Chronic Intermittent Hypoxia Induces Atherosclerosis via Activation of Adipose Angiopoietin-like 4

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***Rationale*: Obstructive sleep apnea is a risk factor for dyslipidemia and atherosclerosis, which have been attributed to chronic intermittent hypoxia (CIH). Intermittent hypoxia inhibits a key enzyme of lipoprotein clearance, lipoprotein lipase, and up-regulates a lipoprotein lipase inhibitor, angiopoietin-like 4 (Angptl4), in adipose tissue. The effects and mechanisms of Angptl4 up-regulation in sleep apnea are unknown.**

***Objectives*: To examine whether CIH induces dyslipidemia and atherosclerosis by increasing adipose Angptl4 via hypoxia-inducible factor-1 (HIF-1).**

***Methods*: *ApoE*$^{-/-}$ mice were exposed to intermittent hypoxia or air for 4 weeks while being treated with Angptl4-neutralizing antibody or vehicle.**

***Measurements and Main Results*: In vehicle-treated mice, hypoxia increased adipose Angptl4 levels, inhibited adipose lipoprotein lipase, increased fasting levels of plasma triglycerides and very low density lipoprotein cholesterol, and increased the size of atherosclerotic plaques. The effects of CIH were abolished by the antibody. Hypoxia-induced increases in plasma fasting triglycerides and adipose Angptl4 were not observed in mice with germline heterozygosity for a HIF-1α knockout allele. Transgenic overexpression of HIF-1α in adipose tissue led to dyslipidemia and increased levels of adipose Angptl4. In cultured adipocytes, constitutive expression of HIF-1α increased Angptl4 levels, which was abolished by siRNA. Finally, in obese patients undergoing bariatric surgery, the severity of nocturnal hypoxemia predicted Angptl4 levels in subcutaneous adipose tissue.**

***Conclusions*: HIF-1–mediated increase in adipose Angptl4 and the ensuing lipoprotein lipase inactivation may contribute to atherosclerosis in patients with sleep apnea.**

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Chronic intermittent hypoxia, a hallmark manifestation of obstructive sleep apnea, induces dyslipidemia and atherosclerosis, but the responsible mechanisms are not sufficiently understood.

What This Study Adds to the Field

We have shown that (*1*) depletion of adipose angiopoietin-like 4, an inhibitor of lipoprotein lipase, abolishes intermittent hypoxia–induced dyslipidemia and atherosclerosis in mice; (*2*) adipose angiopoietin-like 4 is regulated by hypoxia-inducible factor-1; and (*3*) angiopoietin-like 4 mRNA levels in subcutaneous adipose tissue correlate with the severity of nocturnal hypoxemia in patients with sleep apnea.

Keywords: sleep apnea; lipoprotein clearance; lipoprotein lipase; adipose tissue; hypoxia-inducible factor-1

Obstructive sleep apnea (OSA) is a highly prevalent disease characterized by chronic intermittent hypoxia (CIH) (1). OSA increases all-cause and cardiovascular mortality (2–5). OSA is an independent risk factor for atherosclerosis (6), and the severity of atherosclerosis correlates with the severity of nocturnal oxyhemoglobin desaturations (7, 8). The mechanisms by which CIH accelerates atherosclerotic disease are unclear but may include hypertension, dyslipidemia, insulin resistance, systemic inflammation, and oxidative stress (6). In rodent models, CIH accelerates the progression of atherosclerosis in C57BL/6J mice on a high-cholesterol diet (9, 10) and in *ApoE*-deficient mice (11, 12) by inducing dyslipidemia (10) and systemic inflammation (11, 13–15).

Dyslipidemia is one of the best-characterized risk factors for atherosclerotic cardiovascular disease. We have previously shown that, in patients with OSA, high fasting levels of very low-density lipoprotein (VLDL) are associated with severe nocturnal oxyhemoglobin desaturations (10). A recent study demonstrated that OSA inhibits clearance of triglyceride-rich lipoproteins, a defect that is improved by treatment with continuous

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positive airway pressure (16). Impaired clearance of triglyceride-rich lipoproteins, which include dietary chylomicrons and liver-synthesized VLDL, leads to excessive formation of atherogenic remnant lipoproteins, thereby accelerating progression of atherosclerosis (17) and conferring increased risk of myocardial infarction, ischemic heart disease, stroke, and death (17–19). Mice exposed to CIH exhibit impaired triglyceride-rich lipoprotein clearance and decreased activity of a key enzyme of lipoprotein clearance, lipoprotein lipase (LPL), in adipose tissue (20). Furthermore, LPL inhibition was associated with increased adipose levels of a potent LPL inhibitor, angiopoietin-like 4 (Angptl4) (20). Angptl4 is transcriptionally regulated by hypoxia-inducible factor (HIF)-1 in several cell types (21–23), but the role of HIF-1 in Angptl4 regulation in adipose tissue is not known. We hypothesized that *1*) CIH accelerates the progression of atherosclerosis by inducing atherogenic hyperlipidemia via adipose Angptl4 and that CIH-induced dyslipidemia and atherosclerosis would be prevented by Angptl4 depletion and *2*) that adipose Angptl4 is regulated by HIF-1. We tested our hypotheses by treating CIH-exposed, *ApoE*-deficient mice with Angptl4-neutralizing antibody (Ab) and by examining adipose Angptl4 levels in mice with partial deficiency of an O_2–regulated α subunit of HIF-1, in HIF-1α overexpressing mice, and in adipocyte cell cultures. To examine the relevance of our hypotheses for human disease, we measured Angptl4 mRNA levels in adipose tissue of patients with OSA. Some of the results of these studies have been previously reported in the form of an abstract (24).

