Physiological Oxygen Levels Differentially Regulate Adipokine Production in Abdominal and Femoral Adipocytes from Individuals with Obesity Versus Normal Weight

Ioannis G. Lempesis 1,2,3,* , Nicole Hoebers 2, Yvonne Essers 2, Johan W. E. Jocken 2, Kasper M. A. Rouschop 4, Ellen E. Blaak 2, Konstantinos N. Manolopoulos 1,3,† and Gijs H. Goossens 2,*,†

1 Institute of Metabolism and Systems Research (IMSR), College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK
2 Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, 6229 ER Maastricht, The Netherlands
3 Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham B15 2TT, UK
4 Radiotherapy, GROW School for Oncology & Reproduction, Maastricht University Medical Centre+, 6229 ER Maastricht, The Netherlands
* Correspondence: lemp.ioan@gmail.com (I.G.L.); g.goossens@maastrichtuniversity.nl (G.H.G.)
† These authors contributed equally to this work.

Abstract: Adipose tissue (AT) inflammation may increase obesity-related cardiometabolic complications. Altered AT oxygen partial pressure (pO2) may impact the adipocyte inflammatory phenotype. Here, we investigated the effects of physiological pO2 levels on the inflammatory phenotype of abdominal (ABD) and femoral (FEM) adipocytes derived from postmenopausal women with normal weight (NW) or obesity (OB). Biopsies were collected from ABD and FEM subcutaneous AT in eighteen postmenopausal women (aged 50–65 years) with NW (BMI 18–25 kg/m2, n = 9) or OB (BMI 30–40 kg/m2, n = 9). We compared the effects of prolonged exposure to different physiological pO2 levels on adipokine expression and secretion in differentiated human multipotent adipose-derived stem cells. Low physiological pO2 (5% O2) significantly increased leptin gene expression/secretion in ABD and FEM adipocytes derived from individuals with NW and OB compared with high physiological pO2 (10% O2) and standard laboratory conditions (21% O2). We compared the effects of prolonged exposure to different physiological pO2 levels on adipokine expression and secretion in differentiated human multipotent adipose-derived stem cells. Low physiological pO2 (5% O2) significantly increased leptin gene expression/secretion in ABD and FEM adipocytes derived from individuals with NW and OB compared with high physiological pO2 (10% O2) and standard laboratory conditions (21% O2). Gene expression/secretion of IL-6, DPP-4, and MCP-1 was reduced in differentiated ABD and FEM adipocytes from individuals with OB but not NW following exposure to low compared with high physiological pO2 levels. Low physiological pO2 decreases gene expression and secretion of several proinflammatory factors in ABD and FEM adipocytes derived from individuals with OB but not NW.

Keywords: adipose tissue; adipokines; inflammation; body fat distribution; obesity pathophysiology; hypoxia

1. Introduction

Excess fat mass in obesity poses a major health risk [1]. The research in the past decades has clearly demonstrated that body fat distribution is a better predictor of cardiometabolic complications than total fat mass, with abdominal obesity increasing and lower-body (gluteofemoral) fat accumulation conferring relative protection against chronic cardiometabolic diseases [2–6]. This seems related to the distinct functional properties of these different AT depots. Many studies in rodents and humans have shown that AT dysfunction in obesity is characterised by adipocyte hypertrophy, mitochondrial dysfunction, reactive oxygen species (ROS) production, impaired lipid metabolism, reduced blood flow, and inflammation, together contributing to an increased risk of developing cardiometabolic diseases and cancer [6–11].

The AT microenvironment impacts metabolic and inflammatory processes [8,9]. We, and others, have previously demonstrated that AT oxygen partial pressure (pO2), which
is determined by the balance between local oxygen supply (determined by adipose tissue blood flow) and consumption (primarily mitochondrial oxygen consumption), may be an important determinant of the AT phenotype and whole-body insulin sensitivity [9,12–14]. Interestingly, differences in adipose tissue blood flow and/or adipose tissue oxygen consumption between individuals with normal weight and obesity, and between upper and lower AT depots, have previously been demonstrated [9,10,12,15,16]. Although AT pO$_2$ is reduced in rodent models of obesity [17–19], conflicting findings on AT pO$_2$ have been reported in humans [9,20–24]. We have previously shown that AT pO$_2$ was higher in individuals with obesity and was positively associated with AT gene expression of proinflammatory markers and whole-body insulin resistance [22,25]. Moreover, we found that AT pO$_2$ was lower in femoral than in abdominal subcutaneous AT in women with obesity [16].

