

Hypoxia induces leptin gene expression and secretion in human preadipocytes: differential effects of hypoxia on adipokine expression by preadipocytes

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Abstract

The effect of hypoxia on the expression and secretion of major adipokines by human preadipocytes has been examined. Hypoxia (1% O₂) led to an increase in the HIF-1 α transcription factor subunit in cultured preadipocytes, as did incubation with the hypoxia mimetic CoCl₂. Leptin mRNA was essentially undetectable in preadipocytes incubated under normoxia (21% O₂), but exposure to 1% O₂, or CoCl₂, for 4 or 24 h resulted in an induction of leptin gene expression (measured by real-time PCR). Immunoreactive leptin was not detected in the medium from normoxic preadipocytes, but was present in the medium from the hypoxic cells. Hypoxia stimulated expression of the GLUT-1 facilitative glucose transporter gene and the vascular endothelial growth factor (VEGF) gene in preadipocytes, as

in adipocytes. PPAR γ and aP2 mRNA levels, markers of adipocyte differentiation, were reduced by hypoxia in both cell types. In marked contrast to adipocytes, interleukin-6 (IL-6), angiopoietin-like protein 4, and plasminogen activator inhibitor-1 expression by preadipocytes was not stimulated by low O₂ tension. Consistent with the gene expression results, VEGF release into the medium from preadipocytes was increased by hypoxia, but there was no change in IL-6 secretion. It is concluded that hypoxia induces human preadipocytes to synthesize and secrete leptin. Preadipocytes and adipocytes differ in their responsiveness to low O₂ tension, maturation of the response to hypoxia developing on differentiation.

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Introduction

White adipose tissue is a major endocrine organ, secreting a wide range of hormones and signaling factors (Trayhurn & Beattie 2001, Rajala & Scherer 2003, Trayhurn & Wood 2004, Rosen & Spiegelman 2006). The tissue is, in particular, the sole, or main, source of two key pleiotropic hormones, leptin and adiponectin. Leptin, a 16 000 M_r cytokine-like protein that acts as a key peripheral signal in the control of appetite was discovered through the cloning of the *ob* gene (Zhang *et al.* 1994, Friedman & Halaas 1998, Harris 2000). A number of factors that regulate leptin production by white adipocytes have been identified, and these include insulin, PPAR γ agonists, and the sympathetic nervous system (Harris 2000, Trayhurn & Beattie 2001). Leptin is a hypoxia-sensitive gene; its expression being stimulated in adipocytes by low O₂ tension (Grosfeld *et al.* 2002b, Lolmède *et al.* 2003, Polotsky *et al.* 2003, Hausman & Richardson 2004), through transcriptional regulation by the hypoxia-inducible transcription factor, HIF-1 (Grosfeld *et al.* 2001, Ambrosini *et al.* 2002). The induction of leptin expression by hypoxia has also been reported in other cell types, including human trophoblast cell lines (Mise *et al.* 1998, Grosfeld *et al.* 2001,

Meissner *et al.* 2005), MCF-7 breast cancer cells (Cascio *et al.* 2008), and ectopic endometriotic stromal cells (Wu *et al.* 2007). Within adipose tissue, leptin expression and secretion appear to occur solely in adipocytes, with preadipocytes viewed as not expressing the gene (Maffei *et al.* 1995, Leroy *et al.* 1996, Wang *et al.* 2005).

Leptin is one of a growing number of hypoxia-sensitive adipocyte genes, both adipokine and non-adipokine (Trayhurn *et al.* 2008). Other major adipokines, murine or human, whose expression is stimulated by hypoxia include angiopoietin-like protein 4 (Angptl4), interleukin-6 (IL-6), macrophage migration inhibitory factor (MIF), and vascular endothelial growth factor (VEGF), while the non-adipokines include the GLUT-1 and GLUT-3 facilitative glucose transporters (Lolmède *et al.* 2003, Hosogai *et al.* 2007, Wang *et al.* 2007, Wood *et al.* 2007, Ye *et al.* 2007). By contrast, adiponectin and haptoglobin expression is inhibited by low O₂ tension (Chen *et al.* 2006, Hosogai *et al.* 2007, Wang *et al.* 2007). Hypoxia-induced changes in adipokine production have been suggested to underlie the development of the inflammatory response in adipose tissue, which occurs in the obese state (Trayhurn & Wood 2004). Direct physiological evidence for local hypoxia in white adipose

tissue in obesity has now been reported in three different obese mouse models (Hosogai *et al.* 2007, Ye *et al.* 2007, Rausch *et al.* 2008), in support of the original concept.