METHODS

In the first series of experiments, $ApoE^{-/-}$ mice were exposed to CIH ($F_{I_{O_2}}$ cycling from 21 to 6.5%, 60 times/h, 9:00 A.M. to 9:00 P.M.) or intermittent air (IA) for 4 weeks (12) while being treated with Angptl4-neutralizing Ab (Lexicon Pharmaceuticals, Inc., The Woodlands, TX) at 30 mg/kg or vehicle as previously described (25). Upon completion of the exposure, Angptl4 gene expression, LPL activity, the fasting plasma lipoprotein profile, and atherosclerosis in the aortic origin and in *en face* preparation of the entire aorta were measured (12, 20). In the second series of experiments, mice that were heterozygous for a germline null (knockout) allele and therefore present with partial global deficiency of HIF-1α ($Hif1a^{+/-}$ mice) (26) and their wild-type (WT) littermates on an outbred C57BL/6J X 129 genetic background were exposed to CIH and IA for 4 weeks, and fasting plasma lipid profile and adipose Angptl4 were measured. In the third series of the experiments, transgenic (Tg) mice expressing a constitutively active form of human HIF-1α with a deleted oxygen degradation domain (HIF-1αΔODD) under the control of the aP2 promoter and their WT littermates on the FVB background (27) were killed at normoxic conditions, and the fasting plasma lipid profile and adipose Angptl4 were measured. For surgical procedures, anesthesia was maintained with 2% isoflurane. The study was approved by the Johns Hopkins University Animal Use and Care Committee and complied with the American Physiological Society Guidelines for Animal Studies.

3T3-L1 cells were differentiated, transfected with HIF-1α siRNA or nontarget siRNA, and exposed to a prolyl hydroxylase inhibitor dimethyloxaloylglycine (DMOG) or vehicle for 24 hours. Angptl4 and HIF-1α mRNA and protein levels were measured.

Twenty-one patients (all women) without significant comorbidities were retrospectively recruited from the Johns Hopkins Bayview Medical Center (JHBMC) Bariatric Surgery Clinic. The protocol was approved by the Western Institutional Review Board. Sleep studies were performed. Angpl4 mRNA was measured in subcutaneous and visceral adipose tissues obtained during bariatric surgery. Angpl4 levels were correlated with indices of sleep-disordered breathing, including the apnea-hypopnea index (AHI) and the average fall in oxyhemoglobin saturation (ΔSp_{O_2}) during apneic/hypopneic episodes, and with fasting serum triglyceride and total cholesterol levels.

All values are reported as means ± SEM after confirming that all continuous variables were normally distributed using the Kolmogorov-Smirnov test. Statistical significance for all comparisons was determined by two-way ANOVA with Bonferroni *post hoc* correction for multiple comparisons. Statistical significance of correlations was ascertained with Pearson and Spearman tests. All tests were two-sided, and the significance level was established at $P < 0.05$. Methods are described in detail in the online supplement.

RESULTS

Angptl4-Neutralizing Antibodies Reverse Effects of CIH in $ApoE^{-/-}$ Mice

We performed our experiment in four groups of $ApoE^{-/-}$ mice: animals exposed to CIH and treated with Angptl4 Ab (CIH-Ab); mice exposed to CIH and treated with vehicle (saline); mice exposed to intermittent air (IA), treated with Angptl4 Ab (IA-Ab), and weight matched to the CIH-Ab group; and mice exposed to IA, treated with vehicle, and weight matched to the CIH-vehicle group. There was no difference in body weight, food intake, liver weight, and epididymal fat weight between the groups. CIH induced a significant increase in systolic blood pressure, whereas Ab had no effect (Table 1).

CIH caused a 2- to 4.5-fold increase in Angptl4 mRNA levels in epididymal fat but not in the heart, skeletal muscle (quadriceps), or the liver (Figure 1), which was consistent with our previous data in WT mice (20). CIH induced a significant decrease in adipose LPL activity (Figure 2A), which was abolished by Ab. Neither CIH nor Ab affected LPL activity in heart tissue. In muscle, CIH had no effect, whereas Ab significantly increased LPL activity. As expected, LPL activity was low at baseline in the liver (28). It was further decreased by CIH, and the decrease was abolished by Ab. CIH did not alter adipose LPL protein levels, whereas Ab increased it (Figure 2B; n = 6 per group; representative samples shown).

We next determined whether Angptl4 depletion attenuates CIH-induced hyperlipidemia. CIH induced a greater than 50% increase in fasting triglycerides (Figure 3A) and a significant increase in total cholesterol (Figure 3B), and both changes were abolished by Angptl4 Ab. CIH increased free fatty acid (FFA) levels, but Ab had no effect (Figure 3C). CIH greatly increased

TABLE 1. FOOD INTAKE, BODY WEIGHT, AND BLOOD PRESSURE IN $APOE^{-/-}$ MICE EXPOSED TO INTERMITTENT AIR OR CHRONIC INTERMITTENT HYPOXIA TREATED WITH ANGPTL-4 ANTIBODY VERSUS VEHICLE FOR 4 WEEKS

Characteristics	IA-Vehicle	CIH-Vehicle	IA-Ab	CIH-Ab
N	15	16	16	17
Mean food intake, g/d	2.63 ± 0.04	2.68 ± 0.03	2.57 ± 0.02	2.75 ± 0.03
Body weight, g				
Day 0	25.5 ± 0.5	26.3 ± 0.4	26.0 ± 0.5	26.0 ± 0.4
Day 28	25.5 ± 0.4	25.7 ± 0.5	26.0 ± 0.4	26.1 ± 0.4
Liver weight				
g	1.15 ± 0.033	0.99 ± 0.023	1.11 ± 0.024	1.13 ± 0.026
% of body weight	4.49 ± 0.10	3.88 ± 0.05	4.14 ± 0.05	4.30 ± 0.07
Epididymal fat				
g	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.02
% of body weight	0.52 ± 0.05	0.60 ± 0.05	0.60 ± 0.03	0.66 ± 0.05
Systolic blood pressure, mm Hg	114 ± 5	123 ± 5*	115 ± 2	123 ± 5*
Diastolic blood pressure, mm Hg	82 ± 4	85 ± 3	85 ± 2	87 ± 2

Definition of abbreviations: Ab = antibody; CIH = chronic intermittent hypoxia; IA = intermittent air.

* $P < 0.05$ versus IA. Remaining comparisons were not significant.

