

# Adipose tissue hypoxia and insulin resistance

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Accepted 21 February 2016

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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.<sup>1,2</sup> 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,<sup>3–5</sup> dysregulated adipocytokines secretion<sup>6</sup> and infiltration of inflammatory cells in adipose tissue.<sup>7,8</sup> We and others have reported increased components of extracellular matrix and fibrosis as well as decreased capillary densities in adipose tissue with the obesity<sup>5,9,10</sup> that is associated with adipose tissue dysfunction.<sup>3</sup> 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.<sup>11</sup> 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<sub>2</sub> diffusion distance. During the weight gain, adipocytes become hypertrophic and their size increases to up to 140–180 µm in diameter<sup>12</sup> which exceeds the diffusion distance for oxygen (100 µm).<sup>13</sup> 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.<sup>14</sup> 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<sub>2</sub>),<sup>15,16</sup> the expression of hypoxic responsive genes,<sup>17</sup> using chemical hypoxic probe (pimonidazole hydrochloride)<sup>16</sup> or measuring lactate levels which is a product of glycolysis in cells.<sup>15</sup> There are several studies supporting adipose tissue hypoxia in obesity using the above mentioned methods. Among the methods investigating hypoxia, the pO<sub>2</sub> assay is more complex but can provide definite information about oxygen level in the tissue. A previous study<sup>16</sup> reported that adipose tissue pO<sub>2</sub> 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.<sup>18</sup>

## 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.<sup>19,20</sup> The studies of various genetic mouse models have suggested an important role of the HIF pathway in adipose tissue inflammation and fibrosis.<sup>21</sup> HIF activation in adipocyte using constitutively active HIF-1α transgenic mice resulted in local inflammation and fibrosis in adipose tissue.<sup>22</sup> On the other hand, disruption of HIF-1α protected mice from high-fat diet (HFD)-induced obesity and improved insulin sensitivity.<sup>23</sup>

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

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<sub>2</sub>) 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 down-regulated (including adiponectin, peroxisome



**To cite:** Rasouli N. *J Invest Med* 2016;**64**:830–832.

**Table 1** Oxygen level in white adipose tissue

Tissue	pO <sub>2</sub> 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 (PPAR $\gamma$ ), proliferator-activated receptor gamma coactivator 1- $\alpha$  (PGC-1 $\alpha$ ), adipocyte Protein 2 (aP2) and uncoupling protein 2 (UCP2)).<sup>27</sup> Several genes associated with the extracellular matrix were upregulated in vivo in adipose tissue of mice following exposure to hypoxia (10% O<sub>2</sub>).<sup>22</sup>

Hypoxic conditions (1% O<sub>2</sub>) in 3T3-L1 adipocytes resulted in a rapid and robust inhibition of insulin signaling through inhibition of insulin receptor phosphorylation<sup>26</sup> 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<sub>2</sub> in obese as compared to lean participants.<sup>24–28</sup> However, Goossens *et al*<sup>29</sup> reported reduced oxygen extraction by adipose tissue resulting in increased adipose tissue pO<sub>2</sub> 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*<sup>29</sup> measured pO<sub>2</sub> continuously in the perfusate of a microdialysis probe whereas others<sup>24–28</sup> used a small Clarke electrode placed directly in touch with adipocytes. The decreased oxygen extraction by adipose tissue of obesity reported by Goossens *et al*<sup>29</sup> 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.<sup>30</sup>

We reported a 26% decrease in pO<sub>2</sub> in adipose tissue of obese as compared to lean participants (39 mm Hg in obese vs 48 mm Hg in the lean,  $p < 0.001$ )<sup>28</sup> which was consistent with the findings in the previous study by Pasarica *et al*<sup>24</sup> (table 1). As expected, adipose tissue pO<sub>2</sub> measured in Denver, CO (altitude 1 mile)<sup>28</sup> was slightly lower when compared to adipose tissue pO<sub>2</sub> measured at sea level by Pasarica *et al*.<sup>24</sup> 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).

