

# Effect of chronic intermittent hypoxia on triglyceride uptake in different tissues

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**Abstract** Chronic intermittent hypoxia (CIH) inhibits plasma lipoprotein clearance and adipose lipoprotein lipase (LPL) activity in association with upregulation of an LPL inhibitor angiopoietin-like protein 4 (Angptl4). We hypothesize that CIH inhibits triglyceride (TG) uptake via Angptl4 and that an anti-Angptl4-neutralizing antibody would abolish the effects of CIH. Male C57BL/6J mice were exposed to four weeks of CIH or intermittent air (IA) while treated with Ab (30 mg/kg ip once a week). TG clearance was assessed by [<sup>3</sup>H]triolein administration retroorbitally. CIH delayed TG clearance and suppressed TG uptake and LPL activity in all white adipose tissue depots, brown adipose tissue, and lungs, whereas heart, liver, and spleen were not affected. CD146+ CD11b<sup>-</sup> pulmonary microvascular endothelial cells were responsible for TG uptake in the lungs and its inhibition by CIH. Antibody to Angptl4 decreased plasma TG levels and increased TG clearance and uptake into adipose tissue and lungs in both control and CIH mice to a similar extent, but did not reverse the effects of CIH. The antibody reversed the effects of CIH on LPL in adipose tissue and lungs. **In conclusion, CIH inactivates LPL by upregulating Angptl4, but inhibition of TG uptake occurs predominantly via an Angptl4/LPL-independent mechanism.**—Yao, Q., M.-K. Shin, J. C. Jun, K. L. Hernandez, N. R. Aggarwal, J. R. Mock, J. Gay, L. F. Drager, and V. Y. Polotsky. **Effect of chronic intermittent hypoxia on triglyceride uptake in different tissues.** *J. Lipid Res.* 2013. 54: 1058–1065.

**Supplementary key words** lipoprotein clearance • brown adipose tissue • white adipose tissue • obstructive sleep apnea • angiopoietin like protein 4 • lipoprotein lipase • lungs • pulmonary microvascular endothelial cells

Obstructive sleep apnea (OSA) leads to chronic intermittent hypoxia (CIH) during sleep (1). OSA causes

significant cardiovascular morbidity and mortality (2–4). Several lines of evidence demonstrated that OSA is associated with dyslipidemia and accelerated atherosclerosis (5–12). A randomized, placebo-controlled clinical trial of continuous positive airway pressure (CPAP) has shown that OSA treatment with CPAP markedly reduces postprandial hyperlipidemia (13). Postprandial hypertriglyceridemia may confer risk for myocardial infarction, ischemic heart disease, stroke, and death (14–20). Therefore, it is conceivable that postprandial hyperlipidemia contributes to the cardiovascular risk of OSA. The pathogenesis of postprandial hyperlipidemia in OSA is unknown.

We developed a mouse model of CIH, which mimics oxyhemoglobin desaturations in patients with OSA (21–23). Using this model, we have recently shown that CIH impairs chylomicron clearance in mice and inhibits a key enzyme of triglyceride-rich lipoprotein clearance, lipoprotein lipase (LPL), in adipose tissue (24). CIH also upregulated a potent LPL inhibitor, adipose angiopoietin-like protein 4 (Angptl4) (24). However, the effect of CIH on triglyceride (TG) uptake by adipose tissue has not been explored; contribution of other organs and tissues to lipoprotein clearance during CIH is unknown. Finally, the role of Angptl4 in CIH-induced metabolic dysfunction has not been determined.

We hypothesized that CIH impairs TG uptake by adipose tissue depots and other organs and tissues via Angptl4. We exposed C57BL/6J mice to CIH for four weeks while treating them with Angptl4-neutralizing antibodies (Ab) or placebo (vehicle solution) and examined plasma clearance and tissue uptake of [<sup>3</sup>H]triolein-Intralipid.

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Abbreviations: Angptl4, angiopoietin-like protein 4; BAT, brown adipose tissue; CIH, chronic intermittent hypoxia; CPAP, continuous positive airway pressure; EPI, epididymal; IA, intermittent air; OM, omental; OSA, obstructive sleep apnea; RP, retroperitoneal; SC, subcutaneous; TC, total cholesterol; TG, triglyceride; WAT, white adipose tissue.