Although preadipocytes are considered not to express leptin, they do express and secrete a number of other inflammation-related proteins (Chung *et al.* 2006, Lacasa *et al.* 2007). Indeed, it is suggested that preadipocytes are able to mount a substantial inflammatory response, particularly following stimulation by lipopolysaccharide (Chung *et al.* 2006), and leptin secretion has been reported to be induced in these cells by the proinflammatory cytokines tumour necrosis factor- α (TNF- α) and IL-1 β (Simons *et al.* 2005). In the present study, we have examined whether leptin production can be induced in human preadipocytes by hypoxia, our recent studies have shown that expression of the metallothionein-3 gene is hypoxia inducible in preadipocytes as well as in adipocytes (Wang *et al.* 2008). The results demonstrate that both the expression and secretion of leptin are induced by low O₂ tension in human preadipocytes; however, the expression of Angptl4, IL-6, and VEGF genes is insensitive to hypoxia, in contrast to adipocytes.

Materials and Methods

Cell culture

Human subcutaneous preadipocytes and culture media were obtained from Zen-Bio (Durham, NC, USA) and cultured as described previously (Wang *et al.* 2007). Cells were from seven female patients of average age 41 years and with a mean BMI of 25. The cells were plated at a density of 40 000/cm² onto a 24-well plate and maintained in preadipocyte medium (PM) containing DMEM/Ham's F-12 (1:1, v/v), 10% fetal calf serum (FCS), 15 mM HEPES, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 0.25 μ g/ml amphotericin B at 37 °C in a humidified atmosphere of 95% air/5% CO₂. The cells were induced at confluence by incubation in differentiation medium composed of adipose medium (AM) supplemented with 0.25 mM isobutyl methylxanthine and 10 μ M of a PPAR γ agonist for 5 days. The cells were then cultured with AM containing DMEM/Ham's F-12 (1:1, v/v), 3% FCS, 100 nM insulin, 1 μ M dexamethasone, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 0.25 μ g/ml amphotericin B (Wang *et al.* 2005). The medium was changed every 3 days.

Preadipocytes (day 0, day of induction) or fully differentiated cells at day 15 post-induction were exposed to 1% O₂ for up to 24 h. The cells were placed in an MIC-101 modular incubator chamber (Billups-Rosenberg, Del Mar, CA (USA) that was flushed with 1% O₂/94% N₂/5% CO₂ and sealed and placed at 37 °C. Control cells were cultured in a standard incubator (21% O₂/5% CO₂). In some experiments, preadipocytes were incubated with 100 μ M CoCl₂ in normoxia. The cells were harvested in 700 μ l TRIzol (Invitrogen), or 150 μ l lysis buffer, per well at the indicated time points. Culture media were also

collected. All incubations at each time point were performed in replicates of up to 6 wells.

RNA extraction and cDNA synthesis

Total RNA was isolated from cells using TRIzol, and 1 μ g RNA reverse transcribed with Reverse-iT 1st Strand Synthesis kit (Abgene, Epsom, UK) in the presence of anchored oligo dT in a total volume of 20 μ l.

Real-time PCR

Quantitative real-time PCRs were carried out in a final volume of 12.5 μ l consisting of 12.5–50 ng reverse-transcribed cDNA mixed with optimal concentrations of primers and probe and qPCR Core kit (Eurogentec, Southampton, UK) in 96-well plates on an Mx3005P detector (Stratagene, USA). The primer and probe sets were designed using Primer Express software (Applied Biosystems) and synthesized commercially (Eurogentec). The sequence and optimal concentrations of primers and probes together with the size of products were as detailed previously (Wang *et al.* 2005, 2007, Wang & Trayhurn 2006). Typically, the amplification started with 2 min at 50 °C, 10 min at 95 °C, and then 40 cycles of the following: 15 s at 95 °C and 1 min at 60 °C.

Human POLR2A was used as an endogenous reference; its expression remained unchanged both in response to hypoxia and during preadipocyte differentiation. Relative quantitation values were expressed using the 2^{- $\Delta\Delta C_t$} method (see User bulletin 2, ABI PRISM 7700, pp 11–15, Applied Biosystems) as fold changes in the target gene normalized to the reference gene (POLR2A) and related to the expression of the untreated controls. The PCR efficiency in all runs was close to 100%, and all samples were analyzed in at least duplicate.