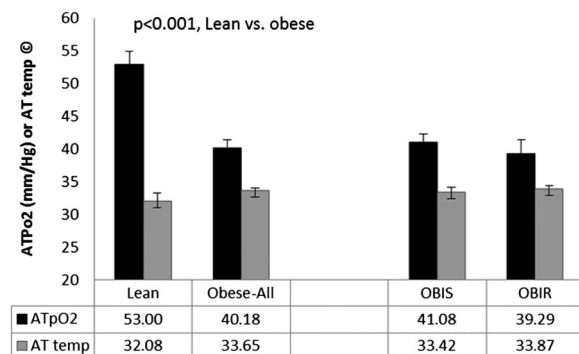
As discussed above, hypoxia has been proposed as a potential link between obesity and insulin resistance because in vitro studies have reported increased gene expression of proinflammatory factors and extracellular matrix components as well as blockade of insulin signaling.<sup>25</sup> It is noteworthy that in vitro studies mostly used severe hypoxic conditions (1% O<sub>2</sub>) as compared to normoxia (21% O<sub>2</sub>) which are very different from physiological range. Adipose tissue pO<sub>2</sub> in humans (40–50 mm Hg) is equivalent to 6–7% O<sub>2</sub> conditions which highlights the importance of studying the significance of different O<sub>2</sub> 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<sup>31</sup> and subsequent reviews<sup>32</sup> 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.<sup>33–34</sup> Our data and others’ have reported that obese participants have increased adipose tissue inflammation and macrophage infiltration when compared to their lean counterparts<sup>7–8</sup> 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.<sup>33</sup>

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.<sup>28</sup> 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<sub>2</sub> was not different between the two groups of obese insulin sensitive and obese insulin resistant participants<sup>28</sup> figure 1. Furthermore, adipose tissue pO<sub>2</sub> correlated only with makers of adiposity (BMI, body fat and waist circumference).<sup>28</sup> Similar to Pasarica *et al*,<sup>24</sup> we did not find any association between adipose tissue pO<sub>2</sub> and insulin



**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 O<sub>2</sub>-signaling pathways, could improve insulin resistance in humans.

**Funding** This work was supported by a Merit Review Grant from the Veterans Administration (N.R.), Denver Research Institute Pilot Grant (N.R.), NIH/NCATS Colorado CTSA UL1 TR001082.

**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** University of Colorado IRB (COMIRB).

**Provenance and peer review** Commissioned; externally peer reviewed.

## REFERENCES

- Centers for Disease Control and Prevention, Obesity Prevalence. 2–18–2016. Ref Type: Online Source.
- Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 2003;289:76–9.
- Sun K, Tordjman JF, Clement KF, et al. Fibrosis and adipose tissue dysfunction. *Cell Metab* 2013;18:470–7.
- Lackey DE, Burk DH, Ali MR, et al. Contributions of adipose tissue architectural and tensile properties toward defining healthy and unhealthy obesity. *Am J Physiol Endocrinol Metab* 2014;306:E233–46.
- Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. *J Cell Biol* 2015;208:501–12.
- Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. *J Clin Endocrinol Metab* 2008;93(11 Suppl 1):S64–73.
- Di Gregorio GB, Yao-Borengasser A, Rasouli N, et al. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* 2005;54:2305–13.
- Weisberg SP, McCann D, Desai M, et al. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;112:1796–808.
- Spencer M, Yao-Borengasser A, Unal R, et al. Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. *Am J Physiol Endocrinol Metab* 2010;299:E1016–27.

