Plasma and Liver Lipid Profiles in Rats Exposed to Chronic Hypobaric Hypoxia: Changes in Metabolic Pathways

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Abstract

Siques, Patricia, Julio Brito, Nelson Naveas, Ruth Pulido, Juan José De la Cruz, Maribel Mamani, and Fabiola León-Velarde. Plasma and liver lipid profiles in rats exposed to chronic hypobaric hypoxia: Changes in metabolic pathways. *High Alt Med Biol* 15:388–395, 2014.—Lipid metabolism under chronic hypoxia (CH) has not received equal attention as intermittent hypoxia (IH). To determine the CH-induced changes in plasma and liver, as well as the mRNA and protein expression of two key enzymes in the triglyceride and cholesterol biosynthesis pathways, SREBP-1 (HMG-CoA reductase) and SREBP-2 (SCD-1), we exposed adult male Wistar rats to CH (4600 m; $n = 15$) for 30 days compared to normoxic rats ($n = 15$). The CH rats exhibited weight loss ($p < 0.001$), higher hematocrit (%), and higher hemoglobin (g/dL) ($p < 0.01$). In the plasma of CH rats, total cholesterol and LDL-cholesterol increased at day 15. VLDL-cholesterol and triglycerides ($p < 0.01$) greatly increased (35%), while HDL-cholesterol decreased ($p < 0.01$). Triglycerides and VLDL-cholesterol remained elevated by 28% at day 30 ($p < 0.01$). Hepatic triglycerides increased two-fold, while total cholesterol increased by 51% ($p < 0.001$; $p < 0.05$). Upregulation of SCD-1 mRNA and protein was observed in the CH rats ($p < 0.01$); however, no differences were observed in HMG-CoA reductase mRNA or protein expression in both groups. In conclusion, CH, like IH, alters lipid profiles by increasing triglycerides in the plasma and liver and upregulating triglyceride biosynthesis without affecting the cholesterol biosynthetic pathway. Additional involved mechanisms require further study because of the importance of lipids in cardiovascular risk.

Key Words: blood lipids; chronic hypoxia; high altitude; HMG-CoA reductase; SCD-1

Introduction

AT HIGH ALTITUDES, THE PARTIAL PRESSURE of oxygen falls due to the decreased barometric pressure in the atmosphere, a condition known as hypobaric hypoxia, leading to a fall in oxygen tension at the tissue level (Grocott et al., 2007).

Although over 80 million people live at high altitudes (Niermeyer et al., 2001) and many studies have been conducted, some issues related to hypobaric hypoxia remain poorly understood or controversial. Surprisingly, one of these unresolved issues is the lipid profile induced by and metabolic

pathways involved in chronic hypobaric hypoxia. Initial reports have shown good lipid levels, low glucose, and fewer coronary events. Moreover, most of the literature regarding hypoxia and lipids comes from studies on intermittent hypoxia (IH), such as obstructive sleep apnea (OSA) and/or neonatal breathing disorders.

Epidemiological studies have suggested that chronic hypobaric hypoxia alters plasma lipid levels; however, drawing proper conclusions is not possible because these studies vary greatly with regard to their results, exposure types, altitudes, populations, and lifestyles of the studied populations (Siques et al., 2007). Controversial and contradictory results about

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total cholesterol and its fractions have also arisen from these findings (Temte, 1996; Jha et al., 2002; Caceres et al., 2004). Hypoxia was shown to increase HDL-cholesterol and reduce LDL-cholesterol (Sharma, 1990; Dominguez Coello et al., 2000), and even these latter authors have ascribed a putative cardiovascular protective effect to hypoxia.

More recent epidemiological studies have provided evidence that high altitude residents of Peru, and more recently Tibet, exhibit hypercholesterolemia and hypertriglyceridemia (Mohanna et al., 2006; Sherpa et al., 2013; Vats et al., 2013). Moreover, young people exposed to high altitude for the first time show a trend toward high triglyceride (TG) values (Siques et al., 2007). Altogether, in populations living in chronic intermittent hypoxia (3,550 m), hypertriglyceridemia was associated with low oxygen saturation and increased pulmonary artery pressure (Brito et al., 2007), suggesting that the hypoxic condition would have a role in lipid metabolism.

