Adipose tissue hypoxia and insulin resistance

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ABSTRACT

Despite the well-established association of obesity with insulin resistance and inflammation, the underlying mechanisms and sequence of events leading to inflammation and insulin resistance remain unknown. Adipose tissue hypoxia has been proposed as one of the possible key events during the process of fat expansion that leads to adipose tissue dysfunction. The focus of this paper is reviewing the evidence on adipose tissue hypoxia in obesity and its relation to insulin resistance.

Obesity is associated with several metabolic disorders including insulin resistance, type 2 diabetes, cardiovascular diseases and cancers. Despite the concerted efforts aimed at curbing the obesity tide, we have witnessed a rising incidence of this pathological phenomenon over the past decades.[1 2] Understanding mechanisms linking obesity with insulin resistance will provide opportunities in preventing the untoward consequences of obesity.

Recent studies have revealed that obesity is not simply an increase in fat mass, but rather a complex association of changes in adipose tissue architecture,[3–5] dysregulated adipocytokines secretion[6] and infiltration of inflammatory cells in adipose tissue.[7 8] We and others have reported increased components of extracellular matrix and fibrosis as well as decreased capillary densities in adipose tissue with the obesity[5 9 10] that is associated with adipose tissue dysfunction.[3] Most of these studies, however, have not been able to determine the primary cause of such changes. To this end, adipose tissue hypoxia has been proposed as one of the possible key events during the process of fat expansion that leads to adipose tissue dysfunction.[11] The focus of this paper is reviewing the evidence on adipose tissue hypoxia in obesity and its relation to insulin resistance.

EVIDENCE SUPPORTING ADIPOSE TISSUE HYPOXIA IN ANIMAL MODELS OF OBESITY

Hypothetically, hypoxia can occur when expanding adipocytes outgrow the normal O_2 diffusion distance. During the weight gain, adipocytes become hypertrophic and their size increases to up to 140–180 µm in diameter[12] which exceeds the diffusion distance for oxygen (100 µm).[13] Therefore, it is quite plausible that hypertrophic adipocytes receive less than adequate oxygen supply. In fact, animal studies have provided strong evidence supporting the presence of adipose tissue hypoxia in obesity.[14] Previous studies have used different methods to demonstrate adipose tissue hypoxia in animal models of obesity including measuring the interstitial partial pressure of oxygen (pO_2),[15 16] the expression of hypoxic responsive genes,[17] using chemical hypoxic probe (pimonidazole hydrochloride)[16] or measuring lactate levels which is a product of glycolysis in cells.[15] There are several studies supporting adipose tissue hypoxia in obesity using the above mentioned methods. Among the methods investigating hypoxia, the pO_2 assay is more complex but can provide definite information about oxygen level in the tissue. A previous study[16] reported that adipose tissue pO_2 measured in *ob/ob* mice was about one-third of that in control mice at 12 weeks of age (15 mm Hg in *ob/ob* vs 48 mm Hg in the lean control mice) (table 1). Adipose tissue hypoxia has been suggested to be the result of impaired vascularization during fat expansion.[18]

EFFECTS OF HYPOXIA ON ADIPOSE TISSUE

Hypoxia increases hypoxia-inducible factor-1 (HIF1), a transcription factor which is inactive when oxygen is abundant but is activated in hypoxic conditions. HIF1 increases the mRNA expression of a wide variety of genes that stimulate erythropoiesis, angiogenesis and glycolysis.[19 20] The studies of various genetic mouse models have suggested an important role of the HIF pathway in adipose tissue inflammation and fibrosis.[21] HIF activation in adipocyte using constitutively active HIF-1α transgenic mice resulted in local inflammation and fibrosis in adipose tissue.[22] On the other hand, disruption of HIF-1α protected mice from high-fat diet (HFD)-induced obesity and improved insulin sensitivity.[23]

Adipose tissue hypoxia is also shown to play an important role in macrophage chemotaxis, adipocytokine dysregulation and impaired insulin signaling.[15 24–26]

Effects of severe hypoxia on adipose tissue gene expression have been investigated using candidate genes approach or array studies. When adipocytes were exposed to hypoxia (1% O_2) for 24 h in vitro, as many as ~1300 genes were differentially expressed. Of these, half were upregulated including leptin, proinflammatory genes (metallothionein-3, matrix metalloproteinases (MMPs) and interleukin 6 (IL6)), vascular endothelial growth factor (VEGF) and glucose transporter 1 (GLUT1) and 650 downregulated (including adiponectin, peroxisome

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Table 1 Oxygen level in white adipose tissue

Tissue	pO_2 mm Hg		Reference
Inspired air (sea level)	160		35
Arterial blood	104		35
	Lean	**Obese**	
White adipose tissue, mice	48	15	16
White adipose tissue, humans	48	39	28
White adipose tissue, humans	55	45	24
White adipose tissue, humans	47	67	29

proliferator-activated receptor gamma (PPARg), proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), adipocyte Protein 2 (aP2) and uncoupling protein 2 (UCP2)).[27] Several genes associated with the extracellular matrix were upregulated in vivo in adipose tissue of mice following exposure to hypoxia (10% O_2).[22]

Hypoxic conditions (1% O_2) in 3T3-L1 adipocytes resulted in a rapid and robust inhibition of insulin signaling through inhibition of insulin receptor phosphorylation[26] suggesting that hypoxia creates a state of insulin resistance in adipocytes in vitro.

Therefore, based on evidence presented, it is plausible that adipose tissue hypoxia is the triggering event in the cascade of macrophage infiltration, inflammation/fibrosis and ultimately insulin resistance in obesity in humans.