Measurement of HIF-1 α by western blotting and ELISA

Cells were solubilized in lysis buffer consisting of 50 mM Tris (pH 7.4), 300 mM NaCl, 10% (w/v) glycerol, 3 mM EDTA, 1 mM MgCl₂, 20 mM β -glycerophosphosphate, 25 mM NaF, 1% Triton X-100, 25 μ g/ml leupeptin, 25 μ g/ml pepstatin, and 3 μ g/ml aprotinin. The total protein of the cells was quantified by the BCA Protein Assay Reagent (Sigma). Fifteen micrograms of the protein were separated by SDS-PAGE and then transferred to a nitrocellulose membrane. The membranes were blocked and probed with polyclonal goat anti-human HIF-1 α (R&D Systems, Abingdon, UK) or monoclonal anti-mouse α -tubulin (Sigma) as the primary antibody, and then subjected to HRP-conjugated anti-goat IgG (R&D Systems) or anti-mouses IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as the secondary antibody. Specific proteins were visualized with the enhanced chemiluminescence reagent (Amersham). Total HIF-1 α in cell lysates was also quantitated with an ELISA kit (R&D Systems), according to the manufacturer's protocol.

Measurement of adipokines by ELISA

IL-6, MIF and VEGF were measured in cell culture media using commercial ELISA kits (R&D Systems), as described previously (Wang *et al.* 2007). The assays were conducted in 96-well microplates, according to the manufacturer's instructions.

Statistical analysis

The statistical significance of differences between groups was assessed by Student's *t*-test.

Results

Hypoxia and leptin expression in preadipocytes

Human preadipocytes were taken at the point at which they would normally be induced to differentiate into adipocytes and exposed to normoxia (21% O₂) or hypoxia (1% O₂) for either 4 or 24 h. A further group of cells was differentiated and exposed to low O₂ tension at day 15 post-induction at which time they contained multiple lipid droplets. Exposure to hypoxia led to an increase in the level of HIF-1 α protein in the preadipocytes (Fig. 1A and B), similar to that previously observed for human adipocytes (Wang *et al.* 2007, Wood *et al.* 2007). Incubation with CoCl₂, a chemical hypoxia mimetic, also led to an increase in HIF-1 α in the preadipocytes and this was particularly marked at 4 h (Fig. 1A and C).

Leptin mRNA was essentially undetectable in the preadipocytes in normoxia, as noted previously by RT-PCR with these cells (Wang *et al.* 2005). However, a distinct signal was obtained by real-time PCR with the preadipocytes incubated under hypoxic conditions, particularly at 24 h (Fig. 2A). In the real-time PCR measurements, the C_t value for leptin mRNA was >35 in the normoxic preadipocytes, and relative to this very low value, leptin mRNA was increased 35-fold at 24 h in the hypoxic cells (Fig. 2A), the mean C_t value being 30.9. A smaller increase (threefold) was evident at 4 h. Hypoxia also induced increases in leptin mRNA level in adipocytes, the increase being 14-fold at 24 h (Fig. 2B). Incubation with CoCl₂ had a similar effect to 1% O₂ in inducing leptin gene expression in preadipocytes (17-fold at 24 h; Fig. 2C).

Leptin was measured in the medium using a highly sensitive ELISA (minimum detectable dose of <7.8 pg/ml) in the cells incubated for 24 h. No detectable leptin was, however, found in the medium of the preadipocytes incubated under normoxic conditions. By contrast, immunoreactive leptin was readily detected in the medium of the preadipocytes incubated under hypoxia (Fig. 2D); the amount of leptin in the medium from the hypoxic cells was ten times the minimum detectable dose for the ELISA. Leptin was also induced by hypoxia in the differentiated adipocytes and the amount was ~60 times more than in the hypoxic preadipocytes (Fig. 2E).