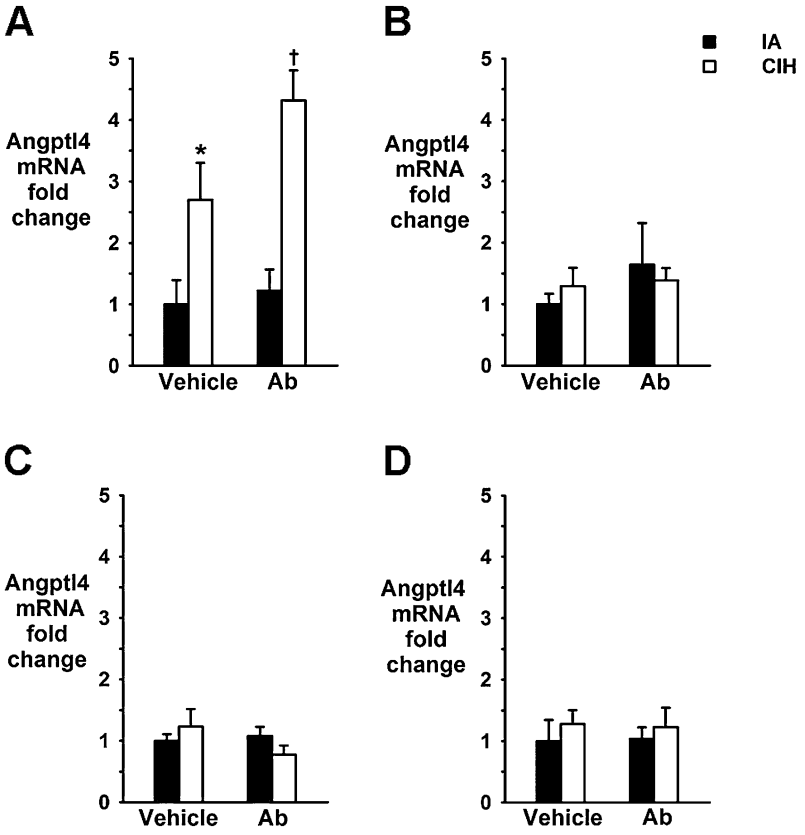

Figure 1. Effect of chronic intermittent hypoxia (CIH) on angiopoietin-like 4 (Angptl4) mRNA in epididymal fat (*A*), heart (*B*), liver (*C*), or skeletal muscle (*D*) of *ApoE*$^{-/-}$ mice. Ab = Angptl4-neutralizing antibodies; Epi fat = epididymal fat; IA = intermittent air. *$P < 0.05$ for CIH-vehicle versus IA-vehicle. †$P < 0.01$ for CIH-Ab versus IA-Ab.

VLDL and LDL cholesterol levels ($P < 0.001$) (Figure 3D, *top panel*), whereas HDL cholesterol was unchanged. Angptl4-neutralizing Ab abolished CIH-induced increases in fasting VLDL cholesterol (Figure 3D, *bottom panel*), whereas the increase in LDL cholesterol was partially attenuated (from a 46% increase in vehicle-treated mice to a 22% increase in Ab-treated mice; $P < 0.05$).

CIH increased the size of atherosclerotic lesions in *en face* preparations of the entire aorta (2-fold increase; $P < 0.001$) (Figures 4A and 4B) and in cross sections of the aortic root (Figures 4C and 4D) and augmented plaque necrosis (Figures 4C and 4E). Angptl4 Ab prevented CIH-induced progression of atherosclerosis (Figure 4).

Adipose Angptl4 Is Regulated by HIF-1

In *ApoE*$^{-/-}$ mice, CIH increased protein levels of HIF-1α and mRNA levels of a canonical HIF-1–regulated gene, vascular endothelial growth factor (VEGF)-A, in epididymal fat (*see* Figure E1 in the online supplement). Therefore, we examined relationships between adipose Angptl4 and HIF-1 in different Tg mouse strains and in cell culture. First, we studied mice with global partial HIF-1α deficiency (*Hif1a*$^{+/-}$). CIH induced similar weight loss in *Hif1a*$^{+/-}$ mice and their WT littermates (Table E1). CIH mice showed a slight increase in omental fat pads, but there was no difference between *Hif1a*$^{+/-}$ and WT mice. CIH increased Angptl4 mRNA and protein levels in epididymal fat of WT mice, and this increase was abolished by partial HIF-1α deficiency (Figures 5A and 5B). In WT mice, CIH raised fasting serum triglyceride and total cholesterol levels (Figures 5C and 5D). HIF-1α deficiency abolished the increase in triglyceride levels but not in cholesterol levels.

We have also used mice overexpressing HIF-1α in adipose tissue. Under normoxic conditions, *HIF-1αΔODD* Tg mice and WT littermates had identical body weights and similar sizes of white adipose tissue pads (*see* Table E1). Tg mice exhibited overexpression of HIF-1α in subcutaneous and omental white adipose tissue, whereas epididymal fat was less affected (27). Tg mice showed higher mRNA and protein levels of HIF-1α and Angptl4 in omental fat and higher fasting serum triglyceride levels compared with WT mice, whereas total cholesterol levels were the same (Figure 6).

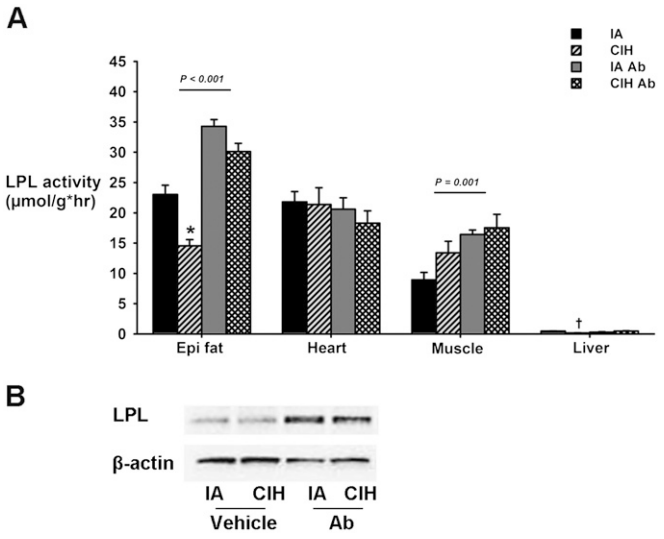

Figure 2. Effect of chronic intermittent hypoxia (CIH) and Angptl4-neutralizing antibodies (Ab) on lipoprotein lipase (LPL) activity in epididymal fat, heart, skeletal muscle, and liver (*A*) and on LPL protein levels (*B*) in epididymal fat (n = 6 per group; representative samples shown) in *ApoE*$^{-/-}$ mice. IA = intermittent air; *$P < 0.001$ for CIH-vehicle versus IA-vehicle. †$P < 0.01$ for CIH-vehicle versus IA-vehicle.