The normal physiological range of AT pO$_2$ in human AT is ~3–11% O$_2$ (~23–84 mmHg) [9,21–23,25]. Therefore, the outcomes of experiments comparing the effects of pO$_2$ below and well above these physiological levels should be interpreted with caution, because the results may not directly translate to the human in vivo situation [9]. Several in vitro studies have demonstrated that the expression and secretion of many adipokines are sensitive to changes in pO$_2$ levels, as extensively reviewed [9,26]. Most of these studies have shown that acute exposure to severe, non-physiological hypoxia (1% O$_2$ for 1–24 h) induces a proinflammatory expression and secretion profile in (pre)adipocytes, while prolonged exposure to mild physiological hypoxia (5% O$_2$ for 14 days) seems to elicit a different adipokine expression/secre tion profile [9,16,27]. Recently, we found that prolonged exposure to low physiological hypoxia decreased proinflammatory gene expression in abdominal and femoral adipocytes derived from women with obesity [16]. The metabolic and inflammatory responses to changes in the AT microenvironment may differ between individuals and AT depots. Thus, oxygen levels might exert distinct effects on AT function in people with different adiposity and in different AT depots. Importantly, however, studies investigating the impact of altered pO$_2$ levels on the inflammatory phenotype of adipocytes derived from people with normal weight and obesity are lacking.

Therefore, the aim of the present study was to investigate the impact of prolonged exposure to various physiological oxygen levels on gene expression and secretion of inflammatory factors within upper and lower body differentiated human multipotent adipose-derived stem (hMADS) cells derived from women with normal weight or obesity.

2. Materials and Methods

2.1. Upper and Lower Body Adipose Tissue Biopsies

Paired abdominal (ABD) and femoral (FEM) subcutaneous AT needle biopsies were obtained from eighteen postmenopausal women (aged 50–65 years) with normal weight (NW: BMI 18–25 kg/m$^2$, $n=9$) or obesity (OB: BMI 30–40 kg/m$^2$, $n=9$) (Table 1). The U.K. Health Research Authority National Health System Research Ethics Committee approved the present study (approval no. 18/NW/0392). Briefly, the biopsy specimens (up to ~1 g) were collected 6 to 8 cm lateral from the umbilicus (ABD AT) and from the anterior aspect of the upper leg (FEM AT) under local anaesthesia (1% lidocaine) after an overnight fast. Samples were immediately rinsed with sterile saline, and visible blood vessels were removed with sterile tweezers. Isolation of hMADS cells followed, as described before [16].

2.2. Human Primary Adipocyte Experiments

Human multipotent abdominal (ABD) and femoral (FEM) adipose-derived stem cells, an established human white adipocyte model [28], were seeded at a density of 2000 cells/cm$^2$ and kept in proliferation medium for seven days. Thereafter, these cells were differentiated under different physiological O$_2$ levels (10% O$_2$, high physiological pO$_2$; 5% O$_2$, low physiological pO$_2$) [9,16,22,29] as well as standard laboratory conditions (room air, 21% O$_2$) for 14 days. Gas mixtures were refreshed every 8 h (to maintain variation <0.1% O$_2$), whereas the medium was refreshed three times per week.
Table 1. Subjects’ characteristics.

| Parameter            | Normal Weight (n = 9) | Obesity (n = 9) | p Value |
|----------------------|-----------------------|-----------------|---------|
| Age (years)          | 56.7 ± 1.8            | 56 ± 1.3        | 0.566   |
| BMI (kg/m²)          | 22.8 ± 0.4            | 34.8 ± 1.3      | <0.001  |
| Waist circumference (cm) | 79.4 ± 3.1       | 105.2 ± 3.8     | <0.001  |
| Hip circumference (cm) | 94.4 ± 2.8          | 119.9 ± 4.8     | <0.001  |
| Waist-to-hip ratio   | 0.84 ± 0.02           | 0.88 ± 0.04     | 0.127   |
| Visceral fat mass (g) | 402.5 ± 118          | 1,325 ± 153.3   | <0.003  |
| Abdominal fat mass (kg) | 10.01 ± 1.48       | 24.4 ± 2.37     | <0.001  |
| Leg fat mass (kg)    | 7.67 ± 0.86           | 16.03 ± 1.43    | 0.001   |
| Fasting glucose (mmol/L) | 4.91 ± 0.10       | 5.10 ± 0.23     | 0.416   |
| 2-hour glucose (mmol/L) | 4.90 ± 0.34        | 4.70 ± 0.33     | 0.684   |
| Fasting insulin (pmol/L) | 28.40 ± 5.80      | 43.30 ± 10.20   | 0.202   |
| HOMA2 IR             | 0.46 ± 0.10           | 0.72 ± 0.20     | 0.187   |
| SBP (mmHg)           | 119.6 ± 4.4           | 133.0 ± 3.1     | 0.039   |
| DBP (mmHg)           | 73.6 ± 4.3            | 81.7 ± 1.9      | 0.153   |

BMI, body mass index; DBP, diastolic blood pressure; HOMA2 IR, Homeostasis Model Assessment 2 Insulin Resistance; SBP, systolic blood pressure. Data are mean ± SEM.