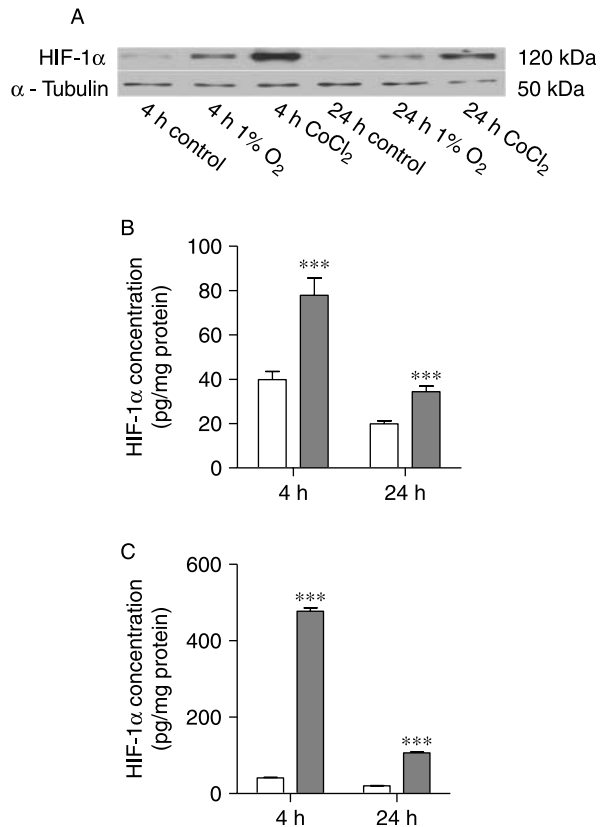


Figure 1 Effect of low O₂ or CoCl₂ treatment on HIF-1 α protein in human preadipocytes. Human preadipocytes were cultured until confluence (day 0) and then subjected to 1% O₂ or 100 μ M CoCl₂ for either 4 or 24 h. Control cells were maintained at 21% O₂ in the absence of CoCl₂. (A) Representative western blot for HIF-1 α and α -tubulin protein. (B) Quantitative changes of HIF-1 α in cells cultured under normoxia (21% O₂, open bars) or hypoxia (1% O₂, filled bars) for 4 or 24 h. (C) Quantitative changes of HIF-1 α in cells cultured under control (open bars) and hypoxia mimetic (CoCl₂, filled bars) conditions for 4 or 24 h. Results are means \pm S.E.M., *n* = 6. ****P* < 0.001 compared with control.

Differential effects of hypoxia on the expression of preadipocyte genes

The effect of hypoxia on the expression of other preadipocyte genes was next examined, and these included several genes that are hypoxia sensitive in human adipocytes (Wang *et al.* 2007). Expression of the GLUT-1 facilitative glucose transporter was induced in preadipocytes by low O₂ tension, the level of the mRNA being increased by 6.5-fold at 24 h (Fig. 3A), and this compares with a 10.3-fold increase in the adipocytes. Two genes characteristic of adipocytes, the PPAR γ nuclear receptor and aP2 (adipocyte lipid-binding protein), were also examined. Hypoxia for 24 h led to a significant reduction in the relative mRNA level of both PPAR γ and aP2 in preadipocytes, as in adipocytes (Fig. 3B and C). As expected, the PPAR γ and aP2 mRNA levels were

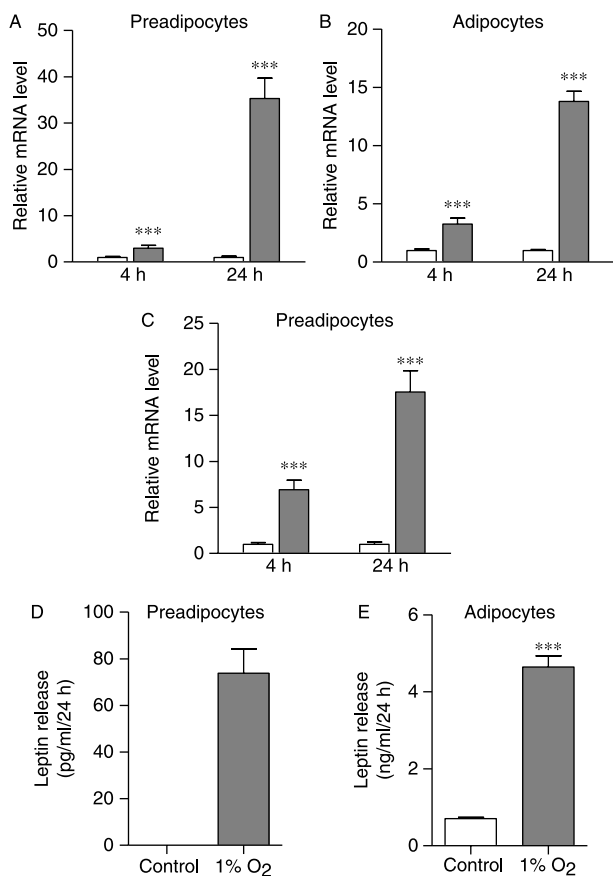


Figure 2 Effect of hypoxia on leptin expression and secretion in human preadipocytes and adipocytes. Human preadipocytes (day 0) or adipocytes (day 15 post-differentiation) were subjected to 1% O₂ or 100 μM CoCl₂ (filled bars) for either 4 or 24 h. Control cells (open bars) were maintained at 21% O₂ in the absence of CoCl₂.

(A) Relative mRNA levels in preadipocytes cultured at 1% O₂ normalized to normoxic controls. (B) Relative mRNA levels in adipocytes cultured at 1% O₂ normalized to normoxic controls. (C) Relative mRNA levels in preadipocytes cultured in 100 μM CoCl₂ normalized to normoxic controls. (D) Leptin secretion (pg/ml) from preadipocytes cultured at 1% O₂ for 24 h compared with normoxic controls. (E) Leptin secretion (ng/ml) from adipocytes cultured at 1% O₂ for 24 h compared with normoxic controls. Results are means ± s.e.m., $n=6$. *** $P<0.001$ compared with control.

very much higher in the adipocytes than the preadipocytes, particularly in the case of the latter (~25- and 24 000-fold respectively).

