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Modest hypoxia significantly reduces triglyceride content and lipid droplet size in 3T3-L1 adipocytes

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ABSTRACT

Background: A previous study has demonstrated that endurance training under hypoxia results in a greater reduction in body fat mass compared to exercise under normoxia. However, the cellular and molecular mechanisms that underlie this hypoxia-mediated reduction in fat mass remain uncertain. Here, we examine the effects of modest hypoxia on adipocyte function.
Methods: Differentiated 3T3-L1 adipocytes were incubated at 5% O_2 for 1 week (long-term hypoxia, HL) or one day (short-term hypoxia, HS) and compared with a normoxia control (NC).
Results: HL, but not HS, resulted in a significant reduction in lipid droplet size and triglyceride content (by 50%) compared to NC ($p < 0.01$). As estimated by glycerol release, isoproterenol-induced lipolysis was significantly lowered by hypoxia, whereas the release of free fatty acids under the basal condition was prominently enhanced with HL compared to NC or HS ($p < 0.01$). Lipolysis-associated proteins, such as perilipin 1 and hormone-sensitive lipase, were unchanged, whereas adipose triglyceride lipase and its activator protein CGI-58 were decreased with HL in comparison to NC. Interestingly, such lipogenic proteins as fatty acid synthase, lipin-1, and peroxisome proliferator-activated receptor gamma were decreased. Furthermore, the uptake of glucose, the major precursor of 3-glycerol phosphate for triglyceride synthesis, was significantly reduced in HL compared to NC or HS ($p < 0.01$).
Conclusion: We conclude that hypoxia has a direct impact on reducing the triglyceride content and lipid droplet size via decreased glucose uptake and lipogenic protein expression and increased basal lipolysis. Such an hypoxia-induced decrease in lipogenesis may be an attractive therapeutic target against lipid-associated metabolic diseases.

1. Introduction

Excessive lipid accumulation in adipocytes is a central feature of obesity and metabolic syndrome. The excess energy is primarily stored as triacylglycerol (TAG) in the lipid droplets (LDs) of mammalian adipose tissue; when the stored energy is required, such as during starvation and exercise, the TAG reserves are hydrolyzed to supply fatty acids (FAs) to various tissues by a process called lipolysis. Thus, physiological strategies, such as exercise training aimed at fat loss through active lipolysis in adipocytes (i.e., fat mobilization) and FA oxidation in muscles (i.e., fat utilization), have become the preferred therapy against metabolic disorders.

Wiesner et al. previously demonstrated that endurance training under a hypoxic condition resulted in a greater reduction in body fat mass in comparison to exercise under a normoxic condition [1]. However, the cellular and molecular mechanisms that underlie this reduced fat mass by hypoxia remain uncertain.

By interacting with one another, LD-associated proteins, such as perilipin, hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), and its co-activator comparative gene identification (CGI)-58 (also called α,β-hydrolase domain-containing [ABHD] 5), are purported to play important roles in regulating fat storage and mobilization [2–7]. In brief, a significant amount of FAs produced by TAG hydrolysis is re-esterified into TAG, leading to the formation of micro-LDs (mLDs) at the endoplasmic reticulum (ER), even during active lipolysis. mLDs are coated with perilipin (peri), HSL, CGI-58 (CGI), and ATGL and are the active sites of lipolysis [5]. Indeed, our recent paper investigated the effects of exercise-inducible factors, including reactive oxygen species (ROS), lactate-inducible signals, increased cellular calcium levels, AMP-activated protein kinase (AMPK), and nitric oxide (NO), on the levels of LD-associated proteins to assess the putative factors that are induced by exercise training to activate lipolysis in differentiated

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3T3-L1 adipocytes. We demonstrated that lipolytic activity was increased by exercise-inducible factors in accordance with the elevated expression of LD-associated proteins [8]. Accordingly, we hypothesized that hypoxia in adipose tissue could enhance lipolytic activity by increasing the levels of LD-associated proteins, thereby diminishing lipid storage in adipocytes.

However, it has been reported that adipose tissue becomes hypoxic in obesity as the tissue mass expands and clusters of adipocytes become distant from the vasculature [9–11]. Trayhurn's group published reports that O_2 tension within white adipose tissue (WAT) is 48 mm Hg in lean mice and 55 mm Hg in humans of normal weight, which is equivalent to 6–7% O_2, and is 15 mm Hg in obese mice, which is equivalent to 2% O_2 [10,12]. Indeed, the hypoxia marker hypoxia inducible factor 1α (HIF-1α) is increased in obese mice [13,14]. Furthermore, the expression and release of adiponectin, which has both anti-inflammatory and insulin-sensing actions, is reduced in response to hypoxia, whereas such inflammation-associated genes as interleukin-6 (IL-6), leptin, and vascular endothelial growth factor (VEGF) are up-regulated [12,15]. Hypoxia also inhibits insulin signaling in adipocytes, which would be associated with the establishment of an insulin-resistant state during obesity [16]. These responses suggest that hypoxia may lead to the metabolic and functional maladaptation of adipose tissue, a notion that contradicts our hypothesis that hypoxia might elicit augmented LD-associated proteins, hence increasing lipolytic activity and diminishing lipid storage in adipose tissue.