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### Experimental animals

In the study, we used a total of 114 male C57BL/6J mice from Jackson Laboratory (Bar Harbor, ME), 6–8 weeks of age at the beginning of the experiments. Fifty animals were used for fasting plasma lipids, tissue LPL activity, gene expression, and protein measurements; 54 mice were used in the [ $H^3$ ]-TG clearance experiment; and 10 mice were used for [ $H^3$ ]-TG clearance by different cell populations of the lungs. Mice were fed with regular chow and exposed to CIH or intermittent air (IA) while being treated with Angptl4-neutralizing antibody or vehicle. The IA group was weight-matched to the CIH group by varying food intake as previously described (21). The study was approved by the Johns Hopkins University Animal Use and Care Committee and complied with Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (25).

### Intermittent hypoxia and Angptl4 antibody treatment

Our mouse model of CIH that mimics the oxygen profile observed in patients with OSA has been previously described (21, 24). During each cycle of intermittent hypoxia, the  $FiO_2$  decreased from 21% to 6.5% over a 30 s period, followed by a rapid return to 21% over the subsequent 30 s period. This regimen of intermittent hypoxia induces oxyhemoglobin desaturations from 99% to 70% 60 times/hour (21, 26). A control group was exposed to an identical regimen of IA. CIH and IA were administered during the light phase (9 AM to 9 PM) to coincide with the mouse sleep cycle. The duration of exposure was four weeks. During the exposure to IA or CIH, mice were treated with Angptl4-neutralizing Ab (14D12 monoclonal antibody, Lexicon Pharmaceuticals, Inc., The Woodlands, TX) (27) in phosphate buffered saline (PBS) at 30 mg/kg (the Ab group) or with PBS alone (the vehicle group) once per week for four weeks. For tissue harvesting, mice were euthanized under 1–2% isoflurane anesthesia after a 4 h fast. All animal euthanizing was performed between 12 PM and 2 PM, during the light phase. Blood was collected by retroorbital puncture. Heart, lung, liver, spleen, brown adipose tissue (BAT), epididymal (EPI), omental (OM), subcutaneous (SC), and retroperitoneal (RP) white adipose tissue (WAT), and skeletal muscle (quadriceps) were immediately snap-frozen in liquid nitrogen. Fasting plasma lipids were measured with total cholesterol and TG kits from Wako (Richmond, VA).

### [ $H^3$ ]triolein-Intralipid TG clearance and tissue uptake

Intralipid (20%, Kabi Pharmacia, Clayton, NC) was diluted in sterile PBS to a final 5% concentration, labeled with 240  $\mu$ Ci of [ $H^3$ ]triolein (Perkin-Elmer NET431001MC) as previously described (28, 29), and administered by retroorbital injection (100  $\mu$ l). Blood samples were taken from the opposite retroorbital sinus 30, 150 and 300 s after the injection under 1–2% isoflurane anesthesia. At the completion of the study (300 s after injection), mice were perfused with 10 ml of PBS through the left ventricle, heart, lungs, liver, spleen, BAT, OM, EPI, SC, and RP WAT, and skeletal muscle (quadriceps) were collected. Radioactivity of plasma and tissue homogenates was determined directly (without prior lipid extraction) in a scintillation counter. The plasma TG clearance and tissue TG uptake were quantified according to Augustus et al. with minor modifications (29). The plasma TG clearance was calculated as the ratio of plasma radioactivity at 150 s and 300 s to plasma radioactivity at 30 s. The tissue TG uptake was calculated as the ratio of the whole organ radioactivity to total plasma radioactivity 30 s after the injection of the label.

After our results showed that CIH selectively suppresses TG uptake in the lungs (see Results), we aimed to determine TG uptake by different cellular populations. For this purpose, 300 s after the retroorbital injection of [ $H^3$ ]-labeled Intralipid, mice were perfused with 10 ml of ice-cold PBS through the right ventricle, the lungs were inflated with a dispase solution (BD Biosciences, CA), isolated, and then digested at 37°C with an enzyme cocktail containing 5 mg of collagenase I and 1 mg of DNase I in 1 ml of DMEM high-glucose medium per lung (Life Technologies, Grand Island, NY). CD11b magnetic beads were used to isolate macrophages in the cell suspensions according to the manufacturer's protocol (Miltenyi Biotec, Auburn, CA) followed by CD146 magnetic bead incubation of the CD11b flow through fraction to isolate endothelial cells. Radioactivity of CD11b+, CD11b– CD146+, and CD11b– CD146– cells was measured in a scintillation counter and normalized per number of cells.

