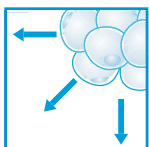


HYPOXIA AND ADIPOSE TISSUE FUNCTION AND DYSFUNCTION IN OBESITY

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Trayhurn P. Hypoxia and Adipose Tissue Function and Dysfunction in Obesity. *Physiol Rev* 93: 1–21, 2013; doi:10.1152/physrev.00017.2012.—The rise in the incidence of obesity has led to a major interest in the biology of white adipose tissue. The tissue is a major endocrine and signaling organ, with adipocytes, the characteristic cell type, secreting a multiplicity of protein factors, the adipokines. Increases in the secretion of

a number of adipokines occur in obesity, underpinning inflammation in white adipose tissue and the development of obesity-associated diseases. There is substantial evidence, particularly from animal studies, that hypoxia develops in adipose tissue as the tissue mass expands, and the reduction in P_{O_2} is considered to underlie the inflammatory response. Exposure of white adipocytes to hypoxic conditions in culture induces changes in the expression of >1,000 genes. The secretion of a number of inflammation-related adipokines is upregulated by hypoxia, and there is a switch from oxidative metabolism to anaerobic glycolysis. Glucose utilization is increased in hypoxic adipocytes with corresponding increases in lactate production. Importantly, hypoxia induces insulin resistance in fat cells and leads to the development of adipose tissue fibrosis. Many of the responses of adipocytes to hypoxia are initiated at P_{O_2} levels above the normal physiological range for adipose tissue. The other cell types within the tissue also respond to hypoxia, with the differentiation of preadipocytes to adipocytes being inhibited and preadipocytes being transformed into leptin-secreting cells. Overall, hypoxia has pervasive effects on the function of adipocytes and appears to be a key factor in adipose tissue dysfunction in obesity.

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I. INTRODUCTION

Historically, white adipose tissue, or white fat, has been viewed as one of the least complex organs in the body. The central perspective for many years was that of a simple fat storage organ in which excess calories are deposited after a meal and from which fuel is released during fasting and prolonged food deprivation. As a consequence, much of the early biochemical interest in adipose tissue centered on the regulation of fat synthesis and breakdown, through lipogenesis and lipolysis, respectively (201). The apparent metabolic simplicity was paralleled by a sense that the morphology of the tissue was also simple, with mature adipocytes being the overwhelmingly dominant cell type. This structural simplicity appears very evident in standard histological sections of white fat. However, over the past 15–20 years, our views of white adipose tissue and its biological

functions have changed radically, and apparent simplicity has been replaced by ever-growing complexity. The complexity has been compounded by recent developments which have indicated that a clear distinction between white and brown adipose depots is itself simplistic.

The major driver behind the growth in interest in white adipose tissue has been the increasing concern with obesity and its implications for public health. In both advanced and developing economies, obesity has become a major health problem. In the United Kingdom, for example (which has one of the highest obesity rates in Europe), ~25% of adults are clinically obese (162), while in the United States the incidence is as high as one-third of the adult population (138). These figures are based on the definition of obesity of a body mass index (BMI) of 30 or more. It is not obesity per se, however, that represents the medical problem, but rather the diseases with which it is associated, and these include insulin resistance and type 2 diabetes, cardiovascular disease, and certain cancers (3, 9, 103, 217a). In the case of type 2 diabetes, the risk of developing this particular obesity-related disorder is increased at least 10-fold in men (and more in women) in those with a BMI of 30, relative to those of ideal weight (103, 105). Obesity is, of course, characterized by a substantial increase in white adipose tissue mass, with BMI being a proxy for body fat content. In lean adults

(men), some 15–20% of total body composition is due to adipose tissue, and this increases to 40% and more in the obese. Thus adipose tissue is quantitatively a very major organ in both lean and obese individuals, and as such a priori would be expected to have a substantial impact on metabolic regulation and whole body physiology.

There have been several pivotal developments that have revolutionized our understanding of white adipose tissue function. The most significant and far-reaching is the recognition, initiated by the discovery of leptin in 1994 (232), that white adipocytes are major endocrine cells that secrete a diverse range of protein signals and factors (52, 158, 167, 193, 194). Another key development is the realization that adipose tissue depots become “inflamed” in obesity, with the view that the inflammatory state leads to the initiation and progression of the major obesity-associated diseases (3, 9, 81, 167, 228). In this review, the concept, which was first advanced in 2004 (198), that white adipose tissue becomes hypoxic as adipocyte size and tissue mass expand in obesity, and that this underlies the development of inflammation and cellular dysfunction, is considered. The basis for adipose tissue hypoxia in the obese state is discussed, together with the accumulating evidence that low oxygen tension has extensive effects on white adipocytes with important implications for adipose tissue functionality in obesity.

II. OVERVIEW OF ADIPOSE TISSUE

A. Cellular Complexity

Before addressing the specifics of hypoxia in adipose tissue, it is important to first consider the extent of the complexity of the tissue. Traditionally, adipose tissue has been considered to be composed of two distinct forms, white and brown, with fundamentally different functions. Brown adipose tissue is specialized for the generation of heat by non-shivering mechanisms, fuelled primarily by the oxidation of fatty acids, through the presence of a specific mitochondrial “uncoupling protein,” UCP1 (uncoupling protein-1), which dissipates the proton gradient across the inner mitochondrial membrane (19, 74, 134, 163). The generation of heat by brown adipose tissue may be evoked for thermoregulatory purposes to enable the maintenance of body temperature, or for the arousal from hibernation (19, 134). Brown adipose tissue thermogenesis also enables the dissipation of excess calories as a mechanism for the maintenance of energy balance, or body weight (19, 20, 75, 168, 169, 192). White adipose tissue, on the other hand, is specialized for fuel storage rather than for the oxidation of stored fatty acids, providing substrate to other tissues such as muscle during fasting and periods of high energy demand. Classically, lipid was considered to be deposited in a single unilamellar droplet within white adipocytes, with multiple droplets in the case of brown fat cells.

It has been argued that it is appropriate to consider the brown and white forms of adipose tissue as comprising a single “adipose organ” (28, 29). Trans-differentiation between brown and white adipocytes has been part of the argument for a single organ (30, 203). However, the view has been challenged by recent evidence that brown adipocytes, in contrast to white, are derived from myogenic progenitor cells (173, 190). The concept of two distinct forms of adipose tissue has also been challenged through the recognition that some fat depots have both white and brown adipocytes, and by the identification of a third form of fat cell, the “brite” adipocyte. These brite cells express UCP1, but do not possess the complete molecular characteristics of brown adipocytes (154, 205). Brite-rich depots can be identified by their combined expression of UCP1 and *Hoxc9* (homeobox 9), while *Zic1* (zinc finger protein in the cerebellum 1) rather than UCP1 is a unique marker of classical brown adipose tissue depots (205). *Tcf21* (transcription factor 21), together with the absence of expression of UCP1, *Zic1*, *Tbx15* (T-box protein 15), and *Shox2* (short stature homeobox 2), can be used to differentiate white adipose depots from brown or brite (205). With the use of these molecular markers, the various adipose tissue depots can be categorized; depots such as the interscapular adipose tissue (and cardiac) of rodents appear to be essentially “brown,” while the epididymal depot is strongly “white” (205).

In addition to different forms of adipocyte, several types of nonadipocyte cell are present in white adipose tissue depots (the focus of this review hereafter is white adipocytes and the essentially white fat depots) (71). These include fibroblastic preadipocytes, the precursor cells of white adipocytes. Adipose tissue also contains vascular endothelial cells, macrophages, and mast and dendritic cells. Macrophages are regarded as being particularly important in the inflammatory response that occurs in the tissue as obesity develops, and the obese state is associated with a major recruitment of these cells (17, 211, 220). In practice, several types of immune cell are present in adipose tissue in addition to macrophages, including lymphocytes, natural killer (NK) cells, and mast cells. Mast cells may be of particular importance to the innate immune response within the tissue (115). Increased infiltration of NK cells and T cells, as well as of macrophages, is evident in adipose tissue of obese subjects (137, 159).

White adipose tissue is innervated by sympathetic nerve endings, the sympathetic system being the principal physiological mediator of lipolysis (66, 183) with marked activation occurring in situations where there is net lipolysis (54, 128). The extent of the sympathetic innervation in white fat is less than that of brown adipose tissue (28). In brown fat, the sympathetic system plays a central role in the initiation and regulation of thermogenesis, including mitochondrial biogenesis and the recruitment of UCP1 (19, 75,

76). The proportion of the total cell content of white adipose tissue due to mature adipocytes varies between depots, ranging from ~30–50% (71). Thus, despite the gross histological appearance, white adipose tissue is not composed overwhelmingly of white adipocytes, in most cases these cells comprising less than half the total cellular content. Nonetheless, adipocytes are the signature cell of adipose tissue and have been the primary focus of cellular studies on the tissue, including of hypoxia. During cell fractionation, the mature adipocytes are separated from the nonadipocyte cells, which constitute what is known as the stromal-vascular fraction.

