type mice and that deletion of *Hif1α* does not alter glucose homeostasis or GSIS [9,11] questioning the role of Hif1α under normal physiological conditions. Furthermore, a number of observations suggest that in contrast to humans, mouse β-cells do not express *Hif2α*. We were unable to detect the Hif2α protein following *Vhl* deletion [9] and another study found Hif1α but not

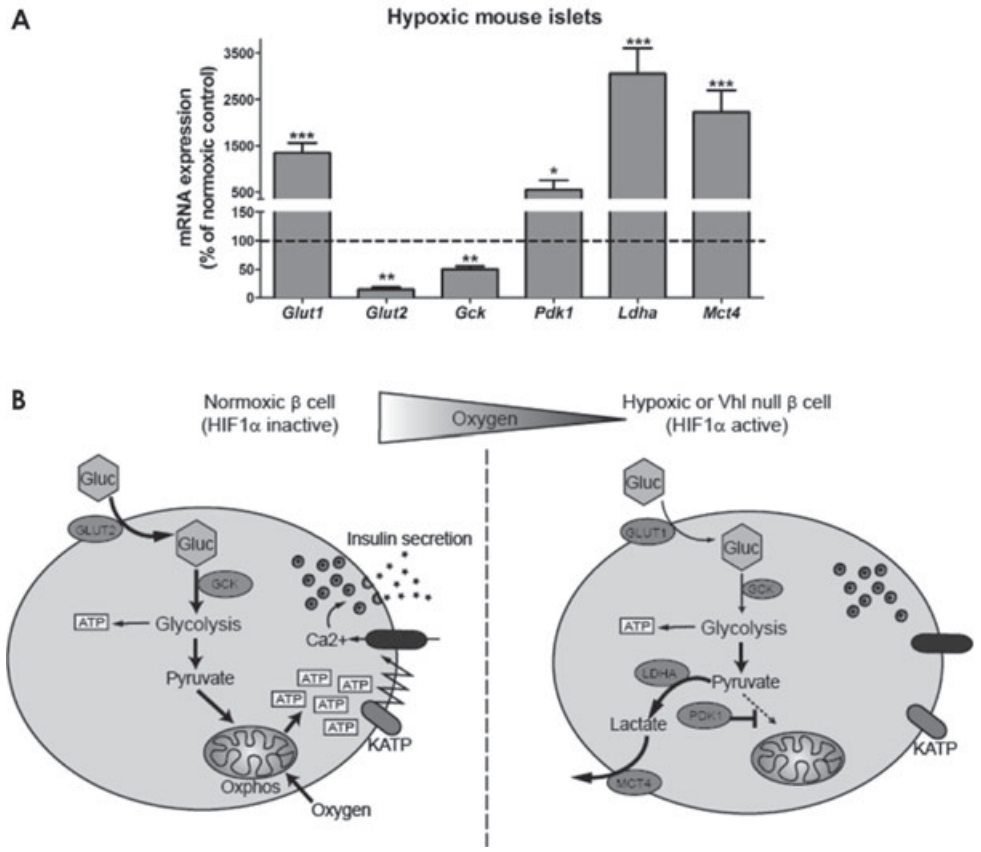

Figure 3. Core gene expression changes with HIF activation and associated disruption of GSIS in the β-cell. This pattern of gene expression changes is responsible for the disruption of glucose sensing and GSIS in mouse islets following HIF activation either because of *Vhl* deletion, pharmacological HIF activation or hypoxic culture. (A) Gene expression changes in mouse islets cultured for 16 h under hypoxic (1% O_2) conditions (J. C. and S. T. G., unpublished results). (B) The effect of HIF-induced gene expression changes on β-cell glucose sensing and GSIS: consensus model from Refs [9–11] as previously outlined [28].

Hif2α to be stabilized in the mouse embryonic pancreas with hypoxic culture or treatment with the PHD inhibitor/Hifα activator dimethyloxalylglycine [32]. Furthermore, we have been unable to detect the presence of the *Hif2α* transcript using Affymetrix arrays of the Min6 mouse β-cell line (J. C., D. J. W, P. H. M and D. Shukla, unpublished results). In addition, a publicly available online β-cell array database compiling consensus data from 19 published array studies lists *Hif2α* (official gene symbol *EPAS1*) as present in human and rat β-cells ($p > 0.95$) but absent from mouse β-cells (http://www.t1dbase.org/page/Overview/display/gene_id/2034). As the majority of studies have failed to detect the *Hif2α* transcript, this indicates that *Hif2α* is not expressed in mouse β-cells and islets. However, the results of the Gunton et al. study cannot be simply written off as off-target effects; therefore, further experiments are required to conclusively test if this gene is expressed in mouse β-cells and if it plays a role in β-cell function or tumourigenesis.