### LPL activity in tissues

LPL activity in heparin eluates from the heart, lung, liver, spleen, BAT, epididymal, omental, subcutaneous, and retroperitoneal WAT, and skeletal muscle was performed according to Nilsson-Ehle and Schotz with minor modification (24, 30). One unit of LPL activity was defined as the release of 1 mmol of free fatty acids (FFA) in 1 h per gram of tissue.

### Real-time PCR

Total RNA was extracted from liver using Trizol (Life Technologies, Rockville, MD), and cDNA was synthesized using Advantage RT for PCR kit from Clontech (Palo Alto, CA). Real-time reverse-transcriptase PCR (RT-PCR) was performed with primers from Invitrogen (Carlsbad, CA) and Taqman probes from Applied Biosystems (Foster City, CA). The sequences of primers and probes for mouse and human 18S were previously described (31). Mouse Angptl4 mRNA had been measured with the Applied Biosystems premade primers and probes. The primers and probes for mouse Angptl4 were designed based on the GenBank sequence NM\_020581.2, forward primer 5'-CAGGACTGGGATGGCAATG-3', reverse primer 5'-GTGTCCTCACCCCCAAAT-3', and the probe 5'-ATTGCTCCAATTTC-3'.

Gene-specific primers of LPL (Mm00434770\_m1) and CD36 (Mm01135198\_m1) were supplied by Applied Biosystems (Foster City, CA). The mRNA expression levels were referenced to 18S rRNA, and the values were derived according to the  $2^{-\Delta\Delta Ct}$  method (32, 33).

### Western blot

The omental adipose tissue and lung tissues were homogenized in RIPA buffer (Sigma, St. Louis, MO). SDS-PAGE and Western blot were performed using Bio-Rad precast gel system. Twenty micrograms of proteins were applied per lane. For CD11b detection, we used primary rabbit polyclonal antibody against mouse CD11b (Abcam, Cambridge, MA) and goat anti-rabbit HRP (KPL). For CD146 detection, we used primary rabbit monoclonal antibody against mouse CD146 (Millipore, Billerica, MA) and goat anti-rabbit HRP (KPL). Actin was detected with mouse monoclonal anti-actin antibody from Sigma (A3853) and goat anti-mouse-HRP (KPL).

### Statistical analysis

All values are reported as means  $\pm$  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 posthoc correction for multiple comparisons. All tests were two-sided, and the significance level was established at  $P < 0.05$ .

## RESULTS

### Basic characteristics

CIH induced weight loss (Table 1). Mice exposed to IA were weight-matched to the CIH group and, therefore, there was no difference in body weight or food intake between the groups. Ab treatment had no effect on body weight or food intake. CIH decreased the amount of OM and SC WAT, whereas liver weight was increased, regardless of Ab treatment. Neither CIH nor Ab affected weight of EPI WAT, BAT, lungs, spleen, or the heart (Table 1).

### Effect of CIH on Angptl4, CD36, and LPL gene expression in different tissues

We have previously shown that CIH upregulates Angptl4 mRNA and protein levels in epididymal WAT (24). In this study, CIH also upregulated Angptl4 in omental WAT (a  $3.2 \pm 1.2$ -fold increase) and lung tissue (a  $3.1 \pm 0.8$ -fold increase), but not in BAT, subcutaneous WAT, heart or liver. mRNA levels of LPL and CD36, a fatty acid transporter (34), were not altered by CIH.

### Effects of Angptl4-neutralizing antibodies and CIH on fasting plasma lipids and TG clearance

CIH increased plasma fasting total cholesterol (TC) and TG levels (Fig. 1). Regardless of IA or CIH exposure, Angptl4 Ab decreased fasting TG and TC. Ab treatment did not diminish the effect of CIH on fasting TG (Fig. 1A), whereas the effect of CIH on fasting cholesterol was attenuated (Fig. 1B). CIH significantly delayed clearance of [ $H^3$ ]triolein-Intralipid (Fig. 2). In the IA group,  $25.6\% \pm 2.3\%$  of [ $H^3$ ] triglyceride was detected in plasma 300 s after injection, whereas in the CIH group,  $36.0\% \pm 5.2\%$  of [ $H^3$ ] triglyceride remained in circulation at this time ( $P < 0.05$ ). Blocking Angptl4 with Ab accelerated the TG clearance, but the effect of CIH was still present.