Most of the current information regarding the effects of hypoxia on lipid metabolic pathways indicates alterations in plasma and hepatic lipid metabolism (Bruder et al., 2005; Li et al., 2005b; Perry et al., 2007). The liver plays a key role in lipid metabolism, including lipid biosynthesis, lipoprotein secretion, and reverse cholesterol transport. Lipid biosynthesis in the liver is regulated by a family of transcription factors, the sterol regulatory element binding proteins (SREBPs), which include SREBP-1 and SREBP-2 (Shimano, 2001). SREBP-1 preferentially regulates enzymes involved in fatty acid synthesis, including stearoyl-CoA desaturase 1 (SCD-1). SREBP-2 regulates cholesterol biosynthesis and uptake, especially through the regulation of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase (Li et al., 2005b).

Under hypoxic conditions, changes in the expression of *SCD-1* and *HMG CoA-reductase*, which encode key enzymes involved in lipid biosynthesis, lead to high lipid levels in the hepatic tissues and plasma (Bruder et al., 2005; Li et al., 2007a, b). Ultimately, hepatic function seems to be altered under hypoxia, leading to liver damage (Savransky et al., 2007b; Qu et al., 2011).

Lipid alterations are strongly associated with cardiovascular risk, which was found to be exacerbated in intermittent hypoxia conditions where higher levels of triglycerides (TG) and cholesterol can ultimately lead to atherosclerosis (Savransky et al., 2007a). Therefore, it seems of utmost importance to study the lipid changes that occur in CH conditions. Consequently, we designed a study to determine if plasma and hepatic lipid profile changes under chronic hypobaric hypoxia (CH) are mediated by the same key enzymes of hepatic lipid biosynthesis as described in studies of OSA. To address this question, the changes in plasma and liver lipid profiles, as well as the mRNA and protein expression of the HMG-CoA reductase and SCD-1 genes in rat livers under CH were assessed.

Materials and Methods

Study groups

A total of 30 adult (3-month-old) male Wistar rats were studied. The rats were randomly assigned to two groups: (i) chronic hypobaric hypoxia (CH; $n = 15$) and (ii) normoxia or the control group (NX; $n = 15$). To avoid secondary changes related to hormonal influences, only adult male rats were included.

Chronic hypoxia exposure

Geographical altitude was simulated in a hypobaric chamber at 428 Torr, equivalent to 4600 m above sea level, maintaining an internal flow of 3.14 L/min of room air for each chamber (0.09 L/min of air per rat). Two similar chambers were used for the entire experiment (one containing 7 rats and one containing 8 rats) to allow better control and decreased time to perform the measurements on alternate days. The control group was placed in the same room ($22 \pm 2^\circ\text{C}$; 12 h light:dark cycle) under ambient conditions comparable to those of the hypoxic groups (Siques et al., 2006). The animals were located in individual cages, with food (10 g/day of pellets per rat) and water available *ad libitum*. The water was provided in bottles that were specifically designed for pressure changes. Housekeeping and replacement of food and water was carried out every 2 days.

To avoid any difference in food intake between the rats under hypoxia and the controls, a protocol to measure the food intake and residual food during hypoxia was developed prior to the experiment. According to this protocol, different amounts of pellets were provided at several different times with different groups of rats under the same hypoxic conditions. As a result, we concluded that 10 g of pellets per day was the mean intake.

