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Rapid communication

Hypoxia increases leptin expression in human PAZ6 adipose cells

A. Grosfeld[1], **V. Zilberfarb**[2], **S. Turban**[1], **J. André**[1], **M. Guerre-Millo**[1], **T. Issad**[2, 3]

[1] INSERM U 465, Paris, France
[2] CNRS UPR 415, Cochin Institute of Molecular Genetics, Paris, France
[3] CNRS UPR 415

Abstract

Aims/hypothesis. Leptin, an adipose tissue-derived cytokine involved in the control of body weight, also participates in a variety of biological functions, including angiogenesis. Because reduced oxygen availability is a major inducer of angiogenesis, we hypothesized that low cellular oxygen tension could regulate leptin expression in adipose cells.
Methods. Differentiated PAZ6 adipocytes were cultured for 48 h in the presence of chemical inducers of cellular hypoxia (cobalt chloride or desferrioxamine) or in an atmosphere containing only 6% oxygen. The effect of hypoxia on the expression of leptin and several adipose genes was assessed by semi-quantitative RT-PCR. The effect of hypoxia on leptin promoter activity was tested in PAZ6 cells transiently transfected with a luciferase reporter construct, containing 1.87 kb of the human leptin promoter. Leptin secretion in the culture medium was determined by radioimmunoassay.
Results. Hypoxia increased leptin mRNA expression, leptin promoter activity and leptin secretion in the culture medium by two- to threefold ($p<0.05$). The expression of the glucose transporter isoform 1 (GLUT-1) mRNA, a well known hypoxia inducible gene, was also increased. In contrast, glucose transporter isoform 4 (GLUT-4), hormone sensitive lipase (HSL), fatty acid binding protein (aP2) and uncoupling protein 2 (UCP2) mRNAs were markedly reduced by hypoxia. In addition, a similar hypoxia-induced increase in leptin mRNA and secretion was observed in primary rat adipose cells.
Conclusion/interpretation. Hypoxia markedly and specifically increased leptin gene expression through activation of the leptin gene promoter, and this resulted in an increased leptin production by human PAZ6 adipocytes. [Diabetologia (2002) 45:527–530]

Keywords Leptin, hypoxia, HIF-1, human adipose cells, uncoupling protein 2.

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A. Grosfeld and V. Zilberfarb contributed equally to this work

Coresponding author: T. Issad, CNRS UPR 415, Cochin Institute of Molecular Genetics, Paris, France, e-mail: issad@cochin.inserm.fr
Abbreviations: UCP2, Uncoupling protein 2; aP2, human fatty acid binding protein; HSL, hormone sensitive lipase; GLUT1, glucose transporter 1; GLUT4, glucose transporter 4; Cyclo, cyclophilin; DFO, desferrioxamine; HIF-1, hypoxia-inducible factor-1; HRE, hypoxia responsive element.

Leptin is a protein synthesized and secreted mainly by the adipose tissue, in relation to fat stores. Although the long-term control of adipose mass is one of the major biological function of leptin, this hormone exerts a wide spectrum of effects on non-neuronal cells. Among those, leptin can stimulate endothelial cells and induce the formation of new vessels [1, 2]. Hypoxia is known to be a major inducer of angiogenesis by stimulating the production of angiogenic factors [3]. Thus, it is possible that in a growing fat pad, hypoxic cells could induce vascularization of the tissue through an increase in leptin release. In agreement with this hypothesis, placental leptin expression is increased under hypoxic conditions [4]. However, there is no information available to date on the effect of

hypoxia on leptin production by the adipocyte. Interestingly, increased serum leptin concentrations have been described in humans after 20 h under hypobaric hypoxia at high altitude [5], and in patients with sleep apnea, a hypoxemic syndrome often associated with obesity [6]. Because adipose tissue is quantitatively the main leptin-producing organ, these observations suggest that leptin expression in adipose cells could be stimulated under hypoxic conditions. To test this hypothesis, we used a cellular model of human adipose cells, PAZ6 adipocytes, which express leptin and other adipocyte genes [7].

