# THE EFFECT OF ACUTE CONTINUOUS HYPOXIA ON POSTPRANDIAL LIPID METABOLISM


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**THESIS**

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## THESIS ABSTRACT

**INTRODUCTION:** Blood lipids, more precisely triglycerides (TG), are important fuel sources that are highly regulated since an exaggerate amount can lead to cardiovascular diseases. TG breakdown after a meal is mainly controlled by an enzyme expressed in adipose tissue called lipoprotein lipase (LPL). Recent evidence in animals report that adipose tissue LPL is inhibited after an exposure to an environment with reduced oxygen content, leading to a raised level of plasmatic TG. The objective of this thesis was to characterize the effects of an acute exposure to hypoxia on the plasmatic lipolytic activity level and on postprandial TG levels in humans. It was hypothesized that postprandial TG level and plasmatic lipolytic activity, a proxy of LPL activity, would be negatively affected by hypoxia. **METHODS:** Postprandial TG, non-esterified fatty acid (NEFA), glucose levels, and postheparin plasmatic lipolytic activity were measured on healthy young men (n=7) exposed for 6 h to either control ($FiO_2$=0.2093) or hypoxia ($FiO_2$=0.1200) in a randomized crossover fashion. **RESULTS:** Exposure to acute hypoxia led to a close to significant ($p = .06$) increase in postprandial plasmatic TG level and significant postprandial NEFA levels. Postprandial glucose levels were not affected by acute exposure to hypoxia. A significant increase in postheparin plasmatic lipolytic activity was observed after acute hypoxia exposure as compared to the control condition. **CONCLUSION:** Acute hypoxia in healthy men tend to negatively affects postprandial TG level while increasing plasmatic lipolytic activity. These results lend support to the increased blood lipid levels reported in individuals exposed to lower partial pressure of oxygen during sojourn at high altitude.

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## PREFACE

The work presented in this thesis is my own and I take full responsibility for its content. The thesis article in section 3.1 was co-authored by Bimit Mahat, Clare Lindon, Dr. Jean-François Mauger, and Dr. Pascal Imbeault. The certificates of ethical approval for this study from the University of Ottawa Health Sciences and Sciences Research Ethics Board are included in the appendix. This study was funded by the Natural Sciences and Engineering Research Council of Canada attributed to Dr. Pascal Imbeault.

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## CHAPTER 1: INTRODUCTION

Antoine Laurent Lavoisier, considered by many as the father of modern chemistry, was the first to identify the importance of oxygen to life in late 18$^{th}$ century. Since then, oxygen is known to have a major role in vegetal and animal respiration. At the cellular level, mitochondria utilize oxygen as the terminal electron acceptor to produce ATP through the electron transport chain via the biochemical process of oxidative phosphorylation. A dysfunction of oxidative phosphorylation leads to severe conditions or even death. Certain circumstances can lead to insufficient supply of oxygen to the different tissues, a condition called hypoxia. Since humans are large animals, the cardiorespiratory system ensures the primary function of extracting oxygen from the atmosphere and delivering it to the mitochondria of cells. A convenient marker of oxygenation of the whole body is oxyhemoglobin, the oxygen-loaded form of hemoglobin. Hemoglobin is the predominant protein found in red blood cells that binds to oxygen. Its regular oxygen saturation, in a healthy individual at sea level, is approximately 97-99%.

Exposure to high altitude can lead to a decrease in the oxygen content of the human body, a phenomenon called hypoxemia ($SaO_2 \leq 90\%$). Therefore, hypoxemia is simply a decrease in the blood oxygen saturation ($SaO_2$) of hemoglobin which may lead to hypoxia in tissues. Altitude is defined by the vertical distance to sea level. Due to the reduction in atmospheric pressure with altitude, oxygen ($O_2$) availability is diminished and it represents a stress for the human organism when not acclimatized. Atmospheric pressure ($PO_2$) will be reduced in proportion to altitude. The reduction in $PO_2$ with altitude implies a reduction in the arterial partial pressure of oxygen ($P_{arterial}O2$). As seen in **Figure 1**, a decrease in $PO_2$ is directly related to a decrease in $P_{arterial}O_2$ in the blood stream. Therefore, moving from sea level (≤500 m) to high altitude (≥3,000 m) (Bartsch, Saltin, & Dvorak, 2008) leads to hypoxemia. Johnson et al. previously reported that oxygen saturation in healthy individuals varies between 72% and 82% when exposed to 3800m above sea level (Johnson, Popa, Prisk, Edwards, & Sullivan, 2010). This study highlights the large inter-individual response to altitude exposure. However, at high altitude, everybody will be under hypoxic stress.

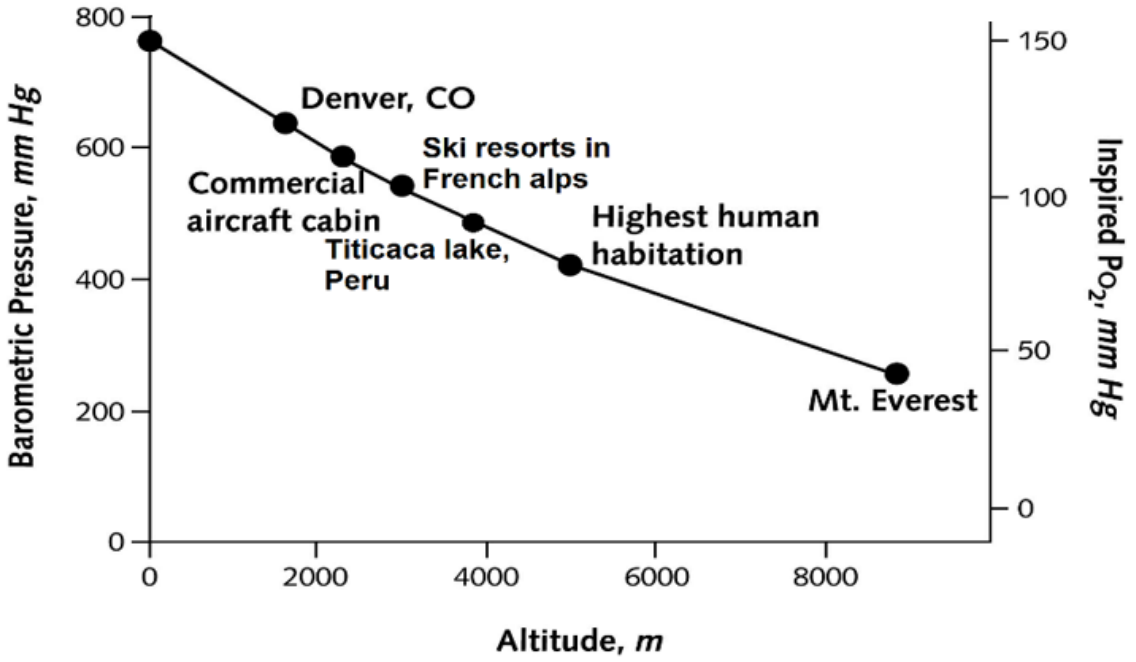


**Figure 1.** Relationship between altitude, inspired $PO_2$ and barometric pressure. A reversed relationship is seen between altitude and barometric pressure (or inspired $PO_2$). (**Modified** from; the physiologic basis of high-altitude diseases, (West, 2004), Annals of internal medicine).

High altitude exposure occurs mainly under three conditions: mountain trekking, exposure for sports/work and living at high altitude. In 1998, there were 13 500 000 people living above 3500m which represented about 0,002% of the world population and were mainly located in the Andes and Himalaya (J. E. Cohen & Small, 1998). Among the modifications in phenotypes due to permanent exposure to high altitude, it is often reported that cardiovascular adaptation occurs, such as an increase in hemoglobin (West, 1990). It has also been reported that people living at high altitude, commonly called highlanders, tend to have worse blood lipid profiles and a higher than normal prevalence for hypertriglyceridemia than lowlanders (**Table 1**) (Gonzales & Tapia, 2013; Hirschler, Maccallini, Aranda, Molinari, & San Antonio de los Cobres Study, 2012; Maccallini et al., 2012; Malaga, Zevallos-Palacios, Lazo Mde, & Huayanay, 2010; Mohanna, Baracco, & Seclen, 2006; Rise et al., 2008; Sherpa et al., 2011; Temte, 1996). Given the popularity of mountain trekking and/or high altitude exposure for sports performance to elevations above 4,000 m, and the degree of hypoxemia known to occur at such altitudes, several studies have characterized the physiological consequences of altitude exposure on an important segment of the fuel commonly

used during prolonged work that are triglycerides. In 1969, Whitten and colleagues first reported that an ascension to high altitude induced an increase of approximately 44% in plasma TG levels (Whitten & Janoski, 1969). Further studies, assessed in environmental chambers and on the field confirmed these results (Siques et al., 2007; P. M. Young et al., 1989). However, other studies concluded the opposite, showing that plasmatic TG level is decreased when exposed to high altitude (de Mol et al., 2012; Ferezou, Richalet, Coste, & Rathat, 1988; Ferezou et al., 1993; Stowhas et al., 2013). These discrepancies are not completely understood, but may likely be explained by diverse variables that affect TG levels such as physical activity levels and/or weight loss induced by the ascent and/or trekking or genetics. When these confounders are taken into account, as reported in controlled studies, plasmatic TG levels increase by approximately 45-50 % with high altitude (Siques et al., 2007; Whitten & Janoski, 1969; P. M. Young et al., 1989).