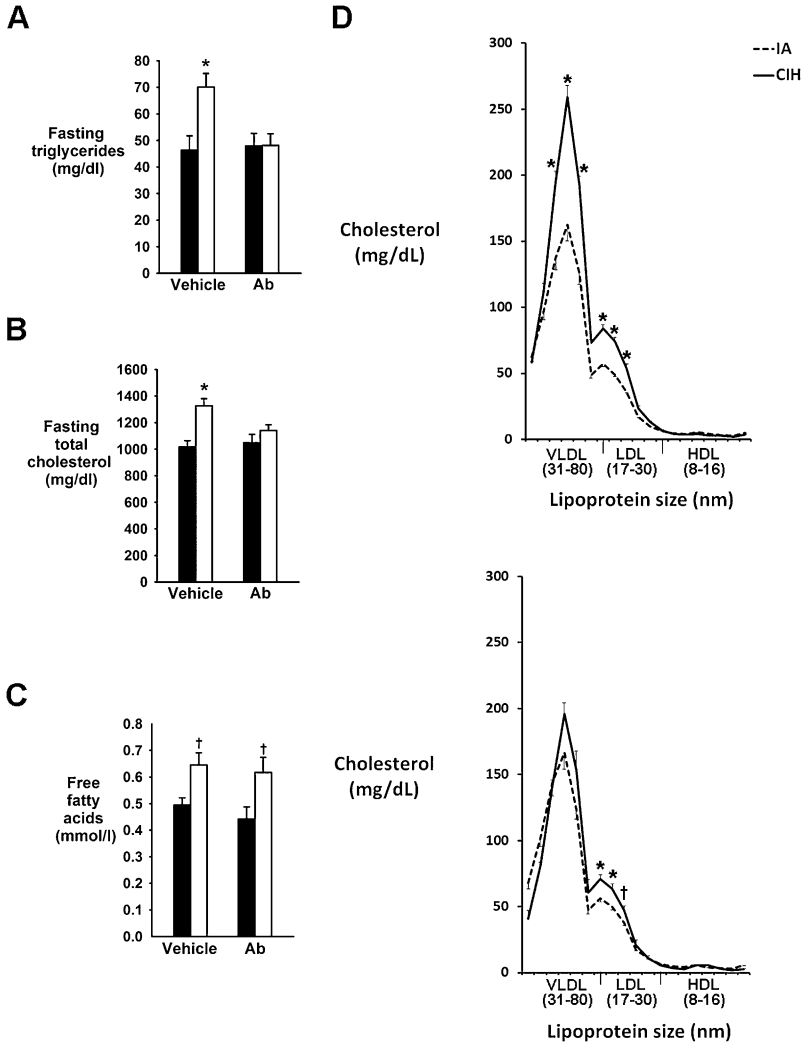

Figure 3. Effect of chronic intermittent hypoxia (CIH) and angiopoietin-like 4 (Angptl4) antibodies (Ab) on serum fasting levels of triglycerides (*A*), total cholesterol (*B*), and free fatty acids (*C*), on the fasting cholesterol profile (*D*; *upper panel*: vehicle treated; *lower panel*: Ab treated) in $ApoE^{-/-}$ mice. IA = intermittent air. *$P < 0.001$ versus IA groups. †$P < 0.01$ versus IA groups.

In cell culture experiments, we exposed 3T3-L1 cells to the nonselective prolyl hydroxylase inhibitor DMOG or vehicle for 24 hours. DMOG induces constitutive expression of HIF-1α and 2α proteins by inhibiting O_2–dependent degradation (29, 30). To examine the role of HIF-1α in Angptl4 regulation, DMOG exposure was performed in the presence and absence of HIF-1α siRNA, which depleted HIF-1α mRNA (Figure 7A). As expected, DMOG increased HIF-1α protein levels and expression of VEGF-A mRNA (Figures 7B and 7C). DMOG also significantly increased mRNA and protein levels of Angptl4 (Figures 7D and 7E). All effects of DMOG on HIF-1α, VEGF-A, and Angptl4 expression were abolished by transfection of cells with HIF-1α siRNA.

Adipose Angptl4 Levels in Patients with OSA Correlate with Nocturnal Hypoxemia

We took advantage of the availability of sleep studies as well as adipose tissue biopsies in obese subjects who underwent bariatric surgery (Table E2). Their body mass index (BMI) varied between 41 and 63 kg/m^2, and the severity of OSA varied from an AHI of 0 to 71.3 events/h. Angptl4 mRNA levels in subcutaneous adipose tissue significantly correlated with the severity of nocturnal oxyhemoglobin desaturation ($P < 0.05$) (Fig. E2) but did not correlate with the BMI or AHI (not shown). Angptl4 mRNA levels in visceral fat and Angptl4 serum levels were not associated with indexes of sleep apnea (Figure E2 and Table E2). When our patient population was dichotomized according to median splits of nocturnal oxyhemoglobin desaturation and the AHI, patients with $\Delta Sp_{O_2} > 4.3\%$ had 2-fold higher Angptl4mRNA levels in subcutaneous adipose tissue than those with $\Delta Sp_{O_2} \leq 4.3\%$, and patients with the AHI > 16.6/h had a 1.8-fold higher Angptl4 mRNA levels than those with the AHI ≤ 16.6 hours (Figure 8). There was no difference in the BMI or Angptl4 expression in visceral adipose tissue between the groups. Fasting serum triglyceride levels correlated with Angptl4 expression in visceral adipose tissue (Pearson $r = 0.496$, $P = 0.022$; Spearman $r = 0.4327$, $P = 0.057$) but not in subcutaneous fat (Fig. E2). There was no significant relationship between fasting serum total cholesterol and adipose Angptl4.

DISCUSSION

We have previously shown that CIH induces atherosclerosis and dyslipidemia in mice (10, 12, 20). The purpose of this study was to examine the role of the LPL inhibitor Angptl4 in the pathogenesis of CIH-induced atherosclerosis. The main novel finding of the study was that Angptl4 depletion with neutralizing Ab prevented CIH-induced progression of atherosclerosis in $ApoE^{-/-}$ mice. Angptl4 depletion also abolished CIH-induced decreases in adipose LPL activity and increases in plasma VLDL cholesterol and triglyceride levels. In addition, we explored the role of HIF-1 in up-regulation of adipose Angptl4 and found that both CIH and HIF-1α overexpression increased