### Effects of CIH and Angptl4-neutralizing antibodies on TG uptake

TG uptake was detected in multiple organs and tissues 300 s after the injection, with particularly high levels in the

liver (Fig. 3, insert). CIH significantly decreased TG uptake by BAT (by 44%), EPI WAT (by 64%), OM WAT (by 48%), and SC WAT (by 77%, Fig. 3). CIH decreased TG uptake by the lungs (56%), whereas heart, liver, and spleen were unaffected. Angptl4 depletion significantly increased TG uptake by all adipose tissue depots, lungs (a 4.4-fold increase), and the spleen (a 2.2-fold increase), but the inhibitory effect of CIH on TG uptake in adipose tissue and lungs was still present, despite Ab treatment. Neither CIH nor Ab affected TG uptake by heart, liver (Fig. 3), or skeletal muscle (quadriceps not shown). Adipose tissue and the lungs were the only tissues in which TG uptake was significantly affected by both CIH and Angptl4.

### Effects of CIH and Angptl4-neutralizing antibodies on tissue LPL activity

The highest LPL activity was detected in heart tissue, whereas, as expected, the lowest levels were in the liver, which does not express active LPL in the adult state (35). CIH significantly decreased LPL activity in BAT, EPI, OM, SC, and RP WAT, and the lungs (Fig. 4), whereas heart, skeletal muscle, liver, and spleen were not affected. Angptl4-neutralizing Ab increased LPL activity in all adipose tissue depots, lungs, liver, and spleen, but not in the heart and skeletal muscle tissue. Similar to the TG uptake data, adipose tissue and lungs were the only tissues in which LPL activity was significantly affected by both CIH and Angptl4. In contrast to TG uptake, Angptl4 depletion completely abolished the inhibitory effect of CIH on LPL activity in WAT and lungs (Fig. 4).

### Effect of CIH on TG uptake by different cellular populations in the lungs

Given that the lungs accounted for a significant fraction of TG uptake and that lung tissue was the only tissue, besides adipose, in which CIH decreased TG uptake and LPL activity (Figs. 3 and 4), we attempted to identify the cellular populations involved. We used CD11b as a macrophage marker (36, 37) and CD146 as an endothelial cell marker (38–41) in single-cell suspension of the lungs from mice

TABLE 1. Food intake, body weight, and weights of adipose tissue depots and organs in C57BL/6J mice exposed to IA or CIH while treated with Angptl4 antibody or vehicle for four weeks