HIF and Islet Angiogenesis

HIF is well known to promote pro-angiogenic gene expression including *VEGF* and *PDGF* [33]. Therefore, it is perhaps not surprising that we observed an increase in islet vascular density following *Vhl* deletion in the pancreas or β-cells of mice (figure 4, [9]). This increase in vascularization could be attributed to Hif1α activation as we were unable to detect Hif2α, suggesting a different situation to that in the mouse liver, where pro-angiogenic gene expression is predominantly regulated by Hif2α [34].

HIF-independent Effects of *Vhl* Deletion

VHL interacts with a number of other proteins [23], some of which may be involved in β-cell function. One potential HIFα-independent function of VHL is to ubiquitinate calcium-independent protein kinase C isoforms [35,36], which may contribute to the role of VHL as a tumour suppressor. However, any functional implications of such interactions in the β-cell are probably subtle, as the profound defect in GSIS observed following *Vhl* deletion was completely rescued by concomitant deletion of the *Hif1α* gene [9,11]. The resulting mice with *Vhl* and *Hif1α* null β-cells had normal glucose tolerance and GSIS indicating that any potential HIF1α-independent actions of VHL are not important for β-cell function under the normal physiological conditions tested.

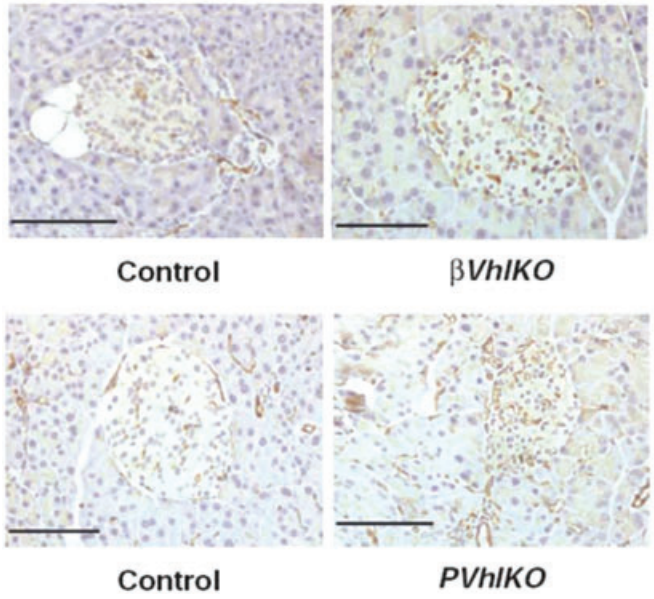

Figure 4. CD31 staining of pancreatic sections from β*Vhl*KO and P*Vhl*KO mice. CD31 staining (brown HRP) shows increased vascularization of *Vhl* null islets. Scale bar 100 micron. Image is reproduced from Ref. [9].

HIF, Pancreatic Development and β-Cell Differentiation

Although the above studies show that Hif1α activation clearly impairs GSIS coupling in β-cells, there are other potential effects of hypoxia and HIF activation on β-cell development and function. The role of physiological hypoxia in embryonic development is well known as the mammalian embryo develops in a lower oxygen environment than adult tissues [37]. There are a number of studies demonstrating that Hif1α, Hif2α or Arnt/Hif1β are required for normal development of the placenta, heart and endochondrial bone formation, as excellently reviewed elsewhere [37]. Therefore, there is potential for hypoxia and the Vhl/Hif system to regulate pancreatic development.

Oxygen tension and Hif1α activity have recently been implicated in the repression of Ngn3 expression and inhibition of embryonic β-cell differentiation [32]. However, studies with β-cell-specific *Vhl* knock-out mice found normal β-cell mass during adulthood indicating that Hif1α activation in β-cells does not alter their state of differentiation or turnover *per se* [9,11]. Aside from a direct role in maintaining β-cell differentiation, the study by Heinis et al. raises the possibility that pools of β-cell progenitors are maintained in a proliferative state by hypoxia and Hif1α activation during pancreatic development. Moreover, it is plausible that the formation of hypoxic gradients in the developing pancreas, proportionate to the distance from the nearest blood vessel, would form a gradient of Hif1α activity which may determine the degree of differentiation within the developing organ. Our studies would suggest that such a progenitor would be a cell type other than pancreatic and duodenal homeobox 1 (Pdx1)-positive, as Pdx1cre-mediated deletion of *Vhl* resulted in Hif1α activation in all pancreatic cell types and resulted in a normal β-cell mass at 12 weeks of age [9]. However, when using the Pdx1cre mouse to delete *Vhl*, only 70% of β-cells at 12 weeks of age showed positive Hif1α staining suggesting that either cells from a Pdx1-negative progenitor pool repopulate the islet during development, diluting out the deleted cells to 70%, or the Pdx1cre transgene is only expressed above the threshold required for loxP site recombination in 70% of β-cells during development and leading into adulthood. Therefore, further studies are needed to directly test if such a progenitor pool is regulated by hypoxia and Hif1α *in vivo*.