Anatomical and physiological parameters

Both groups were exposed to their respective conditions for a 30-day period, and the following parameters were measured right after chamber descent: weight (W, every 4 days), systolic blood pressure (SBP, mm Hg) and heart rate (HR, beats/min). Hematocrit (Ht, %), hemoglobin (Hb, g/dL), and plasma lipid levels were measured at baseline and at 15 and 30 days. The weight was measured using an AccuLab V-1200[®] electronic balance (Marrero, LA). For blood pressure and heart rate measurements, an inflatable tail-cuff and a pressure sensor (RTBP1003-220, Kent Scientific, Torrington, CT) were used following a previously validated method (Johns et al., 1996). The signal was sent through a pre-amplifier to a Workbench data acquisition system, and after three consecutive measurements, the average value was recorded. On the days of measurement, the animals were acclimated to a movement-limiting Plexiglas chamber for 10 min. The data were collected and analyzed using the RTBP-001-DS worksheet (Kent Scientific). Blood samples obtained from cardiac puncture—under anesthesia (0.3 mg Ketamine)—were used for the hematological measurements. The main reason for using this technique is that hematocrits reach so high value and viscosity, that taking samples from a peripheral vessel is almost impossible. After this procedure, the rat was comfortably placed in a separate cage under veterinary care. Once, it was awake and able to mobilize, drink water, and eat some food, the rat was replaced to the chamber. An Eppendorf AG[®] microcentrifuge, Hamburg; Germany, was used for the hematocrit measurement. The hemoglobin concentration was measured using a Coulter Electronics Counter, Cell Dyn 3700[®] (Abbott, Santa Clara, CA).

Plasma and hepatic lipid profiles

Plasma lipids were measured on Day 0, Day 15, and Day 30. Plasma triglycerides (TG), total cholesterol (T-Chol), LDL-cholesterol (LDL-Chol), HDL-cholesterol (HDL-Chol),

and VLDL-cholesterol (VLDL-Chol) were measured using the Vitros DT60 II Chemistry System (Johnson & Johnson, Minnesota, MN). At the end of the protocol, the rats were euthanized with an overdose of ketamine (7 mg/kg) and the livers were surgically removed, dissected into four separated portions, and immediately frozen in liquid nitrogen and stored at minus 70°C for further study.

The first 50-mg portion of each liver was homogenized using a Stir-Pak®, (Barrington, IL). T-Chol and TG were extracted in a chloroform-methanol mixture (2:1), as described by Folch et al. (1957) and used by Yokode et al. (1990) and Li et al. (2005b), and measured with the same Vitros DT60 II Chemistry System.

Total RNA isolation and semi-quantitative RT-PCR analysis

For RT-PCR analysis, total RNA was isolated by homogenization of the second liver portion using the SV Total RNA Isolation System (Promega Corp., Madison, WI), with DNase treatment according to the manufacturer's instructions. The RNA quantity and quality were evaluated spectrophotometrically (Thermo Electron Corporation GENESYS 6®, San Diego, CA), and the integrity was assessed using agarose gel stained Gel-Red, (Sigma, St Louis, MO). cDNA synthesis was performed on 1 µg of total RNA using the Improm II Reverse Transcription System kit (Promega). The PCR reactions were performed using cDNA from each sample, and the cycling conditions were 95°C for 2 min, followed by 30 cycles of 95°C for 20 sec, 60°C for 60 sec, and 72°C for 60 sec. The sequences of primers for rats oligonucleotides were designed based on the Genbank; NCBI; PCR primers were the following: for SCD-1, 5'-GCT CAG CCA AAT GCT GTG TTG TCT-3' (forward) and 5'-TGG AAC ATG GGC TGC ATC AAA-3' (reverse); for HMG-CoA Reductase, 5'-ATT GGC CAA GTT TGC CCT GAG TTC-3' (forward) and 5'-ACA TCT TCA GCC AGA CCC AAG-3' (reverse); and for β -actin, 5'-GCA TAC CTC ATG AAG ATC CTG ACC-3' (forward) 5'-GGC ATA GAG GTC TTT ACG GAT GTC-3' (reverse). The PCR products were loaded onto 1.5% agarose gels. The optical densities of the stained cDNA bands were semi-quantified, and the results were expressed as means of SCD-1 and HMG-CoA reductase normalized to β -actin. Randomly, some experiments were performed three times with similar results.