VEGF mRNA level was increased in response to hypoxia at 4 and 24 h in both preadipocytes and adipocytes (Fig. 4A). However, the response to low O₂ tension was less in preadipocytes; the increase at 24 h being 3.2-fold compared with a 23-fold increase in the adipocytes. The expression of three other adipokine genes, namely Angptl4, IL-6, and PAI-1, was also examined. The mRNA level of each of these adipokines was increased in adipocytes exposed

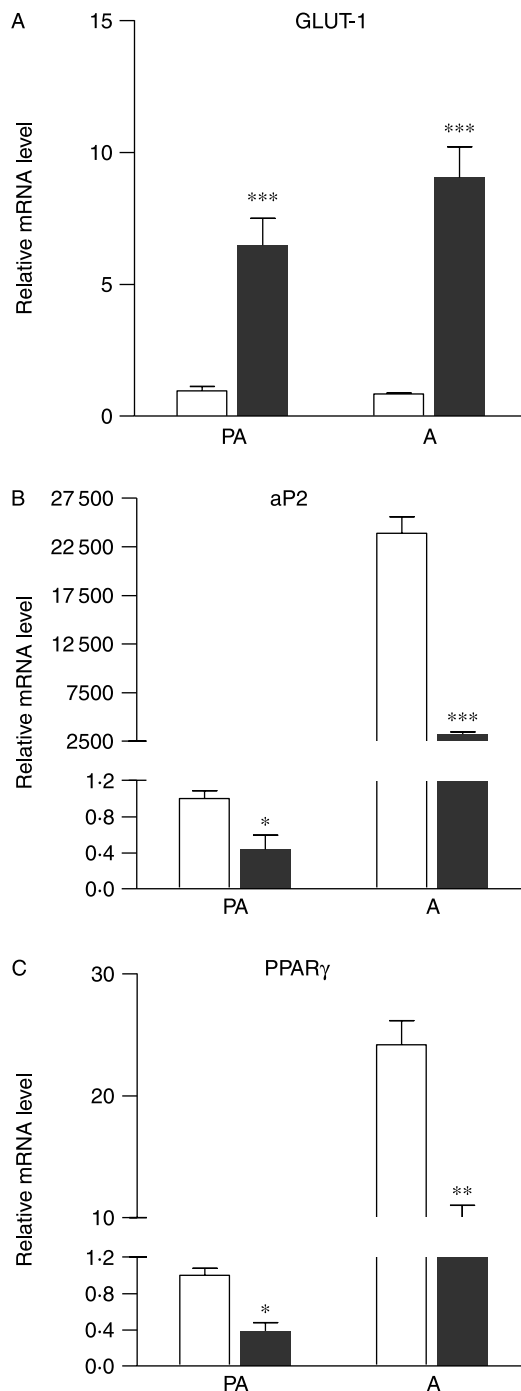


Figure 3 Differential effect of hypoxia on gene expression in human preadipocytes and adipocytes. Human preadipocytes (day 0) or adipocytes (day 15 post-differentiation) were subjected to 1% O₂ (filled bars) for 24 h. Control cells were maintained at 21% O₂ (open bars). (A) GLUT-1, (B) aP2, and (C) PPARγ mRNA levels relative to normoxic preadipocytes are shown. PA, preadipocytes; A, adipocytes. Results are means ± s.e.m., $n=6$. * $P<0.05$, ** $P<0.01$ *** $P<0.001$ compared with PA normoxic control.