Another potential role for hypoxia response pathways in pancreatic/islet development is via interaction with the Notch signalling pathway. Hif1α can directly interact with the Notch intracellular domain (activated Notch receptor) and potentiate the transcription of Notch target genes thereby regulating cell differentiation [38]. In addition, FIH is able to directly hydroxylate Notch in an oxygen-sensitive manner, which is thought to regulate the availability of FIH for HIFα asparagine hydroxylation and therefore modulate the hypoxic response [39]. The studies conducted by Heinis et al. clearly implicate the Notch pathway as involved in the hypoxic suppression of Ngn3 and β-cell differentiation, and therefore this aspect of HIF and Notch signalling warrants further study in pancreatic development.

HIF and Islet Tumours

The role of the VHL protein as a tumour suppressor is well documented [23,40]. Indeed, the protein is named after independent descriptions by von Hippel (1904) and Lindau (1927) of a rare hereditary cancer syndrome. VHL disease is a classic Knudson two-hit disease, whereby somatic inactivation of *VHL* in certain tissues, in addition to an inherited mutant allele, results in highly vascular tumours commonly found in the retina, central nervous system or kidney. In addition, endocrine tumours of the adrenal gland and pancreatic islet (non-secreting) are

well-recognized features of VHL disease [41]. It was studies of renal clear cell carcinoma cell lines, isolated from VHL patients, that led to the seminal finding that VHL regulates HIF1α stability [22]. Therefore, it would be reasonable to predict that *Vhl* deletion in the pancreas or β-cell may result in tumour formation. However, in three independent models of *Vhl* deletion in the β-cell or pancreas, no evidence of tumour formation was found [9–11]. There are several possible reasons for the absence of tumours in these mice. First, there are species differences in the site of VHL tumour formation in mice and humans; for example, VHL liver hemangiomas are common in mice but rare in humans, whereas renal tumours are common in humans but rare in mice. Second, genetic background is known to influence VHL tumour incidence in mice (as discussed below). Third, the timing and site of deletion within the organ may be critical for the initiation of tumour formation. Finally, the lack of tumour formation may be because of the activation of Hif1α but not Hif2α in *Vhl* null β-cells [9], as Hif2α expression is required for the formation of malignant renal carcinomas (in humans) or liver hemangiomas (in mice) during VHL disease [34,42]. As discussed above, a number of studies suggest that mouse β-cells do not express Hif2α. In contrast, we have recently performed arrays on isolated human islets prepared for transplant and found that the *HIF2α/EPAS* gene to be detected in all nine islet preparations tested (J. C. and S. T. G., unpublished results). However, it should be noted that this does not conclusively prove that *HIF2α* is in human β-cells, as islets are composed of multiple endocrine cell types, and also retain endothelial cells and macrophages postisolation. If *HIF2α* is indeed in β-cells, then this suggests that human β-cells/islets may be more prone to tumour formation following VHL inactivation or other conditions leading to the activation of HIFα. This observation also suggests that a cautious approach should be taken when using pharmacological agents that may activate HIF2α in the islet.

A recent study has reported that *Vhl* deletion in the pancreas (using a Pdx1cre mouse) results in postnatal lethality of some animals, but the cause of death could not be established upon histological inspection of pancreata [43]. Of the few surviving mice, pancreatic cysts and a loss of exocrine tissue was observed at 16–18 months of age. It is unclear as to why this phenotype was present in these mice but not in our studies using Pdx1cre mice to delete *Vhl* [9], although this may be because of the following key differences between the studies. First, genetic background is likely to be key: global *Vhl* heterozygote mice show no evidence of tumour formation when bred on C57BL6/Sv129 mixed background [44], whereas when bred on a BALB/C background the majority of mice develop vascular abnormalities including liver hemangiomas [45]. Our mice were maintained on a mixed C57BL6/Sv129 background, whereas those of Shen et al. were produced on C57BL/6, A/J or Balb/C and, intriguingly, markedly less lethality was observed in the C57BL/6 background. Second, Shen et al. used a different Pdx1cre mouse [Tg(Pdx1-cre)89.1Dam] to our study [Tg(Pdx1-cre)1Herr], which was generated using a different length Pdx1 promoter and is known to be expressed slightly earlier during development and to show a more complete pattern of expression in the pancreas [46].