Elucidating the cellular and molecular mechanisms that underlie the reduction in fat mass under hypoxia is very important for the application of endurance exercise programs under hypoxia to obese patients. Thus, the aim of this study was to provide insight into the cellular and molecular mechanisms underlying the effects of hypoxia on the remodeling of adipocytes, mainly in terms of basal and catecholamine-stimulated lipolytic activity based on the levels of LD-associated proteins. Because cell culture studies in hypoxic adipocytes have compared cells at 21% O_2 with those at 1% O_2, which represents relatively severe hypoxia [12], we investigated the effects of incubation at 5% O_2, which is slightly less than the O_2 tension within WAT and hence may represent physiological hypoxia, as short-term (24 h) and long-term (1 week) hypoxia in 3T3-L1 adipocytes.

2. Materials and methods

2.1. Cell culture

All reagents for cell culturing were obtained from Wako (Osaka, Japan), unless otherwise indicated. The cell culture procedure was the same as in our previous report [5] (see Supplemental materials).

2.2. Experimental design

At 5 days after the induction of differentiation, the 3T3-L1 adipocytes were divided into three groups. In the normoxia control (NC) group, the cells were incubated normally with the growth medium at 21% O_2 for 1 week. The cells in the long-term hypoxia (HL) group were incubated with the growth medium at 5% O_2 for 1 week. In the short-term hypoxia (HS) group, the cells were incubated with the growth medium at 21% O_2 for 6 days and then 5% O_2 for one day. At day 12, the cells in all groups were used for biochemical analyses.

2.3. Lipolytic stimulation

For the biochemical studies, lipolytic stimulation was applied to differentiated 3T3-L1 adipocytes, as in our previous report [5] (see Supplemental materials).

2.4. Optical microscopy

All reagents for microscopy were obtained from Wako (Osaka, Japan), unless otherwise indicated. Differentiated 3T3-L1 cells

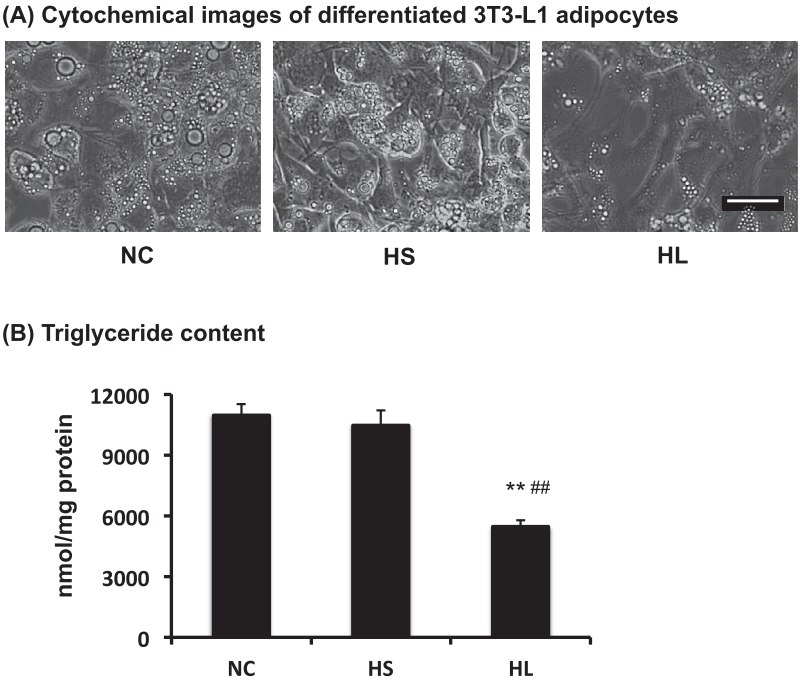

Fig. 1. The effects of hypoxia on lipid storage in 3T3-L1 adipocytes images of the optical microscopy (A) and TAG content (B) are shown. Well-differentiated 3T3-L1 adipocytes in the NC group possessed abundant, large LDs, whereas numerous small LDs were observed in the HS group; in the HL group, the cells possessed a small number of small-sized LDs. The TAG content in the HL group was significantly decreased compared to the NC group and HS group. $^{**}p < 0.01$ vs. NC; $^{\#\#}p < 0.01$ vs. HS. Bar, 100 μm.

were fixed with PBS containing 3.7% paraformaldehyde (PFA) for 15 min at room temperature. To assess cell viability, the differentiated 3T3-L1 cells were stained with trypan blue dye, according to the manufacturer's instructions, and then fixed with PBS containing 3.7% PFA for 15 min at room temperature. After washing with PBS, the cells were mounted and observed using light and fluorescence microscopy (Biozero, Keyence, Osaka, Japan) [5].

2.5. Western blotting

3T3-L1 cells were washed with PBS and directly dissolved in heated SDS–PAGE sample buffer [5]. Aliquots of the extracts were subjected to SDS–PAGE and transferred to a nitrocellulose membrane. The blots were probed with an antibody (see Supplemental materials). The signals were detected using the ECL method (GE Healthcare Life Science, Fairfield, CT, USA).

2.6. Measurement of triacylglycerol (TAG) storage

3T3-L1 cells were grown in 12-well dishes. Differentiated cells at day 12 were washed twice with Hank's buffer and harvested in a lysis buffer (50 mM Tris–HCl [pH 7.4], 150 mM NaCl, 2 mM EDTA, and 1% Triton X-100). TAG was measured using a TG E-test kit (Wako) [5].