Western blotting analysis

The third portion of the liver tissue from each animal was homogenized in homogenization buffer containing 50 mM Tris-HCl, 150 mM NaCl, 100 mM NaF, 2 mM Na₃VO₄, 1% Triton X-100, 1 mM dithiothreitol, 0.1 mM phenyl methyl sulfonyl fluoride, and 1 mM leupeptin, followed by centrifugation at 10,000 g for 10 min. The supernatant was removed, and protein quantification was performed using a Bradford assay. Equal amounts of protein (40 µg) were resolved on 8%–12% SDS-PAGE gels and transferred to a PVDF membrane. The nonspecific binding sites on the membrane were blocked using 5% nonfat dry milk in TBS-T buffer (10 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20, pH 7.4) for 1 hour. The membranes were incubated with primary antibody against SCD-1 and HMG-CoA reductase (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour and washed three times for 10 min each with TBS-T, followed by

FIG. 1. Comparative weight of rats during the 30 days of exposure in the studied groups: Normoxic (NX) and chronic hypoxia (CH). The values are the means $\pm$ confidence intervals (CI). * $p < 0.01$ or # $p < 0.05$ NX vs. CH.

incubation with a horseradish peroxidase-conjugated secondary antibody (1:5000, Santa Cruz Biotechnology) for 1 hour and three additional washes with TBS-T. The blots were visualized using a West Pico chemiluminescence system (Pierce®, Rockford, IL; U.S.A.) and then analyzed using the Image J software. The SCD-1 and HMG-CoA reductase expression levels were normalized to the β -actin expression levels.

This protocol was approved by the Ethics Committee of Universidad Arturo Prat and the animals were handled according to international protocols under veterinary care.

Data analysis and statistics

The results were entered into a database and were analyzed using SPSS 17.0 (SPSS, Inc., Chicago, Ill, U.S.A.). The means, standard deviations, standard errors, and confidence intervals were calculated for each parameter. The normality

TABLE 1. NORMOXIC (NX) AND CHRONIC HYPOXIA (CH) AT DAY 0, 15, AND 30

Variable	NX ($\bar{X} \pm SE$)	CH ($\bar{X} \pm SE$)
SBP (mmHg)		
Day 0	177.0 $\pm$ 1.2	178.1 $\pm$ 1.0
Day 15	178.6 $\pm$ 0.8	180.3 $\pm$ 1.2
Day 30	177.7 $\pm$ 1.1	181.4 $\pm$ 1.0* [#]
HR (beat/min)		
Day 0	373.7 $\pm$ 4.4	372.6 $\pm$ 4.7
Day 15	375.1 $\pm$ 4.1	371.5 $\pm$ 6.7
Day 30	367.9 $\pm$ 5.2	375.2 $\pm$ 4.9
Htc (%)		
Day 0	42.8 $\pm$ 1.6	45.3 $\pm$ 1.4
Day 15	43.9 $\pm$ 1.3	61.6 $\pm$ 2.3* [†]
Day 30	43.7 $\pm$ 0.7	61.8 $\pm$ 1.9* [†]
Hb (mg/dl)		
Day 0	14.8 $\pm$ 0.4	15.8 $\pm$ 0.4
Day 15	15.3 $\pm$ 0.3	21.0 $\pm$ 0.7* [†]
Day 30	15.1 $\pm$ 0.2	21.7 $\pm$ 0.2* [†]

Heart rate (HR; beat/min); Hematocrit (Htc; %); Hemoglobin (Hb; mg/dL); Systolic blood pressure (SBP; mmHg).

The values are the means ($\bar{X}$) $\pm$ standard error (SE). * $p < 0.01$ between NX vs. CH, [†] $p < 0.01$ day 15 or 30 vs. basal (day 0) and # $p < 0.05$ day 15 or 30 vs. basal (day 0).