EVIDENCE OF ADIPOSE TISSUE HYPOXIA IN OBESE HUMAN

There is limited and conflicting data on adipose tissue hypoxia in humans. We and others have reported decreased adipose tissue pO_2 in obese as compared to lean participants.[24 28] However, Goossens *et al*[29] reported reduced oxygen extraction by adipose tissue resulting in increased adipose tissue pO_2 in obese versus lean participants. Perhaps inconsistent results were due to small sample size of these studies or differences in methods used to measure oxygen tension. Goossens *et al*[29] measured pO_2 continuously in the perfusate of a microdialysis probe whereas others[24 28] used a small Clarke electrode placed directly in touch with adiposytes. The decreased oxygen extraction by adipose tissue of obesity reported by Goossens *et al*[29] is in contrast with a recent study in mice which showed HFD-induced obesity resulted in uncoupling of adipocyte respiration leading to increased oxygen consumption and relative adipocyte hypoxia.[30]

We reported a 26% decrease in pO_2 in adipose tissue of obese as compared to lean participants (39 mm Hg in obese vs 48 mm Hg in the lean, $p<0.001$)[28] which was consistent with the findings in the previous study by Pasarica *et al*[24] (table 1). As expected, adipose tissue pO2 measured in Denver, CO (altitude 1 mile)[28] was slightly lower when compared to adipose tissue pO_2 measured at sea level by Pasarica *et al*.[24] However, overall, the degree of adipose tissue hypoxia in obese participants was not as prominent as adipose tissue hypoxia reported in animal models of obesity (table 1).

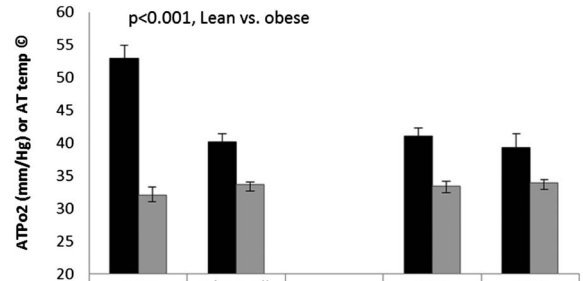
As discussed above, hypoxia has been proposed as a potential link between obesity and insulin resistance because in vitro studies have reported increased gene expression of proinflammatory factors and extracellular matrix components as well as blockade of insulin signaling.[25] It is noteworthy that in vitro studies mostly used severe hypoxic conditions (1% O_2) as compared to normoxia (21% O_2) which are very different from physiological range. Adipose tissue pO2 in humans (40–50 mm Hg) is equivalent to 6–7% O_2 conditions which highlights the importance of studying the significance of different O2 levels in adipose tissue.

ADIPOSE TISSUE HYPOXIA AND INSULIN RESISTANCE IN HUMANS

It is yet to be understood whether adipose tissue hypoxia is just a natural consequence of obesity or does play a role in insulin resistance in humans. Elegant studies in mice that involved a knockout of collagen VI suggested that a restrictive extracellular matrix in the face of expanding adipocytes resulted in hypoxia, inflammation and insulin resistance[31] and subsequent reviews[32] have suggested that individual participants are capable of either 'healthy' or 'pathological' adipose tissue expansion, depending on the ability of the tissue to allow vascular expansion without excessive restriction of the extracellular matrix. Many of these concepts were based on mouse data involving a fairly rapid adipose tissue expansion, as opposed to humans who usually develop obesity over the course of many years.

Obese insulin sensitive (metabolically healthy obese) participants who comprise up to 30% of all obese people are an intriguing model to investigate potential factors leading to insulin resistance (such as hypoxia) independent of obesity. The components involved in transitioning from an insulin sensitive state to insulin resistance are still largely unknown but current evidence points to certain phenotypic differences between obese insulin sensitive and typical obese insulin resistant people.[33 34] Our data and others' have reported that obese participants have increased adipose tissue inflammation and macrophage infiltration when compared to their lean counterparts[7 8] but recent data have indicated that there are differences in adipose tissue inflammation among obese people. As such, the obese participants with insulin resistance have significantly higher levels of adipose tissue inflammation and macrophage infiltration when compared to equally-obese participants who are not insulin resistant.[33]

In a recent study, we compared levels of adipose tissue oxygenation not only between obese and lean participants but also investigated levels of hypoxia among obese people who had similar body mass index (BMI) but significantly different levels of insulin sensitivity.[28] Our obese insulin sensitive (OBIS) group had similar insulin sensitivity levels to the lean control group despite their BMI being similar to the obese insulin resistant (OBIR) group. When adipose tissue oxygenation was measured using a combined temperature and oxygen probe, adipose tissue pO_2 was not different between the two groups of obese insulin sensitive and obese insulin resistant participants[28] figure 1. Furthermore, adipose tissue pO_2 correlated only with makers of adiposity (BMI, body fat and waist circumference).[28] Similar to Pasarica *et al*,[24] we did not find any association between adipose tissue pO_2 and insulin



	Lean	Obese-All		OBIS	OBIR
ATpO2	53.00	40.18		41.08	39.29
AT temp	32.08	33.65		33.42	33.87

Figure 1 Adipose tissue oxygenation was decreased in obesity but not different between obese insulin resistant (OBIR) and obese insulin sensitive (OBIS).

sensitivity. These results suggest that adipose tissue hypoxia is likely a consequence of fat expansion and may not be a causal factor in insulin resistance in humans.

In summary, adipose tissue hypoxia is present in obesity with the evidence being particularly strong in animal models but there are data supporting this concept in humans as well. Whether adipose tissue hypoxia is causing changes in adipose tissue structure or function thus leading to insulin resistance is unknown. Further studies are needed to investigate whether reversing hypoxia, or attenuating the O2-signaling pathways, could improve insulin resistance in humans.

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