Thermoneutrality modifies the impact of hypoxia on lipid metabolism

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Thermoneutrality modifies the impact of hypoxia on lipid metabolism. Am J Physiol Endocrinol Metab 304: E424–E435, 2013. First published December 18, 2012; doi:10.1152/ajpendo.00515.2012.—Hypoxia has been shown to rapidly increase triglycerides in mice by decreasing plasma lipoprotein clearance. However, the usual temperature of hypoxic exposure is below thermoneutrality for mice, which may increase thermogenesis and energy requirements, resulting in higher tissue lipid uptake. We hypothesize that decreased lipid clearance and ensuing hyperlipidemia are caused by hypoxic suppression of metabolism at cold temperatures and, therefore, would not occur at thermoneutrality. Twelve-week-old, male C57BL6/J mice were exposed to 6 h of 10% O2 at the usual temperature (22°C) or thermoneutrality (30°C). Acclimation to 22°C increased lipid uptake in the heart, lungs, and brown adipose tissue, resulting in lower plasma triglyceride and cholesterol levels. At this temperature, hypoxia attenuated lipid uptake in most tissues, thereby raising plasma triglycerides and LDL cholesterol. Thermoneutrality decreased tissue lipid uptake, and hypoxia did not cause a further reduction in lipid uptake in any organs. Consequently, hypoxia at thermoneutrality did not affect plasma triglyceride levels. Unexpectedly, plasma HDL cholesterol increased. The effect of hypoxia on white adipose tissue lipolysis was also modified by temperature. Independent of temperature, hypoxia increased heart rate and glucose and decreased activity, body temperature, and glucose sensitivity. Our study underscores the importance of ambient temperature for hypoxia research, especially in studies of lipid metabolism.

ACUTE HYPOXIA, as might be encountered in the setting of high altitude (2, 20, 74) or respiratory disorders such as obstructive sleep apnea (18, 63), is associated with elevated plasma triglycerides (TG). To date, the potential mechanisms by which hypoxia increases plasma lipids have not been studied in humans. However, rodents exposed to various forms of acute hypoxia (42, 46) develop hyperlipidemia, allowing for translational mechanistic studies. In mice, we showed recently that TG increase within 6 h of hypoxic exposure and that the magnitude of TG elevation correlated directly with the severity of hypoxia (36). Hypoxia decreased lipid uptake in brown adipose tissue (BAT) and white adipose tissue (WAT) (36) while reducing lipoprotein lipase (LPL) activity and the fatty acid transporters of these tissues. Furthermore, hypoxia lowered rates of hepatic VLDL secretion. Thus, we discovered that hypoxic hyperlipidemia in mice was caused by decreased tissue lipid uptake rather than increased hepatic secretion of lipoproteins.

In this study, we examine in greater depth the contribution of BAT to the development of hypoxic hyperlipidemia. Since BAT is the major site of thermogenesis in rodents (11), it is conceivable that hypoxic hyperlipidemia is dependent upon ambient temperature (Tb). Our previous study was conducted at a Tb of 22°C, so our findings could be explained by the fact that BAT is highly active at 22°C (10), 2 hypoxia inactivates nonshivering thermogenesis in BAT (61), and 3 BAT rapidly catabolizes circulating TG in mice exposed to cold (3). BAT lipid uptake can be minimized by acclimatizing mice to a Tb that obviates their need for nonshivering thermogenesis [thermoneutral zone of 26–34°C (10)]. We hypothesized that lack of BAT activity would increase plasma lipids and that hypoxia under these circumstances would not further increase lipids. To test this hypothesis, we examined the effects of acute hypoxia on lipid metabolism at the usual laboratory temperature (22°C) or at thermoneutrality (30°C). We also examined whether Tb and hypoxia interact to influence other physiological parameters such as body temperature (Tb), heart rate, activity, and glucose tolerance.

MATERIALS AND METHODS

Ethical approval. The study was approved by the Johns Hopkins University Animal Care and Use Committee and complied with the American Physiological Society Guidelines for Animal Studies.

Animals. One-hundred eighty male C57BL6/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were procured at 6 wk of age and acclimated to experimental cages, five per cage, and fed ad libitum. Experiments were performed when mice reached 12–14 wk of age. Cages were placed in a temperature-regulated room at 22°C or in a neonatal incubator (Draeger 8000 IC) set to 30°C for thermoneutrality. The room lights were programmed to maintain a 12:12-h light-dark cycle, with lights on from 0900 to 2100.