2.7. Release of glycerol and FAs

Lipolytic activity was analyzed in differentiated 3T3-L1 adipocytes as in our previous report [5] (see Supplemental materials).

2.8. Measurement of glucose uptake

3T3-L1 cells were grown in 12-well dishes. Differentiated cells were washed twice with Hank's buffer and incubated with DMEM containing 2% fatty acid-free BSA/20 mM HEPES-NaOH (pH 7.4) at 37 °C for 16 h. After incubation, aliquots of the medium were collected and assayed for the glucose content using a Glucose II-test kit (Wako, Osaka, Japan). Glucose uptake was calculated as follows:

$$\text{Glucose uptake}(\text{mg/dl/h}) = (\text{GC}_{\text{fresh}} - \text{GC}_{\text{incubation}})/16,$$

where GCfresh is the glucose concentration in the fresh medium and GCincubation is the glucose concentration in the incubated medium.

2.9. Statistical analysis

The differences between the groups were assessed by a one-way analysis of variance (ANOVA). A Bonferroni/Dunn post hoc test was used in the event of a significant ($p < 0.05$) ratio. All the results are presented as the means ± S.E.M.

3. Results

3.1. Long-term hypoxia decreased the size of LDs and lipid storage in 3T3-L1 adipocytes

We first examined whether long- or short-term hypoxia decreased lipid storage in 3T3-L1 adipocytes. As shown in Fig. 1A, well-differentiated 3T3-L1 adipocytes in the NC group possessed abundant, large LDs, whereas numerous small LDs were observed in the HS group. The most striking observation was that the 3T3-L1 adipocytes in the HL group possessed few LDs and that those LDs were small. Actually, the TAG content in the HL group was significantly decreased compared to the NC and HS groups ($p < 0.01$) (Fig. 1B). Cell viability of the NC, HS, and HL groups was 86.4 ± 1.7%, 79.6 ± 2.9%, and 78.8 ± 1.7%, respectively, with no significant differences among the groups.

3.2. Long-term hypoxia increased basal lipolysis in 3T3-L1 adipocytes

Given the decrease in lipid storage due to long-term hypoxia, we hypothesized that long-term hypoxia would increase basal and/or catecholamine-stimulated lipolytic activity. Glycerol

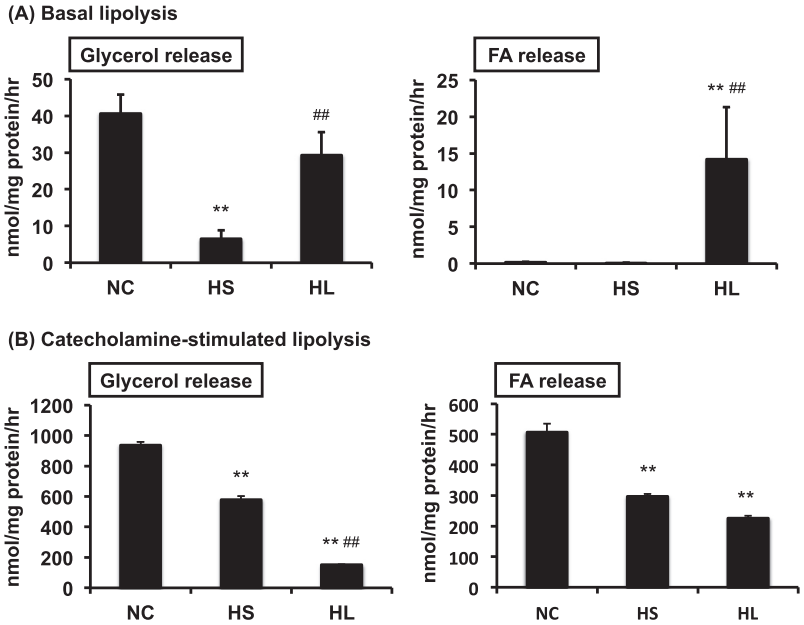

Fig. 2. The effects of hypoxia on basal and catecholamine-stimulated lipolysis Glycerol and FA release into the medium were measured under the basal condition (A) and catecholamine-stimulated condition (B). (A) Glycerol release in the HS group was significantly decreased compared to the NC and HL groups under the basal condition. FA release in the HL group was significantly increased compared to the NC and HS groups under the basal condition. (B) Glycerol release in the HS group was significantly decreased compared to the NC group, and that in the HL group was significantly decreased compared to the NC and HS groups under the catecholamine-stimulated condition. FA release in the HS and HL groups was significantly decreased compared to the NC group under the catecholamine-stimulated condition. $^{**}p < 0.01$ vs. NC; $^{\#\#}p < 0.01$ vs. HS.