**Table 1.** Triglyceride levels in high landers.

| Author | Year | Altitude (m) | Sample size | *Hypertriglyceridemia (%) | TG (mg/dl) | TG (mmol/l) | comments |
|---|---|---|---|---|---|---|---|
| Gonzales et al. | 2013 | 4100 | 506 | 65 | 182,5 | 2,06 | |
| Hirschler et al. | 2012 | 3750 | 303 | 28.8 | 116 | 1,31 | kids |
| Maccallini et al. | 2012 | 3750 | 330 | 29 | 126 | 1,42 | kids |
| Malaga et al. | 2010 | 3600 | 74 | 48,6 | N/A | N/A | |
| Mohanna et al. | 2006 | 4100 | 102 | 53.9 | 192,06 | 2,17 | |
| Santos et al. | 2001 | 2000 | 196 | N/A | 137 | 1,55 | |
| Sherpa et al. | 2011 | 3660 | 371 | 12.2 | N/A | N/A | |
| Temte et al. | 1996 | 3150 | 153 | N/A | 190 | 2,15 | |
| Vinueza et al. | 2010 | 2850 | 1632 | 38.3 | 162,5 | 1,83 | |

*Hypertriglyceridemia is defined as fasting TG levels above 150 mg/dl

Recent animal studies using acute or chronic hypoxia indicate that an exposition to reduced amount of oxygen lead to an increase in plasma TG levels (Drager, Jun, & Polotsky, 2010; Drager et al., 2012; Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012; Li et al., 2005; Siques et al., 2014; Yao et al., 2013). The best hypothesis underlying this phenomenon concerns lipoprotein lipase (LPL), a key enzyme involved in the hydrolysis of triglyceride-rich lipoproteins (Kersten, 2014). LPL is expressed in different tissues such as heart muscle, skeletal muscle and adipose tissue. A reduction in its activity would lead to greater delay of lipoprotein clearance and eventually to hypertriglyceridemia. Jun et al. reported that white adipose tissue LPL activity is decreased by half when animals are acutely exposed to 10% of oxygen, leading to a delayed clearance in triglyceride levels (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012). Drager et al. reported similar results regarding the adipose LPL activity under chronic intermittent hypoxia (Drager et al., 2012). These mechanisms remain to be tested in humans.

The objective of the current thesis is to characterize the effects of an acute exposure to low oxygenation on postprandial plasma TG levels in humans. The proposed hypothesis is that hypoxia will negatively alter TG metabolism by delaying the clearance of blood TG following meals. These observations will expand the field of environmental physiology and nutrition by providing novel insight on how hypoxic exposure modulate TG metabolism in humans. Progression in this domain may lead to the development of research initiatives aiming to the development of strategies to counteract the effects of hypoxia on circulating TG without affecting performance.

## CHAPTER 2: REVIEW OF THE LITERATURE

### General information on TG

TG are composed of a glycerol and three non-esterified fatty acids (NEFA) making them the most energy-dense macronutrient. Therefore, they serve as a key nutrient for ATP production via oxidative phosphorylation. TG are mostly derived from food but can also be produced by the body through de-novo lipogenesis. When in dietary excess, TG are mostly stored in subcutaneous adipose tissue since it represents about 85% of all body adipose tissue (Frayn & Karpe, 2014), but can also be stored in non-adipose tissues such as liver and skeletal muscles. Owing to their water-insoluble property, TG are carried into the vascular system by lipoproteins. More specifically, triglyceride-rich lipoproteins (TRL) carry TG from the liver/small intestine to the rest of the tissues by lipoproteins called very-low density lipoproteins (VLDL) and chylomicrons (CM), respectively. These TRLs are differentiated by the composition of their isoform surface protein, called apolipoprotein B (apoB), with apoB-100 for VLDL and apoB-48 for CM. Overproduction of TRL can be harmful to the body as it can lead to atherosclerosis (Alipour, Elte, van Zaanen, Rietveld, & Castro Cabezas, 2008; Weintraub, Charach, & Grosskopf, 1997). Therefore, both the production and disposal of TRL must be precisely regulated.

### Chylomicron production via the small intestine

The primary function of the small intestine regarding lipids is digestion, absorption, assembly, and secretion of dietary fats [TG, cholesterol, and phospholipids], with a high capacity to respond rapidly and efficiently to large quantities of ingested food (Xiao, Hsieh, Adeli, & Lewis, 2011). Absorption of dietary lipids by the small intestine is modulated by several factors including the amount and types of fat and dietary fiber (Lairon, 2008). Therefore, it is difficult to specify the onset and speed of absorption of the intestines during postprandial lipid metabolism. However, Dubois et al. (1998) reported that the minimal amount of lipids in a meal to significantly increase postprandial CM-TG is 15 g and the peak in blood TG concentration is usually between 2 and 4 h post-meal (Dubois et al., 1998). The postprandial TG level is negatively affected by several factors such as impaired glucose metabolism, high body adiposity but mostly by high fat liver content (Matikainen et al., 2007). However, postprandial CM-TG generally increases dose dependently when the fat content of test meals ranges from 15 to 50 g (Lairon, Lopez-Miranda, & Williams, 2007). Since the CM-TG half-life is ~5 min (J. C. Cohen, 1989), dietary lipid is liberated from CM

relatively soon after they enter the vascular circulation (Katsanos, 2014). As Hussain (2000) proposed, CM are secreted by enterocytes principally when lipids are available in the intestine (Hussain, 2000). However, NEFA may stimulate lipoprotein assembly and secretion through stabilization of apoB-48 and reduction in its intracellular degradation, as first demonstrated by a hamster model with intralipid infusion (Lewis et al., 2004) and then confirmed in humans (Duez et al., 2008). Therefore, it appears that apoB-48 containing particles are continuously secreted from the enterocytes, and at times of excessive TG availability (eg. following a meal), lipid droplets fuse with nascent lipoprotein particles, resulting in the secretion of enormous size CM (Nakajima et al., 2011). It has been previously reported that fasting CM level correlate with fasting TG level (Sakai et al., 2003). Ideal fasting TG level should be lower than 1.7 mmol/L and CM, measured by apoB-48 quantification, should be around 5.2 ± 3.8 µg/ml (0.0052 ± 0.0038 g/L) (Sakai et al., 2003).

## VLDL production via the liver

After a 12h fast, the liver is the key organ that orchestrates lipoprotein regulation in the whole body. The liver is the master gatekeeper of ingested, mobilized, and de novo synthesized lipids, with far greater capacity than the intestine for storage and maintenance of lipid homeostasis during the transition from fed to fasted states (Xiao et al., 2011). It was first suggested that VLDL-TG production is mainly supplied via plasmatic NEFA (Frayn, Summers, & Fielding, 1997). However, Lewis (1997) reported that VLDL-TG may be derived from at least four sources of NEFA/TG varying by the nutritional and hormonal status of an individual, including NEFA spillover, which is described as the loss of plasma TG-derived fatty acids into the venous blood without being directly taken up by the underlying tissue, and fatty acids derived from lipoproteins taken up directly by the liver (Lewis, 1997). The two others sources of NEFA/TG are related directly to the liver, being the de novo lipogenesis, which represents the production of TG from other types of nutrients such as carbohydrates, and the last source of NEFA/TG is the use of cytoplasmic TG. Nevertheless, the liver does not regulate circulating TG alone. In the fasted state, the majority of VLDL-TG is derived from circulating NEFA from adipose tissue lipolysis (Jensen, 2003), which is principally mediated by epinephrine and the low plasmatic insulin concentration (Langin, 2006). In response to epinephrine, adipose tissue intracellular lipolysis leads to the secretion of glycerol and NEFA which are the key building blocks of VLDL-TG (Adebonojo, Coates, & Cortner, 1982). For its part, insulin has a powerful inhibitory effect on VLDL production, thus low concentration of insulin while fasting provides a key state for VLDL production (Pavlic, Xiao,

Szeto, Patterson, & Lewis, 2010). In healthy subjects, following an overnight fast, adipose tissue lipolysis is higher compared with the fed state due to low insulin concentration, leading to higher NEFA secretion and thus a higher VLDL-TG production by the liver (Barrows & Parks, 2006). NEFA from adipose tissue lipolysis comprises 70–80% of VLDL-TG following an overnight fast and may increase to more than 90% during prolonged fasting (Barrows & Parks, 2006). In brief, while fasting, hepatic TG are mostly derived from lipoprotein remnant uptake and re-esterification of circulating NEFAs (from lipolysis of adipose tissue and from TRL fatty acid spillover) (Lewis, 1997).