Materials and methods

Differentiation of human PAZ6 cells and culture conditions were as described previously [7], except that in experiments where leptin mRNA expression or secretion were studied, dexamethasone was removed from the culture medium 48 h before submitting the cells to hypoxic conditions. This markedly reduced basal leptin mRNA expression and allowed a better visualisation of the effects of hypoxia. Chemical hypoxia was induced by adding 100µmol/l of Cobalt chloride ($CoCl_2$) or desferrioxamine (DFO) (Sigma, St Louis, Miss., USA) to the culture medium for 48 h. Culture under low atmospheric oxygen pressure was achieved by placing the culture dishes in a sealed jar containing an oxygen chelator (Oxoid Ltd, Basingstoke, UK) during 48 h. According to the manufacturer, 1 h after sealing the jar, the inside atmosphere contains only 6% O_2.

Total RNA was extracted from PAZ6 cells, as described previously [7]. mRNA expression was assessed by semi-quantitative RT-PCR, using primers described in [7], plus UCP2 (sense 5′TGTGCTGAGCT-GGTGACCTATGAC3′; antisense 5′AAGGGAGCCTCTCGGGAAGTGCAG3′) and GLUT-1 (sense 5′TGCTGGCTGTGGGAGGA3′; antisense 5′GAG-GATGCC-GACGACGAT3′). PCR products were made visible on agarose gels by ethidium bromide staining. As a control, the signals for cyclophilin expression (cyclo) are shown. Leptin concentration in the culture medium was measured by radioimmunoassay (Linco Research, St.Charles, Miss., USA).

Transient transfections of PAZ6 cells were done 9 days after initiation of differentiation, using the calcium phosphate precipitation method with a luciferase reporter construct (5 µg) containing –1.87 kb upstream of the transcription initiation site of the human leptin gene (kind gift of J. Auwerx) and a pRSV-β-galactosidase expression vector (0.5 µg). The fold increase in luciferase normalized to beta-galactosidase activity was at least 30-fold higher than that measured in cells transfected with the promoter-less control vector.

Rat adipose cells were isolated from epididymal adipose tissue by the collagenase method and cultured for 24 h in triplicate, in the absence or presence of 100 µmol/l DFO or $CoCl_2$.

Each hypoxic condition was compared to basal, using the Student's *t* test for paired values. A *p* value of less than 0.05 was considered to be statistically significant.