FIG. 2. Plasma lipid profiles of the studied groups. (A) Triglycerides (TG; mg/dL); (B) Total cholesterol, (T-Chol; mg/dL); (C) LDL-cholesterol, (LDL-Chol; mg/dL); (D) HDL-cholesterol (HDL-Chol; mg/dL); and (E) VLDL cholesterol (VLDL; mg/dL), at basal levels (Day 0), Day 15, and Day 30. The values are means ($\bar{X}$) $\pm$ standard error (SE). * $p < 0.05$ between NX vs. CH, † $p < 0.05$ day 15 or 30 vs. basal (0), # $p < 0.01$ day 15 or 30 vs. basal.

was established using the Kolmogorov–Smirnov test, and the distributions of all parameters were found to be normal. Statistical analysis of the differences between the two conditions was performed using a *t* test of independent variables, and analysis of variable measurements across time was performed using a *t* test of related variables. Pearson's correlations were also performed. The results were considered significant when the *p* value was less than 0.05.

Results

Anatomical and physiological parameters

The CH group lost weight compared to the NX group ($p < 0.01$), a difference that was first observed at a very early time point and was sustained until the end of the study. Re-

sidual food was only left at the first days of the experiment. Rats in the NX group maintained their weight without significant changes (Fig. 1). The Hct and Hb levels in the CH rats increased from Day 15 ($p < 0.01$). All SBP measures were over the normal values, a finding attributed to a probable lack of acclimatization to the white collar effect during the whole experiment. Despite the unusually high levels in both groups, there was an additional significant increase in the CH group at Day 30 ($p < 0.05$), while no changes were observed in the heart rates of the rats (Table 1).

Chronic hypoxia exposure

Plasma lipid profiles. The TG levels were increased, with the largest effect evident on day 15 (a 35% increase) ($p < 0.001$). T-Chol and LDL-Chol levels were transiently

FIG. 3. Liver lipid profiles of the studied groups. (A) Triglycerides (TG; mg/g) and (B) Total cholesterol (T-Chol; mg/g), normalized to the tissue weight, of rats exposed to chronic hypobaric hypoxia (CH) and normoxic controls (NX). The values are the means ($\bar{X}$) $\pm$ standard error (SE). * $p < 0.05$ or ** $p < 0.001$ between NX vs. CH.

increased on day 15 ($p < 0.01$ and $p < 0.001$, respectively). HDL levels transiently decreased on day 15 ($p < 0.05$), while VLDL was elevated at days 15 and 30 ($p < 0.001$) (Fig. 2). In contrast, rats in the NX group exhibited a decrease in LDL-Chol ($p < 0.01$) and an increase in HDL at day 15 and beyond ($p < 0.05$). It was noteworthy to find a correlation between Hct levels and plasma triglycerides at day 30, only in the CH rats ($r^2 = 0.66$; $p < 0.01$).

Liver lipid profile. Hepatic triglycerides were two-fold increased ($p < 0.001$), while T-Chol was increased by 51% ($p < 0.05$) (Fig. 3).

SCD-1 and HMG CoA reductase mRNA and protein expression. Both the mRNA- and protein-level expression of SCD-1 were similarly increased ($p < 0.01$), but the protein exhibited a proportionally larger increase (three-fold). A smaller decrease was observed for HMG CoA reductase, but it was not statistically significant, pNS (Figs. 4 and 5). There was no difference in the mRNA or protein expression of the β -actin housekeeping gene between the two groups.

Discussion

The purpose of this study was to determine changes in plasma and hepatic lipid concentrations and some pathways involved in lipid metabolism following CH exposure in rats. The main findings were an increase in plasma TG and VLDL-Chol after 15 days under CH. Likewise, a remarkable increase in hepatic TG and a moderate increase in hepatic T-Chol were also observed. Additionally, a transient increase in plasma T-Chol and LDL-Chol, along with a decrease in HDL-cholesterol, was observed at day 15 of exposure; at day 30 these levels were again similar to the NX control group. An upregulation of SCD-1 mRNA and protein was clearly seen in the hepatic tissue of the CH rats, but the HMG-CoA reductase mRNA and protein levels were unaffected.

FIG. 4. The expression of genes (mRNA level) involved in lipid metabolism by semi-quantitative RT-PCR assays for SCD-1 and HMG CoA-reductase in the liver tissue of rats exposed to chronic hypobaric hypoxia (CH) and normoxic controls (NX) at day 30. (A) Representative bands for SCD-1, HMG CoA-reductase, and β -actin. Relative density of (B) SCD-1 and (C) HMG CoA-reductase normalized to β -actin. The values are the means ($\bar{X}$) $\pm$ standard error (SE). * $p < 0.01$ CH vs. NX.