Conditions Leading to HIF Activation

It is now clear that activation of HIF1α in the β-cell severely compromises glucose sensing and insulin secretion, by altering gene expression to favour a slower rate of glucose uptake and a switch to predominantly anaerobic glycolysis, with pyruvate shunted into lactate production rather than driving mitochondrial oxidative phosphorylation and the quantitative production of ATP. The next logical questions are what conditions lead to the activation of HIF1α in the β-cell and does HIF1α activation play a role in β-cell dysfunction during type 2 diabetes? The following paragraphs deal with a number of situations, both reported and hypothetical, which may lead to HIF1α activation in the β-cell. These include situations where the β-cell may be under a hypoxic stress, as well as putative hypoxia-independent stimuli that may lead to HIF1α activation.

Islet Graft Hypoxia

The most obvious and relevant situation in which the islet is exposed to a severe hypoxic stress is during islet transplantation. Islet grafts are known to have an insufficient blood supply after transplant and to have a reduced oxygen tension prior to adequate revascularization [47], which may take up to 6 months for vasculature to resemble a normal *in situ* islet [48]. It is also unclear if the transplanted islets ever attain truly adequate vasculature, as the revascularization process requires the in-growth of blood vessels, whereas an islet forming in the developing pancreas will develop and grow in association with blood vessels [49]. In addition, the normal transplant site is the portal vein of the liver which is perfused with relatively deoxygenated blood compared to the oxygen-rich arterial blood supply of the native pancreatic islet, potentially further compounding the hypoxic stress that the transplanted islet is exposed to. It has been reported that transplanted islets show activation of Hif1α, particularly in the days following transplant, and this correlates with impaired graft function [50]. These findings suggest that the activation of HIF1α will impair GSIS and graft function, and that inhibition of HIF1α activation or limiting its effect on GSIS coupling may improve graft function and clinical outcome. Furthermore, streptozotocin-induced diabetic rats that received islet grafts showed improved glucose homeostasis following regular hyperbaric oxygen treatment [51], which may act in part by inactivating HIF1α thereby improving islet function and insulin secretion. Another study has reported that following human islet transplantation to type 1 diabetic recipients, the postprandial c-peptide response negatively correlated with the size of islets transplanted [52]. This observation that larger islet grafts have impaired GSIS may be explained by their probably reduced oxygen tension relative to smaller islets: larger islets will require increased vascular growth and blood oxygen to support their larger mass of cells, therefore assuming the rate of angiogenesis to be equal per islet, larger islets may have a reduced oxygen tension and increased HIF1α activation which would impair GSIS. It is also plausible that HIF1α is required for islet survival and revascularization, by altering β-cell metabolism to favour survival in the hypoxic environment at the expense of GSIS and increasing the expression of pro-angiogenic genes. However, it still remains to be established if

the effects of HIF1α activation in the β-cell are transient and reversible or if they result in permanently altered cell function.

It has previously been reported that hypoxia-regulated genes are elevated in isolated human islets [53]. We have recently performed Affymetrix arrays on human islets prepared for transplant from nine donors as part of the Australian Islet Transplant Consortium. These data were analysed by comparison with a previously published array data set, from the Marselli et al. study cited above, using *in situ* β-cells isolated by laser capture microdissection [53]. We found a number of HIF target genes to be increased in our isolated human islets including *PDK1*, *LDHA* and *MCT4*, with a decrease in glucokinase expression (J. C. and S. T. G., unpublished results). Furthermore, we find after 4 weeks transplant into immunodeficient RAG knock-out mice that this gene expression profile did not normalize, suggesting impaired graft function and a permanence of the hypoxia-like gene expression profile (J. C. and S. T. G., unpublished results). Clearly, more work is required to test the reversibility of hypoxia/HIF-induced gene changes in the β-cell in response to hypoxia and drugs that potentially lead to HIF activation in the islet.