B. Secretory Role of White Adipocytes: Adipokines

White adipocytes are major secretory cells, releasing a multiplicity of lipid and protein entities in addition to the stored fatty acids that are mobilized on lipolysis (52, 158, 193, 194). The lipid secretions include cholesterol and specific prostaglandins, such as PGE₂ and 15-deoxy-PGJ₂ (4, 44, 45), as well as endocannabinoids, including anandamide (39, 58). Adipocytes also store and release the fat-soluble vitamins α -tocopherol and vitamin D₃ (6, 8, 10) and can synthesize active glucocorticoids through the presence of the type 1 form of the enzyme 11 β -hydroxysteroid dehydrogenase, with adipose tissue being an important site in humans of the regeneration of cortisol from cortisone (174). The CYP27B1 gene encoding the 1 α -hydroxylase enzyme that catalyzes the conversion of 25-hydroxycholecalciferol [25(OH)D₃] to 1,25-dihydroxycholecalciferol [1,25(OH)₂D₃] is expressed in adipocytes, with the active vitamin D₃ hormone recently having been shown to be generated in the cells (24, 112). The vitamin D receptor (VDR) is also expressed in adipocytes, and there is growing interest in adipose tissue as a target of 1,25(OH)₂D₃ action (24, 186, 196, 213).

Several major protein hormones are synthesized and secreted by adipocytes, the most prominent of which are leptin and adiponectin (52, 158, 193, 194, 198). Both these hormones are produced primarily, though not exclusively, in fat cells with the circulating level of leptin being directly related to BMI, or body fat (33, 120, 141). Correspondingly, circulating adiponectin levels are reduced in obesity (2, 85). Leptin and adiponectin each have multiple roles, and in the case of leptin, the functions with which it is involved include appetite, angiogenesis, and insulin secretion (12, 16, 40, 64, 149, 181), while adiponectin has insulin-sensitizing, anti-inflammatory, and angiogenic actions (5, 14, 142, 222, 226). These hormones are part of the large family of protein signals and factors released by adipocytes, which collectively are termed “adipokines” (alternatively “adipocytokines”). More than 100 different adipokines have been firmly identified, but proteomic studies indicate that there are many more, with the adipokinome perhaps

numbering several hundred entities (36, 195). The adipokines have served as something of a model for the more recent discovery of the “myokines,” protein factors secreted from myocytes which have autocrine/paracrine actions within skeletal muscle, or act as signals from muscle to other tissues such as white fat (146, 148).

It is emphasized that despite adipocytes being the main site of the synthesis of leptin and adiponectin, the proteins that constitute the adipokinome are in practice not necessarily selective to fat cells. Indeed, in many cases, cells in other tissues are much more important sources of a particular factor. The adipokines encompass a wide range of metabolic functions, as illustrated in **FIGURE 1**, including the regulation of appetite and energy balance, insulin sensitivity, angiogenesis, blood pressure regulation, vascular hemostasis, and inflammation and the immune response (52, 158, 193, 194). There has been particular focus on the role of various adipokines in modulating insulin sensitivity, and tumor necrosis factor (TNF)- α , leptin, adiponectin, retinol binding protein-4 (RBP4), chemerin, and monocyte chemoattractant protein 1 (MCP-1) are among those that have been implicated in this key action (5, 83, 84, 94, 139, 153, 172, 176, 180, 222, 223). There has also been considerable interest in the adipokines involved in the inflammatory response, this reflecting the link between inflammation in adipose tissue and the development of obesity-associated diseases (80, 81, 158, 167, 198). Adipocytes synthesize and secrete a number of classical cytokines and chemokines, including TNF- α , IL-1 β , IL-6, IL-8, IL-10, MCP-1, and macrophage migration inhibitory factor (MIF) (see Refs. 34, 158, 198). Other adipokines linked to the inflammatory response include the angiogenic factor, vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and the acute phase protein plasminogen activator inhibitor-1 (PAI-1). The expression and release of a number of inflammation-related adipokines rise markedly in obesity (80, 158, 198), the notable exception being adiponectin, the production of which falls (2, 85).

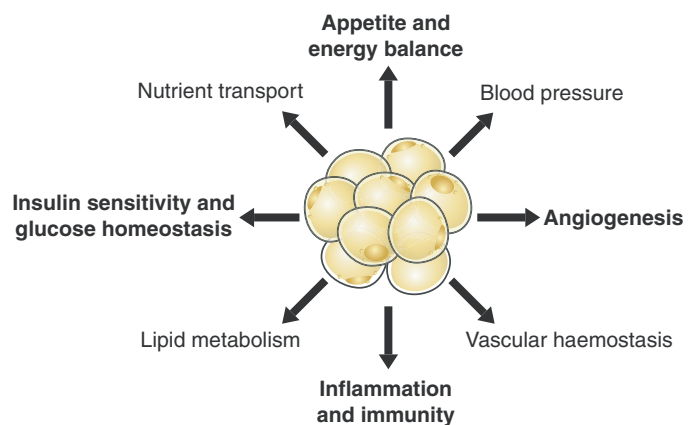


FIGURE 1. Illustration of the major physiological and metabolic processes with which adipose tissue is involved through the secretion of various adipokines from adipocytes. The interactions may be autocrine, paracrine, or endocrine.

The extensive number and range of adipokines implicate adipocytes in multiple interactions within adipose tissue, through both autocrine and paracrine mechanisms. There is also considerable communication from adipose tissue to other tissues via the endocrine actions of specific adipokines, particularly leptin and adiponectin, and bidirectional cross-talk is evident, for example, from skeletal muscle through IL-6 (147, 195).

III. HYPOXIA IN ADIPOSE TISSUE

A. Theoretical Basis for Hypoxia

A central question in adipocyte biology is why the production and release of many inflammation-related adipokines increases in obesity and an inflammatory state develops in the tissue. The initial proposition that adipose tissue depots are hypoxic as tissue mass expands, clusters of adipocytes becoming distant from the vasculature, with this initiating the inflammatory process, was based on the following a priori propositions (198): 1) despite the substantial expansion of adipose tissue mass in obesity, the proportion of the cardiac output and the extent of the blood flow to the tissue are not increased (7, 93, 202); 2) while blood flow to adipose tissue rises postprandially in lean subjects, it does not increase in the obese (60, 95); and 3) large adipocytes, which may be up to 150–200 μm in diameter (182), are larger than the normal diffusion distance of O_2 of 100–200 μm (13). It is emphasized that in some tissues and situations the PO_2 may be close to zero at only 100 μm from the vasculature (13, 48, 55).

The concept that the development of hypoxia underpins the initiation and progression of the inflammatory response in adipose tissue in obesity relates primarily to the direct ef-

fects of a low PO_2 . Alternative mechanisms for the development of inflammation include endoplasmic reticulum stress (61) and oxidative stress (86). In addition, it is increasingly evident that the gut microbiota play an important role in the inflammatory response (18, 62, 124). Hypoxia can underpin both oxidative and endoplasmic reticulum stress (21, 79, 104), providing additional routes by which low O_2 tension can lead to adipose tissue dysfunction.

B. Blood Flow and Oxygenation of Adipose Tissue

Hypoxia and the physiological challenges that this imposes are traditionally associated with exposure, whether acute or chronic, to high altitude or the deep seas. Local tissue hypoxia is also evident in certain pathological situations, including wound healing, ischemic disorders, psoriasis, and obstructive sleep apnea as well as in tumors (13, 179). In the case of solid tumors, the interstitial partial pressure of O_2 may be so low that the cells in the center are essentially anoxic, requiring major metabolic adaptations (13, 179). The O_2 tension in solid tumors can range from 10 mmHg down to almost zero; this compares with a PO_2 in arterial blood of 104 mmHg, while the general level of tissue oxygenation is of the order of 40–50 mmHg (13, 15, 78). Some tissues, in addition to tumors, may have a lower PO_2 than the general level, including the retina, thymus, and brain which have reported values of 2–15, 10, and 0.4–10 mmHg, respectively (15, 41, 227); this is summarized in **TABLE 1**. Cartilage is also markedly hypoxic, with low PO_2 levels being particularly evident in rheumatoid arthritis (46, 117, 119).

White adipose depots are variously described as being “poorly” (71) to “well” vascularized (188). To some extent this depends on the perspective that is applied, particularly

Table 1. Oxygen level in white adipose tissue and other tissues

Tissue	PO_2 , mmHg	Reference Nos.
Inspired air (at sea level)	160	see 11
Alveolar blood from lungs	104	see 13
General tissue oxygenation	40–50	see 78
Brain	0.4–8	41
Retina	2–25	227
Spleen	16	15
Thymus	10	15
Tumors	1–10	see 13
White adipose tissue, lean mice	47.9	224
White adipose tissue, obese mice	15.2	224
White adipose tissue, humans (I)	lean 55.4/obese 44.7	144
White adipose tissue, humans (II)	lean 46.8/obese 67.4	60

PO_2 , oxygen level. Examples are of tissues where hypoxia is evident, including data on white adipose tissue from lean and obese mice and humans.

if the reference point is brown adipose tissue. For example, in rats acclimated to the cold in which nonshivering thermogenesis is maximally activated, up to one-third of the total cardiac output may be channelled to brown fat (at ~1% of body weight); compared with this, the maximal blood flow to white adipose depots is small (49, 50, 189). Oxygen extraction from the vascular network of brown adipose tissue is almost total during maximal rates of thermogenesis, but such extremes of O₂ utilization would not occur with white fat (49, 50, 189). Capillary density is lower in adipose tissue of obese humans compared with lean subjects (144, 185), and this is the case in both visceral and subcutaneous depots (137). Interestingly, while in lean humans the density is greater in the visceral fat than the subcutaneous, there is no difference between the two depots in the obese (137). Reduced capillary density in adipose tissue of the obese is accompanied by the presence of larger vessels (185).