FIG. 5. Protein levels of SCD-1 and HMG CoA-reductase in the liver tissue of rats exposed to chronic hypobaric hypoxia (CH) and normoxic controls (NX) at day 30. **(A)** Representative bands of SCD-1, HMG CoA-reductase, and β -actin, as assessed by Western blotting. Relative density of the protein bands of **(B)** SCD-1 and **(C)** HMG CoA-reductase normalized to the β -actin bands. The values are the means ($\bar{X}$) $\pm$ standard error (SE). * $p < 0.001$ CH vs. NX.

As is widely known, hypoxia consistently elicits a rise in Hct and Hb, (Reeves and Leon-Velarde, 2004; Siques et al., 2006). Several studies in animals and humans assessing lipids and hematological parameters, which were beyond the scope of this study, have shown a possible association between serum iron concentration and TG and T-Chol levels (Choi et al., 2001). Additionally, the concentration of red blood cells is affected by cholesterol synthesis or its mobilization from tissue to plasma. Moreover, TG, T-Chol, and Hb values are influenced by changes in plasma volume (Böttiger and Carlson, 1972). Remarkably, a similar correlation between Hct/Hb and plasma TG levels were found in our current study, in CH. It is plausible to hypothesize that HIF-1 α , a common transcription factor involved in the response to hypoxia, would play a major role in explaining these associations. In fact, HIF-1 α has been found to play a role in early hypertriglyceridemia and hypercholesterolemia (Li et al., 2006).

Lipid alterations in humans depend on many factors such as genetics, lifestyle, living conditions, social environments, and others that are not easily controlled or adjusted for in epidemiological studies. Certainly, one factor is the balance between hypoxia and diet, which seems to have a critical role in atherosclerosis and dyslipidemia during IH, as shown by Savransky et al. (2007a). However, to our knowledge, studies have not been attempted in an animal model under CH with restricted and controlled food intake. In our experiment, the control rats exhibited healthier lipid profiles, with lower LDL-Chol and higher HDL-Chol after day 15, while the CH group exhibited impaired lipid levels. Moreover, ambient temperature would also contribute to hypoxic hyperlipidemia by affecting brown adipose tissue; temperature stability can

reverse or eliminate this effect (Jun et al., 2013), and therefore temperature was controlled in our experiment.

In the current study, the CH group exhibited significant weight loss, a phenomenon consistent with our previous observations (Siques et al., 2006; Brito et al., 2008). It has been suggested that weight loss under any sort of hypoxia exposure may be largely due to altitude anorexia through a leptin-related effect, lipolysis, and an increased metabolic rate (Tschöp et al., 1998; Sierra-Johnson et al., 2008).

In general, although they may not be completely relevant to CH, some of the mechanisms that have been put forth to explain the changes observed in lipid profiles under several kinds of IH include the following: (1) enhanced lipid hydrolysis in extra-hepatic tissue; (2) upregulation of hepatic sterol regulatory element binding protein (SREBP)-1, which controls the *de novo* biosynthesis of fatty acids and TG in liver tissue (Li et al., 2005a); (3) an increased rate or upregulation of lipoprotein secretion or transportation (Li et al., 2005b); (4) an impairment and inhibition of triglyceride-rich lipoprotein clearance and inactivation of adipose lipoprotein (Dräger et al., 2012); and (5) downregulation of reverse cholesterol transport (Li et al., 2007a).

Another mechanism that could impair lipid levels under hypoxia is lipid peroxidation due to oxidative stress, depending on the severity of the IH (Li et al., 2007b). This process would play an important role in atherogenesis by enhancing the oxidation of polyunsaturated fatty acids (Savransky et al., 2007a). Moreover, the tight link between hypoxia inducible factor 1 (HIF-1) and hepatic lipid metabolic pathways in IH must be emphasized, as this association has been established by several authors (Li et al., 2006).