TRL production and disposal is a highly regulated mechanism since TG are the main energy source of the body. In addition, fasting and non-fasting plasmatic TG level can be harmful when dysregulated because it is a predominant factor for cardiovascular diseases (CVD) (Mora, Rifai, Buring, & Ridker, 2008).

## TRL-TG uptake by adipose tissue

Postprandial TRL-TG uptake is mainly regulated by three different tissues; skeletal muscle, cardiac muscle and adipose tissue, with adipose tissue being the primary one while being inactive. Adipose tissue performs a dual function. As previously mentioned, in a fasting state, adipose tissue releases NEFA and glycerol in the blood circulation for VLDL production by the liver. In postprandial state, adipose tissue is the key organ hydrolysing TRL and thus storing TG. TRL hydrolysis is complex and will be described briefly. **Figure 2** illustrates the major steps of TRL hydrolysis.

Since humans eat frequently throughout a day, humans are in postprandial state approximately 18h per day (Dubois et al., 1994). Within 24h following a meal while resting, the adipose tissue will store approximately 70% of the chylomicron fatty acids and the remaining 30% will be oxidised (Jensen, 2003). The efficiency of adipose tissue to hydrolyse TRL is mainly regulated via insulin. Insulin is an anabolic hormone secreted by the β-cells of the pancreas and is the hormone supporting TRL storage (Xiao et al., 2011). Regulation of insulin release is complex since regulation of β-cells are influenced by numerous factors (Szkudelski & Szkudelska, 2015). The first key factor supporting insulin secretion, discovered by Frederick Banting and colleagues in 1920, is the increase of blood glucose. Also, immediately following meal ingestion, L and K cells in the distal small intestine are stimulated, possibly through neuro-hormonal pathways and direct nutrient stimulation, to secrete glucagon-like peptide-1 (GLP-1) and glucose-depedent insulinotropic

polypeptite (GIP), respectively (Baggio & Drucker, 2007). Both incretins stimulates pancreatic β-cells insulin secretion, but with regard to adipose tissue, GIP also promotes energy storage via direct action on adipose tissue (Baggio & Drucker, 2007). Insulin acts on adipocytes principally by signaling exocytosis to GLUT-4, a glucose transporter, but also to vacuoles containing LPL (Mead, Irvine, & Ramji, 2002). Thus, in response to insulin, the Glycosylphosphatidylinositol-Anchored High-Density Lipoprotein-Binding Protein 1 (GPIHBP1) proteoglycan complex from adipose tissue translocate the LPL from the cytosol to the capillary surface (Beigneux et al., 2007) but must stay anchored to the endothelial surface in order to activate the LPL (Olivecrona, 2016). The last key step in TRL management is the LPL-TRL binding. The LPL binds to the GPIHBP1 to allow hydrolysis of the TRL content (Beigneux et al., 2007).

Endothelium-bound LPL activity across body tissues is the rate-limiting step in the disposal of circulating TG (Katsanos, 2014). After an overnight fast, the upregulation of LPL in adipose tissue is slower than the appearance of the chylomicrons and the well-timed blood flow response, which leads to less efficient lipid storage in adipose tissue after the first meal of the day (Ruge et al., 2009). In response to a meal, capillaries in the adipose tissue vasodilate to increase the amount of blood in the underlying tissue, resulting in an increased efficiency to manage TRL (Summers, Samra, Humphreys, Morris, & Frayn, 1996). Furthermore, for the subsequent meals, the efficiency of adipose tissue fatty acids uptake is increase as well as the LPL action increasing by 2-fold (Ruge et al., 2009). Since LPL is rate limiting for plasma TG clearance and adipose tissue uptake of NEFA, the activity of LPL is carefully controlled to adjust NEFA uptake to the requirements of the underlying tissue via multiple mechanisms at the transcriptional and post-translational level (Kersten, 2014). VLDL and CM share the same saturable removal pathways and therefore compete for LPL and cell surface receptors for their removal from circulation (Brunzell, Hazzard, Porte, & Bierman, 1973). It is accepted that TRL accumulate in plasma after dietary fat intake but whether the increase in TRL is caused by CM or VLDL accumulation is still debated. Bjokegren et al. (1996) suggests the delayed lipolysis of the apoB-100 TRL particles is due to competition with CM for the sites of LPL action (Bjorkegren et al., 1996). However, contradictory results show that, in normolipidemic male subjects, 82 ± 4% of the postprandial increase in TRL-TG is attributable to TG in apoB-48 CM (Cohn et al., 1993). Bickerton et al. (2007) showed, with radio label isotopes of TG, that CM-TG is the most nutrient absorbed by adipose tissue while non-fasting, concluding that CM-TG is the preferred substrate for adipose tissue lipid storage (Bickerton et al., 2007). Finally, it has been suggested that the primary determinant of postprandial CM accumulation is the

capacity of the LPL to hydrolyze TG and thereby to convert CM to large CM-remnants (Adiels et al., 2012). However, fasting plasma TG concentration is also a determinant of the increase in plasma TG concentrations in the postprandial period (Katsanos, 2014).

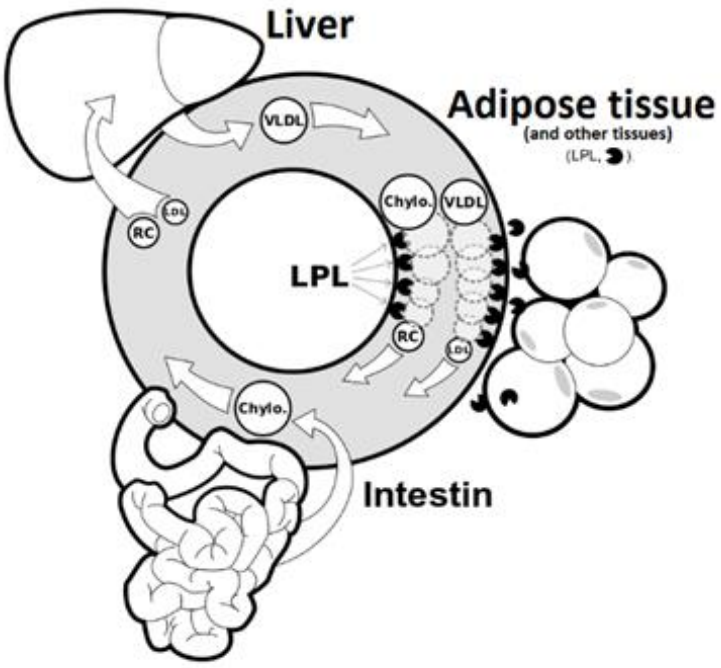


**Figure 2**. Triglyceride-rich lipoprotein (TRL) metabolism. Briefly, small intestine releases chylomicrons when dietary fats are ingested. Liver releases VLDL in a predominant manner during fasting state. Both TRL are mainly hydrolyzed by LPL generated by adipose tissue or other tissues. Following hydrolysis of CM and VLDL, chylomicron remnants (RC) and LDL will be metabolized by the liver to resynthesize VLDL.

Studies on animals have reported the negative effect of hypoxia on TRL disposal (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012). Therefore, it is crucial to determine if the effects are transposable to humans since mounting evidence supports the notion that fasting and non-fasting chylomicronemia is a risk factor for atherosclerosis (Alipour et al., 2008; Mora et al., 2008; Weintraub et al., 1997). The decreased disposal of TRL would be due to the activation of HIF-1 leading to inactivation of adipose tissue LPL. The following paragraphs will elaborate on how hypoxia is sensed by the body and its effects on lipid metabolism.