As noted above, the blood flow to white adipose tissue in obese humans is not increased relative to lean individuals (7, 92, 93, 202). This is also the case for both blood flow and the proportion of cardiac output for obese mice and rats compared with their lean siblings (79, 189, 212). In addition, an early study on dogs observed an inverse relationship between fat cell size and blood flow to adipose tissue (37). While these observations on blood flow suggest that the supply of O₂ to adipose tissue may be restricted in obesity, they do not demonstrate hypoxia per se. This has been directly addressed recently in seminal studies on mouse models of obesity, both by the use of the “Hypoxyprobe” marker system and by direct measurements of O₂ tension (79, 159, 224). Three different types of obese mouse have been employed in these studies: *ob/ob* and KKAy mutants as well as mice with obesity induced by the consumption of a high-fat diet. The Hypoxyprobe investigations, using the hypoxia marker pimonidazole with either immunostaining or western blotting, have demonstrated hypoxia in epididymal and parametrial adipose tissue depots of the obese animals (79, 159, 224).

Immunostaining also demonstrates that hypoxic areas within adipose tissue are colocalized with macrophages, indicating that there is an association between low PO₂ and the accumulation of immune cells (159). This suggests an immediate link between hypoxia and the inflammatory response in adipose tissue. However, the recruitment of macrophages may not necessarily reflect inflammation per se, but rather the need to remove dead and necrotic adipocytes (31). This may link hypoxia to apoptosis and cell survival in adipose tissue.

Direct measurements of O₂ tension in adipose depots with fiber-optic O₂ sensors have similarly recorded hypoxia, in the form of a markedly lower PO₂, in adipose tissue (including epididymal and retroperitoneal depots) of the obese

mice (159, 224). A PO₂ of 48 mmHg was recorded in the white fat of lean mice, a level that is similar to the general level of tissue oxygenation (see **TABLE 1**). Importantly, for obese mice, the PO₂ was threefold lower at 15.2 mmHg (224). Thus there is strong, direct evidence of hypoxia in adipose tissue in rodent obesity. Other, more indirect, indicators of hypoxia in mouse fat depots have also been noted, including raised lactate levels and the increased expression of hypoxia-sensitive genes such as SLC2A1 (facilitative glucose transporter 1, GLUT1), leptin (LEP), and PAI-1 (72, 79, 224). However, since the expression of these genes in vivo may be influenced by multiple factors, increased levels of their mRNAs cannot necessarily be taken to indicate hypoxia.

In the case of humans, in addition to the blood flow data and reduced capillary density, there is also direct evidence of relative hypoxia in adipose tissue in obesity. A study during surgery showed that the PO₂ in subcutaneous adipose tissue of the upper arm is lower in obese than in lean subjects, the difference being ~20 mmHg (93). A further study employing measurements with an O₂ electrode has also indicated that the PO₂ in adipose tissue of overweight/obese subjects is lower than that in the lean; obese 47 mmHg vs. lean 55 mmHg (144). Furthermore, an inverse relationship between percent body fat and PO₂ in the tissue is apparent, together with evidence for macrophage accumulation as the oxygenation level decreases (144). The differences in PO₂ between lean and overweight/obese subjects in this study are not large, however, and certainly less than that between lean and obese mice.

Contrasting results have recently been reported in which the PO₂ in adipose tissue of obese subjects was found to be *higher* rather than lower than in the lean (60). This is particularly surprising since the fasting blood flow to adipose tissue was reduced in the obese relative to the lean in this study, and the obese did not exhibit the postprandial increase in flow that occurs with the lean (60). Furthermore, in vivo measurements of O₂ consumption for the abdominal subcutaneous adipose tissue, as well as markers of mitochondrial function, were lower in the obese, as was the degree of capillarization. It is difficult to satisfactorily reconcile the apparent hyperoxia noted in this study with previous work; possible explanations for the discrepancy include differences in the nature of the groups investigated (such as the stage of obesity) and differences in the techniques employed (O₂ electrode versus optochemical measurement). There is also the possibility of a local response to the perfusion fluid containing ethanol employed in the recent, conflicting study (60).

Overall, the balance of evidence, particularly from experimental animals, strongly indicates that hypoxia occurs in adipose tissue depots in obesity.

IV. HYPOXIA AND ADIPOCYTE GENE EXPRESSION

A. HIF-1 Transcription Factor

The demonstration that white adipose tissue is hypoxic in obese animals has led to a series of studies examining the effects of low O_2 tension on the cellular function of adipocytes. The focus of the early studies has been on the expression of specific adipokine genes, particularly those linked to inflammation and angiogenesis. Both rodent, primarily the classical 3T3-L1 cell line, and human adipocytes have been employed. A significant question in such studies is whether hypoxia-induced alterations in adipokine production are mediated by hypoxia-inducible factor 1 (HIF-1), which is regarded as the key transcription factor in signaling the cellular response to low PO_2 , and frequently described as “the master regulator of O_2 homeostasis” (179). HIF-1, and the other transcription factors that regulate the molecular response to hypoxia, have been the subject of a number of excellent reviews (13, 32, 78, 96, 156, 165, 177), and only an outline will be presented here.

HIF-1 is a heterodimer composed of α and β subunits, with HIF-1 β being constitutively expressed and insensitive to the prevailing level of O_2 (13, 179). The functional transcription factor is recruited under conditions of low O_2 tension through the stabilization of the HIF-1 α subunit, this subunit operating in effect as the molecular O_2 sensor of a cell. HIF-1 α is constantly synthesized in the presence of O_2 and rapidly degraded through the 26S proteasomal system. This involves activated prolyl hydroxylase domain dioxygenases, which hydroxylate HIF-1 α , thereby providing sites for the binding of the von Hippel-Lindau protein (90, 91). Following further hydroxylation, the presence of the von Hippel-Lindau protein enables ubiquitination of the complex and the subsequent targeting of HIF-1 α to proteasomal degradation. The prolyl hydroxylase domain enzymes are inactivated when O_2 tension is low, with the result that HIF-1 α is stabilized since the von Hippel-Lindau is unable to bind.

Chemical hypoxia mimetics, such as cobalt chloride and desferrioxamine, stabilize HIF-1 α in the presence of normal levels of O_2 through inhibition of the prolyl hydroxylase domain enzymes, and incubation with these compounds is frequently employed to determine whether the expression of a particular hypoxia-sensitive gene is regulated through HIF-1 (116, 200, 209, 210). The expression of in excess of 70 genes is understood to be directly regulated by HIF-1, following the binding of the transcription factor to *cis*-acting response elements (179). These genes encode proteins involved in a wide range of cellular functions, including glucose utilization, angiogenesis, apoptosis, extracellular matrix remodeling, and inflammation. Examples of specific genes well-recognized to be transcriptionally regulated by HIF-1 include GLUT1, VEGF, and LEP (179).

In addition to HIF-1 α , there are also HIF-2 α and HIF-3 α subunits that are the product of different genes, but their role in the response to hypoxia appears less extensive than HIF-1 α , and they have been much less studied (96, 165). HIF-2, formed from HIF-1 β and HIF-2 α , appears to be more tissue-specific than HIF-1, and it is suggested that it may respond to different levels of O_2 (145). The HIFs are not, however, the only transcription factors that signal the hypoxic response; several others are recognized, including NF- κ B, cAMP response element-binding protein (CREB), and CHOP/GADD153 (C/EBP homologous protein also identified as growth arrest and DNA damage 153) (21, 35, 96, 135). In practice, there appears to be overlap and cross-talk between different factors, particularly in relation to inflammation and immunity, with NF- κ B, for example, being a transcriptional activator of HIF-1 α (164, 199).

HIF-1 subunits have been directly linked to adiposity, as well as to the response to hypoxia, with increased levels of HIF-1 α being evident in adipose tissue of obese mice (72, 224). Transgenic mice in which HIF-1 β is selectively lacking in adipose tissue show reduced weight gain relative to wild-type controls and resist the development of obesity when fed a high-fat diet (109). They also exhibit reduced energy expenditure and adipocyte size. Genetic ablation of HIF-1 α in mouse embryonic fibroblasts leads to a loss of the inhibitory effect of hypoxia on their differentiation into adipocytes, indicating that HIF-1 is involved in the inhibition of adipogenesis by low O_2 tension (229), as discussed in section VII. On the other hand, transgenic overexpression of HIF-1 α in adipose tissue leads to elevated body fat on a normal diet and increased obesity on a high-fat diet, and these changes are accompanied by larger adipocytes (231).

B. Adipokine Expression and Secretion

A key early study on the effects of hypoxia on adipokine production, which predated the *in vivo* observations of adipose tissue hypoxia in obesity, was that of Lolmède et al. (116). These authors examined the effects of low PO_2 (5% O_2) and the mimetics cobalt chloride and desferrioxamine on the expression and release of several angiogenesis-related factors in 3T3-F442A adipocytes. HIF-1 α levels increased, indicating that murine adipocytes are able to recruit the key hypoxia transcription factor, and the production of leptin, VEGF, and the matrix metalloproteinases MMP2 and MMP9, were each augmented under hypoxic conditions (116). These results demonstrate that hypoxia is markedly proangiogenic in murine fat cells.

