Regulation of HIF-1α activity in adipose tissue by obesity-associated factors: adipogenesis, insulin, and hypoxia

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He Q, Gao Z, Yin J, Zhang J, Yun Z, Ye J. Regulation of HIF-1α activity in adipose tissue by obesity-associated factors: adipogenesis, insulin, and hypoxia. Am J Physiol Endocrinol Metab 300: E877–E885, 2011. First published February 22, 2011; doi:10.1152/ajpendo.00626.2010.—The transcription factor HIF-1α activity is increased in adipose tissue to contribute to chronic inflammation in obesity. However, its upstream and downstream events remain to be characterized in adipose tissue in obesity. We addressed this issue by investigating adipocyte HIF-1α activity in response to obesity-associated factors, such as adipogenesis, insulin, and hypoxia. In adipose tissue, both HIF-1α mRNA and protein were increased by obesity. The underlying mechanism was investigated in 3T3-L1 adipocytes. HIF-1α mRNA and protein were augmented by adipocyte differentiation. In differentiated adipocytes, insulin further enhanced HIF-1α expression. In the all three factors are able to increase HIF-1α protein abundance and transcriptional activity of HIF-1α. HIF-1α gene is expressed constantly in cells. The mRNA increase is not necessary for HIF-1α protein elevation. The transcriptional activity of HIF-1α is regulated by nuclear coactivator and corepressor. The coactivators include p300 and CBP, which catalyze acetylation of histone proteins and initiation of gene transcription (2, 11). The corepressor activity is determined by histone deacetylases (HDACs) (13), which have multiple isoforms. It is not clear which HDAC isoform specifically inhibits HIF-1 activity (25, 29, 41).

VEGF is a major target gene of HIF-1. VEGF promotes angiogenesis, a process that is required for adipocyte differentiation and adipose tissue growth as reviewed (6, 7, 9, 21). Angiogenic inhibitors suppress fat tissue growth in animal models (5, 15, 27, 45) and represent a potential class of antiobesity drugs. Interestingly, adipocytes express a high level of VEGF (8, 34), which provides a molecular mechanism for the high capacity of adipose tissue to induce angiogenesis (64). However, the molecular mechanism for VEGF expression in adipocytes remains poorly understood. The present study explored the HIF-1α activity in adipocytes in response to obesity-associated factors such as preadipocyte differentiation, insulin, and hypoxia. The study suggests that the all three factors are able to induce HIF-1α protein. HIF-1 function is inhibited by the corepressor of HDAC3-silencing mediator for retinoic and thyroid hormone receptors (SMRT).

MATERIALS AND METHODS

Reagents. The 5× HRE (quintuple repeats of a hypoxia-responsive element) luciferase reporter and pLZRS-HIF-1α were described previously (30, 62). The 1,340-bp (–1,286 to +50) VEGF-A promoter-driven luciferase reporter plasmid was a gift from Dr. Alan Knox at the University of Nottingham, City Hospital, Nottingham, UK (4). Antibody to HIF-1α (NB 100/105R) was purchased from Novus Biologicals. Antibodies against glucose transporter 4 (Glu4; ab6549), p-Akt Thr308 (ab38449), tubulin (ab7291), p-IRS-1 Y632 (sc-17196-R), p-GSK-3β (sc-7291), p-IRS1 Y632 (sc-17196-R), p-GSK-3β (sc-11757-R), and specific protein 3 (sc-644X) were obtained from Abcam (Cambridge, UK). Antibody against HDAC1 (H6287) was obtained from Sigma (St. Louis, MO). Antibodies to insulin receptor (IR)α (sc-710), IRβ (sc-711), insulin receptor substrate-1 (IRS-1; sc-7200), Akt (sc-8312), GSK-3β (sc-7291), p-IRS1 Y632 (sc-17196-R), p-GSK-3β (sc-11757-R), and specific protein 3 (sc-644X) were obtained from the Santa Cruz Biotechnology (Santa Cruz, CA). RNAi expression vectors for SMRT, N-CoR, HDAC1, HDAC2, and HDAC3 were kindly provided by Drs. M. A. Lazar and D. Bohmann (24, 56). Isobutyl-