Hypoxia exposures. In all experiments, mice were simultaneously fasted and exposed to 6 h of sustained hypoxia at FiO2 = 0.10 beginning at 0800 and ending at 1400. We began hypoxia 1 h before the lights were turned on so that mice were likely to be feeding or recently sated at the onset of hypoxia. In preliminary experiments, this protocol minimized differences in baseline TG levels. A gas regulator system controlled the flow of nitrogen into customized Plexiglas cages, resulting in a stable hypoxia. To prevent accumulation of carbon dioxide and heat, cage lids were left partially open to allow mixing of gas with the surrounding environment. For all sample collections, injections, and surgical procedures, anesthesia was induced and maintained with 1–2% isoflurane administered by face mask. For time course experiments, mice were briefly removed from cages and anesthetized in a drop jar for ~15 s, and a retroorbital blood sample was collected at 2-h intervals. After each sample collection, mice were quickly returned to hypoxia or control cages.

Telemetry and pulse oximetry. Mice were anesthetized with isoflurane and implanted with transmitters from Data Sciences International (St. Paul, MN). For heart rate, PA-C10 telemeters were placed in the left carotid arteries of five mice terminating in the aortic arch. For temperature and activity, TA-F10 telemeters were surgically implanted in the peritoneal cavity of five separate mice. Signals were converted to analog and captured using PowerLabs 16/35 interfaced with LabChart Pro software from ADInstruments (Colorado Springs,

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CO). Activity was recorded as voltage spikes in response to single mouse movements; number of movements per 5-min interval was used as an estimate of locomotor activity. Mice were given >7 days of recovery prior to data collection. To maintain the grouping of mice as performed in other experiments without telemetry, we placed telemetry into 1 mouse/cage accompanied by four uninstrumented mice. Mouse oxygen saturation (SaO$_2$) and breath rate were measured using a MouseOx neck cuff and StarrLink module from Starr Life Sciences (Pittsburgh, PA). To limit the discomform of the neck cuff, recordings were obtained for 30–60 min of baseline, the first 60 min of hypoxia, and the first 30–60 min of recovery.

**Intraperitoneal glucose tolerance test.** Following 6 h of air or hypoxia exposure at 1400, mice (n = 10/group) were injected with 2 g/kg glucose. Blood was collected from the tail in unanesthetized mice 0, 10, 20, 30, 60, 90, and 120 min after glucose injection. Blood glucose was tested with Accu-Chek Comfort Curve kits from Roche Diagnostics (Indianapolis, IN).

**Lipid clearance, lipoprotein lipase, and hepatic lipase activity.** Plasma TG tissue uptake was assessed using bolus injection of Intralipid (Kabi Pharmacia, Clayton, NC), as described previously (28, 36). For lipoprotein lipase (LPL) activity, mice (n = 10/group) were injected with 200 U heparin/kg body wt, returned to cages for 5 min, and reanesthetized to obtain postheparin plasma (19). Tissue LPL activity assayed using an artificial $[^{3}H]$tri olein lipid emulsion; plasma LPL activity was distinguished from hepatic lipase activity by suppression with 1 M NaCl. A separate group of mice exposed in the same manner to 10 or 21% O$_2$ was used for tissue LPL activity, without the mice undergoing heparin injection. LPL activity in hepatic eluates from the lungs, heart, BAT, WAT, and soleus muscle, and hepatic lipase in hepatic eluates from the liver were tested according to Nilsson-Ehle and Schotz (48), with minor modifications (24). One unit of LPL activity was defined as the release of 1 mmol of free fatty acids (FFA) in 1 h/g tissue.

**Real-time PCR.** Total RNA was extracted from tissues using Trizol (Life Technologies, Rockville, MD), and cDNA was synthesized using AdvantageRT 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 results were quantified according to the 2$^{-ΔΔCΤ}$ method (40, 60).

**Biochemical assays.** Cholesterol, TG, FFA, cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (LDL-C) levels were measured using commercially available kits from Wako Diagnostics (Richmond, VA).