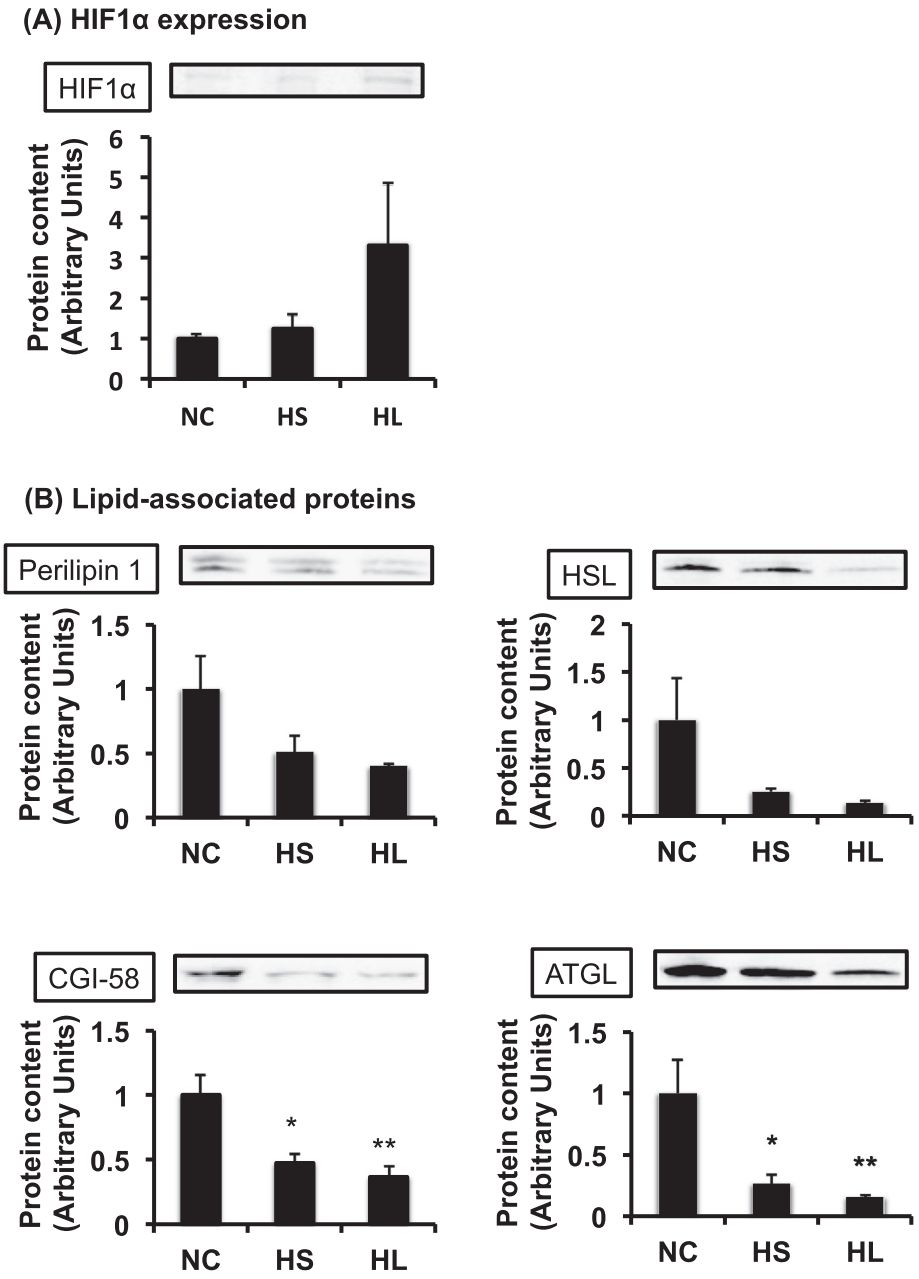

Fig. 3. The effects of hypoxia on the expressions of HIF1α and lipid-associated proteins (A) HIF1α expression is shown. Long-term hypoxia tended to increase the expression of HIF1α. (B) The levels of perilipin 1, HSL, CGI-58, and ATGL were measured by western blotting. Short- and long-term hypoxia tended to decrease the levels of perilipin 1 and HSL and significantly decreased the levels of CGI-58 and ATGL. $^{**}p < 0.05$, $^{**}p < 0.01$ vs. NC.

release and FA release into the medium were measured for cells incubated with a growth medium for 16 h (i.e., basal condition). The amount of glycerol release, which represents total lipolytic activity in adipocytes because glycerol produced during lipolysis is not efficiently phosphorylated and used for TAG synthesis due to the poor activity of glycerol kinase in adipocytes [17,18], was significantly decreased in the HS group (Fig. 2A). Conversely, a significant amount of FA release was obtained in the HL group, whereas little FA release was measured in the NC and HS groups (Fig. 2A).

Catecholamine-stimulated lipolysis demonstrated that short-term hypoxia significantly decreased glycerol release when compared to the NC group, and long-term hypoxia further decreased glycerol release (Fig. 2B). Similarly, FA release in the NC group was significantly higher than that in the HS and HL groups (Fig. 2B).

3.3. Hypoxia decreased lipid-associated proteins in 3T3-L1 adipocytes

By interacting with each other, lipid-associated proteins, such as perilipin 1, HSL, ATGL, and CGI-58, are purported to play important roles in regulating fat storage and mobilization [2–7]. Therefore, we analyzed the expression levels of lipid-associated proteins. Initially, we assessed HIF-1α expression and found that long-term hypoxia tended to increase the level of this protein (Fig. 3A). Further analyses demonstrated that short- or long-term hypoxia tended to decrease the levels of perilipin 1 and HSL and significantly decrease the levels of CGI-58 and ATGL (Fig. 3B).