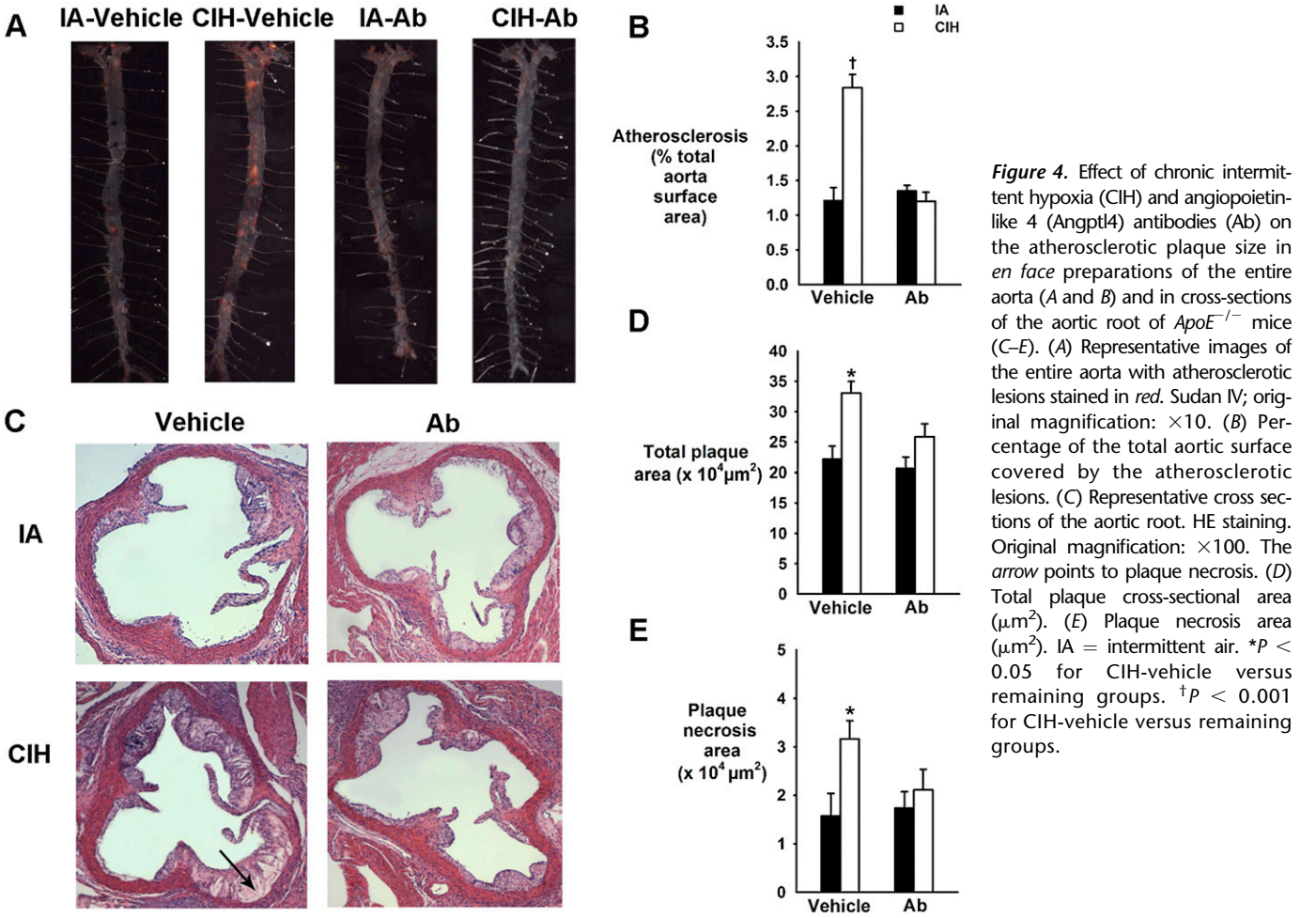

Figure 4. Effect of chronic intermittent hypoxia (CIH) and angiopoietin-like 4 (Angptl4) antibodies (Ab) on the atherosclerotic plaque size in *en face* preparations of the entire aorta (*A* and *B*) and in cross-sections of the aortic root of $ApoE^{-/-}$ mice (*C–E*). (*A*) Representative images of the entire aorta with atherosclerotic lesions stained in *red*. Sudan IV; original magnification: ×10. (*B*) Percentage of the total aortic surface covered by the atherosclerotic lesions. (*C*) Representative cross sections of the aortic root. HE staining. Original magnification: ×100. The *arrow* points to plaque necrosis. (*D*) Total plaque cross-sectional area (μm^2). (*E*) Plaque necrosis area (μm^2). IA = intermittent air. $^*P < 0.05$ for CIH-vehicle versus remaining groups. $^\dagger P < 0.001$ for CIH-vehicle versus remaining groups.

adipose Angptl4 in a similar manner and that this increase was abolished by HIF-1α deficiency. Finally, we showed that in patients with OSA, Angptl4 mRNA levels in subcutaneous adipose tissue correlated with the severity of nocturnal oxyhemoglobin desaturation.

CIH and Angptl4

Severe hypoxia up-regulates Angptl4 in cultured adipocytes, cardiomyocytes, and endothelial cells (21, 22, 31). Constitutive expression of active HIF-1α in endothelial cells and cardiomyocytes results in robust up-regulation of Angptl4 mRNA (21, 22). Functional HIF-1 binding sites were identified in the human Angptl4 gene (23). HIF-1 is a heterodimer, which consists of a constitutively expressed β subunit and an O_2–regulated α subunit (32). HIF-1α activation by sustained hypoxia occurs due to inhibition of O_2–dependent prolyl hydroxylation (29). In contrast, HIF-1α activation by CIH occurs not only through decreased degradation but also via increased HIF-1α biosynthesis (33). CIH increases generation of reactive oxygen species through NADPH oxidase, which increases HIF-1α biosynthesis via mammalian target of rapamycin (34). CIH induces severe hypoxia and oxidative stress in white adipose tissue (35), which may be sufficient to up-regulate HIF-1α levels and induce Angptl4 expression.