**Statistical analysis.** We performed two-way analysis of variance (ANOVA) using the variables of $T_a$ (22 vs. 30°C) and hypoxia (FiO$_2$ = 0.21 or 0.10) with Bonferroni Multiple Comparisons for post hoc comparisons. For intraperitoneal glucose tolerance test, we calculated the area under the curve using a trapezoidal rule, and the area under the curve was compared using two-way ANOVA. Statistics were calculated using GraphPad Prism (GraphPad Software, La Jolla, CA). For continuously measured variables (TG, activity, and heart rate), the effects of time and their interaction with hypoxia or air were examined with mixed-effects linear regression models, using STATA software version 11.0 (STATA, College Station, TX). For all comparisons, a value of $P < 0.05$ was considered significant.

### RESULTS

**Effects of $T_a$ and hypoxia on oximetry, respiratory rate, and core $T_b$.** SaO$_2$ and respiratory rate are shown in Table 1. As expected, initial SaO$_2$ was ~98% in all groups, fell to ~74% during hypoxia, and returned to baseline during recovery. Respiratory rate was lower at thermoneutrality but rose to the same level as mice exposed to hypoxia at 22°C. In terms of $T_b$, we observed influences of $T_a$, hypoxia, and time (Fig. 1A). Before hypoxia (0600–0800), $T_b$ was at its peak in all groups, reflecting the nocturnal circadian rhythm of mice. During this period, $T_b$ was higher in mice housed at the cooler $T_a$ of 22°C ($P < 0.01$). From 0800 to 1400, $T_b$ declined and fell significantly more during hypoxia ($P < 0.01$) regardless of $T_a$. From 1400 to 1600, $T_b$ was at its lowest point in normoxic mice and did not differ with respect to $T_a$. Average $T_b$ was not significantly different between any groups during this period, although $T_b$ in mice recovering from hypoxia briefly exceeded that of the normoxic mice. Hence, mice at both $T_a$ exhibited hypoxemia, hypothermia, and tachypnea during hypoxic exposure. Thermoneutrality accentuated hypoxia-induced tachypnea and diminished the fall in $T_b$ from baseline during the dark phase (0600–0800).

**Effects of $T_a$ and hypoxia on activity.** Mice exposed to 22°C tended to group together, whereas those at thermoneutrality maintained a greater distance between the animals (Fig. 2), a well-known form of behavioral thermoregulation (66), although we did not quantify this behavior in the present study. Activity was highest from 0600 to 0800 in all groups and consistently higher in thermoneutral mice (Fig. 1B). From 0800 to 1400, activity declined in normoxic mice at both $T_a$ but was consistently higher at thermoneutrality ($P < 0.01$). During this period, hypoxia suppressed activity at both $T_a$. From 1400 to 1600, activity recovered in hypoxic mice. In mice at 22°C, activity levels exceeded those of normoxic mice; at thermoneutrality, activity matched that of normoxic mice. Overall, thermoneutrality increased activity, whereas hypoxia suppressed activity.

**Effect of $T_a$ and hypoxia on heart rate.** As shown in Fig. 1C, thermoneutrality significantly lowered heart rate (~100 beats/min relative to mice at 22°C). During exposure to hypoxia, heart rate increased at both $T_a$, but the increase was more pronounced at thermoneutrality, especially in the first hour. The heart rate also varied with time as a function of $T_a$. At 22°C, there was a progressive, modest increase in heart rate in hypoxic mice. At 30°C, the heart rate in hypoxic mice declined over time. During recovery from hypoxia, heart rate returned to prehypoxia levels in mice at both $T_a$. Thus, thermoneutrality lowered overall heart rate, hypoxia increased heart rate, and the magnitude of tachycardia was more pronounced at thermoneutrality.