In Situ Islet Hypoxia

A potential situation for hypoxic stress of the β-cell is during the well-described compensatory increase in β-cell size and number that accompanies insulin resistance [54]. With an enlarging islet mass it is plausible that the vascular density may become insufficient to maintain adequate perfusion/oxygen delivery to the islet. In this situation, regions of the islet may become hypoxic and result in HIF activation and β-cell dysfunction. In support of this hypothesis, it has been reported that islets from Zucker diabetic fatty rats show an insufficient vascular density with age, relative to obese non-diabetic control animals [55] although in this study it is probably because of a loss of vasculature. Moreover, islets from these animals showed increased expression of the HIF target gene *Ldha*, indicative of hypoxia and HIF activation, which will probably act to impair aerobic glucose metabolism and therefore GSIS as discussed earlier in this review. Therefore, these findings warrant a more comprehensive investigation of models of β-cell hyperplasia to analyse vascular density, oxygen tension and HIF activation, and to correlate these parameters with islet function. A similar observation has been made of the enlarged adipose tissue of obese mice with a reduced vascular density and hypoxic regions stained positive with pimonidazole [56,57]. Furthermore, a reduced oxygen tension has been recorded in the subcutaneous fat tissue of obese humans [58] indicating that a rapid expansion of a given tissue can lead to an imbalance between supply and demand for oxygen, somewhat similar to solid tumour growth.

Another situation that may lead to β-cell hypoxia is during hyperglycaemia, as islets consume greater amounts of oxygen when exposed to high glucose [59], potentially leading to a transient lowering of islet oxygen tension and Hif1α activation if oxygen demand is greater than supply [60]. Rat insulinoma cells (INS1) cultured under hyperglycaemic conditions showed evidence for the activation of HIF target genes [60], although it is unclear if this is because of transient hypoxia, hypoxia-independent HIF activation or a mechanism not involving HIF. Results reported by us and others show that deletion of *Hif1α* does not alter GSIS [9,11], which would tend to argue against a role for this transient physiological hypoxia in regulating β-cell function in a normal mouse. However, this may become relevant if mice or islets are challenged with persistent hyperglycaemia or in situations where the islet may already have a restricted oxygen supply (such as following transplantation or β-cell hyperplasia).

Several other clinical situations may result in systemic hypoxia and thus reduce the islet oxygen tension, potentially leading to HIF activation. These include chronic obstructive pulmonary disorder, sleep apnoea and ischaemic diseases such as myocardial infarction. Interestingly, nocturnal intermittent hypoxia has been linked with type 2 diabetes risk in humans [61], although this study did not investigate the mechanism(s) involved.

Hypoxia-independent Activation of HIF

A number of hypoxia-independent mechanisms for HIF activation have been showed in other tissue/cell types, and therefore these could potentially stabilize/activate HIF in the β-cell leading to secretory dysfunction. It is known that hypoxia is paradoxically accompanied by increased mitochondrial reactive oxygen species (ROS) and this has been shown to be required for normal activation of HIF [62–64] possibly via antagonizing prolylhydroxylase activity [65]. Therefore, conditions leading to excess ROS production and oxidative stress may lead to HIF activation in the β-cell and secretory dysfunction. It is well known that exposure of β-cells to hyperglycaemia results in oxidative stress and impaired GSIS [66], as well as HIF target gene expression [60] and therefore this may involve HIF activation.

It has been shown that Hif1α stability and transactivation can be regulated in a redox-dependent manner *in vitro* by the nitric oxide donor *S*-nitroso-*N*-acetylpenicillamine [67], implicating conditions leading to nitric oxide production, such as exposure to pro-inflammatory cytokines, as potentially leading to hypoxia-independent Hif1α activation. Furthermore, there is increasing evidence in the literature for hypoxia-independent activation of Hif1α by pro-inflammatory cytokines. *In vitro* experiments applying transforming nuclear factor α or interleukin 1β to transformed cell lines resulted in the NFκB-dependent stabilization of Hif1α [68,69]. Pro-inflammatory cytokines have long been known to cause β-cell secretory dysfunction [70] as well as playing a pro-apoptotic role in β-cell death. Therefore, when taken together, the above studies suggest that Hif1α activation may be a previously overlooked aspect of cytokine-induced β-cell dysfunction.

Iron chelators such as desferrioxamine or desferasirox are used clinically to treat conditions such as haemochromatosis and have the potential to activate Hif1α in a number of tissues, including the β-cell, by antagonizing the PHD enzymes. In addition, specific PHD inhibitors are currently being trialled for the treatment of anaemia and ischaemia and these will probably activate Hif1α in multiple tissues. Therefore, we suggest that glucose homeostasis and in particular β-cell function should be investigated in these patients, as if Hif1α is activated in

the β-cell, or indeed muscle or liver tissues, this may have unwanted effects on glucose homeostasis.