The role of HIF-1 is supported by several lines of evidence. First, up-regulation of adipose Angptl4 during CIH was abolished in mice with partial global HIF-1α deficiency (Figure 5). Global HIF-1α deficiency may affect Angptl4 levels systemically because it attenuates hypoxic sensitivity of the carotid bodies with loss of downstream sympathetic nervous system (SNS) responses to CIH (36). The SNS is a major regulator of adipose tissue lipolysis (37). CIH increases circulating FFA levels (12) (Figure 3C). FFAs induce Angptl4 gene expression (38). However, very modest elevations of FFAs in CIH may not be sufficient to induce dramatic increases in adipose Angptl4 (compare Figures 1A and 3C). Therefore, it is more likely that global HIF-1α deficiency abolishes Angptl4 up-regulation not through suppression of SNS responses to CIH but directly at the level of adipocytes. Second, in support of the direct effect of HIF-1, mice expressing a constitutively active form of HIF-1α in adipose tissue showed increased Angptl4 levels (Figure 6). Third, activation of HIF-1α in adipocyte cell culture resulted in increased Angptl4 expression, which was abolished by HIF-1α siRNA (Figure 7). Fourth, HIF-1α overexpression in adipose tissue increased levels of serum triglycerides in parallel with up-regulation of adipose Angptl4 (Figure 6), whereas HIF-1α deficiency abolished CIH-induced increases in serum triglycerides and adipose Angptl4, supporting the role of HIF-1α in Angptl4-mediated dyslipidemia (Figure 5). Based on all of the above, we hypothesize that increased HIF-1α levels mediate Angptl4 up-regulation in adipose tissue during CIH. The lack of an increase in Angptl4 levels in liver, skeletal muscle, and heart may be attributable to relatively moderate levels of tissue hypoxia and/or oxidative stress (35), which were not sufficient to stabilize HIF-1α, and low levels of Angptl4 expression compared with adipose tissue (38, 39).

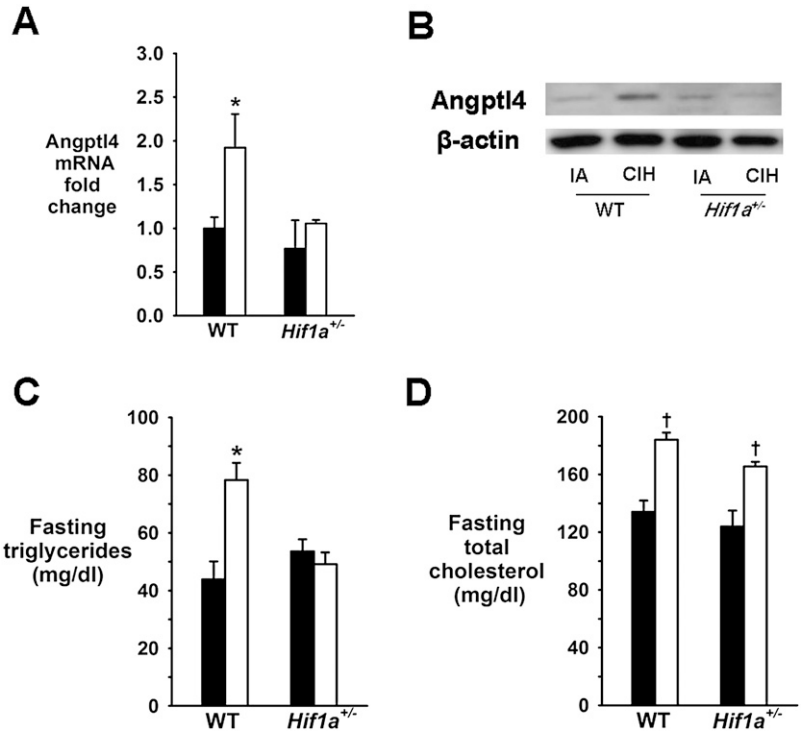

Figure 5. Effect of chronic intermittent hypoxia (CIH) on angiopoietin-like 4 (Angptl4) mRNA levels in epididymal fat (*A*), Angptl4 protein levels in epididymal fat (representative samples) (*B*), fasting serum triglycerides (*C*), and fasting serum total cholesterol in *Hif1a*$^{+/-}$ mice and their wild-type (WT) littermates (*D*). IA = intermittent air. *$P <$ 0.05 for the difference between CIH and IA. $^{\dagger}P < 0.01$ for the difference between CIH and IA.

Angptl4 and LPL Activity during CIH

Angptl4 inhibits LPL by converting active dimers to inactive monomers (40). Neutralizing Ab recognize a LPL binding domain of Angptl4, thereby preventing LPL inactivation (25). Our data demonstrate that up-regulation of Angptl4 in adipose tissue during CIH causes inhibition of adipose LPL. LPL protein levels were not altered by CIH, which is consistent with the posttranslational mode of Angptl4 action (39, 40). Angptl4 Ab reversed adipose LPL inactivation and increased LPL protein level (Figure 2), which can be attributed to increased levels of

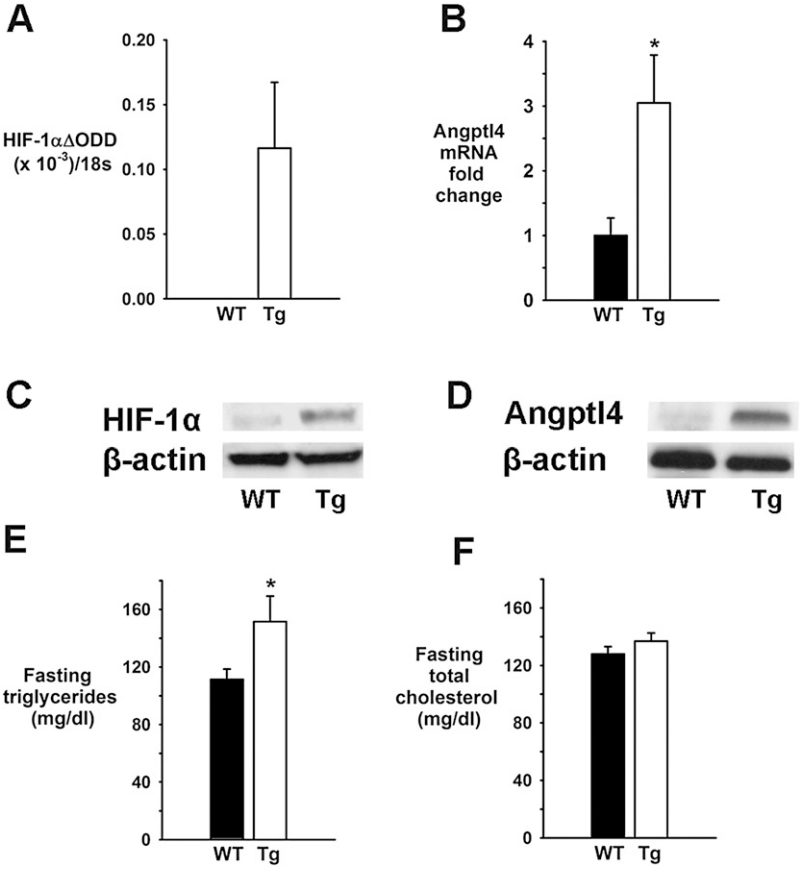

Figure 6. Effect of hypoxia-inducible factor (HIF)-1α overexpression in adipose tissue (a HIF-1α ΔODD mutation) of transgenic (Tg) mice on HIF-1α ΔODD mRNA in omental fat (*A*), angiopoietin-like 4 (Angptl4) mRNA in omental fat (*B*), protein levels of HIF-1α (*C*) and Angptl4 (*D*) (representative samples) in omental fat, fasting serum triglyceride levels (*E*), and fasting serum total cholesterol levels (*F*). *$P <$ 0.05 for the difference between Tg and wild-type (WT) mice.