A further key study showed that hypoxia inhibits the expression and secretion of the major adipocyte hormone adiponectin in 3T3-L1 cells, together with a stimulation of PAI-1 production (23). Since, as noted above, adiponectin has both anti-inflammatory and insulin-sensitizing actions, and may act as an angiogenesis inhibitor (14), the hypoxia-

induced fall in the production of this hormone would be expected to be both pro-inflammatory and pro-angiogenic, while potentially compromising insulin sensitivity (5, 222). Several other studies have examined the effects of hypoxia on the expression of selected genes in murine adipocytes. In addition to those already mentioned, included in the list of those adipokine genes whose expression is increased by low O_2 tension are IL-6, MIF, NAMPT (visfatin), and APLN (apelin) (57, 72, 175, 224). The majority of studies have focused on gene expression, and data are not always available for the secretion of the adipokine protein. Nevertheless, in those cases where the release of an adipokine into the culture medium has been investigated, the changes observed parallel those at the gene expression level.

The effects of hypoxia on adipokine production are not specific to rodent fat cells. Several candidate gene studies have been conducted on human adipocytes, either fibroblastic preadipocytes differentiated in culture to mature fat cells or cells of the Simpson-Golabi-Beymel (SGBS) strain (204). The outcome of these studies is generally similar to those obtained on rodent cells. Thus the expression of the *LEP*, *VEGF*, *IL6*, *PAI1*, *MIF*, *CFD* (adipsin, or complement factor D) and angiopoietin-like protein 4 (*ANGPTL4*) genes is upregulated by hypoxia, while adiponectin (*ADIPOQ*) and haptoglobin (*HP*) expression is decreased (206). Furthermore, HIF-1 α levels rise, indicating that the transcription factor subunit is stabilized under conditions of low O_2 in human, as in murine, adipocytes (72, 118, 206). For those adipokines where the secretion of the protein into the medium has been determined, which include leptin, adiponectin, IL-6, VEGF, PAI-1, and Angptl4, the changes observed parallel those at the level of gene expression (59, 118, 206); the effect of hypoxia on the secretion of key adipokines by human adipocytes is illustrated in **FIGURE 2**. As with murine cells, incubation with cobalt chloride indicates that the hypoxia-dependent transcription of genes such as *LEP*, *VEGF*, *PAI-1*, *MIF*, *HP*, and *ADIPOQ* is transcriptionally regulated through HIF-1 in human adipocytes (206). In contrast, IL-6 expression and secretion appear to be indepen-

dent of HIF-1, at least in human adipocytes, and other transcription factors, particularly NF- κ B, are likely to be involved (206).

Almost all reports to date of the effects on hypoxia on the production of selected adipokines have focused on the consequences of low P_{O_2} as the sole modulator. The potential interaction between hypoxia and other factors, including the response to specific hormones and nutrients, that regulate or influence adipokine expression and secretion have been little considered. An example of such interactions in other cell systems is the potentiation of the hypoxia-induced expression of VEGF by arachidonic acid in mouse embryonic stem cells (110). Synergistic effects of fatty acids (such as arachidonic and lauric acid) and hypoxia on the secretion of Angptl4 by human adipocytes have been noted, both factors alone stimulating production of this adipokine (59). The potential for interaction between lipids and low O_2 tension in modulating adipose tissue function is strong, given the lipid-rich environment of the tissue and the importance of specific fatty acids in inflammation and insulin resistance.

Of major interest in the context of interactions, hypoxia has recently been reported to reduce the response of adipocytes to TNF- α , the stimulatory effect of the cytokine on MCP-1 release being reduced through a downregulation of the NF- κ B signaling pathway (47). This appears to be linked to a decrease in TNF receptor 1 (TNF-R1) expression and indicates an attenuated response to inflammatory stimuli which may reflect a counterregulatory process (47). These observations suggest that the way in which hypoxia interacts with regulatory systems may be complex, and in some cases counterintuitive.

C. Hypoxia and Global Gene Expression

The majority of studies, whether on rodent or human adipocytes, have employed a candidate gene approach with the focus on specific adipokines, as described above. PCR ar-

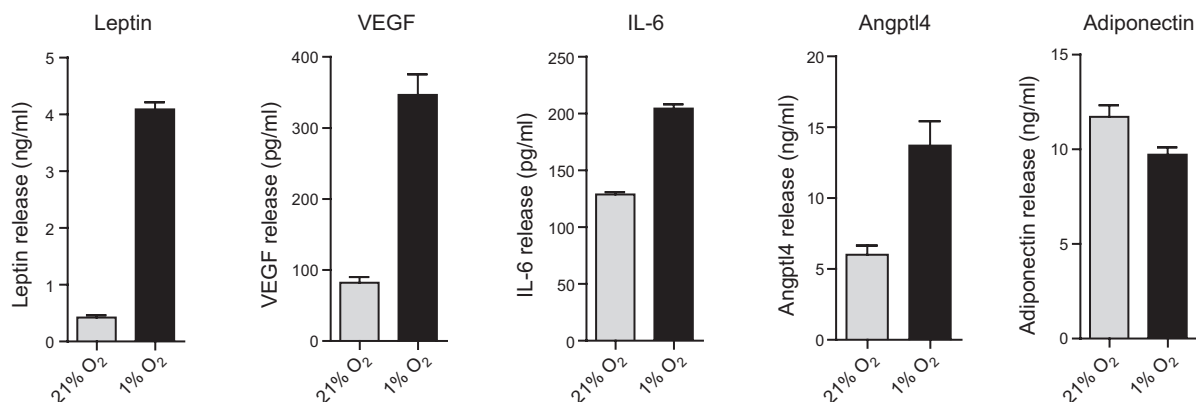


FIGURE 2. Example of the effects of hypoxia on the secretion of key adipokines by human adipocytes in cell culture. The data are derived from studies in which adipocytes were incubated in either 21% or 1% O_2 for 24 h (54, 188). The results are means \pm SE (bars; 6 observations per group), and for each adipokine, the difference between the hypoxic and control cells is statistically significant ($P < 0.01$ or better).

rays have also been utilized in which some 85 genes associated with the hypoxia-signaling pathway were simultaneously probed in human fat cells exposed to 1% O₂ for 24 h (208). The arrays showed that a specific member of the metallothionein gene family, metallothionein-3 (*MT3*), is strongly induced by hypoxia, induction being transcriptionally regulated through HIF-1. Indeed, there was a >600-fold increase in *MT3* mRNA level, although such an apparently large response reflects, to some extent, the fact that expression is minimal in adipocytes incubated under normoxic conditions (208). The metallothioneins are low molecular weight (~6,000 M_r) cysteine-rich, metal-binding proteins, with several presumed functions which include angiogenesis and antioxidant defense (129, 150, 230). A proangiogenic role in adipose tissue would be consistent with the major induction of *MT3* expression by hypoxia. Hypoxia-induced expression of the *MT3* gene was first reported in human astrocytes where it was suggested that *MT3* acts to protect the brain from hypoxic damage (187). A protective action, as well as a proangiogenic role, could similarly underlie the induction of *MT3* expression in adipocytes by low P_O₂.

UCP2 (uncoupling protein-2), *CAT*, and *GPX1* are among the genes that the PCR arrays suggested are downregulated by exposure to low O₂ tension (208). The second two encode catalase and glutathione peroxidase 1, respectively, which are “protective” enzymes involved in oxidative stress and the breakdown of hydrogen peroxidase. PCR arrays are themselves restricted in the number of genes whose expression can be simultaneously probed, and they involve selectivity in what to include; <100 genes in the reported study (208). The effect of hypoxia on global gene expression in human adipocytes has recently been examined using DNA microarrays. In one study, which employed SGBS adipocytes (day 10 postdifferentiation), the expression of just over 500 genes was found to be modulated by exposure to 1% O₂ for 16 h (56). Major cellular functions altered by low O₂ tension included glycolysis, the regulation of cell movement and migration, and the response to nutrient levels (56). However, surprisingly the expression of genes directly associated with inflammation was not generally upregulated by hypoxia in this study. Nevertheless, some individual inflammation-related genes, such as *VEGFA*, exhibited increased expression.

In a study from the author’s group with adipocytes derived from fibroblastic preadipocytes from normal subjects and employing stringent inclusion criteria in the arrays (>2.0-fold change in mRNA level, *P* < 0.01), as many as ~1,300 genes were found to be differentially expressed in response to hypoxia (1% O₂ for 24 h) (126). Of these, half were upregulated (including *LEP*, *IL6*, and *VEGF*) and 650 downregulated (including *ADIPOQ* and *UCP2*). Major genes not previously identified as hypoxia sensitive in adipocytes included *AQP3* (aquaporin 3), *FABP3* and *FABP5*

(which encode fatty acid binding proteins), and *PPARGC1A*. *PPARGC1A* encodes the PPARγ coactivator 1α, PGC-1α, and downregulation of a number of genes controlled by this factor was observed. Bioinformatic analysis indicated that several pathways and functions are modulated by hypoxia, including glucose utilization, lipid oxidation, and cell death. Network analysis points to a marked downregulation of PGC-1α and p38/ mitogen-activated protein kinase (MAPK) signaling in the adipocytes (126).

Included in the genes whose expression was shown on microarrays to be upregulated by hypoxia in human adipocytes is *CCL28* (CC-chemokine ligand 28), which has recently been reported to be induced by low O₂ tension in tumors, promoting angiogenesis and tumor immune tolerance (42). Induction of *CCL28* expression in adipocytes by hypoxia may have a similar function in adipose tissue, particularly given the parallels between expanding adipose tissue and tumor growth. These parallels include an increasing tissue mass with detrimental consequences for the organism, together with the stimulation of angiogenesis and a switch from oxidative to anaerobic metabolism.