2.3. Adipocyte Gene Expression

Total RNA was extracted from hMADS cells using TRIzol reagent (Invitrogen, Breda, The Netherlands), and SYBR-Green-based real-time PCRs were performed to assess the gene expression of leptin, dipeptidyl-peptidase (DPP)-4, interleukin (IL)-6, plasminogen activator inhibitor (PAI)-1, adiponectin, tumour necrosis factor (TNF)α, and monocyte chemoattractant protein (MCP)-1; the adipocyte differentiation markers peroxisome proliferator-activated receptor γ (PPARγ), CCAAT-enhancer binding protein α (C/EBPα), fatty acid synthase (FAS), and perilipin 1 (PLIN1); as well as the hypoxia markers glucose transporter 1 (GLUT1), Bcl-2 interacting protein 3 (BNIP3), and vascular endothelial growth factor A (VEGFA) using an iCycler (Bio-Rad, Veenendaal, The Netherlands). Results were normalised to 18S ribosomal RNA.

2.4. Adipokine Secretion

The medium of the hMADS cells was collected over 24 h, from day 13 (after replacement of medium) to day 14 of differentiation, to determine the secretion of adipokines using high-sensitive ELISAs (leptin and DPP-4 from R&D Systems, Inc., Minneapolis, MN, USA; IL-6 and MCP-1 from Diaclone SAS, Besancon Cedex, France; adiponectin and PAI-1 from BioVendor–Laboratorni medicina a.s., Brno, Czech Republic). If necessary, samples were diluted with the dilution buffer provided by the manufacturer prior to the assay, which was performed in duplicates according to the manufacturer’s instructions.

2.5. Statistical Analyses

Data are presented as mean ± SEM. The effects of exposure to different oxygen levels on adipocyte gene expression and adipokine secretion were analysed using one-way ANOVA or the Friedman test when data were not normally distributed, followed by post hoc comparison using Student’s paired t-tests or the Wilcoxon signed-rank test in case of skewed data. GraphPad Prism version 8 for Windows (GraphPad Software, San Diego, CA, USA) was used to perform statistical analyses. p < 0.05 was considered statistically significant.

3. Results

3.1. The Effects of Oxygen Partial Pressure on Adipocyte Gene Expression

The exposure of differentiated hMADS cells derived from ABD and FEM AT to different $pO_2$ levels induced distinct gene expression patterns. Specifically, exposure to low physiological $pO_2$ (5% $O_2$) increased leptin expression compared with exposure to high physiological $pO_2$ (10% $O_2$) or room air (21% $O_2$) in differentiated ABD and FEM hMADS
derived from individuals with NW as well as OB (all \( p < 0.01 \), Figure 1A). Furthermore, low physiological \( pO_2 \) markedly reduced the gene expression of the proinflammatory factors DPP-4 and IL-6 in both ABD and FEM differentiated hMADS derived from donors with OB (all \( p < 0.01 \)) but not NW compared with high physiological \( pO_2 \) (Figure 1B,C). Low physiological \( pO_2 \) levels did not significantly alter the gene expression of PAI-1, TNF\( \alpha \), or MCP-1 in differentiated ABD and FEM hMADS derived from NW and OB individuals (Figure 1D–G), except for a modest but significant (\( p = 0.041 \)) increase in adiponectin gene expression in FEM differentiated hMADS derived from individuals with obesity (Figure 1E). In addition, high physiological AT \( pO_2 \) (10% \( O_2 \)) increased the PAI-1 (\( p = 0.005 \)) and reduced the adiponectin expression (\( p = 0.010 \)) in FEM differentiated hMADS derived from individuals with OB compared with those at 21% \( O_2 \) exposure. As expected, exposure to physiological oxygen levels, i.e., lower oxygen levels compared with standard laboratory conditions, increased the gene expression of the classical hypoxia markers GLUT1 and VEGFA, and, to a lesser extent, increased that of BNIP3 (Figure S1A–C). Furthermore, exposure to low physiological oxygen levels (5% \( O_2 \)) did not alter the gene expression of adipocyte differentiation markers compared with room air (21% \( O_2 \)) in differentiated hMADS derived from individuals with NW as well as OB (Supplemental Figure S1D–G). In the differentiated hMADS derived from individuals with OB, the gene expression of PPAR\( \gamma \), C/EBP\( \alpha \), and FAS was lower, and expression of PLIN1 higher, following exposure to 5% compared with 10% \( O_2 \).