### Table 1. Oximetry and respiratory rate

<table>
<thead>
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<th>Baseline</th>
<th>Hypoxia or Air</th>
<th>Recovery</th>
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<tbody>
<tr>
<td></td>
<td>22°C</td>
<td>30°C</td>
<td>22°C</td>
</tr>
<tr>
<td>SaO$_2$</td>
<td>0.21</td>
<td>97.7 ± 0.2</td>
<td>98.0 ± 0.2</td>
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<td></td>
<td>0.10</td>
<td>98.2 ± 0.2</td>
<td>97.9 ± 0.2</td>
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<tr>
<td>Respiratory rate</td>
<td>0.21</td>
<td>174 ± 6</td>
<td>144 ± 15†</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>183 ± 7</td>
<td>148 ± 7†</td>
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Values are means ± SE. SaO$_2$, oxygen saturation. *P < 0.05 for air vs. hypoxia; †P < 0.05 for 22 vs. 30°C.
Fig. 1. Body temperature (Tb; A) activity (B), and heart rate (C) of mice exposed to hypoxia at 22 or 30°C. Shaded region represents hypoxic exposure in the hypoxia group (dashed line) or air exposure in control mice (solid line). Right: aggregate mean of baseline (0600–0800), hypoxia/air (0800–1400), or recovery (1400–1600) periods. *P < 0.01 for effect of hypoxia; bracketed P values for significant effects of T<sub>a</sub> are shown. Values are plotted as means ± SE.
Effects of \( T_a \) and hypoxia on glucose and lipid profiles. Glucose, glycerol, FFA, and lipid profiles (Fig. 3) were assessed immediately following hypoxia or air exposure. Relative to mice at 22°C, thermoneutral mice exhibited decreased glycerol and higher TG and cholesterol \((P < 0.05)\). Hypoxia independently increased glucose \((P < 0.05)\). In addition, we noted several striking \( T_a \)-hypoxia interactions. At 22°C, hypoxia increased TG, total cholesterol, and LDL cholesterol and decreased plasma FFA and glycerol. At thermoneutrality, hypoxia increased glycerol and did not affect FFA. Hypoxia had no effect on TG or total cholesterol but lowered LDL-C and unexpectedly increased HDL-C.

Effects of \( T_a \) and hypoxia on glucose tolerance. Acute hypoxia \((43, 73)\) and low \( T_a \) \((67)\) have separately been shown to cause glucose intolerance, so we examined the interaction of these variables on glucose tolerance in mice by intraperitoneal glucose tolerance test (Fig. 4). Thermoneutrality improved glucose tolerance, whereas acute hypoxia worsened glucose tolerance at both \( T_a \) \((P < 0.01)\). Thus, cool \( T_a \) and hypoxia impaired glucose tolerance in an additive but noninteractive manner.

Effects of \( T_a \) and hypoxia on lipid metabolism and hepatic VLDL secretion. As shown in Fig. 5, hypoxia significantly delayed clearance of plasma TG at 22°C. At thermoneutrality, mice exhibited a significantly higher baseline TG, and the rate of decline was not affected by hypoxia. The rate of hepatic secretion of VLDL-TG was not affected by \( T_a \) but was decreased by hypoxia \((P < 0.01; \text{Fig. 6})\). Tissue uptake of \([\text{H}]\)oleate intralipid was greatest at 22°C in the lungs, heart, BAT, and skeletal muscle and was lowered by thermoneutrality \((\text{Fig. 7A})\). Hypoxia inhibited lipid uptake in liver, lung, heart, BAT, and WAT only at 22°C. To examine the role of LPL in these observations, we performed LPL and hepatic lipase (HL) activity assays on tissue homogenates, as shown in Fig. 7B. Thermoneutrality lowered heart and BAT LPL activity and increased WAT LPL activity. Hypoxia independently decreased LPL activity in WAT. Only at 22°C did hypoxia inhibit BAT LPL activity. Postheparin total lipase activity and LPL activity were lower in thermoneutral mice \((\text{Fig. 7C})\), and HL activity trended toward a decrease by hypoxia in 22°C mice \((P = 0.14)\). Therefore, interacting effects of \( T_a \) and hypoxia on lipid uptake appear to be mediated through LPL in the BAT and through LPL-independent mechanisms in other tissues.

Effects of \( T_a \) and hypoxia on transcription of genes of lipid transport in adipose tissue. Previously, we showed that hypoxia in mice at 22°C downregulated transcription of LPL, fatty acid transporter CD36, and the transcription factor peroxisome-proliferator-activated receptor-(PPAR\( \gamma \)) in adipose tissues. Levels of posttranscriptional LPL regulators glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein-1 (GPIHBP1) and angiopoietin-like protein-4 (Angptl4) were not affected \((36)\). Here, we reexamined the effects of hypoxia on adipose tissue gene expression at thermoneutrality. In BAT, as might be anticipated from patterns of lipid uptake and LPL activity, thermoneutrality lowered transcription of LPL, CD36, and PPAR\( \gamma \) \((\text{Fig. 8})\). Hypoxia inhibi-
ited LPL, CD36, and PPARγ transcription only at 22°C. In WAT, LPL, CD36, and PPARγ were inhibited by hypoxia regardless of Ta. Interestingly, transcription of GPIHBP1 and Angptl4 were inhibited only by hypoxia at thermoneutrality.