Conclusions

It is now clear that activation of HIF in the β-cell impairs glucose sensing and GSIS. HIF activation alters β-cell gene expression resulting in reduced glucose uptake and a switch from aerobic glucose metabolism to anaerobic glycolysis, thereby blocking the GSIS triggering signal. This indicates that conditions resulting in islet hypoxia and/or HIF activation, such as islet transplantation, will result in impaired GSIS. However, more work is needed to investigate if HIF activation plays a role in β-cell dysfunction during the progression of type 2 diabetes.

Acknowledgements

Because of the expansive body of work concerning HIF biology, we have been unable to exhaustively cite all research pertaining to the areas discussed in this review. Therefore, we apologize to any researchers whose contributions were not cited.

Conflict of Interests

Patrick Henry Maxwell is a Scientific Founder, Director and equity holder of ReOx Ltd, and has been a member of the Scientific Advisory Board of the Roche Foundation for Anemia Research. S. Grey is a member of the Australian Islet Transplant Consortium.

References

1. Canfield DE, Poulton SW, Narbonne GM. Late-neoproterozoic deep-ocean oxygenation and the rise of animal life. Science 2007; **315**: 92–95.
2. Mason SD, Howlett RA, Kim MJ et al. Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. PLoS Biol 2004; **2**: e288.
3. Mason SD, Rundqvist H, Papandreou I et al. HIF-1{alpha} in endurance training: suppression of oxidative metabolism. Am J Physiol Regul Integr Comp Physiol 2007; **293**: R2059–R2069.
4. Aragones J, Schneider M, Van Geyte K et al. Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. Nat Genet 2008; **40**: 170–180.
5. Zhong L, D'Urso A, Toiber D et al. The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. Cell 2010; **140**: 280–293.
6. Park SK, Haase VH, Johnson RS. von Hippel Lindau tumor suppressor regulates hepatic glucose metabolism by controlling expression of glucose transporter 2 and glucose 6-phosphatase. Int J Oncol 2007; **30**: 341–348.
7. Wang XL, Suzuki R, Lee K et al. Ablation of ARNT/HIF1beta in liver alters gluconeogenesis, lipogenic gene expression, and serum ketones. Cell Metab 2009; **9**: 428–439.
8. Halberg N, Khan T, Trujillo ME et al. HIF 1 alpha induces fibrosis and insulin resistance in white adipose tissue. Mol Cell Biol 2009; **29**: 4467–4483.
9. Cantley J, Selman C, Shukla D et al. Deletion of the von Hippel-Lindau gene in pancreatic β cells impairs glucose homeostasis in mice. J Clin Invest 2009; **119**: 125–135, doi:10.1172/JCI26934.
10. Puri S, Cano D, Hebrok M. A role for Von-Hippel Lindau protein in pancreatic β-cell function. Diabetes 2008; **58**: 433–441.
11. Zehetner J, Danzer C, Collins S et al. PVHL is a regulator of glucose metabolism and insulin secretion in pancreatic beta cells. Genes Dev 2008; **22**: 3135–3146.
12. Gunton JE, Kulkarni RN, Yim S et al. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 2005; **122**: 337–349.
13. Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer 2008; **8**: 851–864.
14. Liu L, Cash TP, Jones RG, Keith B, Thompson CB, Simon MC. Hypoxia-induced energy stress regulates mRNA translation and cell growth. Mol Cell 2006; **21**: 521–531.
15. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992; **12**: 5447–5454.
16. Wenger RH, Stiehl DP, Camenisch G. Integration of oxygen signaling at the consensus HRE. Sci STKE 2005 Oct 18; (306): re12.
17. Lendahl U, Lee KL, Yang H, Poellinger L. Generating specificity and diversity in the transcriptional response to hypoxia. Nat Rev Genet 2009; **10**: 821–832.
18. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 1995; **92**: 5510–5514.
19. Sogawa K, Nakano R, Kobayashi A et al. Possible function of Ah receptor nuclear translocator (Arnt) homodimer in transcriptional regulation. Proc Natl Acad Sci USA 1995; **92**: 1936–1940.
20. Antonsson C, Arulampalam V, Whitelaw ML, Pettersson S, Poellinger L. Constitutive function of the basic helix-loop-helix/PAS factor Arnt. Regulation of target promoters via the E box motif. J Biol Chem 1995; **270**: 13968–13972.
21. Jaakkola P, Mole DR, Tian YM et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 2001; **292**: 468–472.
22. Maxwell PH, Wiesener MS, Chang GW et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999; **399**: 271–275.
23. Frew IJ, Krek W. pVHL: a multipurpose adaptor protein. Sci Signal 2008; **1**: pe30.
24. Ward JP. Oxygen sensors in context. Biochim Biophys Acta 2008; **1777**: 1–14.
25. Schuit FC, Huypens P, Heimberg H, Pipeleers DG. Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes 2001; **50**: 1–11.
26. Schuit F, De Vos A, Farfari S et al. Metabolic fate of glucose in purified islet cells. J Biol Chem 1997; **272**: 18572–18579.
27. Quintens R, Hendrickx N, Lemaire K, Schuit F. Why expression of some genes is disallowed in beta-cells. Biochem Soc Trans 2008; **36**: 300–305.
28. Gribble FM. Intolerant of glucose and gasping for oxygen. Nat Med 2009; **15**: 247–249.
29. Ainscow EK, Zhao C, Rutter GA. Acute overexpression of lactate dehydrogenase-A perturbs beta-cell mitochondrial metabolism and insulin secretion. Diabetes 2000; **49**: 1149–1155.
30. Nicholls LI, Ainscow EK, Rutter GA. Glucose-stimulated insulin secretion does not require activation of pyruvate dehydrogenase: impact of adenovirus-mediated overexpression of PDH kinase and PDH phosphate phosphatase in pancreatic islets. Biochem Biophys Res Commun 2002; **291**: 1081–1088.
31. Brahimi-Horn MC, Chiche J, Pouyssegur J. Hypoxia signalling controls metabolic demand. Curr Opin Cell Biol 2007; **19**: 223–229.