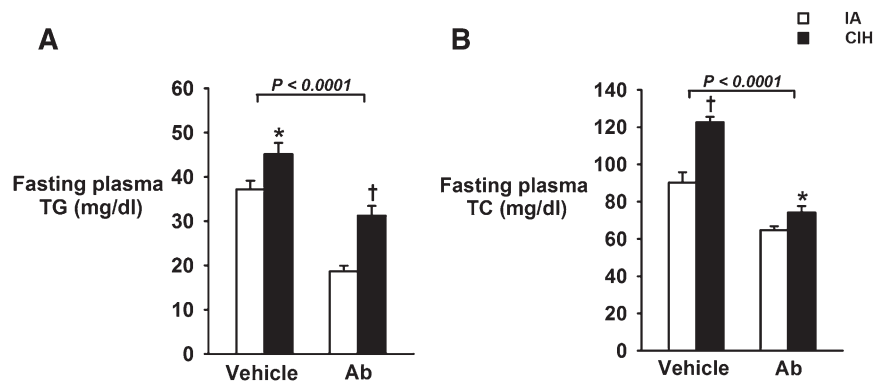
Characteristic	IA - Vehicle	IA - Ab	CIH - Vehicle	CIH - Ab
N	28	30	28	28
Mean food intake (g/mouse/day)	$2.94 \pm 0.13$	$2.83 \pm 0.11$	$3.22 \pm 0.29$	$3.26 \pm 0.28$
Body weight (g):				
Day 0	$24.8 \pm 0.5$	$25.5 \pm 0.5$	$25.3 \pm 0.6$	$25.9 \pm 0.5$
Day 28	$24.4 \pm 0.4$	$24.8 \pm 0.6^c$	$24.1 \pm 0.4^c$	$24.5 \pm 0.6^c$
EPI WAT (g)	$0.49 \pm 0.029$	$0.49 \pm 0.028$	$0.44 \pm 0.017$	$0.44 \pm 0.023$
OM WAT (g)	$0.22 \pm 0.013$	$0.20 \pm 0.012$	$0.18 \pm 0.010^a$	$0.18 \pm 0.009^a$
RP WAT (g)	$0.11 \pm 0.008$	$0.10 \pm 0.007$	$0.11 \pm 0.006$	$0.12 \pm 0.023$
SC WAT (g)	$0.38 \pm 0.020$	$0.35 \pm 0.020$	$0.32 \pm 0.015^a$	$0.32 \pm 0.015^a$
BAT (g)	$0.23 \pm 0.010$	$0.25 \pm 0.020$	$0.21 \pm 0.007$	$0.25 \pm 0.016$
Liver (g)	$0.87 \pm 0.027$	$0.86 \pm 0.014$	$0.93 \pm 0.019^b$	$1.00 \pm 0.021^b$
Lung (g)	$0.14 \pm 0.003$	$0.13 \pm 0.004$	$0.15 \pm 0.006$	$0.14 \pm 0.003$
Spleen (g)	$0.05 \pm 0.003$	$0.06 \pm 0.002$	$0.05 \pm 0.002$	$0.06 \pm 0.002$
Heart (g)	$0.12 \pm 0.002$	$0.11 \pm 0.002$	$0.11 \pm 0.002$	$0.12 \pm 0.002$

<sup>a</sup> $P < 0.05$  for the effects of hypoxia.

<sup>b</sup> $P < 0.001$  for the effects of hypoxia.

<sup>c</sup> $P < 0.001$  versus day 0 body weight.



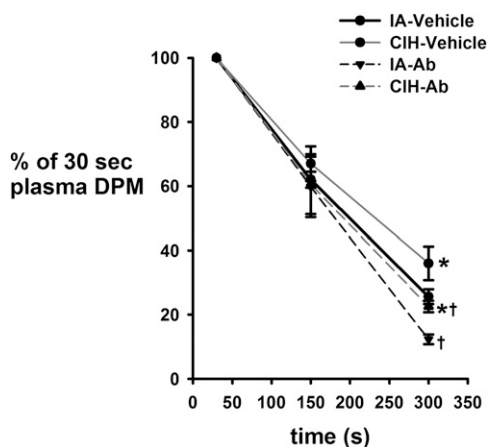


**Fig. 1.** Effect of CIH and Angptl4-neutralizing antibodies on serum fasting levels of (A) TG and (B) TC. \* $P < 0.05$  and † $P < 0.001$  versus IA.

euthanized 300 s after [ $H^3$ ]triolein-Intralipid injection. CD11b<sup>−</sup> CD146<sup>+</sup> pulmonary endothelial cells exhibited a greater than 4-fold TG uptake than CD11b<sup>+</sup> cells and a greater than 10-fold TG uptake than CD11b<sup>−</sup> CD146<sup>−</sup> cells, which included epithelial cells, stromal cells, lymphocytes, and other lung cells (Fig. 5A). CIH decreased TG uptake by CD11b<sup>−</sup> CD146<sup>+</sup> endothelial cells by 42%, whereas TG uptake by CD11b<sup>+</sup> macrophages and by CD11b<sup>−</sup> CD146<sup>−</sup> cells was not affected (Fig. 5A). Western blot confirmed the absence of CD11b<sup>+</sup> cells in the CD11b<sup>−</sup> CD146<sup>+</sup> fraction, whereas CD11b<sup>+</sup> macrophages could express CD146 (42) (Fig. 5B). Thus, pulmonary endothelial cells were predominantly responsible for the TG uptake in the lungs and susceptible to the inhibitory effect of CIH.