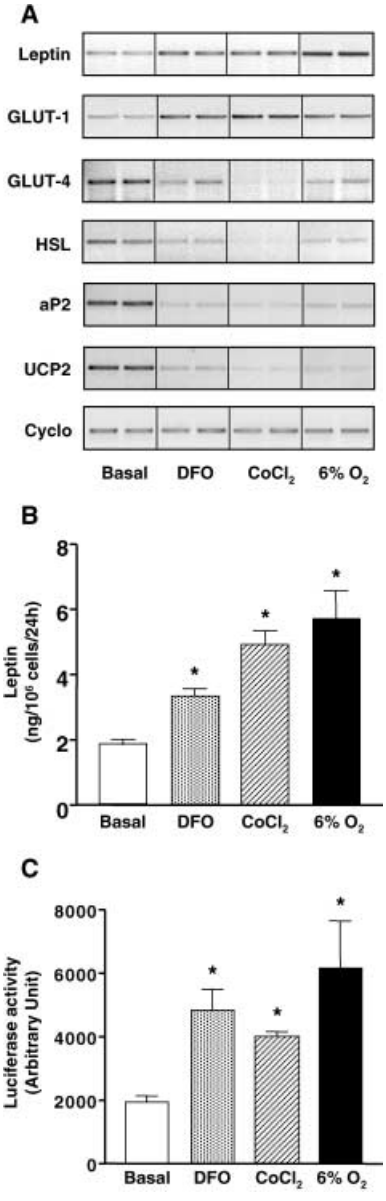

Fig. 1A–C. Effect of hypoxia on the expression of adipocyte markers, leptin release and leptin promoter activity in human PAZ6 cells. PAZ6 cells were incubated for 48 h under normoxic (Basal) or hypoxic ($CoCl_2$, DFO or 6%O_2) conditions. **A** Duplicated culture wells were extracted independently and semi-quantitative RT-PCR was carried out on total RNA. The results are representative of three to seven independent experiments. **B** Leptin secreted in the culture medium was measured by radioimmunoassay. Data are the means ± SEM for three to seven experiments. **C** Cells were transfected with a human leptin promoter reporter construct (–1.87 kb) and a pRSV-beta-galactosidase expression vector. Data represent luciferase normalized to beta-galactosidase activity and are the means ± SEM for three to four experiments. *p<0.05 when compared to basal using Student's *t* test for paired values

Results

Leptin mRNA expression was markedly increased by chemical hypoxia and by culture under low oxygen pressure (Fig. 1A). An increase in GLUT-1 mRNA expression was also observed. In sharp contrast, the expression of GLUT-4, HSL, aP2 and UCP2 was markedly decreased. The stimulation of leptin mRNA expression resulted in a two- to threefold increase in the amount of leptin secreted in the culture medium (Fig. 1B).

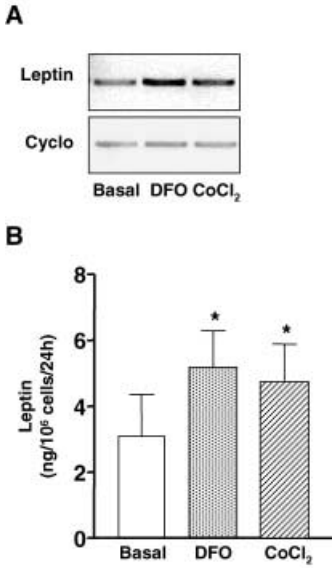

Fig. 2A, B. Effect of hypoxia on leptin gene expression and release in epididymal rat adipose cells. Epididymal rat adipose cells were cultured for 24 h under normoxic (Basal) or hypoxic ($CoCl_2$, DFO) conditions. **A** Semi-quantitative RT-PCR was done on total RNA. The results are representative of three independent experiments. **B** Leptin secreted in the culture medium was measured by radioimmunoassay. Data are the means ± SEM for three independent experiments done in triplicate. *$p<0.05$ when compared to basal using Student's *t* test for paired values

To determine whether hypoxia can directly increase the transcriptional activity of the leptin gene, PAZ6 cells were transfected with a luciferase cDNA under the control of a fragment (1.87 kb) of the human leptin promoter. We observed that the luciferase activity driven by this promoter was stimulated by two to threefold under hypoxic conditions (Fig. 1C).

The effect of hypoxia on leptin expression was also tested in another adipose cell system. Freshly isolated rat epididymal adipose cells were cultured in presence of DFO or $CoCl_2$ for 24 h. The data showed that chemical hypoxia increased leptin mRNA expression (Fig. 2A) and secretion by twofold (Fig. 2B). A similar effect was observed in retroperitoneal adipose cells from the same rats (data not shown).

Discussion

Human immortalised PAZ6 cells have proven to constitute an appropriate model for the study of the regulation of adipose gene expression, including leptin [7]. In addition, the rate of leptin release in PAZ6 cells is in the same order of magnitude as in human primary adipose cells [8].