## High altitude and plasma lipids

**Table 2** provides a summary of studies on the effects of high altitude on plasmatic TG. Throughout the last fifty years, multiple investigators have studied the effects of altitude exposure on circulating TG. Whitten et al. (1969) were one of the first to report lipid levels at high altitude in humans, showing a 44% increase in plasma TG level after 9 days of exposure at Pikes Peak (CO, U.S.A.), 4302m above sea level. Thereafter, multiple investigators tried to reproduce these results with hypobaric chamber and/or terrain studies. EVEREST II was a study in a hyporbaric chamber trying to reproduce the Mount Everest ascent. For 40 days, 8 men were exposed to increase simulated altitude up to Mt. Everest (8848 m) to examine the physiological adaptation to high and extreme altitude. Young et al. (1989) were the investigators in charge of studying lipid levels for EVEREST II. They reported, in accordance to Whitten et al. (1969), an 81% increase in plasma TG level after 40 days of the simulated altitude exposure (P. M. Young et al., 1989). More recently, Siques et al. reported, via a cross-over protocol, a 47% increase in plasma TG level in young military exposed to high altitude for eight months (Siques et al., 2007). However, not all studies supports that high altitude exposure leads to a rise in TG levels. Ferezou et al. (1988) followed a group of 8 people trekking around the Annapurna mountain (Nepal) for 39 days. During the first 12 days, the group was trekking to reach an altitude of 4800 m. Following the trek, the subjects were staying at the same altitude for 3 weeks. It was reported that fasting TG levels decreased by 42% at the end of the stay at altitude. In a following terrain study, Ferezou et al. (1993) brought six participants from sea level to the *observatoire Valot du Mt. Blanc* (4350m) in helicopter to study plasma lipid levels for seven days. They report a 40% decrease in plasma lipid levels on day 7 (Ferezou et al., 1993). In congruence with these results, De Mol et al. (2012) reported a 27% decrease in fasting TG levels following a 12 day trek reaching 4167 m by 12 type 2 diabetes subjects (de Mol et al., 2012). Stowhas et al. (2013) reported, in a cross-over design, a 19% decrease in fasting TG levels following a 2 day exposure to 2590 m (Stowhas et al., 2013). Finally, Leaf and Kleinman (1996) did not measured a significant difference in fasting TG levels followed by a 2 hours exposure to a simulated altitude of 2200 m (Leaf & Kleinman, 1996). The discrepancies related to the effect of high altitude on plasma lipids may likely be due to factors such as the intensity of the hypoxic stress, the length of the stay, the variation in dietary intake and physical activity levels.

**Table 2.** Results summary of the effect of altitude on plasma TG in humans.

| Author | Year | Type of exposure | Altitude reached | Length of altitude exposure | Effects on plasma TG | Activity level |
|---|---|---|---|---|---|---|
| Whitten et al. | 1969 | Terrain | 4265 m | 9 days | 44% increase on day 7 | no exercise |
| Ferezou et al. | 1988 | Trekking | 4800 m | 12 days | 42% decrease on day 33 | exercise |
| Young et al. | 1989 | Hypobaric chamber | 8848 m | 40 days | 81% increase | light exercise |
| Ferezou et al. | 1993 | Terrain | 4350 m | 7 days | 40 % decrease on day 7 (postprandial) | no exercise |
| Leaf et al. | 1996 | Normobaric chamber | 2000 m | 2 hours | no difference | no exercise |
| Siques et al. | 2007 | Terrain | 3550 m | 8 months | 47 % increase | light exercise |
| De Mol et al. | 2012 | Trekking | 4167 m | 12 days | 27% decrease | exercise |
| Stowhas et al. | 2013 | Terrain | 2590 m | 2 days | 19% decrease on day 2 | no exercise |

## Effect of hypoxia on cardiovascular system

One of the most obvious responses of the human body to a reduction in the systemic partial pressure of oxygen is an increase in heart rate. During low oxygen exposure, heart rate is inversely proportional to the oxygen availability (Mazzeo, Wolfel, Butterfield, & Reeves, 1994). Despite the interindividual variability observed in response to hypoxic exposure, the increase in heart rate is generally 10 beats per minute (BPM) faster when exposed to 4000 m above sea level (12.5% of oxygen) as compared to sea level (20.93% oxygen) (Hooper & Mellor, 2011). Additionally, under exposure to continuous low oxygenation ($FiO_2 \approx 0.12$) for 2 to 6 days, blood pressure is increased (Cornolo, Mollard, Brugniaux, Robach, & Richalet, 2004; Peltonen et al., 2012; Wolfel, Selland, Mazzeo, & Reeves, 1994). Sagawa et al. (1993) were one of the first to investigate the acute effect of hypoxia on the cardiovascular system and particularly blood pressure. Sagawa et al. (1993) determined that the principal acute effect of hypoxia was a blood redistribution among the limbs which caused the heart rate to increase, stroke volume to decrease, without increasing blood pressure to maintain mean arterial pressure (Sagawa, Shiraki, Miki, & Tajima, 1993).

## Effect of hypoxia on substrate utilisation

The human body relies on diverse substrate for sustaining its energy production (ATP). Lipids, through oxidative phosphorylation, have the power to generate a lot of ATP compared to carbohydrates. The human body, depending on nutrients availability, and mostly on ATP demand and oxygen availability, utilises mostly lipids, glucose or a mix of both substrates. While at rest and fasting, an individual eating a mixed diet will sustain energy demand through 50 % of the energy derived from lipids and 50 % from circulating glucose. Lipids used to sustain energy demand mainly come from adipose tissue lipolysis and blood glucose comes from glycogenolysis of the liver. However, in postprandial state following a balanced meal, substrate used to sustain energy production will shift according to the nutrients of the meal, generally toward glucose utilisation. When moving to high altitude, it is generally accepted that a turnover from lipids to glucose at rest happens (A. J. Young, 1990). However, based on exercise studies, the shift from lipids to carbohydrates is not observed when relative workload (i.e. % $VO_2$ max) is matched between sea level and high altitude (Lundby & Van Hall, 2002). In addition, it is often reported that resting metabolic rate is increased by 20 to 30% under exposure to low oxygenation

(Butterfield et al., 1992; Hill, Stacey, & Woods, 2011). Therefore, it is suggested that the decrease relative reliance on lipid as a substrate depends on exercise intensity (ATP demand) and/or exposure time to high altitude (Roberts et al., 1996). A shift from lipids to glucose in substrate utilisation might seem impactless but it reduces the oxidation of lipids further amplifying the possible delay in TRL clearance following a meal.

## Effect of hypoxia on nervous system

Exposure to high altitude activates the sympathetic nervous system (SNS), as measured by microneurography (Hansen & Sander, 2003), by circulating catecholamines levels (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012), by urinary catecholamines excretion (Mazzeo et al., 1994) and finally by analysis of the time and frequency domain of the heart rate response to high altitude (Louis & Punjabi, 2009). However, some studies report that plasma and/or urine catecholamine are not affected by high altitude exposure, this contradiction would be due to a higher clearance of epinephrine and norepinephrine at high altitude (Rostrup, 1998). Even if plasma and/or urine measurements of catecholamine are not affected, it does not mean that SNS is not activated. The technique, called microneurography, is used to measure the SNS activity and consists of measuring the nervous system by burst per minute directly in a tissue (eg. muscle). Studies using microneurography on people at high altitude report an increase in sympathetic activity by 3-fold (Hansen & Sander, 2003). Therefore, it can be concluded that hypoxic exposure is associated with a shift in sympathovagal balance toward heightened SNS activity (Hansen & Sander, 2003; Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012; Louis & Punjabi, 2009; Mazzeo et al., 1994). Activation of the SNS leads to an increase in catecholamine efflux by the adrenal medulla (Mesarwi, Sharma, Jun, & Polotsky, 2015). As Mesarwi et al. (2015) described, catecholamine stimulate glucagon secretion, activate glycogenolysis and gluconeogenesis in the liver, and cause the breakdown of muscle glycogen and adipose tissue TG. It was first believed that adipose tissue lipolysis is mediated only by circulating catecholamines, however, it is now reported that adipose tissue is directly innervated by SNS and thus does not require the circulating catecholamines to start lipolysis (Youngstrom & Bartness, 1995). Catecholamines also inhibit insulin secretion and insulin-mediated glucose uptake by skeletal muscle (Mesarwi et al., 2015). During hypoxia, circulating catecholamines act upon α-adrenoreceptors to cause hyperglycemia and glucose intolerance (Jun et al., 2014). Epinephrine also leads to an increase in adipose tissue blood flow (Samra et al., 1996), suggesting a potential mediating effect on TG storage and/or NEFA

secretion. Finally, studies report, based on microneurography, that hypoxia has a long-lasting effect on SNS, measuring elevated burst activity up to 3 days following return to sea level after a 4 weeks period at high altitude (Hansen & Sander, 2003). A sympathovagal balance shift toward SNS activation is thus an important feature of hypoxia leading to impairment in glucose and TG metabolism.

## Activation of HIF-1 under hypoxia

Through evolution, species have developed complex systems to carry oxygen around the body. The precise establishment and regulation of these systems provide a major basis for $O_2$ homeostasis (Semenza, 2000). Oxygen is essential to all living organism (Semenza, 2000) since it is used for oxidative phosphorylation, in which $O_2$ serves as the electron acceptor during ATP formation. Oxygen is thus essential for ATP production. Therefore, humans have a highly regulated mechanism to sense small fluctuations of oxygen tension in tissues.