## DISCUSSION

In this study, we examined mechanisms of triglyceride clearance impaired by CIH. The main novel finding of our study is that CIH inhibited TG uptake and LPL activity in all adipose tissue depots and lungs, delaying



**Fig. 2.** Effect of CIH and Angptl4-neutralizing antibodies on clearance of [ $H^3$ ]triolein-Intralipid. Results are normalized to the plasma radioactivity 30 sec after injection according to Refs. 34, 72. \* $P < 0.05$  for the effect of CIH; † $P < 0.001$  for the effect of Ab.

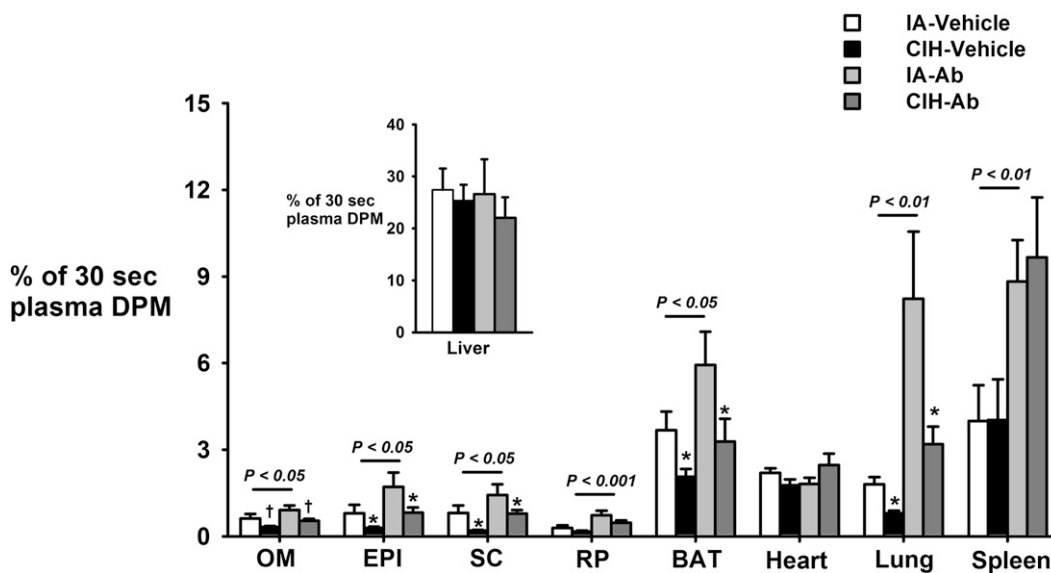
lipoprotein clearance. In addition, we report several other novel findings. First, Angptl4-neutralizing antibody increased LPL activity in all adipose tissue depots and lungs, abolishing the inhibitory effect of CIH. Second, Angptl4-neutralizing antibody increased TG uptake in all adipose tissue depots, but the inhibitory effect of CIH was not reversed, which suggests that CIH suppresses TG clearance via an Angptl4/LPL-independent mechanism. Third, pulmonary endothelial cells were responsible for the majority of the TG uptake in the lungs, and CIH inhibited TG uptake by pulmonary endothelial cells, but not by other lung cellular populations.

## CIH and Angptl4

Consistently with our previous findings (24), CIH suppressed TG clearance. We have now shown that CIH-induced suppression of LPL activity and TG uptake occurs in all adipose tissue depots as well as in the lungs. LPL inactivation by CIH is likely attributable to upregulation of a potent LPL inhibitor, Angptl4 (43–50). The effect of CIH on LPL is posttranscriptional given that mRNA levels were unchanged, which is consistent with the effect of Angptl4. The N-terminus of Angptl4 binds to LPL anchored to the surface of endothelial cells, converting active LPL dimers to inactive monomers (43, 50, 51). We have previously shown that CIH induces Angptl4 in epididymal fat (24). In the present study, we report that the effect of CIH on Angptl4 is tissue-dependent, affecting OM fat and the lung, but not other tissues. Expression of Angptl4 is regulated by hypoxia inducible factor 1 (HIF-1) (52–57). We have recently shown that CIH-induced upregulation of Angptl4 in visceral fat is abolished by heterozygous deficiency of HIF-1 $\alpha$ , whereas constitutive overexpression of HIF-1 $\alpha$  increases Angptl4 levels (58). Although mechanisms of selective upregulation of Angptl4 in OM and EPI WAT and lungs are unknown, we hypothesize that differential regulation of HIF-1 $\alpha$  in different organs may play a role.