In this study, we observed that hypoxia markedly stimulates leptin expression in PAZ6 cells, as well as the expression of GLUT-1, a ubiquitous hypoxia-inducible gene. Activation of hypoxia-inducible genes is generally mediated by stabilisation of the hypoxia-inducible factor-1 (HIF-1). This process involves HIF-1 hydroxylation by a prolyl-4-hydroxylase activity [3]. Transcription of hypoxia-inducible genes is regulated by hypoxia response elements (HRE) which contain binding sites for HIF-1 [3]. The 1.87 kb of the human leptin promoter fragment used in this study contains a consensus HRE motif (5′-RCGTG-3′) conserved among several hypoxia-inducible genes [3]. This observation favours the involvement of HIF-1 in the induction of leptin gene by hypoxia in human adipocytes.

In addition to increasing leptin and GLUT-1 expression, hypoxic culture conditions resulted in a reduction in the expression of several genes (GLUT-4, HSL, aP2 and UCP2). This indicates that the stimulatory effect of hypoxia on leptin and GLUT-1 mRNA expression is highly specific. Besides HIF-1 proline hydroxylation, another pathway, which involves the production of reactive oxygen species (ROS), has been proposed as an alternative mechanism for oxygen sensing and HIF-1 regulation [3]. Therefore, inhibition of UCP2 expression, which is known to be an important regulator of ROS production [9], could play a role in hypoxia signalling. On the other hand, cellular hypoxia also evokes inflammatory responses, and a decrease in UCP2 expression could participate in these processes.

Activation of leptin gene expression by hypoxia clearly resulted in an increase in leptin release by PAZ6 cells. This effect was not restricted to this cellular model, but extends to primary adipose cells. What could be the physiological significance of hypoxia-mediated induction of leptin secretion by the adipose cell? Because leptin has been shown to be an angiogenic factor [1], [2], a local effect of the cytokine could be to stimulate the development of new vessels during normal or pathological adipose tissue growth. Therefore, increased leptin production under hypoxic conditions would reflect an adaptive mechanism that promotes angiogenesis, allowing proper oxygenation of the adipose tissue. However, it has been shown recently that leptin induces angiopoietin-2 expression in adipose tissue, suggesting that locally, leptin has an angiostatic rather than an angiogenic effect [10]. Thus, hypoxic cells, through the release of leptin, could induce a feed-back mechanism that will oppose to further adipose tissue growth by inhibiting vascularization of the adipose pad (through local angiopoietin-2 production) while promoting vascularization of other tissues.

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References

1. Sierra-Honigmann MR, Nath AK, Murakami C et al. (1998) Biological action of leptin as an angiogenic factor. Science 281: 1683–1686
2. Bouloumie A, Drexler HC, Lafontan M, Busse R (1998) Leptin, the product of Ob gene, promotes angiogenesis. Circ Res 83: 1059–1066
3. Zhu H, Bunn HF (2001) Signal transduction: How Do Cells Sense Oxygen? Science 292: 449–451
4. Grosfeld A, Turban S, Andre J et al. (2001) Transcriptional effect of hypoxia on placental leptin. FEBS Lett 502: 122–126
5. Tschop M, Strasburger CJ, Hartmann G, Biollaz J, Bartsch P (1998) Raised leptin concentrations at high altitude associated with loss of appetite. Lancet 352: 1119–1120
6. Ip MS, Lam KS, Ho C, Tsang KW, Lam W (2000) Serum leptin and vascular risk factors in obstructive sleep apnea. Chest 118: 580–586
7. Zilberfarb V, Siquier K, Strosberg AD, Issad T (2001) Effect of dexamethasone on adipocyte differentiation markers and tumour necrosis factor-alpha expression in human PAZ6 cells. Diabetologia 44: 377–386
8. Wabitsch M, Jensen PB, Blum WF et al. (1996) Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45: 1435–1438
9. Arsenijevic D, Onuma H, Pecqueur C et al. (2000) Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat Genet 26: 435–439
10. Cohen B, Barkan D, Levy Y et al. (2001) Leptin induces angiopoietin-2 expression in adipose tissues. J Biol Chem 276: 7697–7700