Wang and Semenza (1995) first characterized the metabolic pathway which senses variation in oxygen tension inside the body (Wang, Jiang, Rue, & Semenza, 1995). Hypoxia-inducible factors (HIF) are the class of transcription factors linked to oxygen sensing. In the HIF family, there are 3 members, HIF-1, HIF-2 and HIF-3 each having a specific action on oxygen sensing. HIF-1 is the main transcription factor sensing oxygen at the cellular level (Semenza, 2000). In 2014, Semenza reported that HIF-1 was directly involved in the expression of more than 1 000 genes (Semenza, 2014). HIF-1 activates the transcription of genes encoding enzymes, transporters, and mitochondrial proteins that decrease O2 utilisation, again functioning as a master regulator to switch cells from oxidative metabolism to glycolytic metabolism (Semenza, 2014). For example, HIF-1-dependant measures to decrease O2 demand include the downregulation of mitochondrial oxidative phosphorylation via inhibition of pyruvate dehydrogenase (Murray & Horscroft, 2015). HIF-1 acts at the cellular level to signal low oxygenation and by controlling oxygen-related gene expression. As seen in **figure 3**, HIF-1s are made of 2 subunits, alpha ($\alpha$) and beta (b). Both of them are essential for HIF-1 to be able to activate/inhibit the expression of genes. However, the biological activity of HIF-1 is determined by the expression and activity of the HIF-1a subunit (Semenza, 2000). As Semenza (2000) reported, under non-hypoxic conditions, HIF-1a appears to be ubiquitinated and subject to proteasomal degradation. In other words, availability of oxygen leads to hydroxylation of HIF-1a by prolyl hydroxylase resulting in its degradation by the proteasome (Myre & Imbeault, 2014;

Semenza, 2000). Under low-oxygenation conditions, propyl hydroxylase is inactivated and therefore HIF-1a is not hydroxylated. Stable HIF-1a is thus translocated to the nucleus. To activate transcription of target genes, HIF-1a dimerizes with HIF-1b and the heterodimer binds to DNA (Semenza, 2000).

Among the genes expressed by HIF-1, there is one of particular interest in lipid metabolism, angiopoietin like-4 (ANGPTL-4). Expression of ANGPTL-4 is under transcriptional control of peroxisome proliferator-activated receptors (PPARs) (Kersten, 2014). ANGPTL-4 is a direct target of HIF-1a in adipose tissue (Kersten, 2014). ANGPTL-4 is secreted by numerous cells including hepatocytes, adipocytes, (cardio) myocytes, endothelial cells, intestinal epithelial cells, and macrophages (Kersten, 2014). Compelling evidence indicates that ANGPTL4 inhibits LPL by promoting the conversion of active LPL dimers to inactive LPL monomers (Sukonina, Lookene, Olivecrona, & Olivecrona, 2006). In vitro studies have suggested that ANGPTL4 enzymatically catalyzes the dimer to monomer conversion whereas in vivo studies suggest that ANGPTL4 disables LPL by binding LPL monomers, thereby driving the LPL dimer–monomer equilibrium toward inactive monomers (Lichtenstein & Kersten, 2010). Because LPL is a critical determinant of plasma TG clearance and resultant tissue uptake of fatty acids, the activity of LPL needs to be carefully regulated in order to match the rate of uptake of plasma TG-derived fatty acids to the needs of the underlying tissue and the ability of the tissue to dispose of the fatty acids, all while being confronted with huge fluctuations in the quantity of TG-rich lipoproteins (Kersten, 2014). Therefore, an up-regulation of ANGPTL-4 under low oxygenation would lead to a delayed plasma TG clearance due to an inactivation of adipose tissue LPL.

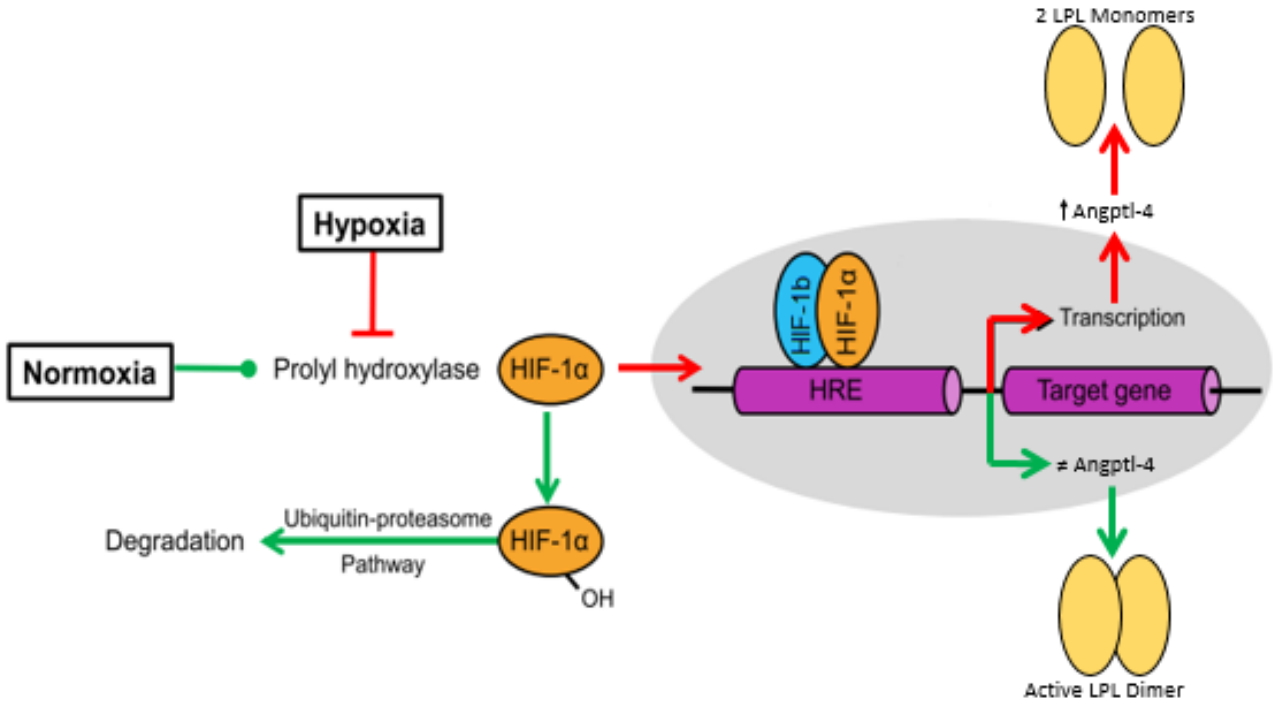


**Figure 3.** Potential mechanism inactivating LPL under low oxygenation**.** Briefly, under normoxia, prolyl hydroxylase uses oxygen to hydroxylateHIF-1α which initiates the ubiquitin-proteasomal pathway and eventually leads to HIF-1α degradation. However, under hypoxia, the lack of oxygen inhibits the hydroxylation of HIF-1α. HIF-1α thus translocates to the nucleus of the cell and dimerizes with HIF-1b. The HIF-1 dimer then binds to the hypoxia response element (HRE) and as a master transcription regulator, it promotes the transcription of target genes. The gene of interest here is *ANGPTL-4,* which the translation leads to a protein of the same name and is a powerful LPL inhibitor. It is hypothesized that ANGPTL-4 breaks the LPL dimer conformation and thus inactivates it. (Modified from Myre & Imbeault, (2014))

Studies on tissue oxygenation demonstrate that the decrease in oxygen tension varies between tissues (Rupp et al., 2013). It was previously reported that there is a tissue-specific sensitivity to hypoxia, where the central nervous system is more challenged compared to muscles in humans (Rupp et al., 2013). A recent study on mice measured the oxygen pressure in different tissues, including adipose tissue, while exposing the animals to hypoxia. When the animals were expose to $FiO_2$ = 10%, they report a 66% decrease in $O_2$ partial pressure in adipose tissue (Reinke, Bevans-Fonti, Drager, Shin, & Polotsky, 2011). Even if no investigators reproduced the results on humans,

this information is important to support that the decrease in oxygen tension is not only located in the blood but also in the different tissues.

## Dysregulation of lipoprotein metabolism under hypoxia

The reduced clearance of postprandial triglycerides following an acute or chronic hypoxic stress have been highlighted in animal studies (**Table 3**). Jun and colleagues (2012) determined that 2h of hypoxic stress is the minimum amount of time required for the TG raising effect to happen under an hypoxic stress. Principally, the severity of the increase in lipids is proportional to the severity of the hypoxic stimulus (Drager et al., 2010) reaching a maximum when $FIO_2$ equals 10 percent (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012). It was previously hypothesized that the hypertriglyceridemia following an exposure to hypoxic conditions resulted from impaired removal of circulating triglycerides (Muratsubaki, Enomoto, Ichijoh, & Yamamoto, 2003). Recently, Drager et al. (2012) demonstrated that the accumulation in plasma TG is predominantly localized in TRL confirming the delay in clearance of TRL postprandially. To better show that the clearance of TRL is the main cause of TG accumulation, Jun et al. (2012) switched mice previously under hypoxic conditions to normoxia while progressively fasting. It resulted in an acceleration of the plasmatic TG clearance.