32. Heinis M, Simon M-T, Ilc K et al. Oxygen tension regulates pancreatic β-cell differentiation through hypoxia-inducible factor 1α. Diabetes 2010; **59**: 662–669.
33. Kaelin WG Jr. The von Hippel-Lindau protein, HIF hydroxylation, and oxygen sensing. Biochem Biophys Res Commun 2005; **338**: 627–638.
34. Rankin EB, Rha J, Unger TL et al. Hypoxia-inducible factor-2 regulates vascular tumorigenesis in mice. Oncogene 2008; **27**: 5354–5358.
35. Iturrioz X, Durgan J, Calleja V et al. The von Hippel-Lindau tumour-suppressor protein interaction with protein kinase C delta. Biochem J 2006; **397**: 109–120.
36. Okuda H, Saitoh K, Hirai S et al. The von Hippel-Lindau tumor suppressor protein mediates ubiquitination of activated atypical protein kinase C. J Biol Chem 2001; **276**: 43611–43617.
37. Dunwoodie SL. The role of hypoxia in development of the mammalian embryo. Dev Cell 2009; **17**: 755–773.
38. Gustafsson MV, Zheng X, Pereira T et al. Hypoxia requires notch signaling to maintain the undifferentiated cell state. Dev Cell 2005; **9**: 617–628.
39. Coleman ML, McDonough MA, Hewitson KS et al. Asparaginyl hydroxylation of the Notch ankyrin repeat domain by factor inhibiting hypoxia-inducible factor. J Biol Chem 2007; **282**: 24027–24038.
40. Kaelin WG, Iliopoulos O, Lonergan KM, Ohh M. Functions of the von Hippel-Lindau tumour suppressor protein. J Intern Med 1998; **243**: 535–539.
41. Woodward ER, Maher ER. Von Hippel-Lindau disease and endocrine tumour susceptibility. Endocr Relat Cancer 2006; **13**: 415–425.
42. Raval RR, Lau KW, Tran MG et al. Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. Mol Cell Biol 2005; **25**: 5675–5686.
43. Shen HCJ, Adem A, Ylaya K et al. Deciphering von Hippel-Lindau (VHL/Vhl)-associated pancreatic manifestations by inactivating *Vhl* in specific pancreatic cell populations. PLoS One 2009; **4**: e4897.
44. Gnarra JR, Ward JM, Porter FD et al. Defective placental vasculogenesis causes embryonic lethality in VHL-deficient mice. Proc Natl Acad Sci USA 1997; **94**: 9102–9107.
45. Haase VH, Glickman JN, Socolovsky M, Jaenisch R. Vascular tumors in livers with targeted inactivation of the von Hippel-Lindau tumor suppressor. Proc Natl Acad Sci USA 2001; **98**: 1583–1588.
46. Heiser PW, Lau J, Taketo MM, Herrera PL, Hebrok M. Stabilization of β-catenin impacts pancreas growth. Development 2006; **133**: 2023–2032.
47. Carlsson PO, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. Diabetes 2001; **50**: 489–495.
48. Rooth P, Dawidson I, Lafferty K et al. Prevention of detrimental effect of cyclosporin A on vascular ingrowth of transplanted pancreatic islets with verapamil. Diabetes 1989; **38**(Suppl. 1): 202–205.
49. Lammert E, Gu G, McLaughlin M et al. Role of VEGF-A in vascularization of pancreatic islets. Curr Biol 2003; **13**: 1070–1074.
50. Miao G, Ostrowski RP, Mace J et al. Dynamic production of hypoxia-inducible factor-1alpha in early transplanted islets. Am J Transpl 2006; **6**: 2636–2643.
51. Juang JH, Hsu BR, Kuo CH, Uengt SW. Beneficial effects of hyperbaric oxygen therapy on islet transplantation. Cell Transpl 2002; **11**: 95–101.