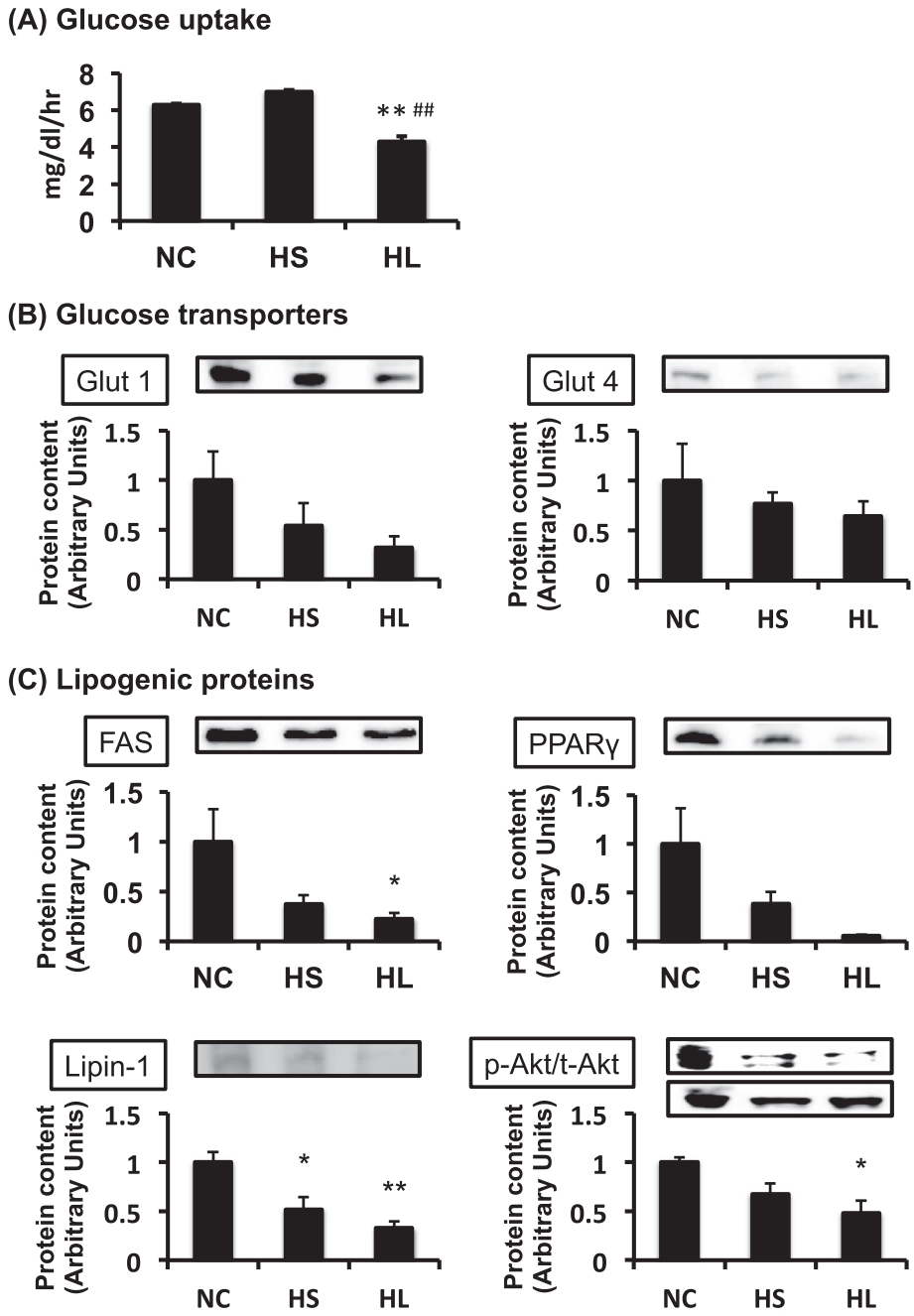

Fig. 4. The effects of hypoxia on basal glucose uptake, glucose transporters, and the expressions of lipogenic proteins (A) The glucose uptake under the basal condition is shown. Long-term hypoxia significantly decreased glucose uptake under the basal condition. (B) The levels of Glut 1 and Glut 4 are shown. Hypoxia tended to decrease the levels of glucose transporters. (C) The levels of FAS, PPAR-γ, and lipin-1 and the p-Akt/t-Akt ratio are shown. Long-term hypoxia significantly decreased the levels of FAS and lipin-1 and the p-Akt/t-Akt ratio. Hypoxia tended to decrease the levels of PPAR-γ. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. NC; $^{\#\#}p < 0.01$ vs. HS.

3.4. Hypoxia decreased basal glucose uptake and lipogenic proteins in 3T3-L1 adipocytes

We next examined whether hypoxia decreased *de novo* lipogenesis, thereby decreasing lipid storage by measuring glucose uptake under the basal condition, as glucose is a precursor of glycerol-3-phosphate. As expected, long-term hypoxia significantly decreased glucose uptake under the basal condition (Fig. 4A), and the expression of Glut 1 and Glut 4 tended to decrease (Fig. 4B). In addition, we assessed the expression levels of lipogenic proteins [19–21] and found that long-term hypoxia significantly decreased the levels of FAS and lipin-1 and the p-Akt/t-Akt ratio (Fig. 4C); hypoxia similarly tended to decrease the levels of PPAR-γ (Fig. 4C).