**Table 3.** Animal studies reporting the effect about acute/chronic hypoxia exposure on TG metabolism.

| Researchers | Year | Type of Oxygenation | Exposition time | Results on WAT LPL activity | Results on fasting Blood TG | Comments |
|---|---|---|---|---|---|---|
| Li et al. | 2005 | IH | 5 days | N/A | increase by 40% | C57bl/6j mice, not thermoneutral |
| Drager et al. | 2012 | IH | 4 weeks | decrease by 80% | increase by 200% | C57bl/6j mice, not thermoneutral |
| Jun et al. | 2012 | CH | 6 hours | decrease by 60% | increase by 240% | C57bl/6j mice, not thermoneutral |
| Drager et al. | 2013 | IH | 4 weeks | N/A | Increase by 50% | C57bl/6j mice, not thermoneutral |
| Jun et al., | 2013 | CH | 6 hours | decrease by 60% | No differences | C57bl/6j mice, thermoneutral |
| Yao et al. | 2013 | IH | 4 weeks | decrease by 50% | increase by 30% | C57bl/6j mice, not thermoneutral |
| Siques et al. | 2014 | CH | 30 days | N/A | increase by 35% | Winstar rats, not thermoneutral |

IH refers to intermittent hypoxia. CH refers to constant hypoxia

Variability in results between studies might be explained by thermoneutrality of the animals. Hypoxia-induced changes in lipid metabolism depend highly upon ambient temperature. 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, which is the thermoneutrality for mice, had minimal elevations in plasma TG and LDL-C levels following hypoxic stress (Jun et al., 2013). Jun et al. (2013) report that effects of hypoxia on TG and cholesterol metabolism at 22°C were eliminated or reversed by thermoneutrality in mice (Jun et al., 2013). Thermoneutrality is therefore an important factor to consider when working with a hypoxic environment. Only one study reported the ambient temperature under the hypoxic stress even if it seems like an important factor (Jun et al., 2013). Finally, all of the previous studies were assessed on animals, whether mice or rats, and might not fully represent what really happens in humans.

## Adipose tissue metabolism dysregulation under hypoxia

As mentioned previously, there is an activation of SNS under hypoxia which leads to increase white adipose tissue (WAT) lipolysis (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012). β-adrenoreceptors, receptors for catecholamines, located on the surface of adipocytes respond to this activation of SNS under hypoxic stress by increasing lipolysis (Mesarwi et al., 2015). Thus, after exposure to low oxygen concentration, there is an increase in plasma NEFA (Drager et al., 2010). Drager and colleagues also reported that accumulation of NEFA following hypoxic exposure induces angiopoietin like-4 (angptl-4) gene expression. However, very modest elevations of NEFA under hypoxia may not be sufficient to induce dramatic increases in adipose ANGPTL-4 (Drager et al., 2013).

Several animal studies have also reported a reduction in adipose tissue LPL activity in response to exposure to low oxygen concentration (Drager et al., 2012; Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012; Yao et al., 2013). Drager and colleagues first quantified LPL activity suppression at 0.5 fold under chronic intermittent hypoxia (Drager et al., 2012). Endothelium-bound lipoprotein lipase (LPL) activity across body tissues is the rate-limiting step in the disposal of circulating TG (Katsanos, 2014). Thus, by decreasing LPL activity, hypoxia induces a delayed plasma TG clearance (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012; Yao et al., 2013). Jun and colleagues (2012) first proposed the previously described mechanism to explain this phenomenon. Drager and colleagues (2012) reported that the drastic decrease in adipose LPL activity is not accompanied by a decrease in plasma postheparin LPL activity, suggesting a tissue-

specific effect of hypoxia on LPL in vivo (Drager et al., 2012; Yao et al., 2013). This tissue-specific effect of hypoxia on LPL may be related to ANGPTL-4, which was reported to be increased by 2 to 4.5 fold in WAT but not in cardiac skeletal muscle or liver (Drager et al., 2013). It nevertheless remains unknown why hypoxia up-regulates ANGPTL-4 exclusively in adipose tissue, although the severity of tissue hypoxia and oxidative stress with ensuing activation of HIF-1a may play a role (Drager et al., 2013). Supporting evidence shows that intermittent nocturnal hypoxemia, characterized by obstructive sleep apnea (OSA), is associated with increased ANGPTL-4 mRNA levels in subcutaneous adipose tissue in obese humans (Drager et al., 2013). To further approve the mechanism, Drager and colleagues (2013) demonstrated that the deleterious effect of hypoxia on fasting TG can be overturned by treating the animals with an ANGPTL-4 antibody.

Catecholamines were also reported to have an effect on LPL activity. In vitro studies indicate that catecholamines reduce adipocyte LPL activity via downregulation of LPL gene transcription (Raynolds et al., 1990). Catecholamines, released under hypoxic stress, would add their effect to inhibit LPL and delay the clearance of postprandial lipids. Finally, tumor necrosis factor alpha (TNF-a), a cytokine marker of inflammation expressed in obstructive sleep apnea patients, a medical condition inducing hypoxic stress in the body (Mesarwi et al., 2015), represses LPL activity by downregulating LPL transcription and thereby LPL mRNA (Kersten, 2014).

## Liver metabolism dysregulation under hypoxia

The liver is extremely sensitive to low-oxygen conditions and it was reported that its oxygen tension is reduced by up to 65% under acute hypoxia (Reinke et al., 2011). The liver's role towards TRL regulation is primarily VLDL secretion and recycling CM remnants. Drager and colleagues (2010) first reported and was later reviewed by Geerling et al. (2014) that a hypoxic environment up-regulates lipoprotein secretion by the liver due to the SNS activation (Drager et al., 2010; Geerling et al., 2014). It is hypothesized that hypoxia may directly activate sterol regulatory element binding protein (SREBP) transcription factors in the liver to produce hyperlipidemia (Li et al., 2005). Drager et al. (2012) reported, after hypoxia exposure, plasma TG accumulation is almost exclusively VLDL particles (Drager et al., 2012). Finally, dyslipidemia under hypoxia may be a consequence of hypoxia-induced upregulation of lipid biosynthetic pathways in the liver (Drager et al., 2010).

## SUMMARY

Following a meal, the small intestine is the key organ managing the production of TRL in order to store dietary lipids in adipose and/or non-adipose tissues. While fasting, the liver is the key organ involved in TRL production and disposal. Both TRL, intestine produced CM and liver produced VLDL, compete for the same removal pathway and, postprandially, is limited by adipose tissue LPL (**Figure 2**).

It was previously reported that a sojourn to high altitude affects circulating lipids. As seen in **Table 2**, high altitude experimental and terrain studies reported mixed results on the effect of hypoxia on circulating TG probably due to important confounding factors such as exercise, weight loss and the altitude reached during the sojourn.

Recent animal studies are consistent in reporting that hypoxia reduces adipose tissue LPL activity, a key limiting enzyme involved in the management of postprandial TG levels, by up to 5 fold (**Table 3**). Accordingly, these same studies reported an increase in plasma TG levels after exposure to low oxygenation. To our knowledge, the effects of an acute exposure to low oxygenation on postprandial plasma TG levels in humans remains to be investigated.

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## 2.1 Specific Problem

High altitude exposure happens frequently either for sports/leisure or work related. It was previously reported that hypoxic exposure in animals affects negatively postprandial TG levels due to inhibition of the key TRL hydrolyzing enzyme, LPL (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012). Increased postprandial plasmatic TG level is a risk factor for cardiovascular disease in humans (Pirillo, Norata, & Catapano, 2014). This thesis investigates whether an exposition to reduced fraction of oxygen leads to increased plasmatic TG levels in humans and determine if whole body lipolytic activity is impaired by hypoxia.

## 2.2 Objectives

To investigate the effects of an acute exposure to low fraction of oxygen on 1) postprandial plasmatic TG levels; 2), cardiovascular responses and 3) substrate utilisation healthy young men.

## 2.3 Hypotheses

Primary Hypothesis: Acute exposure to normobaric hypoxia will increase plasmatic TG levels due to a decrease in plasma lipolytic activity.

Secondary Hypothesis: Acute exposure to low oxygen will decrease SaO2 and increase heart rate.

Tertiary Hypothesis: Acute exposure to low oxygen will not affect the substrate utilisation.

## 2.4 Limitations

There are several possible limitations to this study. First, since we do not have access to a direct measurement of adipose tissue oxygenation, we do not know if adipose tissue was exposed to hypoxia and for how long. Second, the cohort of this study is only composed of seven young healthy male individuals. Therefore, the results might not reflect other demographic population and might not be representative of the whole population. We have to be careful when generalising to the public. Thrid, the analysis of TG made doesn't reflect the different fractions of lipoproteins. Unfortunately, we did not have access to the equipment needed to further analyse the different fractions of TRL. Due to limited participation in the study, we were not able to conduct adipose tissue biopsies and must only speculate on adipose tissues LPL activity and expression. Finally, parameters such as TG or NEFA constantly fluctuate during the day and from one day to another. Even if we tried our best to control them with our pre-experimental

procedures, differences are seen and we must correct for it. Thereby, further investigations will be needed to fully determine the effect of hypoxia on plasmatic TG metabolism.