52. Lehmann R, Zuellig RA, Kugelmeier P et al. Superiority of small islets in human islet transplantation. Diabetes 2007; **56**: 594–603.
53. Marselli L, Thorne J, Ahn YB et al. Gene expression of purified beta-cell tissue obtained from human pancreas with laser capture microdissection. J Clin Endocrinol Metab 2008; **93**: 1046–1053.
54. Weir GC, Bonner-Weir S. A dominant role for glucose in beta cell compensation of insulin resistance. J Clin Invest 2007; **117**: 81–83.
55. Li X, Zhang L, Meshinchi S et al. Islet microvasculature in islet hyperplasia and failure in a model of type 2 diabetes. Diabetes 2006; **55**: 2965–2973.
56. Hosogai N, Fukuhara A, Oshima K et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 2007; **56**: 901–911.
57. Ye J, Gao Z, Yin J, He Q. 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 2007; **293**: E1118–E1128.
58. Pasarica M, Sereda OR, Redman LM et al. Reduced adipose tissue oxygenation in human obesity. Diabetes 2009; **58**: 718–725.
59. Wang W, Upshaw L, Strong DM, Robertson RP, Reems J. Increased oxygen consumption rates in response to high glucose detected by a novel oxygen biosensor system in non-human primate and human islets. J Endocrinol 2005; **185**: 445–455.
60. Jonas JC, Bensellam M, Duprez J, Elouil H, Guiot Y, Pascal SM. Glucose regulation of islet stress responses and beta-cell failure in type 2 diabetes. Diabetes Obes Metab 2009; **11**(Suppl. 4): 65–81.
61. Muraki I, Tanigawa T, Yamagishi K et al. Nocturnal intermittent hypoxia and the development of type 2 diabetes: the Circulatory Risk in Communities Study (CIRCS). Diabetologia 2010; **53**: 481–488.
62. Guzy RD, Hoyos B, Robin E et al. Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metabolism 2005; **1**: 401–408.
63. Brunelle JK, Bell EL, Quesada NM et al. Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. Cell Metab 2005; **1**: 409–414.
64. Mansfield KD, Guzy RD, Pan Y et al. Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-[alpha] activation. Cell Metab 2005; **1**: 393–399.
65. Kaelin WG Jr. ROS: really involved in oxygen sensing. Cell Metab 2005; **1**: 357–358.
66. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J Biol Chem 2004; **279**: 42351–42354.
67. Park Y-K, Ahn D-R, Oh M et al. Nitric oxide donor, ({+/−})-S-nitroso-N-acetylpenicillamine, stabilizes transactive hypoxia-inducible factor-1{alpha} by inhibiting von Hippel-Lindau recruitment and asparagine hydroxylation. Mol Pharmacol 2008; **74**: 236–245.
68. Jung Y, Isaacs JS, Lee S, Trepel J, Liu ZG, Neckers L. Hypoxia-inducible factor induction by tumour necrosis factor in normoxic cells requires receptor-interacting protein-dependent nuclear factor kappa B activation. Biochem J 2003; **370**: 1011–1017.
69. Jung YJ, Isaacs JS, Lee S, Trepel J, Neckers L. IL-1beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. FASEB J 2003; **17**: 2115–2117.
70. Sandler S, Andersson A, Hellerstrom C. Inhibitory effects of interleukin 1 on insulin secretion, insulin biosynthesis, and oxidative metabolism of isolated rat pancreatic islets. Endocrinology 1987; **121**: 1424–1431.