A HYPOXIA RESPONSE HAS BEEN REPORTED in adipose tissue during obesity by several laboratories (58). This finding provides a cellular mechanism underlying the chronic inflammation and adipose tissue dysfunction in obesity (50, 58). Adipose tissue hypoxia has led our attention to hypoxia marker genes such as hypoxia inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF). HIF-1α is a transcription factor whose activity is induced by hypoxia, and it has been used as an indicator of adipose tissue hypoxia (19, 23, 42, 60). Its qualification as a hypoxia-specific marker remains to be evaluated in adipose tissue dysfunction in obesity (50, 58). Adipose tissue hypoxia has led our attention to hypoxia marker genes such as hypoxia inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF). HIF-1α is a transcription factor whose activity is induced by hypoxia, and it has been used as an indicator of adipose tissue hypoxia (19, 23, 42, 60). Its qualification as a hypoxia-specific marker remains to be evaluated in adipose tissue dysfunction in obesity (50, 58).

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methylxanthine (15879), dexamethasone (D8893), human insulin solution (I278), collagenase (C6885), and CoCl2 (60818) were obtained from Sigma. Chemical inhibitors for MEK (098059, P-215), and p38 MAPK (SB203580, S-8307) were also purchased from Sigma. Phosphatidylinositol 3-kinase (PI3K) inhibitor LY-294002 (ST-420), JNK inhibitor SP-600125 (EI-305), mammalian target of rapamycin (mTOR) inhibitor rapamycin (A-275), and PKC inhibitor calphostin C (EI-198) were from Biomol (Plymouth Meeting, PA). Actinomycin D was purchased (11466; Calbiochem, EMD Biosciences, La Jolla, CA).

**Methods.**

**Results.**

Hypoxia treatment. In a sealed chamber, in which air was replaced with a gas of 1% O2, 5% CO2, and 94% N2, the cells were maintained in serum-free medium for 8 h before and during the hypoxia treatment to avoid insulin effect in the serum. The chamber was kept in a water bath at 37°C to maintain the temperature. The humidity was generated by water in the chamber. The control cells were under normoxia conditions (CO2 incubator) in a humidified 37°C incubator with 21% O2 and 5% CO2.

**Quantitative real-time RT-PCR.** The mRNA levels of VEGF-A (Mm_00437304_m1), HIF-1α (Mm_00468869_m1), and HIF-1β (Mm_00507836_m1) were determined using TaqMan probes with the 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The total RNA was extracted using the TRIzol protocol (Sigma). Mouse ribosome 18S rRNA_1 (without intron-exon junction) was used as a control for normalization of mRNA. The forward primer (5'-GGGAATCACGGTGTTGATC-3'), reverse primer (5'-CTGCGCTCTCTTGGTGGTGA-3'), and probe (5'-AGCTGAGAACG-3') were made for the ribosome 18S rRNA_1 by Applied Biosystems. Each experiment was repeated at least three times.

**Western blot.** The whole cell lysate, nuclear extracts, and cytoplasmic extracts were made as described elsewhere (17, 59). Western blotting was conducted according to protocols used previously (16).

The detection system contains horseradish peroxidase-conjugated secondary antibodies (catalog no. NA934V or NA931; Amersham Biosciences) and chemiluminescence reagent (catalog no. NEL-105; PerkinElmer Life Sciences) for generation of the chemiluminescent signal.

**Chromatin immunoprecipitation assay.** Differentiated 3T3-L1 adipocytes were cultured in a 100-mm cell culture plate and treated with hypoxia. The cells were treated with formaldehyde and collected for extraction of chromatin. The chromatin immunoprecipitation (ChIP) assay was performed as described elsewhere (16). The chromatin DNA was broken into fragments at 400–1,200 bp in length and immunoprecipitated with ChIP antibodies to HIF-1α (ab-1; Abcam). IgG was used as a control in immunoprecipitate for the nonspecific signal. The DNA in the immunoprecipitate product was quantified in qRT-PCR. The PCR primers were designed to cover the HIF-1 binding site (–975/–968) in the mouse VEGF gene promoter (37): forward, 5'-GAGGTTGCGGCGAGGCAC-3'; reverse, 5'-CAGTG-GGGGAGTGGACGC-3'. The relative signal strength was used to indicate interaction of HIF-1α and HDAc3 with the VEGF promoter DNA.