## CHAPTER 3: METHODS AND RESULTS

A full description of the methods used to assess this project can be found in **Chapter 3, Method section of the article**.

Briefly, after written consent from participant was obtained, participants were exposed randomly to either 6 h of normobaric hypoxia ($fiO_2$= 0.1200) or normoxia ($fiO_2$= 0.2093) in a crossover fashion. During the exposition, participants were fed at specific intervals. Blood was collected at specific intervals. Using repeated measures analysis of variance, we were able to determine the effect of hypoxia on circulating TG levels and other physiological parameters.

3.1 Thesis Article: To be submitted to the Journal of Applied Physiology and formatted accordingly.

# The effect of acute continuous hypoxia on postprandial triglyceride levels in healthy men

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**Running head:** Hypoxia and plasmatic TG level

**Key words:** Triglycerides; lipase activity; normobaric hypoxia

**Author contributions:** Etienne Chassé conceived and designed experiments; Etienne Chassé, Clare Lindon, Bimit Mahat and Jean-François Mauger performed experiments; Etienne Chassé, Bimit Mahat, Jean-François Mauger analysed data; Etienne Chassé and Pascal Imbeault interpreted data; Etienne Chassé drafted manuscript; Etienne Chassé, Bimit Mahat, Jean-François Mauger and Pascal Imbeault edited, revised, and approved final draft of the manuscript.

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## ABSTRACT

**INTRODUCTION:** Blood lipids, more precisely triglycerides (TG), are important fuel sources that are highly regulated since an exaggerated amount can lead to body malfunctions including cardiovascular diseases. TG breakdown following a meal is mainly controlled by the lipolytic action of an enzyme expressed in adipose tissue called lipoprotein lipase (LPL). Recent evidence in animals and human cells report that adipose tissue LPL is inhibited after an exposure to an environment with reduced oxygen content, leading to a raised level of plasmatic TG. The objective of this study was to characterize the effects of an acute exposure to normobaric hypoxia on postprandial TG levels and the plasmatic lipolytic activity in healthy humans. It was hypothesized that postprandial TG levels and plasma lipolytic activity would be negatively affected by hypoxia. **METHODS:** Postprandial TG, non-esterified fatty acid (NEFA), glucose levels, and postheparin plasmatic lipolytic activity were measured in healthy young men (n=7) exposed for 6 h to either control ($FiO_2$=0.2093) or normobaric hypoxia ($FiO_2$=0.1200) in a randomized cross-over fashion. **RESULTS:** Exposure to acute hypoxia led to a close to significant (p = .06) increase in postprandial TG levels as well as greater postprandial NEFA levels. Postprandial glucose levels were not affected by acute exposure to hypoxia. A significant increase in postheparin plasmatic lipolytic activity was observed after acute hypoxia exposure as compared to the control condition. **CONCLUSION:** Acute hypoxia in healthy men tends to negatively affect postprandial TG levels while increasing plasmatic lipolytic activity. These results lend support to the increased blood lipid levels reported in animals exposed acutely to lower partial pressures of oxygen.

## INTRODUCTION

Throughout the last fifty years, multiple investigators have studied the effects of high altitude/ low oxygenation (>3500 m) exposure on plasma TG levels by using either terrain studies or simulated-altitude environments. An earlier terrain study by Whitten et al. (1969) reported a 44% increase in plasmatic TG levels after 9 days of exposure at 4265 m (Whitten & Janoski, 1969). Conversely, using terrain studies at high altitude, Férézou et al. (1988 and 1993) showed that TG levels are decreased by approximately 40 % in individuals exposed to terrestrial high altitude for more than seven days (Ferezou et al., 1988; Ferezou et al., 1993). Finally, Siques et al. (2007) reported, in a terrain study with a cross-over design a 47 % increase in TG levels following an 8-month acclimatization (Siques et al., 2007). These contradicting results on the effect of terrestrial high altitude on TG levels may be explained by confounders such as exercise and/or weight loss induced by the ascent and/or cold exposure. To take into account some of the aforementioned confounders on plasma lipid profile during a sojourn at altitude, some studies have used controlled chambers simulating altitude conditions. In this regard, during the EVEREST II study, 6 men were exposed to increased simulated altitude for 40 days through decreased partial pressure of air (hypobaric hypoxia) up to the altitude equivalent of Mt. Everest (8848 m), and showed an 81% increase in fasting plasma TG levels (P. M. Young et al., 1989). To our knowledge, no study focused on postprandial plasmatic TG levels after simulated high altitude exposure in humans.

Blood lipids, more precisely TG, are important fuel sources that are highly regulated (Lewis et al., 1990). Plasma TG levels represent the production of TG-rich lipoproteins (TRLs) by both the liver (as very low density lipoproteins, or VLDLs) and the intestine (as chylomicrons (CM)), as well as the disposal of TRLs and their remnants in the periphery (Nielsen & Karpe, 2012; Williams, 2008). The typical Western diet, one remains in a postprandial state for more than 18 h per day (Lairon et al., 2007), involving elevated plasmatic TG for almost the entire day. Following

a meal, the small intestine manages the production of chylomicrons (CM) in order to store excess dietary TG in adipose and/or non-adipose tissues (Xiao et al., 2011). At rest, TG breakdown after a meal is mainly controlled and limited by an enzyme expressed in adipose tissue called LPL (Kersten, 2014). A previous study in animals reported that plasma TG rapidly increases in direct proportion to the severity of hypoxia (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012). These changes appear to be caused mainly by the suppression of adipose tissue LPL activity by more than 50% (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012). To confirm whether an impaired clearance in postprandial triglyceride levels is translated in humans acutely exposed to hypoxia, we investigated the effects of an acute exposure to normobaric hypoxia on postprandial TG levels and plasmatic lipolytic activity in healthy individuals. We hypothesized that acute exposure to low oxygen concentration would lead to an exaggerated elevation in postprandial TG level and decreased plasmatic lipolytic activity.

## METHODS

### *In vivo* experiment

#### Subjects

Seven healthy young men were recruited from the University of Ottawa population. Study subjects provided written consent and the study protocol was approved by the Research and Ethics Board of the University of Ottawa. Exclusion criteria included: hypertension, cardiovascular diseases, diabetes, habitual sleep duration of less than 7 hours per night, allergies to lactose, and current smoking status.

#### Anthropometric and metabolic measurements

Body weight was determined with a standard beam scale (HR-100, BWB-800AS; Tanita, Arlington Heights, IL) after urination and height was measured using a standard stadiometer (Perspective Enterprises, Portage, Michigan, USA). Percentage of fat mass (%FM), total fat mass (FM) and fat free mass (FFM) were measured using dual energy X-ray absorptiometry (DXA) (General Electric Lunar Prodigy, Madison, Wisconsin; software version 6.10.019). Resting energy expenditure (REE) was measured in a thermoneutral dark room for 30 min using a Vmax Encore 29 System metabolic cart (VIASYS Healthcare Inc, Yorba Linda, CA) following a 12h overnight fast.

**Experimental protocol and procedures**

This study was a randomized cross-over study consisting of two experimental sessions. Prior to each experimental session, volunteers were counseled to sleep at least 7 hours per night, to restrain from any exercises, caffeine and alcohol for at least 36 hours, and to consume a provided standardized evening dinner between 7:00 PM and 8:00 PM (680 kcal; 54% from carbohydrates, 22% from fat, and 24% from protein). On study days, volunteers presented themselves at the laboratory at 7:30 AM after a 12-hour overnight fast. Participants were allowed only to drink water. Weight and blood pressure measurements were performed after urination but before an intravenous line was inserted in the antecubital vein for blood sampling. Volunteers were thereafter asked to consume the first of twelve servings of the liquid meals, (35% of calories from fat, 55 % from carbohydrates and 10 % from protein, providing 40 % of their estimated daily energy expenditure obtained by REE (1925 kcal (156)) during a preliminary session multiplied by a physical activity factor of 1.375) (Harris & Benedict, 1918), and were then exposed to either hypoxia ($FIO_2$ = 0.12) or ambient air (normoxia) for 6 hours. In order to maintain TG levels at a postprandial level, volunteers consumed ~45 mL of the liquid meal (1/12 of the whole meal) every thirty minutes in both experimental conditions. Volunteers remained in a semi-recumbent position, and occupied themselves by watching television. Conversation with the evaluators was

limited. Sleep was not allowed. Six mL of blood was collected every hour following standard procedures. A heparin injection (Sandoz Canada Inc, Qc, Canada, LOT ET 4161) at a dose of 60 unit per kg of body weight was assessed after completion of 6 h of experimental conditions but 20 min prior to the last blood sample in order to analyse the plasmatic lipolytic activity.