For the purpose of this current study on CH, we only assessed the plasma and hepatic lipid levels, along with the role of two key liver enzymes in the SREBP-1 and SERBP-2 pathways in lipid alterations. Based on our results, the transient increase in plasma T-Chol and LDL-Chol observed at day 15 may be the result of an ongoing sympathetic response to hypoxia, which can last through 90 days of exposure in humans (Kanstrup et al., 1999; Calbet, 2003). Moreover, Jun et al. (2013) showed a dose-dependent increase in circulating norepinephrine and epinephrine levels. However, at the end of the study, the plasma T-Chol and LDL-Chol levels were normal, which is consistent with our observation and with the literature that HMG-CoAR was unaffected (Li 2007a). Nonetheless, the discreet increase in liver T-Chol found in this study may be attributed to the downregulation of reverse cholesterol transport that has been described for moderate IH only (Li et al., 2007b). Likewise, the increase in TG and VLDL-Chol in the plasma, as well as the dramatic TG increase and discreet T-Chol increased in the liver, are also consistent with previous work on IH but have not been well described for hypobaric CH.

Furthermore, we propose that a *de novo* biosynthesis plays a role in the lipid alterations observed under chronic hypobaric hypoxia conditions, which mainly explains the TG increase, because we observed an upregulation of SCD-1 at both the mRNA and protein levels. Thus, monounsaturated fatty acids endogenously synthesized by SCD-1, which ultimately are transported to the blood stream, would serve as the main substrates for the synthesis of hepatic triglycerides in CH, as well as in IH. Nevertheless, some other mechanisms that have been described for IH could also explain the TG increase observed in CH rats. For example, IH impairs chylomicron clearance in mice and inhibits lipoprotein lipase (LPL), a key enzyme of triglyceride-rich lipoprotein clearance, during the uptake of TG in adipose tissue by upregulating adipose angiopoietin-like protein 4 (Angpl4), a potent LPL inhibitor. However, TG uptake in adipose tissue and lungs is primarily impaired through an LPL-independent mechanism (Drager et al., 2012; Yao et al., 2013).

Conversely, HMG-CoA reductase, which is a key enzyme in the cholesterol biosynthesis pathway (SREBP-2) that catalyzes the reduction of HMG-CoA to mevalonate (Ness and Chambers, 2000), was not upregulated at either the mRNA or the protein level in our experiment. Similarly, IH was shown to have no effect on the expression of enzymes in the SREBP-2 and HMG-CoA pathways in either lean or obese mice. However, in lean mice, IH was shown to decrease the protein levels of the HDL receptor SR-B1, which is a regulator of cholesterol uptake in the liver (Li 2005b).

Our results are fairly consistent with more recent epidemiological studies that have reported larger changes in TG than in TChol (Mohanna et al., 2006; Sherpa et al., 2013), while they contradict initial reports that stated that living in CH would lead to less risk of significant cardiovascular events. Moreover, several of the mechanisms described for the lipid alterations that occur during IH may be common and could explain the changes observed in our study, provided that IH is similar to CH. This idea may be supported by the finding that more severe hypoxia leads to greater changes in lipids (Li et al., 2007b); however, we should keep in mind that in humans, as well as animals, there are adaptive and acclimatization mechanisms that might overturn this comparison.

Finally, the importance of these findings extend to the clinical and public health fields, not only as a matter of concern for cardiovascular disease because of the potential increase or acceleration in atherosclerosis but also as they relate to the damage of specific organs such as the liver (steatosis, fibrosis, and liver failure).

Conclusion

In conclusion, we have demonstrated that chronic hypobaric hypoxia affects plasma and hepatic lipid levels similarly to IH. Mainly, TG and VLDL-Chol levels are increased in the plasma, and TG and T-Chol levels are increased in liver tissue. The underlying mechanism involved in this alteration seems to be mediated by the upregulation of SCD-1 mRNA and protein expression. Further studies are needed to determine whether other mechanisms that have been previously described for IH are also involved and to understand their contributions.

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Author Disclosure Statement

The authors have no conflicts of interest or financial ties to disclose.

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