**DISCUSSION**

The central finding of this study was that hypoxia-induced changes in lipid metabolism depend highly upon Ta. Specifically, mice acclimated to 22°C had relatively low plasma TG and LDL-C levels that were increased by hypoxia, whereas mice acclimated to 30°C had relatively high plasma TG and LDL-C levels that were not further increased by hypoxia. Underlying these changes was the observation that cooler Ta stimulated lipid uptake in several tissues, and hypoxia attenuated this stimulation, especially in BAT. In the discussion below, we will elaborate upon the effects of temperature and hypoxia and address the limitations and clinical implications of this study.

**Effects of ambient temperature.** For clothed humans, 22°C is a comfortable indoor temperature and falls within the human thermoneutral zone (41). For mice, the thermoneutral zone is 26–34°C (10). When mice are challenged with Ta outside of this range, maintenance of Tb requires large amounts of energy, given...
the large surface area/volume ratio of rodents (51, 62). Golozoubova et al. (29) showed that oxygen consumption (\(V\hat{O}_2\)) in mice increases by \(\frac{1}{2}\) by 50% at 22°C compared with thermoneutrality. An increase in \(V\hat{O}_2\) of this magnitude occurs near 0°C in minimally clothed humans (34, 53). In many studies the metabolic, cardiovascular, and immunological differences between humans and mice, hitherto thought to reflect species differences, were eliminated by thermoneutrality (37).

![Figure 4](image_url)

**Fig. 4.** Glucose tolerance of mice exposed to hypoxia at 22 or 30°C. **A:** blood glucose level of mice after injection of 2 g/kg glucose ip. **B:** area under the curve (AUC) of glucose in each group of mice. Hypoxia independently impaired glucose tolerance (*\(P < 0.01\)) and lowered \(T_a\) (*\(P < 0.05\)). Values are plotted as means ± SE.

![Figure 5](image_url)

**Fig. 5.** Triglyceride (TG) levels of mice exposed to hypoxia at 22 or 30°C. **A:** TG level of mice during progressive fasting. **B:** AUC of TG in each group of mice. Hypoxia prolonged lipemia at 22°C (*\(P < 0.01\)) but had no effect at 30°C. Overall, TG levels were higher at 30°C. Values are plotted as means ± SE.
The hypothalamus stimulates shivering and nonshivering thermogenesis, huddling, and other processes to defend $T_h$. In part, these responses are facilitated by increased outflow of the sympathetic nervous system (SNS) and the hypothalamic-pituitary axis (25, 68). This study reveals the integrated hypermetabolic response of mice to cold $T_a$ in terms of both physiology and lipid metabolism. The physiological manifestations of elevated $V_O2$ included a 20% faster respiratory rate, 32% faster heart rate, and 1°C warmer $T_h$. In addition, activity was suppressed, which may reflect the competing energy costs of thermoregulation. Glucose intolerance also occurred, which is a well-known consequence of SNS activation in rodents (67) and humans (16).

Elevated $V_O2$ was also manifested in whole body lipid metabolism. Cold $T_a$ stimulated WAT lipolysis, mobilizing FFA to meet the energy demands of other tissues. In fact, fatty acid uptake doubled in the heart and lungs and tripled in BAT, where expression of fatty acid transport genes was also markedly increased. In a more extreme example of this phenomenon, Barteldt et al. (3) showed that BAT lipid uptake increased 10-fold in mice at $T_a = 4°C$ (compared with $T_a = 22°$), which rapidly reduced plasma lipids. Together with their study, it can be seen that $T_a$ below thermoneutrality stimulates FFA mobilization and uptake by organs critical for oxygen delivery (heart, lungs) and maintenance of $T_h$ (BAT). Furthermore, the contribution of BAT to lipid uptake increases greatly as $T_a$ decreases. As we will discuss below, this hypermetabolic state predisposed mice to hyperlipidemia during hypoxia.