## CIH, LPL, and TG uptake

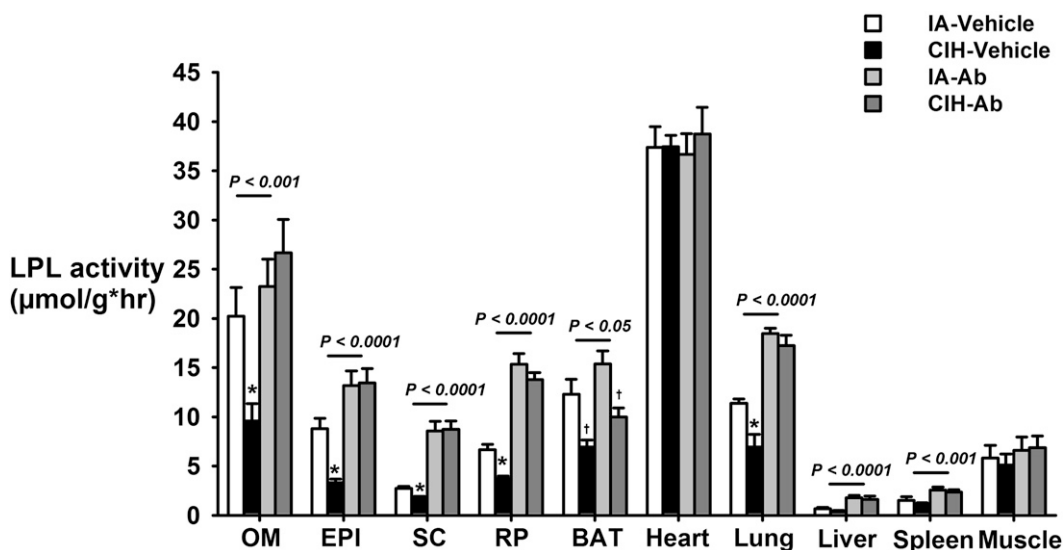
Angptl4-neutralizing Ab completely reversed CIH-induced inhibition of LPL in all WAT depots and the lung. Neutralizing Ab bind to specific epitope at the N-terminus



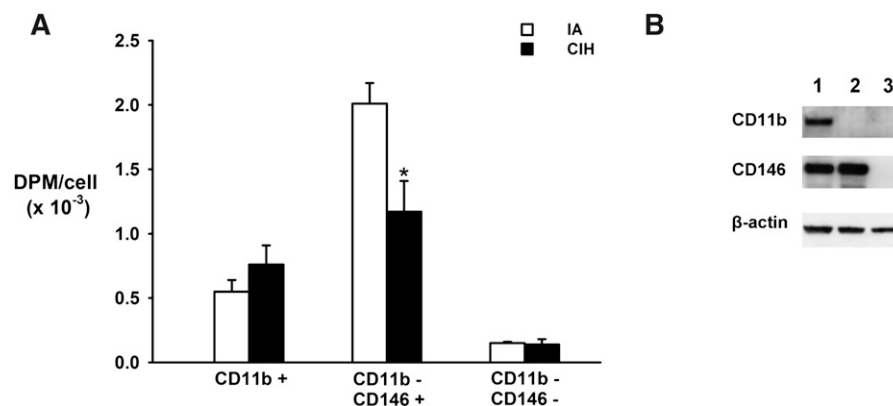
**Fig. 3.** Effect of CIH and Angptl4-neutralizing antibodies on the [ $H^3$ ]triolein-Intralipid uptake in OM, EPI, SC, and RP WAT, BAT, heart, lung, liver, and spleen 300 sec after injection. The results are normalized to the plasma radioactivity 30 sec after injection according to Refs. 34, 72). The liver data are depicted on the insert. \* $P < 0.05$  and † $P < 0.001$  for the effect of hypoxia.