Overall, despite some specific differences, the published microarray studies indicate that hypoxia has pervasive effects on gene expression in adipocytes; **FIGURE 3** illustrates some of the key genes whose expression is modulated by low O₂ tension in adipocytes.

V. SUBSTRATE AND GENERAL METABOLISM

A. Glucose Utilization

As noted above, microarray studies suggest that glucose utilization through the glycolytic pathway is likely to be

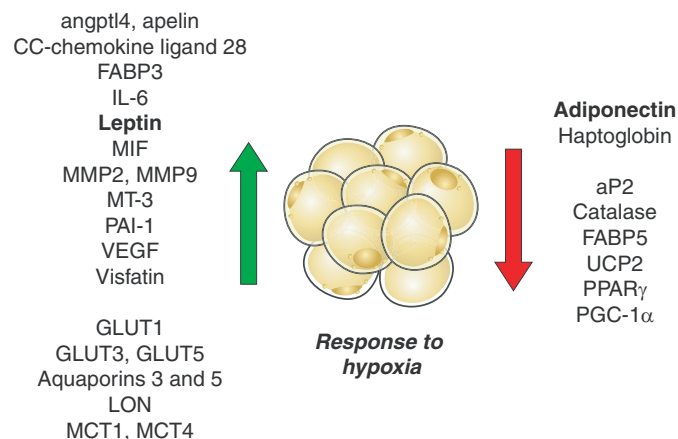


FIGURE 3. Illustration of the effects of hypoxia on the expression of key genes in human adipocytes (↑ increased, ↓ decreased expression in response to hypoxia). Genes encoding both adipokines and proteins involved in general cellular metabolism are shown. The commonly used, or protein, name rather than the gene name is given for clarity.

strongly upregulated in adipocytes by hypoxia (56, 126). This represents, of course, a well-recognized cellular response to low O₂ tension, reflecting a switch from aerobic to anaerobic metabolism. Such changes are particularly evident in tumors that exhibit markedly elevated rates of glycolysis (13, 78, 125). Genes in the glycolytic pathway whose expression has been shown by either PCR arrays or microarrays to be upregulated in adipocytes in response to hypoxia include *HK2* (hexokinase 2), *PFKP* (phosphofructokinase, platelet), and *GPI* (glucose-6-phosphate isomerase) (56, 126, 208). Elevated levels of key glycolytic enzymes in lysates of 3T3-L1 adipocytes have also been demonstrated by proteome analysis (26).

Increased rates of glucose utilization through glycolysis implies increased hexose transport. The facilitative transporter GLUT1, responsible for basal glucose uptake in many cells, is highly sensitive to hypoxia (179); indeed, induction of its expression is frequently utilized as a marker for the cellular response to low P_O₂. GLUT1 gene expression is markedly increased in adipocytes, both murine and human, in response to hypoxia (23, 79, 116, 175, 206, 224), and this is accompanied by a substantial increase in GLUT1 protein (217). GLUT1 is not the only facilitative glucose transporter in white adipocytes, however, with several other GLUTs being expressed. Transcripts for GLUT3, GLUT5, GLUT10, and GLUT12 are evident in human adipocytes, as well as GLUT1 and the insulin-sensitive transporter GLUT4 (215, 217).

Of the various transporters, GLUT1, GLUT3, and GLUT5 have each been shown to be upregulated in terms of gene expression in response to hypoxia, with there being no response to low P_O₂ in the case of GLUT4, GLUT10, and GLUT12, at least over periods of up to 24 h (217). However, in the case of GLUT3 and GLUT5, the changes at the transcript level appear not to be mirrored by increases in the amount of the transporter protein. Thus GLUT1 is the key hypoxia-inducible glucose transporter in fat cells. Intriguingly, while most studies on hypoxia in adipocytes have employed an incubation period of 24 h or less, a study involving longer periods of exposure to low P_O₂ indicated that the increase in GLUT1 is sustained over several days, but by 48 h there was a substantial fall in GLUT4 gene expression (151). This observation, if mirrored at the protein level which preliminary results indicate is the case (216), suggests that the insulin sensitivity of adipocytes in terms of GLUT4-mediated glucose uptake may be compromised by chronic exposure to hypoxia.

The increase in GLUT1 protein indicates that the capacity for basal glucose uptake is enhanced in adipocytes, as in other cell types, under hypoxic conditions. Functional studies using 2-deoxy-D-glucose have demonstrated that glucose uptake is indeed raised on exposure to hypoxia. This increased uptake is blocked in the presence of cytochalasin B,

indicating that it is transporter mediated (160, 217). Interestingly, transgenic mice with a selective knockdown of HIF-1 β in adipose tissue exhibit a decrease in 2-deoxy-D-glucose uptake in their adipocytes, and this is accompanied by a reduction in GLUT1 and GLUT4 gene expression (109). Furthermore, knockdown of HIF-1 β in 3T3-L1 adipocytes has been shown to result in a fall in GLUT1 and GLUT4 protein (109). These observations highlight the importance of the HIF-1 system in modulating glucose uptake in adipose tissue in response to hypoxia.

A corollary of increased glucose uptake and catabolism through the glycolytic pathway is that lactate production would be expected to rise, as is well-documented in tumors (55). Indeed, lactate release by adipocytes (murine and human) increases markedly under hypoxic conditions (116, 152), and lactate levels are elevated in white adipose tissue of obese mice (79). Lactate is transported across cell membranes by specific proton-linked monocarboxylate transporters, the MCTs (67–69). The *MCT1*, *MCT2*, and *MCT4* genes are each expressed in human adipocytes, and hypoxia leads to increased expression of both *MCT1* and *MCT4*, changes which appear to be transcriptionally regulated by HIF-1; however, only *MCT1* appears to be increased at the protein level (152). Thus the rise in lactate production under hypoxic conditions involves the recruitment of *MCT1* for removal of the monocarboxylate from adipocytes.

Lactate is increasingly recognized as an important metabolic signal and not simply the end product of the anaerobic catabolism of glucose (166). Functions attributed to lactate include the stimulation of inflammation in macrophages and L6 cells (70, 171), the induction of insulin resistance in muscle (25), and as an inhibitor of lipolysis in adipocytes (114). Recent evidence suggests that the inhibition of lipolysis by lactate, which is through a specific G protein-coupled receptor, GPR81, mediates the classical antilipolytic action of insulin (1, 166). White adipose tissue is an important site of lactate production, the amount released increasing in obesity (38, 79, 224). There is a direct correlation between fat cell size and the proportion of glucose that is converted to lactate, with up to 70% of the glucose used by adipocytes being metabolized to lactate in the obese (38, 123).

The high lactate production by adipose tissue in obesity is likely to be a direct reflection of the hypoxia in the tissue. A greater inhibition of lipolysis and an accentuation of the antilipolytic effect of insulin might be expected as one of the implications of the elevated lactate release. However, insulin resistance in adipose tissue is a characteristic of the obese state (103), potentially as a direct consequence of hypoxia, and as such, the lactate-mediated antilipolytic of insulin may be attenuated (see next section). A recent clinical study has in fact reported that the ability of insulin to inhibit lipolysis is directly correlated with the O₂ tension within

white fat (143). Thus adipose tissue hypoxia may impair the antilipolytic action of insulin and dysregulate the local lactate autocrine/paracrine loop in the tissue (166).

B. Insulin Sensitivity

A decline in insulin sensitivity is widely recognized to occur in obesity (82, 103, 105). Reference has already been made to the fall in GLUT4 production in adipocytes following prolonged exposure to hypoxia and the potential implications of this for insulin-stimulated glucose uptake. Whether there is also a decrease in the translocation of GLUT4 to the plasma membrane in response to insulin is unclear. Changes in the production of specific adipokines in low P_{O_2} are also likely to impact on insulin sensitivity, both locally within adipose tissue and distally in other tissues. For example, the reduction in adiponectin secretion (23, 206), given the insulin-sensitizing action of this hormone, may lead to insulin resistance. Similarly, the increase in the synthesis and release of IL-6 may contribute to resistance, given the link between this cytokine and insulin sensitivity (107, 108, 170).

Importantly, a direct effect of hypoxia in inducing marked insulin resistance in 3T3-L1 adipocytes has been documented (160, 225). Within 24 h of exposure to low O_2 tension, 2-deoxy-D-glucose experiments indicate that there is an essentially complete loss of insulin-stimulated glucose uptake. This is a consequence of an inhibition in the insulin-induced phosphorylation of the insulin receptor- β and of insulin receptor substrate 1 (IRS-1), together with impairment of other components of the insulin signaling pathway in adipocytes (160, 225). The downregulation of insulin signaling in response to hypoxia is driven by both HIF-1 and HIF-2, since overexpression of HIF-1 α or HIF-2 α mimics the effects of low P_{O_2} (160). Correspondingly, inhibition of these two HIF subunits attenuates the hypoxia-induced downregulation of the insulin signaling pathway. Collectively, hypoxia may lead to marked insulin resistance in adipose tissue through multiple, and potentially additive, routes: the insulin signaling pathway, the synthesis of GLUT4, and alterations in the production of adipokines linked to insulin sensitivity. Indeed, hypoxia may well play a central role in the development of insulin resistance in the tissue in obesity.