4. Discussion

A previous study has demonstrated that endurance training under hypoxia resulted in greater reduction in body fat mass compared to exercise under normoxia [1]. Accordingly, we hypothesized that hypoxia in adipose tissue could enhance lipolytic activity by increasing the levels of LD-associated proteins, thereby diminishing lipid storage in adipocytes. We, for the first time, found that incubation with 5% O_2 for 1 week prominently reduced the lipid droplet size and triglyceride content (by 50%) in 3T3-L1 adipocytes compared to normoxia, whereas the levels of such LD-associated proteins as perilipin, CGI-58, HSL, and ATGL significantly or tended to decrease with long-term hypoxia. As a consequence, catecholamine-stimulated lipolysis was significantly

decreased by long- and/or short-term hypoxia. Under the basal condition, glycerol release in response to long-term hypoxia was restored to the normoxic control levels, whereas FA release was drastically increased in response to long-term hypoxia. Because augmented FA release from adipose tissue is a risk factor for FA elevation in the circulation, which contributes to systemic insulin resistance in obesity [14,16], augmented FA release under the basal condition by long-term hypoxic exposure might be implicated in the development of insulin resistance in adipocytes.

The glycerol produced by lipolysis in adipocytes is not efficiently reused as the partner of FA re-esterification but is largely released from the cells due to the poor activity of glycerol kinase, which is essential for the production of glycerol 3-phosphate, a key substrate for TAG synthesis [17,18]. Therefore, glycerol release into the medium represents the lipolytic activity of adipocytes [5]. Under the basal condition, glycerol release was significantly decreased by short-term (24 h) hypoxia, a result that does not agree with previous studies in which glycerol release was significantly increased in response to short-term (16 h or 24 h) hypoxia in 3T3-L1 adipocytes [14,16]. The possible reason for the difference in glycerol release in response to short-term hypoxia may be that the present study used 5% O_2, whereas the previous studies used 1% O_2, a level that represents relatively severe hypoxia [12]. Glycerol release in response to long-term hypoxia, however, was not different from that of the normoxic control. The level of perilipin 1 expression might mediate basal lipolysis at the molecular signaling level [2,22]. In the present study, we found that perilipin 1 expression tended to decrease in response to both long-term and short-term hypoxia, concomitant with the decrease in HSL and ATGL, pivotal lipases in adipocytes. Hence, it is difficult to clearly explain the restored lipolytic activity in response to long-term hypoxia in terms of molecular signaling.

Although glycerol release into the medium, which represents the lipolytic activity of adipocytes, did not decrease under the basal condition, FA release was drastically elevated in response to long-term hypoxia. We previously found that FAs are in large part re-esterified under typical lipolytic conditions and are released into the medium when re-esterification is blocked [5]. In the present experimental setting, glycerol 3-phosphate, the partner of FA re-esterification and the key substrate for TAG synthesis, is continuously supplied *de novo*, mostly through glycolysis but not by lipolysis at the intracellular LDs. In this study, glucose uptake was reduced in response to long-term hypoxia, despite no statistical changes in the expression of Glut 1 and Glut 4. This result can be interpreted that the supply of glycerol 3-phosphate decreased due to reduced glucose uptake, resulting in reduced FA re-esterification and increased FA release into the medium.

It is known that endurance training under hypoxia results in a greater reduction in body fat mass compared to exercise under normoxia [1]. Thus, we expected that catecholamine-stimulated lipolysis might be enhanced concomitant with increased levels of LD-associated proteins. However, catecholamine-stimulated glycerol and FA release was significantly decreased in response to short-term and long-term hypoxia, with reduced protein expression of such LD-associated proteins as CGI-58 and ATGL. Our recent paper demonstrated that lipolytic activity was increased by exercise-inducible factors, including ROS, lactate-inducible signals, increased cellular calcium levels, AMPK, and NO, in accordance with the elevated expression of LD-associated proteins [8]. Although it is not evident that hypoxia is an "exercise-inducible factor" in adipocytes, we show for the first time that hypoxia reduces LD-associated proteins, thereby decreasing catecholamine-stimulated lipolysis. Conceivably, hypoxia may augment whole-body glucose metabolism, in particular, increasing glucose transport into skeletal muscle, whereas fatty acid utilization is compromised [23–25]. Carbohydrates are thought to be the preferred fuel in hypoxia due to the higher yield of energy per mole of oxygen: the energy equivalent of oxygen is 18.7 kJ/l, 19.6 kJ/l, and 21.1 kJ/l for protein, fat, and carbohydrate, respectively [26]. Indeed, a low content of intramyocellular LDs was found in skeletal muscle biopsies from high-elevation residents, suggesting a shift in muscle metabolism toward a preferred reliance on carbohydrates as substrates [27,28].