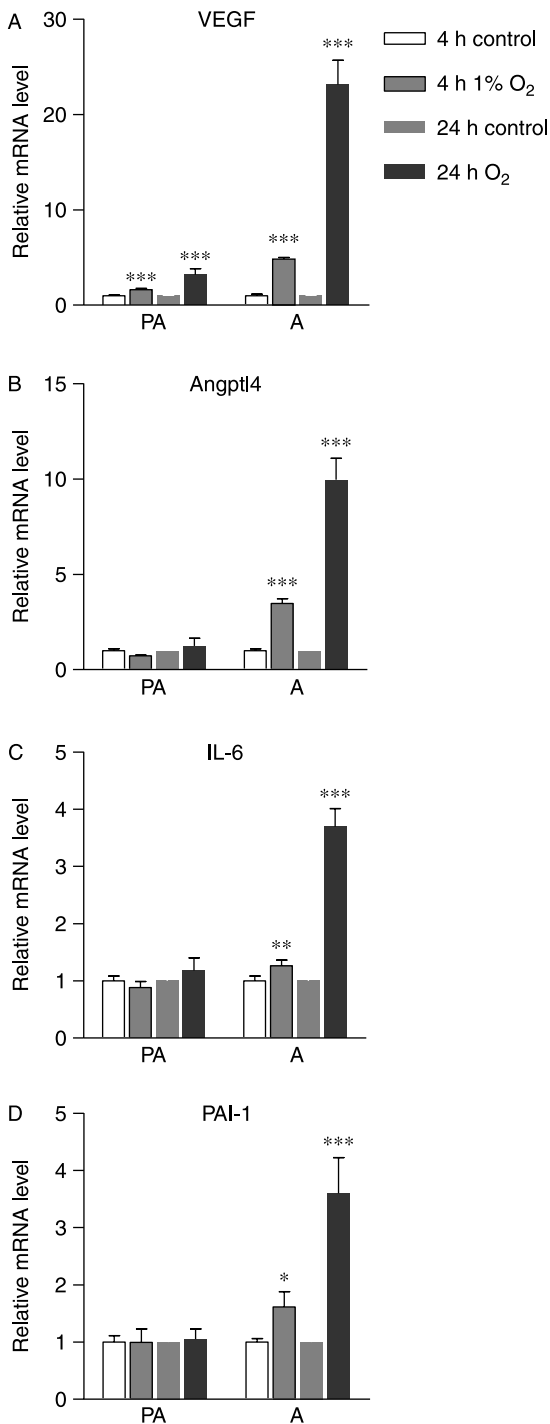


Figure 4 Differential effect of hypoxia on adipokine gene expression in human preadipocytes and adipocytes. Human preadipocytes (day 0) or adipocytes (day 15 post-differentiation) were subjected to 1% O₂ for 4 or 24 h. Control cells were maintained at 21% O₂. (A) VEGF, (B) Angptl4, (C) IL-6, and (D) PAI-1 mRNA levels relative to normoxic controls are shown. PA, preadipocytes; A, adipocytes. Results are means \pm s.e.m., $n=6$. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with normoxic controls.

to hypoxia, the increases being greater at 24 than at 4 h (Fig. 4B–D). By contrast, hypoxia had no effect on the mRNA level of these three genes in preadipocytes, at either 4 or 24 h of exposure.

The effects of hypoxia on the secretion into the medium of VEGF and IL-6 by preadipocytes were next determined. VEGF release was stimulated by exposure to hypoxia in the preadipocytes, as in adipocytes, paralleling the changes in gene expression (Fig. 5A). Nevertheless, the scale of the increase was greater in the adipocytes (6.6-fold) than the preadipocytes (2-fold); this mostly reflected the higher 'basal' release in normoxia from the preadipocytes. On the other hand, while hypoxia resulted in increased release of IL-6 from adipocytes, there was no hypoxia-induced change in the secretion of this cytokine from preadipocytes (Fig. 5B). This is again consistent with the mRNA measurements.

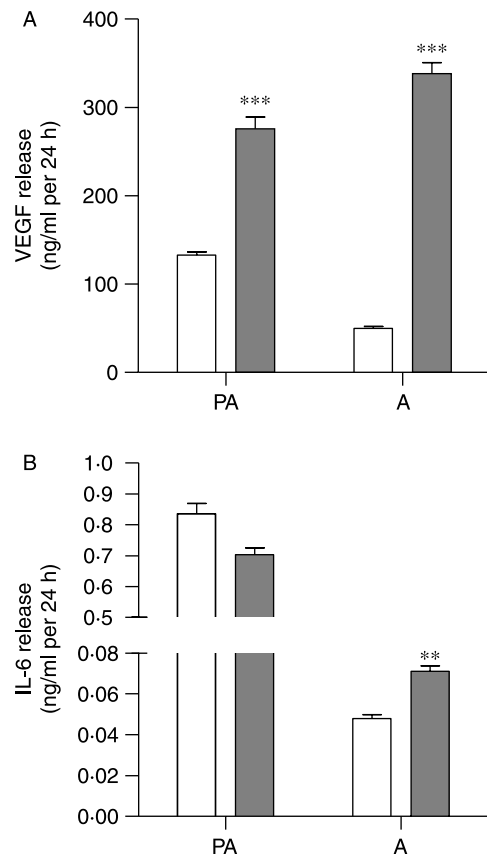


Figure 5 Differential effect of hypoxia on adipokine protein secretion from human preadipocytes and adipocytes. Human preadipocytes (day 0) or adipocytes (day 15 post-differentiation) were subjected to 1% O₂ (filled bars) for 24 h. Control cells (open bars) were maintained at 21% O₂. (A) VEGF and (B) IL-6 release into the culture medium was measured by ELISA. PA, preadipocytes; A, adipocytes. Results are means \pm s.e.m., $n=6$. ** $P<0.01$, *** $P<0.001$ compared with PA normoxic control.