**Statistical analysis.** Each experiment was conducted at least three times. Western blot was quantified with the Image J program, and the representative blots are presented. A mean value ± SE of three independent experiments is presented for the reporter assay, ChIP assay, and mRNA assay. The data were analyzed using Student’s t-test, with significance at P < 0.05.

**RESULTS**

HIF-1α and VEGF increased in obese mice. In an earlier study, we observed that HIF-1α protein is elevated in adipose tissue of obese mice (60). In an effort to characterize the HIF-1α activity, we repeated the experiment. HIF-1α protein was determined in the epidydimal fat tissue homogenization in a Western blot. The obese mice exhibited a fourfold increase in HIF-1α protein (P < 0.001; Fig. 1A). The protein is associated with a 100% increase in VEGF mRNA in the adipose tissue (Fig. 1B), suggesting enhanced function of HIF-1 in the adipose tissue. The plasma VEGF protein was measured using an ELISA assay. The VEGF protein was elevated in both dietary obese mice (C57BL/6J) and genetically obese mice (ob/ob in C57BL/6J) (Fig. 1, C and D). The data suggest that the HIF-1α protein level may account for the VEGF expression in adipose tissue in the obese condition. Our
data support that adipocyte VEGF expression is associated with the plasma VEGF elevation in obesity (33, 49).

**Induction of HIF-1α activity by adipocyte differentiation.** Although the upstream events of HIF-1 activation have been studied extensively in many types of cells, the work had not been done in adipocytes (26, 46). In some studies, HIF-1α was tested in adipocytes for hypoxia-induced gene expression (53, 54, 60). There is little information about nonhypoxia signal in the regulation of HIF-1α in adipocytes. To address this issue, we examined the impact of preadipocyte differentiation on HIF-1α activity. In 3T3-L1 cells, VEGF expression was used to monitor function of HIF-1α during differentiation. VEGF mRNA rose from the beginning of differentiation to reach its highest level at the end of the differentiation period (day 8; Fig. 2A). As a VEGF activator, HIF-1α protein was elevated 10-fold in the differentiated cells (P < 0.001; Fig. 2B).

**Fig. 1.** Hypoxia-inducible factor-1 (HIF-1) function indicated by plasma vascular endothelial growth factor (VEGF) in obese mice. A: HIF-1α protein in the adipose tissue. Tissue homogenizer was made from epididymal fat of dietary obese mice and examined for HIF-1α protein in a Western blot. B: VEGF mRNA in white adipose tissue (WAT) of dietary obese mice. mRNA was quantified with quantitative real-time PCR (qRT-PCR) in epididymal fat of mice on high-fat diet (HFD) for 12 wk. C: plasma VEGF protein in dietary obese mice. The protein was determined using an ELISA assay in samples collected from mice on HFD at 12 wk (n = 10). D: Plasma VEGF in ob/ob mice. The test was conducted at 6 and 9 wk of age (n = 7). In the bar graph, each data point represents means ± SE. *P < 0.05, **P < 0.001 (compared with control).

**Fig. 2.** HIF-1α regulation by cell differentiation. A: VEGF mRNA during adipogenesis of 3T3-L1. Total mRNA was prepared from cells collected at times as indicated and quantified for VEGF mRNA by qRT-PCR. B: HIF-1α protein in the whole cell lysate of differentiated 3T3-L1 cells. The protein was determined in a Western blot. C: proteins in the insulin-signaling pathway. The proteins were determined in the whole cell lysate of differentiated 3T3-L1 cells in a Western blot. D: phosphorylation status of the signaling proteins. Phosphospecific antibodies were used in the assay of whole cell lysate, as described in MATERIALS AND METHODS. In the chart, each data point represents means ± SE (n = 3). *P < 0.05, **P < 0.001 (compared with control). IR and -IR, insulin receptor-α and -β, respectively; IRS-1, insulin receptor substrate-1.
To understand the mechanism underlying the HIF-1α induction by adipogenesis, we examined the insulin-signaling pathway. It was reported that HIF-1α is induced by insulin in several cell types (51, 63), but the adipocyte was not examined in those studies. HIF-1α protein reflects a balance between synthesis and degradation. HIF-1α protein synthesis is induced by PI3K in the insulin-signaling pathway. PI3K is activated by many other hormones/growth factors. Insulin is the most relevant hormone in the adipocyte differentiation model, in which insulin is required for adipogenesis. In the insulin-signaling pathway, several molecules were examined for their protein expression at 4 h (Fig. 3, A and B). mRNA reached the peak at 4 h, and exhibited a reduction thereafter with time (Fig. 3A). The protein peaked at 4 h, and the level remained for 24 h (Fig. 3B). Glut1 is a HIF-1 target gene. Its protein expression was significantly (Fig. 2C). Phosphorylation status of the proteins was examined in response to hypoxia. The data suggest that insulin signal may be required for HIF-1α induction during adipocyte differentiation.