It is likely that hypoxia decreased *de novo* lipogenesis, thereby decreasing lipid storage. Again, long-term hypoxia significantly decreased glucose uptake under the basal condition. In addition, long-term hypoxia significantly decreased the levels of FAS and lipin-1 and the p-Akt/t-Akt ratio and tended to decrease the levels of PPAR-γ. Mylonis et al. demonstrated that the exposure of human cells to 1% O_2 for 48 h caused TAG and LD accumulation via the increased expression of lipin-1, which might enhance the conversion of phosphatidic acid to diacylglycerol [20]. A possible reason for the opposite response with regard to the expression of lipin-1 in response to hypoxia may be that the present study used 5% O_2, whereas the previous studies used 1% O_2. In addition, the previous study used non-adipocyte cells, whereas the present study used 3T3-L1 adipocytes, and the metabolic response to hypoxia could be different between these cells. A limitation of this study is that we are not able to exclude the possibility that hypoxia inhibits adipocyte differentiation, thereby decreasing lipogenesis and TAG storage. In fact, Kim et al. demonstrated that hypoxia attenuated adipocyte differentiation by inhibiting PPAR-γ expression [29], and hypoxic exposure in the present study also tended to decrease PPAR-γ expression. Overall, the present results suggest that hypoxia might decrease lipogenesis in adipocytes.

We conclude that hypoxia would have a direct impact on reduced triglyceride content and lipid droplet size by decreasing glucose uptake and the expression of lipogenic proteins and increasing basal lipolysis. However, as augmented FA release from adipose tissue is a risk factor for FA elevation in the circulation, which contributes to systemic insulin resistance in obesity [14,16], augmented FA release under the basal condition by long-term hypoxic exposure might be implicated in the development of insulin resistance in adipocytes. Therefore, the adaptation of adipocytes to a current hypoxic condition *per se* may not be beneficial physiologically and pathologically. Nonetheless, the beneficial adaptation of exercise training under hypoxia is not restricted to adipose tissue but extends to other metabolically crucial tissues, such as skeletal muscle and the liver [30–33]. In addition, exercise and its inducible factors could in turn elicit favorable responses in adipocytes (i.e., increased lipolysis-associated proteins and lipolytic activity) [8]. Further *in vivo* studies using exercise training under hypoxia are warranted to understand the beneficial effects of hypoxia-induced decreases in lipogenesis in a more complex systemic whole body and for controlling obesity.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.09.034.

References

[1] S. Wiesner, S. Haufe, S. Engeli, H. Mutschler, U. Haas, F.C. Luft, J. Jordan, Influences of normobaric hypoxia training on physical fitness and metabolic