An impaired response to hypoxia has recently been reported in mature subcutaneous adipocytes of diabetic *db/db* mice compared with their wild-type siblings (77). VEGF and heme oxygenase 1 (HMOX1) gene expression was not stimulated by hypoxia in the adipocytes from the mutant mice. However, whether this is directly related to the diabetic state of the donor mice, is a consequence of the leptin receptor mutation, or is due to differences in fat cell size between the different groups of animals is not clear. Furthermore, the overall response to low O_2 tension in the

adipocytes from the control mice was limited, with no effect on the expression of several adipokines being evident, including leptin (77).

In a study on obese humans, higher HIF-1 α mRNA levels have been observed in omental, though not subcutaneous, adipose tissue of subjects who were insulin resistant relative to those who were insulin sensitive, suggesting that the tissue of the former group is more hypoxic (102). This difference was associated with increased macrophage accumulation in the tissue, together with increased expression of factors that act as chemoattractants to immune cells (102). Caution has, however, to be exercised in interpreting HIF-1 α mRNA levels, since they may not correlate with changes in the amount of the functional HIF-1 α protein (197).

C. Lipid and Oxidative Metabolism

The increased glucose utilization through the glycolytic pathway in hypoxia is a reflection of the absolute requirement for increased anaerobic metabolism in response to the lack of O_2 . The corollary is that substrate flux through lipid oxidation, and indeed all oxidative metabolism, will fall. Analysis of microarray data indicates that the expression of genes associated with lipolysis and lipid oxidation is indeed strongly altered by hypoxia (126). Similarly, several genes encoding proteins involved in oxidative phosphorylation, including *ATP5D* (ATP synthase, H^+ transporting, mitochondrial F1 complex, delta subunit), *COX5A* (cytochrome *c* oxidase subunit Va) and *CYTB* (mitochondrially encoded cytochrome *b*) are downregulated in human adipocytes following exposure to a low P_{O_2} (126). One of the most strongly downregulated genes is *PPARGC1A*, the encoded protein PGC-1 α being linked with mitochondrial biogenesis (191).

One of the key adaptations to reduced P_{O_2} at the mitochondrial level is a HIF-1-dependent switch at complex IV from the COX4-1 to the COX4-2 subunit of cytochrome *c* oxidase (53, 178). This switch, which has been demonstrated in several cell types and is catalyzed by the mitochondrial protease LON, has been shown by gain-of-function and loss-of-function studies to result in an increase in the efficiency of oxidative phosphorylation (53, 178). There is, however, a penalty for this change in that the generation of reactive oxygen species rises. In studies on human adipocytes, an increase in COX4-2 and LON mRNA level, together with a fall in COX4-1 mRNA, has been noted in response to hypoxia, consistent with a switch towards more tightly coupled oxidative phosphorylation (Wang and Trayhurn, unpublished data). In contrast, a decrease in COX4-2, with no change in COX4-1, expression has recently been noted in mouse adipocytes (109); it is unclear why different responses have been observed between human and murine cells.

Although basal lipolysis in murine adipocytes, as determined by glycerol release, was originally reported to be unaltered by hypoxia (116), other studies have suggested that it is increased (225). An increase would be consistent with hypoxia-induced insulin resistance leading to a loss of the antilipolytic action of the hormone. Fatty acid uptake by adipocytes, on the other hand, is decreased by hypoxia (225). This, together with increased lipolysis, may underlie the elevation of plasma free fatty acid levels in obesity (51).

D. Extracellular Matrix and Fibrosis

There is growing recognition of the importance of the extracellular matrix in the architecture and function of adipose tissue, and the consequences of dysregulation for tissue malfunction (73, 98, 122). It is suggested that the energy cost to adipocytes of maintaining, and remodeling, the extracellular matrix is considerable (122). Fibrosis develops in adipose tissue in obesity (65, 73), and a model has recently been suggested in which adipose tissue hypoxia, as one of the earliest events in adipose tissue dysfunction, induces a local state of fibrosis in the tissue (65). This in turn leads to the inflammation and the metabolic derangements linked to obesity, particularly insulin resistance. Selective knock-down of HIF-1 β in adipose tissue is, however, reported to have no effect on diet-induced inflammation and fibrosis in the tissue (109).

Several genes associated with the extracellular matrix are upregulated in vivo in adipose tissue of mice following whole body exposure to hypoxia (10% O₂), including COL1A1 (collagen, type I, alpha 1) and COL3A1 (collagen, type III, alpha 1) (65). Furthermore, >50 genes in the tissue are reported to be upregulated in a HIF-1-dependent manner in transgenic HIF-1 α mice, including several procollagens and the enzyme lysyl oxidase (LOX) which plays a central role in collagen cross-linking (65). Interrogation of microarray datasets from human adipocytes indicates that the expression of the *LOX* gene is stimulated by hypoxia, as is the *COL13A1* gene encoding collagen type XIII alpha (126). Other genes functionally linked with the extracellular matrix that are regulated in adipocytes by hypoxia include the matrix metalloproteinases MMP2 and MMP9, which are involved in matrix turnover and tissue remodeling (116).

A reduction in capillary density in adipose tissue of the obese accompanied by larger vessels has already been noted, and it is suggested that these changes inhibit angiogenesis and limit the expandability of adipose tissue (185). Collagen V levels appear to be increased in adipose tissue of the obese and are colocalized with large vessels and fibrotic areas within the tissue; elastin, on the other hand, is decreased (185). In a study on collagen VI knockout mice, the absence of this extracellular matrix protein, which is normally highly enriched in adipose tissue, was shown to result

in continued expansion of adipocytes together with multiple metabolic changes, including a reduction in tissue and systemic inflammation (98).

VI. THE SIGNIFICANCE OF DIFFERING O₂ LEVELS

In *in vitro* studies on the cellular response to hypoxia, it is common to employ 1% O₂ (although 2 or 5% O₂ are also sometimes used). This is considered to constitute severe hypoxia, but is generally justified as an experimental tool to establish “proof of principle.” However, as noted earlier, measurements suggest that *in vivo* the P_{O₂} in white adipose tissue of obese mice is as low as 15 mmHg, which is equivalent to 2% O₂ (224). Thus incubating adipocytes in 1% O₂ does not represent a substantial departure from the level of oxygenation to which the cells may be subject *in vivo*. It should also be noted, as discussed earlier, that physiologically several other tissues, including the retina, parts of the brain, and tumors have a similar, or lower, O₂ tension (13, 41, 227).

The P_{O₂} in adipose tissue of lean mice, on the other hand, at between 45 and 50 mmHg, is similar to that of the general level of tissue oxygenation (13, 78) and is equivalent to ~6.5% O₂ (224). This is considerably lower than the 21% O₂ under which adipocytes, and other cells, are routinely incubated as the reference O₂ level and which is termed “normoxia.” The effects of different levels of O₂, ranging from 21 to 1%, on the expression and secretion of adipokines from human adipocytes has recently been examined (214), and is illustrated in **FIGURE 4**. The results indicate that for several adipokines there is a direct dose-dependent effect of O₂ tension, both in terms of gene expression and the secretion of the mature protein. This is particularly evident for leptin and VEGF, and for both these adipokines, there are marked increases in mRNA and protein release between 21 and 10% O₂, with further increases as P_{O₂} is decreased. For some adipokines, including leptin, the peak response is evident at 5% O₂ with little further effect at lower O₂ concentrations. The major part of the response was generally found to be between 10 and 3% O₂, which is effectively the physiological range (214).

The effects of differing O₂ concentrations on glucose uptake and lactate efflux have also been examined, together with the expression of their respective transporters (214). Each of these parameters shows a similar dose-dependent response as is found for adipokine production. However, 2-deoxy-D-glucose uptake and GLUT1 gene expression continue to increase as O₂ tension falls from 5 to 1%, indicating that the demand for glucose does not appear to plateau in the same manner as adipokine synthesis. Given the evidence for the direct induction of insulin resistance in adipocytes by exposure to 1% O₂ (160, 225), an important question is the threshold level of O₂ tension at which insulin

Gene expression	↑ <i>LEP</i> ↓ <i>ADIPOQ</i> ↑ <i>SLC2A1</i>	↑ <i>VEGFA</i> ↑ <i>ANGPTL4</i> ↑ <i>SLC16A1</i>	↑ <i>IL6</i>
Adipokine secretion	↑ leptin	↑ VEGF ↑ IL-6	↓ adiponectin
Glucose utilization		↑ 2-DG uptake	↓ lactate release

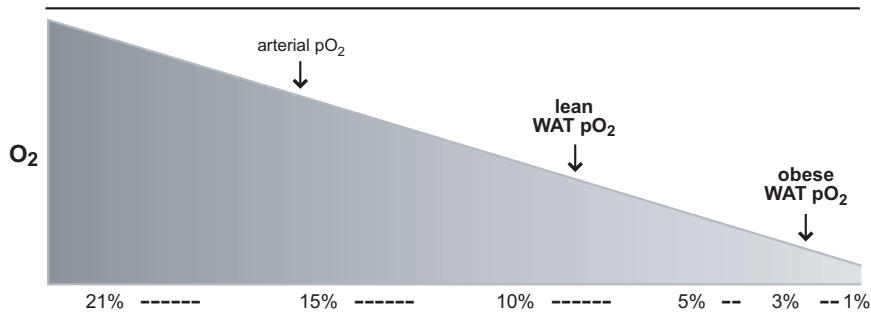


FIGURE 4. Illustration of how different levels of oxygen (between 21% and 1% O_2) influence gene expression, adipokine secretion, and the metabolic function of human adipocytes in culture. The figure is derived from data in References 196 and 206 and shows the level of O_2 at which significant changes in the given parameter are initiated relative to the adipocytes maintained under “normoxia” (21% O_2). 2-DG, 2-deoxy-D-glucose; *ADIPOQ*, adiponectin; *SLCA1*, GLUT1; *SLC16A1*, MCT1.

sensitivity is compromised and overt resistance ensues. It is possible, of course, that insulin sensitivity is gradually modulated across the entire physiological range of O_2 concentrations.