Discussion

The present study has examined the effects of hypoxia on the expression of major adipokine genes in preadipocytes. Leptin is considered not to be produced by preadipocytes, its expression and release only occurring following differentiation into adipocytes, including in human cell systems (Maffei *et al.* 1995, Leroy *et al.* 1996, Wang *et al.* 2005). The present results are consistent with this view; no leptin being detectable in the medium from preadipocytes incubated under normoxia and with only a minimal level of leptin mRNA in the cells. However, incubation under hypoxia resulted in a marked induction of leptin gene expression in the preadipocytes and immunoreactive leptin was readily detectable in the medium. Thus, preadipocytes can be induced to produce leptin in response to low O₂ tension.

Hypoxia has been previously shown to induce the expression of leptin in several other cell types that do not normally synthesize the hormone, such as breast cancer and human trophoblast cell lines (Mise *et al.* 1998, Grosfeld *et al.* 2001, Meissner *et al.* 2005, Cascio *et al.* 2008, Wu *et al.* 2007). The stimulation of leptin production in differentiated adipocytes, both murine and human, by low O₂ tension has been demonstrated in several studies and this was also observed here (Grosfeld *et al.* 2002b, Lolmède *et al.* 2003, Polotsky *et al.* 2003, Hausman & Richardson 2004, Wang *et al.* 2007). Although preadipocytes were induced to synthesize leptin by hypoxia, the amount secreted from these cells was much less than from adipocytes; it is, of course, possible that more prolonged exposure to hypoxia would lead to greater levels of production from preadipocytes.

The present results suggest specifically that preadipocytes could contribute to the overall leptin signal generated in adipose tissue in obesity as a consequence of the induction by hypoxia. Although quantitatively, any contribution from the preadipocytes to the circulating levels of the hormone can be no more than minor, there might be a significant local paracrine role for leptin released from these cells. Hypoxia has recently been shown to occur in adipose tissue in different obese animal models (Hosogai *et al.* 2007, Ye *et al.* 2007, Rausch *et al.* 2008), supporting the proposition that low O₂ tension underlies the inflammatory response in adipose tissue, which leads to the development of obesity-associated diseases (Trayhurn & Wood 2004). Previous studies have focused on the response of mature adipocytes to hypoxia, and the effects of low O₂ tension on the other cell types that are resident in adipose tissue have not been reported.

Preadipocytes are able to mount a substantial inflammatory response (Chung *et al.* 2006, Fain 2006, Lacasa *et al.* 2007). They are highly responsive to lipopolysaccharide with increased expression of a number of inflammation-related cytokines and chemokines, including IL-6, IL-8, and TNF- α , and it is argued that the release of inflammatory signals from these cells is important in triggering the loss of insulin responsiveness in adipocytes (Chung *et al.* 2006). TNF- α

and IL-1 β , which may be released from adipocytes or macrophages within adipose tissue (or from preadipocytes), have been shown to induce leptin expression and secretion from human preadipocytes as part of the inflammatory response (Simons *et al.* 2005), similar to the present results with hypoxia.

Some basal leptin secretion from preadipocytes was evident in this particular study (Simons *et al.* 2005). However, the cells were used at 21 days after confluence, in contrast to our work where the preadipocytes were employed as soon as they became confluent (day '0'). In other studies, we have found, with the same source of preadipocytes (Zen-Bio) maintained until day 15 post-confluence, evidence for some spontaneous, though limited (a few percent of cells exhibiting tiny lipid droplets), differentiation into adipocytes on the basis of Oil Red O staining for lipid (Wang, unpublished observations); this is likely to account for the basal leptin secretion observed by Simons *et al.* (2005). The preadipocytes used here at day '0' did not demonstrate any lipid by Oil red O staining, while >95% of the differentiated cells contained fat droplets.