**Effect of hypoxia on lipid metabolism at 22°C.** At 22°C, hypoxia inhibited lipid uptake in all tissues other than skeletal muscle, causing a rapid elevation of plasma TG and LDL-C. This parallels the manner in which hypoxia prevents the augmented $V_O2$ of cold (7, 32). An equivalent degree of hypoxia lowered lipid uptake at 22 but not at 30°C. This fact argues against insufficient oxygen for aerobic metabolism (47) and suggests a regulated decrease in lipid uptake at the cooler $T_a$. Most likely, hypoxia inhibits hypothalamic control of $T_h$, which mediates a systemic decrease in $V_O2$ (26). Thereafter, a combination of end-organ and systemic effectors decreased lipid flux in various tissues. End-organ pathways are exemplified by BAT, where lipid uptake fell in concert with LPL activity and the transcription of CD36 and PPARγ. Hypoxia inhibits PPARγ in adipose tissue (50, 69), which controls the expression of LPL and CD36 (70). Alternatively, decreases in fatty acid transport gene expression in BAT may follow the lowering of plasma FFA (55). In some tissues, such as the lungs, hypoxia lowered lipid uptake without affecting LPL activity. In such cases, blood flow, hormonal signaling, or fatty acid transport may have been responsible for decreasing tissue lipid uptake.

Hypoxia inhibited WAT lipolysis at 22°C. Hypoxic inhibition of cold-stimulated lipolysis has been reported by others, but mechanisms are not known (4, 5). Possibly, blunted adrenoreceptor sensitivity (15), accumulations of adenosine and lactate (22), or reductions in blood flow to WAT caused by hypoxia (39) and SNS stimulation (23, 58) are responsible for reduced efflux of FFA from WAT. Since hypoxia stimulates the SNS, which activates WAT lipases, local WAT factors must have prevailed over sympathetic stimulation. In our previous study, hypoxia stimulated WAT lipolysis even at 22°C (36). Differences in the age of mice (8 vs. 12 wk), circadian factors (lights were turned on simultaneously with the onset of hypoxia), and less rigorous control over $T_h$ in the prior study might account for differences.

**Effects of hypoxia on lipid metabolism at 30°C.** Seminal experiments demonstrated that hypoxia suppresses $V_O2$ in small mammals in a $T_a$-dependent manner, with thermoneutrality markedly attenuating or abolishing this response (27, 32). Since humans exhibit a stable or elevated $V_O2$ during acute hypoxia (8, 33, 56, 57), thermoneutrality in effect “humanizes” the murine response. Indeed, lipid uptake in tissues was already low and not further reduced by hypoxia at 30°C. In addition, we observed several unanticipated changes in plasma lipids and cholesterol. First, hypoxia lowered plasma LDL-C. This most likely resulted from a fall in hepatic VLDL secretion without the suppression of lipid uptake that occurred at 22°C. Second, hypoxia increased plasma HDL-C. In humans, Ferezou et al. (21) showed that ascent to 4,300 m decreased postprandial lipemia and increased HDL-C. In another study, a simulated ascent to 12,000 ft. for 8.5 h increased HDL-C (14). Although the mechanisms and cardiovascular implications of HDL-C elevation by hypoxia are not clear, thermoneutral $T_h$ will be a prerequisite for further studies in mice. Third, hypoxia increased lipid uptake by the heart without a detectable increase in LPL activity. Hypoxia has been shown to induce translocation of CD36 to the membrane of myocardocytes (12) that would foster LPL-independent FFA uptake. Alternatively, higher levels of tracer could reflect lower myocardial β-oxidation. In either case, the opposite effect of hypoxia at 22 and 30°C reveals competing systemic and end-organ factors for lipid uptake by the heart. Given the importance of substrate metabolism for efficient myocardial function (38, 71), cardiovascular studies in mouse models of hypoxia must consider the effects of $T_a$. Finally, hypoxia stimulated WAT lipolysis at 30°C, similar to the effect of acute hypoxia in humans (35, 49, 56). Perhaps the relative vagal tone (65) of the mice at thermoneutrality predisposes them to greater SNS stimulation by hypoxia. Despite the mobilization of

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**Fig. 6.** Hepatic TG secretion in mice exposed to hypoxia at 22 or 30°C. Mice were fasted for 6 h under normoxia or hypoxia and injected with Triton WR-1339 to prevent TG hydrolysis. TG secretion rates were similar at both $T_a$ under normoxic conditions, and hypoxia independently decreased hepatic TG secretion.
FFA, hepatic VLDL secretion was not increased, perhaps because of concurrent ketone oxidation (44, 75) or catecholamine-mediated suppression of lipoprotein secretion (6, 13).