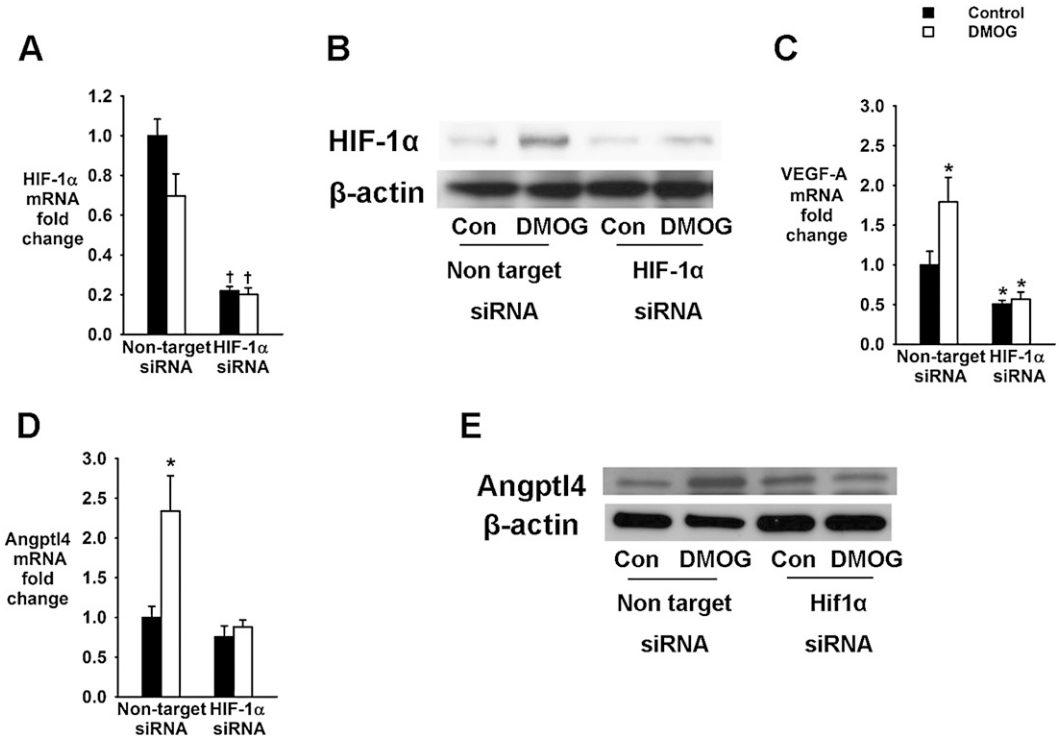

Figure 7. Effects of hypoxia-inducible factor (HIF)-1α protein stabilization with a prolyl hydroxylase inhibitor dimethyloxaloylglycine (DMOG) in 3T3-L1 adipocytes transfected with HIF-1α or nontarget small interfering RNA (siRNA). (*A*) HIF-1α mRNA levels. (*B*) HIF-1 protein levels (representative bands). (*C*) Vascular endothelial growth factor (VEGF)-A mRNA levels. (*D*) Angiopoietin-like 4 (Angptl4) mRNA levels. (*E*) Angptl4 protein levels (representative bands). $^{*}P < 0.05$ for the difference with control cells transfected with nontarget siRNA. $^{\dagger}P < 0.001$ for the difference with control cells transfected with nontarget siRNA. Con = control.

LPL dimers. The latter have higher affinity for cell surface proteoglycans than monomers and therefore are more stable (40). Neither CIH nor Ab affected LPL activity in skeletal muscle or the heart, suggesting that the effect of adipose Angptl4 is local. In addition, LPL is regulated by multiple mechanisms (28), and it is conceivable that CIH affects LPL in skeletal and cardiomyocytes via other pathways counterbalancing any impact of Angptl4. Overall, our data suggest that CIH inhibits adipose LPL via Angptl4.

Angptl4 and Dyslipidemia during CIH

Our previous and present work showed that CIH increases plasma levels of triglyceride-rich lipoproteins by delaying lipoprotein clearance (20). LPL plays a pivotal role in the clearance of VLDL and chylomicrons (28). The current study revealed that depletion of Angptl4 abolished CIH-induced increases in plasma VLDL, suggesting that Angptl4-mediated inactivation of LPL is the principal mechanism of VLDL dyslipidemia during CIH. Angpl4 Ab also attenuated the CIH-induced increase