of Angptl4, blocking its ability to inactivate LPL (27, 59). Our findings implicate OM and lung Angptl4 in inhibition of LPL during CIH. Angptl4 Ab decreased fasting lipid levels, accelerated TG clearance, and increased TG uptake by all adipose tissue depots and lung tissue, suggesting that Angptl4-mediated inhibition of LPL contributed to dyslipidemia of CIH. However, Angptl4 Ab-treated mice still exhibited decreased TG tissue uptake during CIH, despite restoration of LPL activity. The latter strongly suggests that inhibition of LPL is not the principal mechanism of impaired TG tissue uptake in hypoxic mice. What would be potential LPL-independent mechanisms? One possibility is that CIH inhibited transport of FFA derived

from LPL-mediated lipolysis of TG-rich lipoproteins. CIH did not alter expression of an FFA transporter CD36 (34), but other FFA transporters could have been affected, including fatty acid binding and transport proteins (60). CIH may affect nonreceptor-mediated FFA uptake. Another possibility would be that TG uptake is influenced by changes in tissue perfusion during hypoxic exposure. CIH activates sympathetic nervous system (61–63), which can lead to peripheral vasoconstriction and relatively poor perfusion of adipose tissue (64, 65). Catecholamines can also inhibit LPL posttranscriptionally (35, 66, 67). Interestingly, CIH-induced inhibition of LPL in BAT was minimally affected by Angptl4 Ab,



**Fig. 4.** Effect of CIH and Angptl4-neutralizing antibodies on LPL activity in OM fat, EPI fat, SC fat, RP fat, BAT, heart, lung, liver, spleen, and muscle in C57BL/6J mice. \* $P < 0.05$  and † $P < 0.01$  for the effect of hypoxia.



**Fig. 5.** Effect of CIH on [ $H^3$ ]triolein-Intralipid uptake by (A) CD11b+ cells, CD11b- CD146+ cells, and CD11b+ CD146+ cells isolated from the lungs of C57BL/6J mice 300 sec after injection. (B) CD11b and CD146 Western blot in CD11b+ cells (lane 1); CD11b- CD146+ cells (lane 2); and CD11b- CD146- cells (lane 3) isolated from single-cell lung tissue suspension. \* $P < 0.05$  versus IA.

which could be ascribed to abundant sympathetic innervations of BAT (68). We conclude that CIH induces dyslipidemia by suppressing lipoprotein clearance and TG uptake in visceral adipose tissue and the lungs primarily via an LPL-independent mechanism.

#### Role of the lungs in TG clearance during CIH

Liver, spleen, and the heart were responsible for significant portion of TG uptake, but there was no effect of CIH. An unexpected finding of our study was that lungs contributed significantly to lipoprotein clearance and that TG uptake by the lung was inhibited by CIH.

Could our results be influenced by a route of administration of TG? TG were delivered via the retroorbital venous plexus, and consequently, to the superior vena cava, the right heart, and directly to the lungs. Chylomicrons formed from TG in fatty meal enter the systemic venous circulation with lymph flow via the thoracic duct. Thus, although the retroorbital route bypasses intestinal absorption, Intralipid pharmacokinetics resembles that of chylomicrons in fatty meal and should not have a major adverse impact on our results. TG-rich lipoproteins can be used for surfactant biosynthesis in the lungs (69, 70), but impact of CIH on surfactant biosynthesis has not been studied.

CD146+ CD11b- cells were responsible for the vast majority of TG uptake in the lung. CD146 is a validated marker of pulmonary microvascular endothelial cells (38–41). Therefore, our data strongly suggest that pulmonary microvascular endothelial cells participate in postprandial TG clearance. CIH inhibited TG uptake by the pulmonary endothelium (Fig. 5). LPL inactivation was not a key mechanism suppressing TG clearance by the pulmonary endothelium, because CIH decreased pulmonary TG uptake despite restoration of the enzyme activity with Angptl4-neutralizing Ab (Figs. 3 and 4). We hypothesize that changes in pulmonary blood flow during CIH play a role. Hypoxic pulmonary vasoconstriction may decrease the area of the vascular bed available for TG uptake (71) and can be implicated in CIH-induced inhibition of TG clearance, independent of Angptl4.

#### Conclusions

CIH induces postprandial hyperlipidemia by decreasing TG uptake in adipose tissue and the lungs. CIH inactivates adipose and lung LPL via upregulation of Angptl4. However, reversal of LPL inhibition with Angptl4-neutralizing Ab does not abolish CIH-induced impairment in TG uptake. We conclude that CIH inhibits TG uptake in adipose tissue and the lungs primarily via an LPL-independent mechanism.

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