- Spencer M, Unal R, Zhu B, et al. Adipose tissue extracellular matrix and vascular abnormalities in obesity and insulin resistance. *J Clin Endocrinol Metab* 2011;96:E1990–8.
- Goossens GH, Blaak EE. Adipose tissue dysfunction and impaired metabolic health in human obesity: a matter of oxygen? *Front Endocrinol (Lausanne)* 2015;6:55.
- Brook CGD, Lloyd JK, Wolf OH. Relation between age of onset of obesity and size and number of adipose cells. *Br Med J* 1972;2:25–7.
- Helminger G, Yuan FF, Dellian MF, et al. Interstitial pH and pO<sub>2</sub> gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. *Nat Med* 1997;3:177–82.
- Ye JM, Doyle PJ, Iglesias MA, et al. Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation. *Diabetes* 2001;50:411–17.
- Hosogai N, Fukuhara A, Oshima K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* 2007;56:901–11.
- Ye J, Gao Z, Yin J, et al. 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:E1118–28.
- Rausch ME, Weisberg S, Vardhana PF, et al. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int J Obes (Lond)* 2008;32:451–63.
- Corvera S, Gealekman O. Adipose tissue angiogenesis: impact on obesity and type-2 diabetes. *Biochim Biophys Acta* 2014;1842:463–72.
- Semenza GL. Targeting hypoxia-inducible factor 1 to stimulate tissue vascularization. *J Invest Med* 2016;64:361–3.
- 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;12:5447–54.
- Lin Q, Yun Z. The Hypoxia-Inducible Factor Pathway in Adipocytes: The Role of HIF-2 in Adipose Inflammation and Hypertrophic Cardiomyopathy. *Front Endocrinol (Lausanne)* 2015;6:39.
- Halberg N, Khan T, Trujillo ME, et al. Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in White adipose tissue. *Mol Cell Biol* 2009;29:4467–83.
- Jiang C, Qu AF, Matsubara TF, et al. Disruption of hypoxia-inducible factor 1 in adipocytes improves insulin sensitivity and decreases adiposity in high-fat diet-fed mice. *Diabetes* 2011;60:2484–95.
- Pasirica M, Sereida OR, Redman LM, et al. Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. *Diabetes* 2009;58:718–25.
- Ye J. Emerging role of adipose tissue hypoxia in obesity and insulin resistance. *Int J Obes (Lond)* 2009;33:54–66.
- Regazzetti C, Peraldi P, Gremeaux T, et al. Hypoxia decreases insulin signaling pathways in adipocytes. *Diabetes* 2009;58:95–103.
- Mazzatti D, Lim FL, O'Hara A, et al. A microarray analysis of the hypoxia-induced modulation of gene expression in human adipocytes. *Arch Physiol Biochem* 2012;118:112–20.
- Lawler HM, Underkofler CM, Kern PA, et al. Adipose tissue hypoxia, inflammation and fibrosis in obese insulin sensitive and obese insulin resistant subjects. *J Clin Endocrinol Metab* Published Online First: 12 Feb 2016. doi: <http://dx.doi.org/10.1210/jc.2015-4125#sthash.aIMw3Bke.dpuf>
- Goossens GH, Bizzarri AF, Venticlef NF, et al. Increased adipose tissue oxygen tension in obese compared with lean men is accompanied by insulin resistance, impaired adipose tissue capillarization, and inflammation. *Circulation* 2011;124:67–76.
- Lee YS, Kim JW, Osborne O, et al. Increased adipocyte O<sub>2</sub> consumption triggers HIF-1alpha, causing inflammation and insulin resistance in obesity. *Cell* 2014;157:1339–52.
- Khan T, Muise ES, Iyengar P, et al. Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. *Mol Cell Biol* 2009;29:1575–91.
- Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest* 2011;121:2094–101.
- McLaughlin T, Deng A, Gonzales O, et al. Insulin resistance is associated with a modest increase in inflammation in subcutaneous adipose tissue of moderately obese women. *Diabetologia* 2008;51:2303–8.
- Stefan N, Kantartzis K, Machann J, et al. Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med* 2008;168:1609–16.
- Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. *Physiol Rev* 2013;93:1–21.