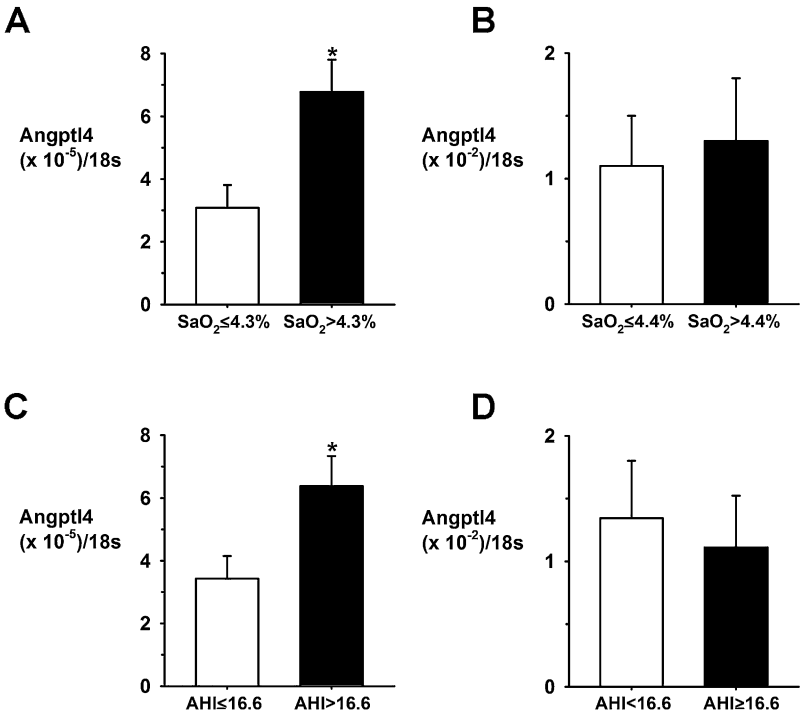

Figure 8. Adipose Angptl4 mRNA levels in obese patients grouped according to median splits of the average fall in oxyhemoglobin saturation (ΔSp_{O_2}) during apneic/hypopneic episodes and the apnea-hypopnea index (AHI). (*A*) Angiopoietin-like 4 (Angptl4) mRNA levels in subcutaneous adipose tissue in patients with $\Delta Sp_{O_2} \leq 4.3\%$ (n = 8) and $> 4.3\%$ (n = 7). (*B*) Angptl4 mRNA levels in visceral adipose tissue in patients with $\Delta Sp_{O_2} \leq 4.4\%$ (n = 11) and $> 4.4\%$ (n = 10). (*C*) Angptl4 mRNA levels in subcutaneous adipose tissue in patients with AHI $\leq$ 16.6/h (n = 8) and $>$ 16.6/h (n = 7). (*D*) Angptl4 mRNA levels in visceral adipose tissue in patients with the AHI $<$ 16.6/h (n = 11) and $\geq$ 16.6/h (n = 10). $^{*}P < 0.05$.

in LDL cholesterol and the incomplete inhibition indicates that other factors may also contribute to CIH-induced LDL hyperlipidemia. Indeed, hepatic VLDL secretion may also be increased by CIH via up-regulation of hepatic stearoyl coenzyme A desaturase (9, 10). Thus, we found that Angptl4-mediated suppression of adipose LPL plays a pivotal role in triglyceride-rich lipoprotein hyperlipidemia during CIH.

Angptl4 and CIH-induced Atherosclerosis

Multiple mechanisms may contribute to accelerated atherosclerosis during CIH, including proatherogenic dyslipidemia, hypertension, vascular inflammation, and oxidative stress (9–14). Shear stress of elevated blood pressure damages the endothelium allowing macrophage infiltration of the arterial intima. Macrophages take up oxidized LDL, becoming foam cells that form the atherosclerotic lesions. Macrophage foaming occurs due to HIF-1α–dependent accumulation of sterols and triglycerides and decreased cholesterol efflux (41). The main finding of our study is that Angptl4-mediated inactivation of adipose LPL and resulting hyperlipidemia is the key mechanism in the progression of atherosclerosis during CIH. In fact, Angptl4 deficiency may confer a partial protection against atherosclerosis in *ApoE*-deficient mice at baseline (42). Other mechanisms of atherogenesis triggered by CIH were not sufficient to induce the progression of atherosclerosis when Angptl4 was depleted. Thus, the progression of atherosclerosis during CIH occurs due to inefficient clearance of atherogenic, triglyceride-rich lipoproteins.

Clinical Implications

An E40K loss-of-function variant of the *ANGPTL4* gene in humans was associated with substantially reduced plasma triglyceride levels, increased plasma HDL levels, and reduced risk of stroke, peripheral vascular disease, and carotid atherosclerosis (39, 43, 44). Human OSA is associated with non-HDL hyperlipidemia and triglyceridemia and atherosclerosis (6). OSA has been associated with increased postprandial hyperlipidemia (16), which is consistent with LPL inhibition. In addition, circulating LPL concentrations in patients with OSA are decreased in proportion to the severity of the disease (45). Our human data revealed that Angptl4 levels in subcutaneous adipose tissue directly correlated with nocturnal hypoxemia in patients with OSA and that subcutaneous adipose Angptl4 levels were increased in patients with more severe OSA, independent of BMI (Figure 8). Taken together with our murine data, these data suggest that up-regulation of adipose Angptl4 may play a role in the progression of atherosclerosis in OSA.

Limitations of the Study

Our study had several limitations. First, murine CIH mimics oxyhemoglobin desaturation in severe OSA and induces sleep fragmentation but does not model other features of OSA, such as snoring, hypercapnea, and transpulmonary pressure swings. Second, it remains unknown why CIH up-regulates Angptl4 exclusively in adipose tissue, although the severity of tissue hypoxia and oxidative stress with ensuing activation of HIF-1α may play a role. Third, Angptl4 Ab reversed CIH-induced hypertriglyceridemia and hypercholesterolemia in $ApoE^{-/-}$ mice, whereas HIF-1α knockout and overexpression affected only plasma triglyceride levels in $HIF\text{-}1a^{+/-}$ and *HIF-1aΔODD* mice. This phenomenon is likely explained by differences in the lipoprotein profile, with the most of cholesterol confined to the VLDL fraction in $ApoE^{-/-}$ mice and to the HDL fractions in other strains. Fourth, we did not observe correlations between visceral adipose Angptl4 levels and the AHI or nocturnal hypoxemia in patients with OSA. Differences between our human and mouse data may be related to the fact that human data were obtained in severely obese individuals, whereas mouse experiments were performed in lean mice. Obesity *per se* leads to adipose tissue hypoxia and up-regulation of HIF-1α in the absence of OSA or IH (46–48). In addition, factors other than hypoxia factors could influence Angptl4 levels in visceral fat (e.g., high levels of FFA compared with subcutaneous fat) (38, 49). Fifth, we lacked data on the severity of atherosclerosis in our human subjects. However, an association between OSA and atherosclerosis (7, 8) and attenuation of markers of atherosclerosis after continuous positive airway pressure (50) have been previously reported.

Conclusions

CIH causes atherosclerosis via HIF-1–mediated up-regulation of adipose Angptl4, which inactivates adipose LPL and inhibits clearance of triglyceride-rich lipoproteins in mice. Intermittent nocturnal hypoxemia is associated with increased Angptl4 mRNA levels in subcutaneous adipose tissue in obese humans, but the role of adipose Angptl4 in the progression of atherosclerosis in patients with OSA is yet to be confirmed.

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