There are several important implications from these observations on the effect of different O_2 levels. First, adipocytes appear highly sensitive to O_2 tension, their metabolic functions being titrated by even relatively small changes in O_2 level within the normal physiological range. Second, so-called “normoxia” is in effect “hyperoxia,” with each of the measured parameters being at a minimum at 21% O_2 ; this results in an exaggeration of the apparent scale of response to “hypoxia.” Third, it would seem appropriate to incubate cells at a lower P_{O_2} than is customarily done. Indeed, there is a question of whether some observations from cell culture studies in practice reflect a response to hyperoxia. In this regard, it is appropriate to contrast the focus on employing “physiological” conditions in in vitro work with respect to pH (7.4) and temperature (37°C), except when these are the variables under investigation.

VII. HYPOXIA AND THE STROMAL-VASCULAR CELLS: MACROPHAGES AND PREADIPOCYTES

By virtue of their size, large adipocytes would be expected to be the cells within adipose tissue that are most subject to hypoxia. Nevertheless, the extent to which other cell types in the tissue may be modulated by hypoxia needs to be considered. Hypoxia has been shown to increase the secre-

tion of key cytokines, and of VEGF, from the combined cells of the stromal-vascular fraction (SV) of human adipose tissue (137, 161). The cytokines whose release is stimulated include TNF- α , IL-6, IL-10, and CCL-2 (MCP-1), while increases in the expression of genes such as *VEGF* also occur. Inhibition of JNK (c-Jun NH₂-terminal kinase) and p38 attenuates the hypoxia-induced increase in cytokine secretion. The SV fraction is, of course, composed of different types of cell, effectively all the cells within adipose tissue with the exception of mature adipocytes. It is therefore difficult to attribute the hypoxic response in the SV fraction as a whole to a specific cell type. However, the effects were reported to be enhanced in SV fractions enriched in the macrophage marker CD14 and absent in fractions that were CD14 depleted. This implies that macrophages are the major source of inflammatory cytokines within the SV under hypoxic conditions, as they are in normoxia (137).

Macrophages are central players in the inflammatory response in white adipose tissue. Direct studies on macrophages, or macrophage cell lines, indicate that they respond strongly to hypoxia with a stimulation of the production of a range of cytokines and other inflammation-related factors (111, 132, 224). In a recent study, hypoxia has been reported to upregulate TLR-4 (Toll-like receptor 4) expression via HIF-1 in RAW264.7 cells, providing a mechanism for the stimulation of the production of inflammatory mediators by enhancing the response to lipopolysaccharide (100, 101). Interrogation of microarray datasets does not suggest, however, any such hypoxia-induced upregulation of TLR-4 expression in adipocytes.

Preadipocytes, as the precursors of adipocytes, are key cells within adipose tissue, and their response to hypoxic conditions has been explored from different perspectives. A major effect of low O₂ tension on preadipocytes is to inhibit their differentiation to adipocytes (21, 99, 113, 229, 233). Expression of the PPAR γ nuclear transcription factor is downregulated in preadipocytes by hypoxia, through HIF-1, and this is likely to be the central mechanism through which adipogenesis is inhibited when O₂ tension is reduced (99, 207, 229). PPAR γ expression is also inhibited by hypoxia in mature adipocytes (79, 207), and this may be the explanation, at least in part, for the hypoxia-induced changes in the expression of genes such as adiponectin. These effects may in turn involve C/EBP homologous protein (CHOP), given the role of this factor in inhibiting adipocyte differentiation (87), and CHOP expression is increased in adipose tissue of obese animals and in 3T3-L1 adipocytes exposed to hypoxia (79). The downregulation of PPAR γ and inhibition of adipocyte differentiation in a low O₂ environment may in practice provide a brake on fat cell recruitment in obesity. This would be consistent with the concept that fat cell number stays constant in adult obese (and normal weight) subjects, the number of adipocytes being set in childhood and adolescence (184).

As well as being the precursors of mature adipocytes, preadipocytes are significant inflammatory cells in their own right, expressing and releasing a range of inflammation-related factors, particularly in response to stimulation by macrophage-derived mediators (27, 43, 97, 106, 136). Hypoxia leads to the stabilization of HIF-1 α , leading to the accumulation of HIF-1, in preadipocytes as in adipocytes (118, 207). Exposure of preadipocytes to low P_O₂ has been shown to modulate the expression of several genes that are hypoxia-sensitive in adipocytes, including *VEGF*, *FABP4* (aP2), and *GLUT1*, and VEGF secretion is stimulated (207), as is the release of PAI-1, IL-4, and IL-6 (118). However, intriguingly the expression of several genes that are hypoxia sensitive in adipocytes, including *IL6* and *ANG-PTL4*, are insensitive to reduced O₂ tension in preadipocytes. Maturation of the full cellular response to hypoxia would seem to be dependent on adipocyte differentiation, because of the absence (or presence) of other transcriptional regulators of HIF-1 target genes (207).

One of the most intriguing aspects of the response of preadipocytes to hypoxia relates to leptin. Preadipocytes are considered not to express the *LEP* gene, expression generally occurring at around 3–4 days after the induction of differentiation in cell culture. Indeed, the appearance of leptin is in effect a late marker of adipocyte differentiation. In several cell types, including trophoblast-derived BeWo cells and breast cancer cell lines, which normally show very little or no leptin expression, exposure to hypoxia leads to the induction of leptin synthesis (22, 63, 127, 131, 218). Similarly, incubation of human preadipocytes in low P_O₂

results in the marked induction of leptin gene expression, and leptin is secreted into the medium (207). Thus hypoxia turns preadipocytes into leptin-secreting endocrine cells. However, the amount of leptin released by preadipocytes is considerably less than by adipocytes and is unlikely to make a significant contribution to the circulating level of the hormone. Preadipocyte-derived leptin may, instead, play a specific local autocrine/paracrine role within adipose tissue.

The studies on leptin production by preadipocytes compared cells incubated in 1% O₂ with those under the so-called “normoxic” conditions. As discussed above, physiologically the O₂ tension to which cells in adipose tissue are exposed will normally be equivalent to no more than 7% O₂. This raises the possibility that in vivo preadipocytes constitutively synthesize leptin and secrete the hormone. If this is the case, the cell culture studies reflect an artefactual suppression of leptin expression as a consequence of hyperoxia. Examination of the response of preadipocytes to differing levels of O₂, similar to the investigations on mature adipocytes, would be of considerable interest.

VIII. CONCLUDING COMMENTS

There is now substantial evidence, both direct and indirect, indicating that white adipose tissue depots become hypoxic as tissue mass expands during the development of obesity. The sheer size of large adipocytes limits the availability of O₂, especially for those fat cells that are distant from the capillaries. The evidence for hypoxia is particularly strong in animal models with the direct demonstration of reduced P_O₂ in adipose tissue of different types of obese mouse (79, 159, 224). Similarly, much of the available data in humans, including from studies on tissue vascularization and blood flow as well as O₂ tension, also strongly support the proposition of adipose tissue hypoxia in obesity (7, 92, 93, 95, 144, 202). The recent suggestion that adipose tissue in obese humans exhibits hyperoxia rather than hypoxia is intriguing (60), and further investigations are warranted to clarify the position.

Obesity has been the focus and driver of much of the interest in the biology of adipose tissue over the past decade. However, it is not the only condition in which there is a considerable increase in adipose tissue mass, although it is one of the most extreme. The tissue is highly dynamic in the sense that changes in mass of an order of magnitude can occur, and this far exceeds the alterations that take place with other tissues (except in profound cachexia). Physiological states where there may also be a substantial increase in adiposity include pregnancy, in anticipation of the need to fuel the costs of lactation, and the fattening that occurs prehibernation and premigration for those species that undergo seasonal hibernation and migration, respectively (193). In the case of some hibernating species, such as Richardson’s ground squirrel (*Spermophilus richardsonii*), body

weight may double during the 4- to 5-mo period between the emergence from hibernation in the spring and the reentry into hibernation in late summer, with almost all of this increase being due to adipose tissue (130). Whether hypoxia develops with the rapid increase in adipose tissue mass pre-hibernation (or premigration and during pregnancy) is a question of some interest; there is little reason to suppose, a priori, that reduced PO_2 in the expanding tissue would be unique to obesity.

White adipocytes exhibit extensive metabolic and functional adaptations to low PO_2 , as illustrated in **FIGURE 5**. The expression of in excess of 1,000 genes is altered, with similar numbers being downregulated as are upregulated (126). HIF-1 is the key transcription factor regulating the expression of some of these genes. However, other transcription factors are clearly involved in view of the large number of genes that are hypoxia-sensitive in adipocytes. These transcriptional regulators may include HIF-2 and NF- κ B, the former having been linked with the hypoxia-induced inhibition of insulin signaling (160), and the potential role of these and other factors in the pathways involved in the response of adipocytes to hypoxia needs to be explored, as does the cross-talk between different signals.