Additional evidence for the absence of differentiation of the preadipocytes into adipocytes was obtained from the reference genes aP2 and PPAR γ . aP2, in particular, is a strong marker of adipocyte differentiation (Ailhaud 2001), and the level of the mRNA for this gene was higher in the adipocytes than the preadipocytes by a factor of many thousands. PPAR γ mRNA level was also considerably lower in the preadipocytes. We were further concerned to establish whether the preadipocytes that were obtained from a commercial source might be contaminated by macrophages, these being important in the inflammatory response in adipose tissue in obesity (Weisberg *et al.* 2003, Xu *et al.* 2003). The preadipocyte preparations were therefore screened for the expression of two macrophage markers: Mac-1 and F4/80. However, the mRNA of both markers was undetectable, indicating that there was no macrophage contamination. A previous study has confirmed that these same commercial preparations do not contain endothelial cells (Simons *et al.* 2005).

aP2 and PPAR γ mRNA levels were reduced by hypoxia in both human adipocytes and preadipocytes, and previous studies have indicated that PPAR γ gene expression is inhibited by hypoxia in murine adipocytes (Yun *et al.* 2002, Kim *et al.* 2005, Hosogai *et al.* 2007). The inhibition by low O₂ tension in the present study was greater in the adipocytes than preadipocytes. Adipocyte differentiation has been shown to be inhibited by incubation under hypoxic conditions and the reduction in PPAR γ gene expression appears to be a key part of the mechanistic explanation for this (Yun *et al.* 2002, Carriere *et al.* 2004, Kim *et al.* 2005, Zhou *et al.* 2005).

Expression of the facilitative glucose transporter, GLUT-1, and the key angiogenic signal, VEGF, was stimulated by hypoxia in both preadipocytes and adipocytes. These genes are well recognized to be highly hypoxia inducible and their induction by low O₂ tension has been shown previously in adipocytes (Lolmède *et al.* 2003, Hosogai *et al.* 2007, Wang *et al.* 2007, Wood *et al.* 2007, Ye *et al.* 2007). The response to

hypoxia was, however, blunted in the preadipocytes relative to that of adipocytes, particularly for VEGF; this was evident both at the level of gene expression and the secretion of the protein into the medium.

Examination of the expression of three further hypoxia-sensitive adipokines indicated that there are major differences between adipocytes and preadipocytes in response to incubation under low O₂ tension. While Angptl4, IL-6, and PAI-1 mRNA levels were each markedly increased in adipocytes by hypoxia, in agreement with our previous studies on human fat cells (Wang *et al.* 2007), there was no hypoxia-induced modulation of the expression of these genes in preadipocytes. Thus, preadipocytes show a reduced, and more selective, response to hypoxia in terms of gene expression than adipocytes. In preadipocytes, the expression of some genes (such as leptin) is readily induced, others (such as VEGF) are induced but the response is blunted, while others (such as Angptl4 and IL-6) are insensitive to hypoxia. One consequence is that *in vivo* hypoxia would have a more restricted effect on inflammation-related adipokine production in obesity by preadipocytes than adipocytes. Indeed, the induction of leptin production could be seen as a special case; however, expression of the metallothionein-3 gene is readily induced in preadipocytes by hypoxia, as in adipocytes (Wang *et al.* 2008).

Despite the differential effects of hypoxia on gene expression in adipocytes and preadipocytes, both types of cell exhibit recruitment of the HIF-1 α transcription factor subunit, and thus the ability to produce functional HIF-1. Transcription of some of the hypoxia-sensitive genes examined in the present study, particularly GLUT-1 and leptin, is recognized to be HIF-1 dependent through hypoxia response elements (Grosfeld *et al.* 2002a, Rocha 2007, Semenza 2007), and studies with hypoxia mimetics have indicated the involvement of HIF-1 in their expression in adipocytes (Lolmède *et al.* 2003, Wang *et al.* 2007). In the present study, leptin expression in preadipocytes was induced by CoCl₂, indicating that there is functional HIF-1-dependent gene transcription in these cells.

Previous studies in other cellular systems have demonstrated cell-specific responses, with HIF-1 acting either as an activator or a repressor of Angpt1 and Angpt2 gene expression (Kelly *et al.* 2003, Semenza 2007). This is likely to depend on prior programming of the cell, with the presence or absence of other transcriptional regulators of HIF-1 target genes (Kelly *et al.* 2003, Semenza 2007). There are, of course, several transcription factors beyond HIF-1 involved in the intracellular signaling of hypoxia, including NF- κ B and CREB (see Cummins & Taylor 2005). However, the extent to which these other transcription factors are implicated in response to hypoxia in adipocytes, or preadipocytes, is unknown.

In conclusion, the present study has shown that leptin synthesis and release can be readily induced in human preadipocytes by hypoxia. Preadipocytes, however, show more limited responses to low O₂ tension compared with adipocytes, with maturation of the responses to hypoxia

occurring during or after differentiation. Preadipocytes may, nonetheless, contribute to the production of certain adipokines in hypoxic adipose tissue in obesity, and particularly of leptin.

Declaration of Interest

The authors declare that there are no conflicts of interest that would prejudice the impartiality of the work reported herein.

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