**Figure 1.** Adipokine and inflammatory markers’ gene expression in hMADS cells following differentiation under different \( pO_2 \) (21% vs. 10% vs. 5% \( O_2 \)) (\( n = 9 \) paired samples): (A) leptin, (B) dipeptidyl-peptidase (DPP)-4, (C) interleukin (IL)-6, (D) plasminogen activator inhibitor (PAI)-1, (E) adiponectin, (F) tumour necrosis factor (TNF)\( \alpha \), and (G) monocyte chemoattractant protein (MCP)-1. Data are expressed as mean ± SEM. * \( p < 0.05 \).

### 3.2. The Effects of Oxygen Partial Pressure on Adipokine Secretion

Next, we investigated whether exposure to different \( pO_2 \) levels elicited functional changes in adipokine secretion from differentiated ABD and FEM hMADS. We found that adipokine secretion from both differentiated ABD and FEM hMADS was significantly affected by changes in oxygen availability (Figure 2). Specifically, low physiological \( pO_2 \) (5% \( O_2 \)) exposure increased leptin secretion in differentiated ABD and FEM hMADS derived from individuals with OB compared with exposure to high physiological \( pO_2 \) (10% \( O_2 \); ABD,
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$p = 0.009; \text{FEM, } p = 0.021$), and in differentiated ABD and FEM hMADS derived from individuals with NW compared with exposure to room air (21% $\text{O}_2$: ABD, $p = 0.014; \text{FEM, } p = 0.006$) (Figure 2A). Furthermore, DPP-4 secretion was significantly lower following exposure to low (5% $\text{O}_2$) compared with high (10% $\text{O}_2$) physiological $\text{pO}_2$ in differentiated ABD ($p = 0.027$) and FEM hMADS ($p = 0.004$) and IL-6 secretion in differentiated FEM hMADS only ($p = 0.007$), derived from donors with OB but not NW (Figure 2B,C). Moreover, low physiological $\text{pO}_2$ (5% $\text{O}_2$) reduced MCP-1 secretion ($p = 0.030$) but did not alter PAI-1 secretion from differentiated ABD hMADS derived from individuals with OB compared with 10% $\text{O}_2$ (Figure 2D,E). Finally, low physiological $\text{pO}_2$ (5% $\text{O}_2$) reduced both MCP-1 ($p = 0.028$) and PAI-1 ($p = 0.003$) secretion from differentiated FEM hMADS derived from donors with NW compared with 21% $\text{O}_2$ (Figure 2D,E). Adiponectin secretion was not detectable, and these data are therefore not reported.

Figure 2. Adipokine and inflammatory markers’ secretion in hMADS cells following differentiation under different $\text{pO}_2$s (21% vs. 10% vs. 5% $\text{O}_2$) ($n = 9$ paired samples). (A) Leptin, (B) dipeptidyl-peptidase (DPP)-4, (C) interleukin (IL)-6, (D) plasminogen activator inhibitor (PAI)-1, and (E) monocyte chemoattractant protein (MCP)-1. Data are expressed as mean $\pm$ SEM. * $p < 0.05$.

4. Discussion

In the present study, we investigated the impact of oxygen tension on adipokine gene expression and secretion in differentiated human multipotent ABD and FEM adipose-derived stem cells from women with NW or OB. Here, we demonstrate that low physiological $\text{pO}_2$ decreased gene expression and secretion of the proinflammatory factors DPP-4 and IL-6 in both differentiated ABD and FEM hMADS derived from individuals with OB, while these responses were not present in differentiated hMADS cells from NW individuals. Our findings highlight that the changes in $\text{pO}_2$ within the human physiological range in the adipocyte microenvironment contribute to alterations in the AT inflammatory phenotype and that these effects may differ between individuals with normal weight and obesity.