Changes in the expression of some adipocyte genes over the 16- or 24-h exposure period employed in the published microarray studies (56, 126) may, of course, be secondary to other changes, rather than being a direct effect of low PO_2 . Further interrogation of microarray datasets is likely to yield insight into the effects of hypoxia on adipocyte function beyond the major pathways and networks that have been identified to date.

A key metabolic change in response to low PO_2 is the switch from oxidative metabolism to anaerobic glycolysis with a marked increase in glucose uptake and utilization. This adaptation to hypoxia in adipocytes is characteristic of most, if not all, cells and has been well-documented in tumors where the PO_2 may be so low that essentially anoxic conditions prevail (13, 78). One of the consequences of increased glucose metabolism through glycolysis is a rise in lactate release, and this may impact on the downstream processes that are regulated by the metabolite. These may include, for example, an augmentation of the inflammatory stimulus to macrophages through TLR-4 signaling (171) as well as the induction of insulin resistance in skeletal muscle (25). The response to hypoxia that is particularly characteristic of adipocytes relates to the production of key adipokines. The

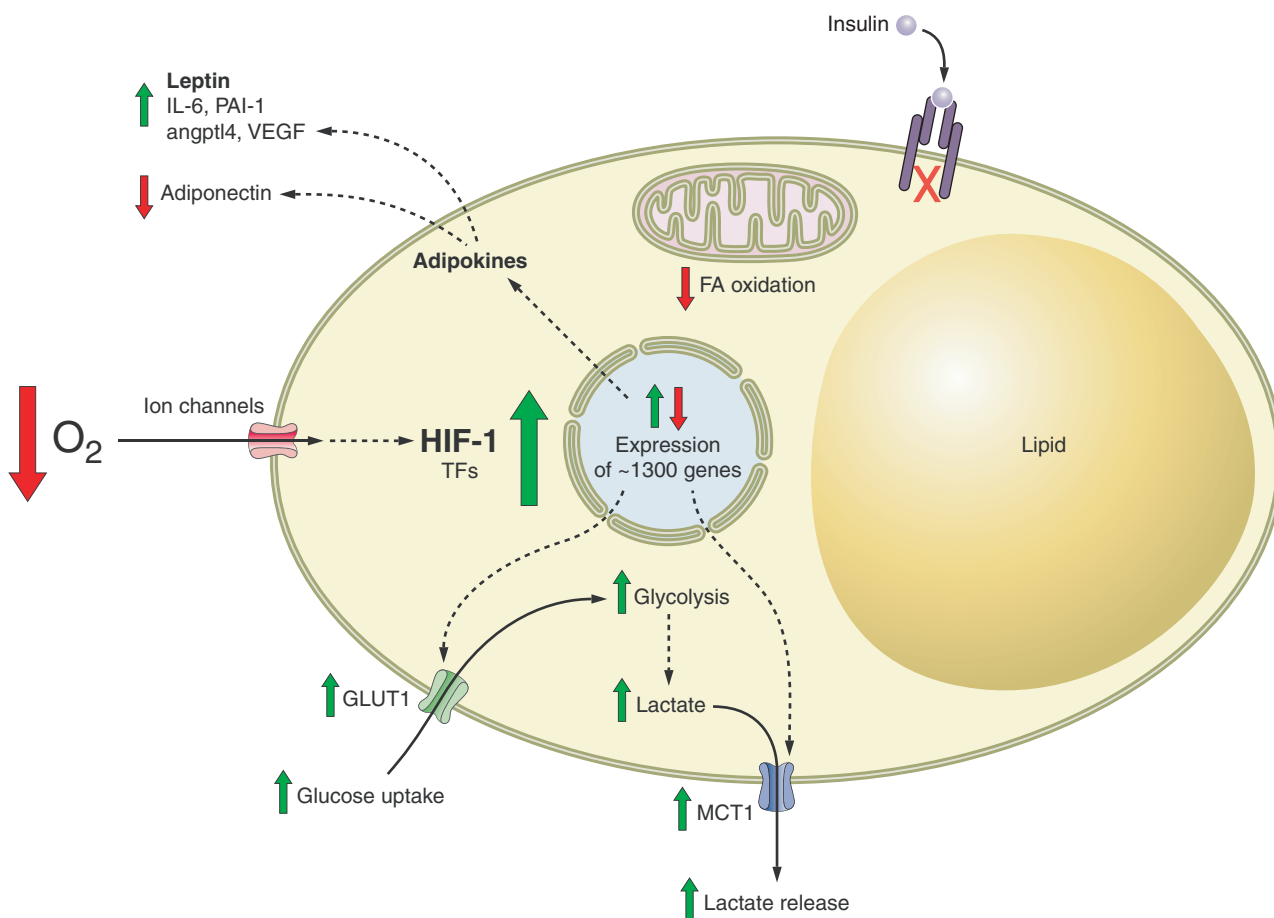


FIGURE 5. Schematic representation of the effects of hypoxia on key functions of white adipocytes. The effect of low O_2 tension on the production of selected adipokines and on glucose uptake and utilization is shown, together with effects on other processes. FA, fatty acid; TF, transcription factors (additional to HIF-1).

expression and secretion of both the signature adipocyte hormones, leptin and adiponectin, are altered by low PO_2 , leptin production being increased and adiponectin decreased (23, 116, 206). As such, hypoxia may be the principal reason for the increase in leptin and reduction in adiponectin levels in plasma in obesity. These changes in hormone production would be expected to have a detrimental impact on insulin sensitivity and to be proinflammatory.

Hypoxia induces the synthesis of several angiogenic factors by adipocytes, particularly VEGF, but also leptin and apelin, and this reflects the classical response to low O_2 tension, with the intent of growing the capillary network and thereby enhancing blood delivery and tissue oxygenation to hypoxic areas. However, the angiogenic stimulus is clearly unable to compensate sufficiently to reverse the adipose tissue hypoxia. Hypoxia-independent angiogenesis in adipose tissue has been noted in mice in the context of acclimation to the cold (221). The general view is that angiogenesis in adipose tissue is driven through inflammation (219), and a broader inflammatory response is evident with increased production of specific cytokines and chemokines (79, 206, 224), and this too is presumed to relate to the need to increase blood flow to hypoxic tissue. Chronic inflammation, with alterations in the production of key adipokines, is detrimental, however, leading to the development of the key obesity-associated diseases (80, 167, 228). Insulin resistance in adipocytes is likely to be one of the first consequences of hypoxia, and there appear to be several routes by which this can be initiated, including changes in inflammation-related adipokines, but most importantly through a direct inhibition of insulin signaling (160, 225). Fibrosis is emerging as one of the defects that develops in adipose tissue in obesity, and in one scenario hypoxia is viewed as the initiating event in adipose tissue dysfunction, followed by fibrosis and inflammation (65).

An important question physiologically is the extent to which the O_2 tension falls in adipose tissue before what would be regarded as a hypoxic response begins to be established in adipocytes. However, the O_2 level employed in cell culture and other *in vitro* studies is much higher than the prevailing PO_2 in adipose tissue, even in lean animals, and what is apparent from dose-response data is that a hypoxic response relative to normoxia is well-established at physiological levels of oxygenation (214). A particularly pertinent issue is the level of PO_2 at which insulin resistance is initiated in adipocytes.

The focus of this review has been the effects of chronic hypoxia, but there are situations where reductions in PO_2 may be intermittent. The clearest example is with obstructive sleep apnea, which because of recurring episodes of upper airway collapse results in bouts of intermittent hypoxia (11). Obstructive sleep apnea, the prev-

alence of which is high in the obese (103), is associated with insulin resistance, the metabolic syndrome, and cardiovascular abnormalities (11, 89, 157). In *in vivo* studies, intermittent hypoxia has been found to induce insulin resistance in mice, and this is accompanied by an upregulation of leptin gene expression in adipose tissue (88, 155). Intermittent hypoxia appears to inhibit the secretion of adiponectin from 3T3-L1 adipocytes in culture, similar to the effects of chronic exposure to a low PO_2 , but paradoxically ADIPOQ gene expression is upregulated (121). In PC12 cells, HIF-1 α levels are reported to increase more following intermittent compared with chronic hypoxia (133), and if this is also the case for adipocytes, it would of interest to establish whether it is accompanied by increased transcriptional activation of HIF-1-sensitive genes. It is, of course, possible that intermittent hypoxia during episodes of obstructive sleep apnea in the obese may not impact on adipose tissue, because there is little additional effect on a tissue that is already hypoxic.

Preventing and treating obesity has become a major public health priority, but after several decades of increasingly intensive research activity, progress has been very limited, as the continuing escalation in the number of those who are obese demonstrates. While it would be clearly desirable to prevent obesity in the first place, this involving complex social and cultural issues as well as biological insight, much of the medical concern is primarily with the health consequences of being obese, rather than obesity *per se*. Substantial progress is more likely to be made in identifying successful strategies for treating the major obesity-associated diseases, particularly insulin resistance and the metabolic syndrome, than with preventing obesity itself. The hypoxia that develops in adipose tissue as the mass of the tissue expands provides a focus for understanding and potentially treating the disease sequelae of obesity. Reversing hypoxia, or attenuating the O_2 -signaling pathways, whether through the HIFs or other regulatory factors, presents novel opportunities and targets, providing adiposity is not itself enhanced.

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