Elevation of HIF-1α by insulin. To test the role of insulin, we examined HIF-1α during insulin treatment of differentiated adipocytes. In the time course study, insulin induced HIF-1α mRNA and protein in a time-dependent manner, with peak expression at 4 h (Fig. 3, A and B). mRNA reached the peak at 4 h, and exhibited a reduction thereafter with time (Fig. 3A). The protein peaked at 4 h, and the level remained for 24 h (Fig. 3B). Glut1 is a HIF-1 target gene. Its protein expression was enhanced by hypoxia (Fig. 3B). The transcriptional activity of HIF-1 was further tested using a luciferase reporter construct whose activation is under the control of hypoxia response element (HRE). In the transient transfection assay, insulin induced the reporter activity in a way that is comparable with hypoxia (Fig. 3C), suggesting that HIF-1α mediates insulin signal to activate expression a hypoxia response gene.

**HIF-1α elevation by hypoxia.** Insulin and hypoxia were compared in the stimulation of HIF-1α activity in adipocytes. To this point, HIF-1α was examined in response to hypoxia. HIF-1α protein and nuclear translocation were investigated in adipocytes. Total HIF-1α protein was increased in a time course study under hypoxia treatment. The protein increase exhibited at 0.5 h and reached a peak at 8 h (Fig. 4A). A similar pattern of increase was observed in the nuclear HIF-1α protein (Fig. 4B). The nuclear translocation of HIF-1α protein was investigated using immunofluorescent staining (Fig. 4C). Prior to hypoxia treatment, HIF-1α was located in the cytosol. Under the hypoxic condition, HIF-1α protein was detected in the
nucleus. HIF-1α mRNA was examined at four time points between 0.5 and 24 h in adipocytes. We did not observe mRNA change for HIF-1α in adipocytes (Fig. 4D). These data suggest that hypoxia is able to induce total protein and nuclear translocation of HIF-1α in adipocytes in a time-dependent manner. The increase in HIF-1α protein is independent of mRNA level in response to hypoxia.

**PI3K and mTOR in hypoxia-induced HIF-1α activity.** The HIF-1α protein is regulated by several serine kinases, including PI3K, mTOR, and ERK (43). To investigate these kinases in the HIF-1α regulation by hypoxia in adipocytes, we inhibited the kinases individually with chemical inhibitors. The hypoxia effect was significantly reduced by pretreatment of 3T3-L1 adipocytes with inhibitors to PI3K (LY-294002) or mTOR (rapamycin) (Fig. 5A). The inhibitors to MEK (PD098059) and p38 (SB-203580) were also tested. They did not show any effect on hypoxia-induced HIF-1α (Fig. 5B). The HIF-1α inhibition was associated with suppression of the transcriptional activity of HIF-1 (Fig. 5, C and D). VEGF-Luc and HRE-Luc reporters were used in the functional assay for HIF-1α. In response to the PI3K and mTOR inhibitors, their activities were significantly reduced. In the reporter assay, inhibitors to JNK (SP-600125) and PKC (calphostin C) were tested together with those for MEK and p38. All of them failed to block the HIF-1 activities (Fig. 5, C and D). These data suggest that the PI3K-Akt-mTOR pathway is required for the hypoxia-induced HIF-1α activity in adipocytes.