To determine whether the amount of oxygen present in the AT microenvironment affects the gene expression of adipokines, we exposed differentiating hMADS cells from ABD and FEM AT to low (5%) and high (10%) physiological $\text{pO}_2$ levels in human AT [9,16,21–24]. As expected, low physiological $\text{pO}_2$ levels increased the gene expression of several hypoxia
markers. Strikingly, we show for the first time that low physiological pO₂ during adipogenesis consistently decreased the expression and secretion of the proinflammatory markers IL-6 and DPP-4 in both differentiated FEM and ABD hMADS derived from individuals with OB, but not NW. Moreover, the present data suggest that these cells maintain a memory of origin (i.e., a normal-weight or obese microenvironment) in vitro, even after 14 days of exposure to the same experimental conditions. In agreement with our findings, we have previously reported that in vivo ABD AT pO₂ was positively associated with the AT gene expression of several proinflammatory markers [22] and that low physiological pO₂ exposure reduced the gene expression of IL-6 and DPP-4 in adipocytes derived from women with obesity [16]. In addition, the present results show that low physiological pO₂ levels consistently increased leptin gene expression and secretion in differentiated ABD and FEM hMADS derived from donors with NW or OB. Leptin is an important regulator of appetite and energy expenditure, providing important feedback in relation to energy storage in the body through the hypothalamus, and is involved in multiple physiological processes such as the regulation of immunity [9,30–32]. Changes in leptin secretion due to altered oxygen tension in the AT microenvironment may thus affect these processes. Notably, pO₂-induced alterations in adipokine gene expression were paralleled by comparable changes in adipokine secretion. Importantly, the modest effects of pO₂ levels on adipocyte differentiation, if present at all, do not seem to explain the observed changes in adipokine expression and secretion, exemplified by the opposing effects of low pO₂ on the expression and secretion of leptin and the proinflammatory markers IL-6 and DPP-4. Famulla et al. [27] previously showed increased DPP-4, adiponectin, and IL-6 secretion following prolonged exposure to high physiological pO₂ (10% O₂), while low physiological pO₂ (5% O₂) tended to reduce the secretion of adiponectin. These differences between studies may at least partly be explained by differences in donor characteristics.

A strength of the present study is the paired comparisons between differentiated adipose-derived multipotent stem cells derived from ABD and FEM AT of individuals with NW and OB. Previous studies examining the effects of pO₂ levels on adipocyte inflammation have either used cell lines, adipose-derived multipotent stem cells from a single donor, or a pool of stem cells obtained from different donors. Because our findings demonstrate that the impact of changes in the AT microenvironment (i.e., different physiological pO₂ levels) on adipokine expression and secretion depends on the characteristics of the donors, future studies in the field of AT biology should take this ‘memory-of-origin effect’ into account. Secondly, in contrast to many studies showing that acute exposure to severe (non-physiological) hypoxia evokes a proinflammatory response in murine and human (pre)adipocytes [13,14], we aimed to mimic physiological in vivo conditions in terms of pO₂ levels as well as the prolonged exposure duration in the present study. This study also has some limitations. We examined the effects of various oxygen levels in cells derived from postmenopausal women. Therefore, our findings cannot be translated to other subgroups of the population such as men or individuals of different age. Furthermore, we used a targeted approach to examine the gene expression and secretion of several adipokines. Future studies using an untargeted approach (e.g., microarray analysis, RNA sequencing, or proteomics) are warranted.

5. Conclusions

In conclusion, the present findings demonstrate that physiological oxygen levels regulate adipokine expression and secretion in differentiated ABD and FEM hMADS. Differentiated hMADS cells derived from women with OB display lower expression and secretion of several (proinflammatory) adipokines at low (5% O₂) compared with high (10% O₂) physiological oxygen tension. Except for the effects on leptin expression, no significant effects of low compared with high physiological oxygen levels were observed in differentiated hMADS cells derived from individuals with NW. Our findings thus indicate that pO₂ levels alter the expression and secretion of several adipokines in differentiated human ABD and FEM hMADS, and that donor characteristics determine experimental outcomes.
This has important implications for future mechanistic in vitro studies in the field of AT biology. For example, the outcomes of studies in which the effects of certain interventions on adipocyte inflammation and related biological mechanisms are investigated may depend on the microenvironmental oxygen tension. Furthermore, our findings highlight that it is important to report detailed the characteristics of the cell donor(s) in studies examining human adipocyte biology.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cells11223532/s1, Figure S1: Adipocyte differentiation and hypoxia markers’ gene expression in adipose tissue-derived mesenchymal stem cells following differentiation under different pO$_2$s (21% vs. 10% vs. 5% O$_2$).

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and the U.K. Health Research Authority National Health System Research Ethics Committee approved the present study (approval no. 18/NW/0392).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data presented in this manuscript are available from the corresponding author on reasonable request.

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