Overall, changes in lipid metabolism between mice and humans during acute hypoxia are similar when each species is studied in its respective thermoneutral zone. Some human studies show that hypobaric hypoxia increases TG and decreases HDL-C, but in volunteers exposed for days (2) or weeks (59) to high altitude and after overnight fasting. These more subacute changes have yet to be studied in rodents at thermoneutrality.

Fig. 7. Tissue lipid uptake and lipoprotein lipase (LPL) activity of mice exposed to hypoxia at 22 or 30°C. A: radioactive [1H]oleate intralipid levels in tissues following air or hypoxia exposure. B: LPL activity in corresponding tissue homogenates. C: pre- and postheparin lipase activity. *P < 0.05 for effect of hypoxia; bracketed P values for significant effect of T are shown. BAT, brown adipose tissue; WAT, white adipose tissue; HL, hepatic lipase.
Temperature-independent effects of hypoxia. Many effects of hypoxia occurred to a similar extent at 22 and 30°C, and therefore, we can exonerate their involvement in hyperlipidemia at 22°C. These effects included hyperventilation, hyperglycemia, tachycardia, glucose intolerance, reduced WAT LPL activity and lipid transport capacity, and reduced hepatic TG secretion. Many of these effects stem from arterial chemoreceptor and stimulation of the SNS (1, 49, 52, 54, 64). In terms of heart rate, some studies report that hypoxia causes bradycardia, particularly below thermoneutrality (7, 9). In this study, mice slept much of the light phase, lowering their heart rate, which may have been a precondition for relative tachycardia during hypoxia. Hypoxia caused hypothermia at both 22 and 30°C (30, 45), but by different mechanisms, as revealed by patterns of lipid uptake. At 22°C, hypoxia principally inhibited BAT nonshivering thermogenesis. At 30°C, BAT lipid uptake was unaffected by hypoxia, suggesting that hypothermia resulted from greater heat dissipation (31, 72). This is an important distinction, because it shows that decreased Tb per se does not cause hyperlipidemia.

Limitations. Several caveats should be acknowledged when interpreting our findings. First, we did not measure VO₂ in this study. However, many studies describe elevated VO₂ at 22°C (17, 29), and separate studies demonstrate suppression of VO₂ by hypoxia (26). Second, we did not directly assess cholesterol transport. It is reasonable to assume that plasma LDL-C levels should be negatively related to rates of circulating lipoprotein hydrolysis and positively with hepatic VLDL secretion. However, experiments specifically examining cholesterol transport are warranted, especially for HDL-C. Third, experiments were performed in group-housed mice during the light phase, when they tended to be asleep. Effects of Ta were probably mitigated by these conditions, and certain parameters such as heart rate may have been highly dependent upon sleep-wake state. Fourth, we did not examine other tissues such as the brain, other types of muscle, or various adipose tissue depots. Such tissues may exhibit differing patterns of lipid uptake. Finally, we studied mice that were recently fed at the start of hypoxia and did not deliver oral or IV boluses of lipid. More compre-
hensive tests of lipid clearance were performed in our recent study (36). However, the postabsorptive state was not studied. Muratsubaki et al. (46) showed that sated but not fasted rats developed hypertriglyceridemia during acute hypoxia (although the Tₐ of the experiments was not specified).

Conclusion. Tₐ is known to alter the metabolic impact of hypoxia in rodents. In this study, effects of hypoxia on TG and cholesterol metabolism at 22°C were eliminated or reversed by thermoneutrality. Figure 9 is a schematic relating changes in plasma lipids to the competing influences of Tₐ and hypoxia in various tissues. We conclude that Tₐ must be carefully considered in the design and interpretation of experiments that involve hypoxia in mice.

GRANTS

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DISCLOSURES

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

REFERENCES


HYPOXIA AND THERMONEUTRALITY