**Enhanced transcriptional activity of HIF-1α.** To determine interaction of HIF-1 with target gene in adipocytes, we examined HIF-1 in the VEGF gene promoter. The protein-DNA interaction was examined at the proximal HRE using the ChIP assay. In response to hypoxia, the interaction was enhanced in adipocytes (Fig. 6A). The interaction was associated with VEGF mRNA expression in response to hypoxia (Fig. 6, B and C), which was observed in both primary adipocytes and 3T3-L1 adipocytes. The role of HIF-1α in VEGF transcription was tested in HIF-1α-null cells. In the wild-type mouse embryonic fibroblasts (MEFs), VEGF mRNA was elevated fourfold under hypoxia (Fig. 6D). In the HIF-1α-knockout MEFs, the increase in VEGF mRNA was reduced to onefold under the same condition, suggesting that the HIF-1α is required for a full response of VEGF gene to hypoxia. To test the role of gene transcription in the mRNA induction, we examined VEGF mRNA stability, in

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**Fig. 5.** Signaling for HIF-1α elevation. A: phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) in the control of HIF-1α activity. Differentiated 3T3-L1 cells were serum starved and pretreated with LY-294002 (LY) and rapamycin (Rap) for 0.5 h and then treated with hypoxia for 8 h. The HIF-1α protein was examined in the nuclear extract in a Western blot. B: ERK and p38. The nuclear HIF-1α was determined with suppression of the transcriptional activity of HIF-1 (Fig. 5, C and D). VEGF-Luc and HRE-Luc reporters were used in the functional assay for HIF-1α. In response to the PI3K and mTOR inhibitors, their activities were significantly reduced. In the reporter assay, inhibitors to JNK (SP-600125) and PKC (calphostin C) were tested together with those for MEK and p38. All of them failed to block the HIF-1 activities (Fig. 5, C and D). These data suggest that the PI3K-Akt-mTOR pathway is required for the hypoxia-induced HIF-1α activity in adipocytes.
which the control and hypoxia-treated cells were compared using the actinomycin D protocol. The mRNA half-life was ~4 h in both conditions (Fig. 6D), suggesting no change in mRNA stability under hypoxia. These data support a role of HIF-1α-mediated VEGF transcription in the VEGF protein expression in adipocytes.

HIF-1α activity was inhibited by HDAC3 and SMRT. In addition to the protein abundance, the transactivation function of HIF-1 is also regulated by HDAC (31). However, it was not known which isoform of HDAC acts in adipocytes (25, 29, 41). In this study, we explored the role of three HDACs (HDAC1, HDAC2, and HDAC3) in the regulation of HIF-1 function. The study was conducted by knocking down HDACs in cells through transient transfection of RNAi expression vectors. The HDAC-1 transcriptional activity was monitored using the VEGF luciferase reporter. Knockdown of HDAC3 led to a significant induction in the transcriptional activity of HIF-1, as shown by the elevated reporter activity (Fig. 7, A and B). Knockdown of either HDAC1 or HDAC2 had no significant effect. The efficacy of HDAC knockdown was confirmed in Western blot. Our results suggest that HDAC3 is the major isoform in the inhibition of HIF-1 in adipocytes.

SMRT and nuclear corepressor (NCoR) are components in the HDAC3-containing corepressor complex. Their activities were examined using the RNAi knockdown system. Whereas knockdown of SMRT induced the HIF-1 activity (Fig. 7, A and B), knockdown of NCoR had minimal impact on the HIF-1 activity. These data suggest that SMRT is a component of the corepressor complex for HIF-1. The efficacy of SMRT or NCoR knockdown was confirmed in Western blot (data not shown).

**DISCUSSION**

In the current study, the upstream signals that regulate HIF-1α in adipocytes were investigated, with a focus on

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Fig. 6. HIF-1α in transcriptional expression of VEGF. A: chromatin immunoprecipitation (ChIP) assay for HIF-1α in the VEGF gene promoter. The assay was conducted in differentiated 3T3-L1 adipocytes after hypoxia treatment for 8 h. B: hypoxia induction of VEGF mRNA in primary adipocytes. C: VEGF induction in 3T3-L1 adipocytes. D: VEGF mRNA in HIF-1α-knockout (KO) cells. Mouse embryonic fibroblasts from HIF-1α-KO mice were treated with hypoxia for 8 h in the experiment. E: VEGF mRNA stability in differentiated 3T3-L1 adipocytes. In the bar graph, each data point represents means ± SE. *P < 0.05, **P < 0.001 (n = 3–5). WT, wild type.