risk markers in overweight to obese subjects, Obesity (Silver Spring) 18 (2010) 116–120.
[2] D.L. Brasaemle, Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis,, J. Lipid Res. 48 (2007) 2547–2559.
[3] J.G. Granneman, H.P. Moore, Location, location: protein trafficking and lipolysis in adipocytes, Trends Endocrinol. Metab. 19 (2008) 3–9.
[4] J.G. Granneman, H.P. Moore, R. Krishnamoorthy, M. Rathod, Perilipin controls lipolysis by regulating the interactions of AB-hydrolase containing 5 Abhd5) and adipose triglyceride lipase (Atgl), J. Biol. Chem. 284 (2009) 34538–34544.
[5] T. Hashimoto, H. Segawa, M. Okuno, H. Kano, H.O. Hamaguchi, T. Haraguchi, Y. Hiraoka, S. Hasui, T. Yamaguchi, F. Hirose, T. Osumi, Active involvement of micro-lipid droplets and lipid droplet-associated proteins in hormone-stimulated lipolysis in adipocytes, J. Cell Sci. 125 (24) (2012) 6127–6136.
[6] T. Yamaguchi, Crucial role of CGI-58/alpha/beta hydrolase domain-containing protein 5 in lipid metabolism, Biol. Pharm. Bull. 33 (2010) 342–345.
[7] R. Zechner, P.C. Kienesberger, G. Haemmerle, R. Zimmermann, A. Lass, Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores, J. Lipid Res. 50 (2009) 3–21.
[8] T. Hashimoto, K. Sato, M. Iemitsu, Exercise-inducible factors to activate lipolysis in adipocytes, J. Appl. Physiol. 115 (2) (2013) 260–267.
[9] K. Sun, C.M. Kusminski, P.E. Scherer, Adipose tissue remodeling and obesity, J. Clin. Invest. 121 (2011) 2094–2101.
[10] P. Trayhurn, Hypoxia and adipose tissue function and dysfunction in obesity, Physiol. Rev. 93 (2013) 1–21.
[11] J. Ye, Emerging role of adipose tissue hypoxia in obesity and insulin resistance, Int. J. Obes. (Lond) 33 (2009) 54–66.
[12] I.S. Wood, T. Stezhka, P. Trayhurn, Modulation of adipokine production, glucose uptake and lactate release in human adipocytes by small changes in oxygen tension, Pflugers Arch. 462 (2011) 469–477.
[13] Q. He, Z. Gao, J. Yin, J. Zhang, Z. Yun, J. Ye, Regulation of HIF-1{alpha} activity in adipose tissue by obesity-associated factors: adipogenesis, insulin, and hypoxia, Am. J. Physiol. Endocrinol. Metab. 300 (2011) E877–885.
[14] J. Yin, Z. Gao, Q. He, D. Zhou, Z. Guo, J. Ye, Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue, Am. J. Physiol. Endocrinol. Metab. 296 (2009) E333–342.
[15] N. Hosogai, A. Fukuhara, K. Oshima, Y. Miyata, S. Tanaka, K. Segawa, S. Furukawa, Y. Tochino, R. Komuro, M. Matsuda, I. Shimomura, Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation, Diabetes 56 (2007) 901–911.
[16] C. Regazzetti, P. Peraldi, T. Gremeaux, R. Najem-Lendom, I. Ben-Sahra, M. Cormont, F. Bost, Y. Le Marchand-Brustel, J.F. Tanti, S. Giorgetti-Peraldi, Hypoxia decreases insulin signaling pathways in adipocytes, Diabetes 58 (2009) 95–103.
[17] S.N. Leroyer, J. Tordjman, G. Chauvet, J. Quette, C. Chapron, C. Forest, B. Antoine, Rosiglitazone controls fatty acid cycling in human adipose tissue by means of glyceroneogenesis and glycerol phosphorylation, J. Biol. Chem. 281 (2006) 13141–13149.
[18] L. Reshef, Y. Olswang, H. Cassuto, B. Blum, C.M. Croniger, S.C. Kalhan, S.M. Tilghman, R.W. Hanson, Glyceroneogenesis and the triglyceride/fatty acid cycle, J. Biol. Chem. 278 (2003) 30413–30416.
[19] T. Hashimoto, Y. Endo, Cyclic restricted feeding enhances lipid storage in 3 T3–L1 adipocytes, Lipids Health Dis. 12 (2013) 76.
[20] I. Mylonis, H. Sembongi, C. Befani, P. Liakos, S. Siniossoglou, G. Simos, Hypoxia causes triglyceride accumulation by HIF-1-mediated stimulation of lipin 1 expression, J. Cell Sci. 125 (2012) 3485–3493.
[21] J.L. Yecies, H.H. Zhang, S. Menon, S. Liu, D. Yecies, A.I. Lipovsky, C. Gorgun, D.J. Kwiatkowski, G.S. Hotamisligil, C.H. Lee, B.D. Manning, Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways, Cell Metab. 14 (2011) 21–32.
[22] J.T. Tansey, C. Sztalryd, J. Gruia-Gray, D.L. Roush, J.V. Zee, O. Gavrilova, M.L. Reitman, C.X. Deng, C. Li, A.R. Kimmel, C. Londos, Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity, Proc. Natl. Acad. Sci. USA 98 (2001) 6494–6499.
[23] K. Katayama, K. Goto, K. Ishida, F. Ogita, Substrate utilization during exercise and recovery at moderate altitude, Metabolism 59 (2010) 959–966.
[24] K.R. Kelly, D.L. Williamson, C.E. Fealy, D.A. Kriz, R.K. Krishnan, H. Huang, J. Ahn, J.L. Loomis, J.P. Kirwan, Acute altitude-induced hypoxia suppresses plasma glucose and leptin in healthy humans, Metabolism 59 (2010) 200–205.
[25] D.C. Wright, P.C. Geiger, J.O. Holloszy, D.H. Han, Contraction- and hypoxia-stimulated glucose transport is mediated by a Ca2+-dependent mechanism in slow-twitch rat soleus muscle, Am. J. Physiol. Endocrinol. Metab. 288 (2005) E1062–1066.
[26] K.R. Westerterp, Energy and water balance at high altitude, News Physiol. Sci. 16 (2001) 134–137.
[27] H. Hoppeler, M. Vogt, Muscle tissue adaptations to hypoxia, J. Exp. Biol. 204 (2001) 3133–3139.
[28] P.W. Hochachka, L.T. Buck, C.J. Doll, S.C. Land, Unifying theory of hypoxia tolerance. molecular/metabolic defense and rescue mechanisms for surviving oxygen lack, Proc. Natl. Acad. Sci. USA 93 (1996) 9493–9498.
[29] K.H. Kim, M.J. Song, J. Chung, H. Park, J.B. Kim, Hypoxia inhibits adipocyte differentiation in a HDAC-independent manner, Biochem. Biophys. Res. Commun. 333 (2005) 1178–1184.
[30] A. Ascensao, I.O. Goncalves, J. Lumini-Oliveira, I. Marques-Aleixo, E. Dos Passos, S. Rocha-Rodrigues, N.G. Machado, A.C. Moreira, P.J. Oliveira, J.R. Torrella, J. Magalhaes, Endurance training and chronic intermittent hypoxia modulate in vitro salicylate-induced hepatic mitochondrial dysfunction, Mitochondrion 12 (2012) 607–616.
[31] Y.C. Chen, S.D. Lee, C.H. Kuo, L.T. Ho, The effects of altitude training on the AMPK-related glucose transport pathway in the red skeletal muscle of both lean and obese Zucker rats, High Alt. Med. Biol. 12 (2011) 371–378.
[32] S. Schmutz, C. Dapp, M. Wittwer, A.C. Durieux, M. Mueller, F. Weinstein, M. Vogt, H. Hoppeler, M. Fluck, A hypoxia complement differentiates the muscle response to endurance exercise, Exp. Physiol. 95 (2010) 723–735.
[33] J.S. Wang, M.H. Wu, T.Y. Mao, T.C. Fu, C.C. Hsu, Effects of normoxic and hypoxic exercise regimens on cardiac, muscular, and cerebral hemodynamics suppressed by severe hypoxia in humans, J. Appl. Physiol. 109 (2010) 219–229.