Fig. 7. Inhibition of HIF-1α function by histone deacetylase (HDAC)3 and silencing mediator for retinoic and thyroid hormone receptors (SMRT). Functions of HDAC1, HDAC2, HDAC3, SMRT, and nuclear corepressor (NCoR) in the regulation of HIF-1 activity were examined using VEGF-luc reporter in the 293 cells. The corepressor proteins were knocked down with RNAi that was expressed from plasmid vectors cotransfected. The reporter activity was induced by either HIF-1α overexpression or hypoxia treatment. After transfection for 24 h, the cells were treated with hypoxia for 8 h in serum-free medium. A: VEGF-luc activity induced by HIF-1α expression. B: VEGF-luc activity induced by hypoxia. In the bar graph, each data point represents means ± SE. *P < 0.05 (n = 3).
obesity-associated factors such as cell differentiation, insulin, and hypoxia. HIF-1α activity is enhanced in mRNA and protein in adipose tissue in obesity. To understand the mechanism underlying the HIF-1α change, we tested the three obesity-associated factors. All of them were able to enhance HIF-1α protein expression, but only two of them (adipogenesis and insulin) were able to increase HIF-1α mRNA. Hypoxia did not induce HIF-1α mRNA in adipocytes. The increase in HIF-1α protein is likely a result of inhibition of protein degradation since the mRNA change occurs after the protein. Our data confirm that adipogenesis increases HIF-1α mRNA and protein in the mouse preadipocyte 3T3-L1, as reported by Floyd et al. (14), but is opposite of the observation in human preadipocytes by Wang et al. (53). This discrepancy might be related to differences in species (mouse vs. human) or experimental conditions. In the search for the mechanism of HIF-1α expression during adipogenesis, we found that expression of insulin receptor was enhanced by adipogenesis. The insulin receptor makes adipocytes to react actively to insulin with HIF-1α mRNA and protein expression. This effect of the insulin-signaling pathway is demonstrated in mature adipocytes. IGF (insulin-like growth factor) may stimulate the HIF-1α activity as well since it activates the insulin receptor. Given that both adipogenesis and insulin levels are elevated in obesity, the two factors should contribute to the HIF-1α up-regulation in adipose tissue. Hypoxia exists in adipose tissue in obesity (60, 61) and contributes to the HIF-1α activity in adipocytes (53, 60), but hypoxia only stimulates the HIF-1α protein elevation in adipocytes. In addition to the three factors above, inflammation signal may induce HIF-1α expression in obesity. Activation of the IKK2-NF-κB pathway by TNFα leads to HIF-1α mRNA expression (44). These lines of evidence suggest that HIF-1α is not an exclusive biomarker for hypoxia in adipose tissue under obesity. To demonstrate hypoxia in adipose tissue in obesity, other parameters such as interstitial oxygen pressure, the chemical hypoxia probe (pi-monomiazole HCl), or lactate level are required (23, 60). Hypoxia induces lactate production through inhibition of mitochondria (61) and induction of monocarboxylate transporter 1 expression (39).

We tested several kinases that are reported to regulate HIF-1α. Our results suggest that the PI3K-Akt pathway is required by insulin and hypoxia in the stimulation of HIF-1α activities. The kinase inhibitors to PI3K and mTOR blocked HIF-1α protein activities in cytoplasm and nucleus in adipocytes. ERK is reported to enhance HIF-1 activity in nonadipocytes (43). However, this ERK activity is not supported by our data.

We examined HIF-1α downstream events by investigating VEGF gene promoter in adipocytes. VEGF secretion from adipocytes is associated with the plasma VEGF elevation in overweight or obese subjects (20, 33–35, 49). However, the VEGF production had not been comprehensively examined in adipocytes. The current study suggests that in obesity there are at least three factors to stimulate VEGF transcription in adipocytes. The first is adipogenesis. Adipocyte differentiation increases VEGF expression, which is consistent with the report by Claffey et al. (8). In obesity, adipogenesis is accelerated in response to quick turnover rate of adipocytes in adipose tissue (22). The adipogenesis will lead to more VEGF expression through HIF-1α. The second is insulin stimulation. Obesity leads to hyperinsulinemia from insulin resistance. We show that insulin induces HIF-1α expression in mRNA and protein in adipocytes, which is translated into the VEGF transcription. The third is hypoxia response. In adipocytes, HIF-1α activity is increased by hypoxia for VEGF expression, which is consistent with other reports (38, 42, 60). VEGF expression is also enhanced by inflammation (TNFα) (52) and sympathetic nerve signal (57). In obesity, all of these factors may contribute to VEGF production by adipose tissue. Our data show that stability of VEGF mRNA is not changed by hypoxia in adipocytes.

Our data suggest that HIF-1α is an activator of VEGF gene, but HIF-1α is not sufficient to induce VEGF expression in adipocytes in obesity. In an earlier study, we observed that VEGF mRNA was increased in epididymal fat of diet-induced obese mice but not ob/ob mice, although HIF-1α activity was enhanced in both models (60). This observation raised a question about role of HIF-1α in the control of VEGF expression in adipocytes. Here, we show that HIF-1α interacts with the VEGF gene promoter DNA in the ChIP assay, and VEGF expression is reduced significantly in HIF-1α-null cells. These data suggest that HIF-1α is a major transcriptional activator of VEGF gene in adipocytes. However, in ob/ob mice, this HIF-1α activity is not sufficient to induce VEGF expression. There may be an increase in VEGF expression in subcutaneous fat in ob/ob mice, which leads to VEGF elevation in plasma. VEGF transcription is controlled by several other transcription factors in addition to HIF-1α, such as STAT3 (36, 55), peroxisome proliferator-activated receptor (PPARγ) coactivator-1α (PGC-1α) (1), and PPARγ (12). STAT3 is activated by leptin, which is absent in ob/ob mice. STAT3 activity may be decreased in ob/ob mice from leptin deficiency. This condition may prevent VEGF expression in response to HIF-1α. PGC-1α activity is enhanced by sympathetic nerve signal (cAMP) and inflammation (TNFα) (40, 57), both of which stimulate VEGF expression.

Our result suggests that HDAC3 and SMRT form a corepressor for HIF-1α in adipocytes, which was not found in the literature. In nonadipocytes, HIF-1α activity is regulated by HDACs, and HDAC isoforms in the regulation of HIF-1α are highly controversial (25, 29, 41). HDAC3, one of HDAC isoforms, belongs to the class I HDACs (HDAC1, HDAC2, HDAC3, and HDAC8) (10, 48). It was reported that HDAC1 suppresses the transcriptional activation of HIF-1α since HDAC1 expression was enhanced by hypoxia (28). HDAC1-3 was reported to inhibit the HIF-1α activity through an interaction with von Hippel-Lindau (31). Class II HDACs (HDAC4, HDAC6, and HDAC7) were reported to enhance the transcriptional activity of HIF-1α through a direct protein association (25, 41). The class II isoforms include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC10. In the current study, the activity of HDAC1-3 was examined using an RNAi-based gene knockdown strategy. The data suggest that in adipocytes HDAC3 is the primary isoform of HDACs in the inhibition of HIF-1 function, whereas HDAC1 and HDAC2 do not play a major role.

SMRT and NCoR are two different corepressor components that do not have any enzymatic activity. They act through triggering the catalytic activities of HDACs in the corepressor complex (18). Our data suggest that SMRT, but not NCoR,
interacts with HDAC3 in the suppression of HIF-1 activity in adipocytes.

In summary, HIF-1α is increased in both mRNA and protein in adipose tissue in obesity. There are at least three factors to stimulate HIF-1α activity in obesity: adipogenesis, insulin, and hypoxia. In adipocytes, all of the three factors enhance HIF-1α protein. The PI3K-Akt pathway is required for HIF-1α activation by those factors. HIF-1α is a major transcriptional activator for VEGF gene, but it is not sufficient for activation of VEGF gene expression in adipose tissue. In adipocytes, the transcriptional activity of HIF-1α is inhibited by the corepressor composed of HDAC3 and SMRT. In adipose tissue, the increase in HIF-1α mRNA is likely a result of adipogenesis and hyperinsulinemia in obesity but not adipose tissue hypoxia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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