Pulse rate (HR) and oxyhemoglobin saturation ($SaO_2$) were recorded every 2 seconds during both experimental sessions using a Masimo, radical 7 unit (Masimo, Irvine, CA, USA). A mean average was calculated with all the values for an experimental session. Blood pressure (BP) was measured following a 5-10 min rest period after the arrival of the participant to the laboratory, at mid-experiment (T180) and at the end of the experimental session (T360) with an automatic sphygmomanometer (American Diagnostic Corporation, E-sphyg 2, Hauppage NY, USA) following the Canadian Society of Exercise Physiology (CSEP) standard procedures (CSEP, 2013). Mean arterial pressure (MAP) was also calculated with the following formula: MAP = (1/3*systolic pressure) + (2/3*diastolic pressure).

**Energy expenditure and fuel utilisation**

$\dot{V}O_2$ and production of $\dot{V}CO_2$ were measured intermittently (30 min on/ 30 min off) using a calibrated Vmax Encore 29 System metabolic cart (VIASYS Healthcare Inc, Yorba Linda, CA) and are expressed in STPD. Total carbohydrate ($Rcho_{ox}$) and lipid ($Rfat_{ox}$) oxidations rates (g/min) were calculated as described previously (Haman et al., 2002), with the following formulas:

$Rcho_{ox}$ (g / min) = 4.59 $VCO_2$ (l / min) – 3.23 $VO_2$ (l/min)

$Rfat_{ox}$ (g/min) = -1.70 $VCO_2$ (l / min) + 1.70 $VO_2$ (l/min)

$Rpro_{ox}$ was set at 20 % since participants were fed with a mixed meal (Labayen, Forga, & Martinez, 1999).

**Normobaric hypoxic exposure**

Each of the two experimental sessions was performed in a climate-controlled chamber. During normoxia sessions, only ambient air was used. During hypoxia sessions, $O_2$ extractors (oxygen extractor model CAT12 by Altitude Control Technologies, Lafayette, Colorado, USA), connected to a climate-controlled chamber (volume of approximately 64 $m^3$) allowed for a stabilized $FIO_2$ level of 0.1200 to be reached. Temperature fluctuated between 27°C and 29°C during the experimental sessions and relative humidity remained stable at approx. 45%. Hypoxia was generally well-tolerated and presented no adverse effects apart from mild headaches in 4 participants.

**Fasting and postprandial plasma metabolic parameters**

Plasma was obtained by centrifugation at 3200 rpm for 12 minutes at 4°C immediately after blood collection. Commercially available colorimetric enzymatic assays were used to measure plasma triglycerides, glucose, and non-esterified fatty acid (NEFA) levels (Wako Chemicals USA Inc, VA, USA) as previously described (Imbeault, Depault, & Haman, 2009). Plasmatic lipolytic activity was measured on postheparin blood using a novel technique developed by Basu et al. (2011) with the Enzchek fluorescent substrate (Basu, Manjur, & Jin, 2011).

**Statistical Analysis**

SPSS version 12 for Windows was used for data analysis (SPSS Inc. Chicago, IL, USA). Analysis of variance (ANOVA) with repeated measures were performed with condition and time as within subject's parameters. Alpha was set at .05. When Mauchly's test of sphericity was significant, Grennhouse-Geisser correction was used. Errors bars in Figures 1, 2 and 3 were adjusted to a within subject 95% confidence interval, as previously described (Cousineau, 2005).

## RESULTS

**Characteristics of participants**

The study sample consisted of 7 healthy men whose characteristics are summarized in Table 1. Participants' weights were stable (±0.625 kg) between both experimental sessions, which were separated by 7 ± 4 days on average.

**Heart Rate and Oxyhemoglobin**

**Table 2** displays the variations in heart rate and oxyhemoglobin saturation during both experimental sessions. Mean heart rate was significantly higher by 13 bpm in hypoxic conditions ($p<0.01$), when compared to normoxic conditions. Mean oxyhemoglobin saturation levels were significantly lower by more than 15% ($p<0.01$) during hypoxic compared to normoxic conditions. Neither MAP, systolic pressure, nor diastolic pressure differed between experimental conditions (data not shown).

**Substrate oxidation**

**Figure 1** presents the average postprandial substrate utilisation rate and energy expenditure over 6 hours under each experimental condition. On average, $Rcho_{ox}$, $Rfat_{ox}$ and energy expenditure did not differ between experimental conditions. Substrate utilisation and energy expenditure were stable during the course of both experimental conditions (data not shown).

**Plasma metabolic parameters**

Postprandial plasma TG, NEFA and glucose levels during normoxic and hypoxic sessions are shown in **Figure 2.** TG levels rose in a significant manner in response to meals in both experimental conditions, and this increase tended to be greater under hypoxia (Cond x Time interaction at $p = 0.06$). NEFA fasting levels were significantly higher during normoxia than hypoxia ($p < 0.05$). When fasting NEFA levels were excluded from the analysis, NEFA levels were significantly greater in

hypoxia than normoxia conditions ($p < 0.05$) irrespectively of time. Glucose levels varied similarly in response to meals in both experimental conditions (Condition x Time interaction, NS). Irrespectively of time, glucose levels were significantly greater in hypoxia than normoxia conditions ($p < 0.05$). Also, glucose levels significantly increased over time, regardless of experimental condition ($p < 0.05$). As seen in **Figure 3**, postheparin plasma lipolytic activity following 6 hours of exposure to each condition was significantly higher under hypoxia than normoxia ($p< 0.05$).

## DISCUSSION

This study is, to our knowledge, the first to determine the effect of acute normobaric hypoxia on postprandial TG levels in young healthy adults. In a randomized cross-over fashion with an innovative protocol maintaining elevated postprandial TG levels, we report that acute normobaric hypoxia tends to increase postprandial TG levels despite an increase in postheparin plasmatic lipolytic activity. Using indirect calorimetry, our results also indicate that the rise in postprandial TG levels observed under acute normobaric hypoxia is not derived by an alteration in energy expenditure, neither from a reduction in lipid oxidation rate. Together, these results partly confirm the hypothesis stating that acute hypoxia negatively impacts postprandial TG levels, a phenomenon likely explained by the impairment in triglyceride synthesis and/or removal.

### Oxyhemoglobin saturation and Heart Rate responses to hypoxia

Exposure to a reduced fraction of oxygen ($FiO_2$=0.12) for 6 h led to a significant increase in HR, a significant decrease in oxyhemoglobin saturation, and had no effect on blood pressure **(Table 2)**. These results are concordant with those reported in a previous review by Hooper and Mellor (2009) indicating that heart rate increases at rest and during exercise upon altitude exposure, and this response increases with altitude level (Hooper & Mellor, 2011). It is generally accepted that

during high altitude exposure, there is an increase in sympathetic drive which, in turn, increases heart rate. It was previously reported that sympathetic bursts measured by microneurography can increase up to 3-fold under high altitude exposure (Hansen & Sander, 2003).

As expected, oxyhemoglobin saturation was decreased during exposure to a reduced fraction of oxygen **(Table 2)**. Johnson et al. (2010) previously reported a 76 % oxyhemoglobin saturation at an altitude of 3800 m (Johnson et al., 2010). In our study, FiO2 under the hypoxic condition was equivalent to approx. 4800m. Therefore, our results on oxyhemoglobin levels under acute exposure to normobaric hypoxia support the current literature indicating that there are minor differences in peripheral oxygen saturations measured by pulse oximetry between hypobaric hypoxia and normobaric hypoxia (Coppel, Hennis, Gilbert-Kawai, & Grocott, 2015).

**Substrate utilisation**

When exposed chronically to high altitude, it is recognized that there is a shift toward glucose utilisation and decreased reliance on fat utilization at rest (Roberts et al., 1996). Therefore, the respiratory exchange ratio (RER) would increase, approaching a value of 1. As previously reported by Brooks et al. (1991), the shift in substrate utilisation does not occur instantly when exposed to high altitude (Brooks et al., 1991). There is indeed a difference between acute and chronic exposure to high altitude on substrate utilisation, probably due to a time delay in the shift toward glucose utilisation. Our results indicate that an exposure of 6 h to normobaric hypoxia does not modify substrate oxidation rate, nor energy expenditure (EE), supporting previous literature findings in this regard (Brooks et al., 1991; Roberts et al., 1996)**(Figure 1)**.

**Plasma metabolic parameters**

Plasmatic TG levels increased over time in both experimental conditions and tended to be greater under normobaric hypoxia ($p = 0.06$, effect size $n^2 = 0.54$, **Figure 2A**). Plasma steady-state TG levels

reflect the production of TRLs as VLDLs and/or CMs, and the disposal of TRLs and their remnants in the periphery (Nielsen & Karpe, 2012; Williams, 2008). In healthy individuals in a postprandial state, insulin acutely suppresses VLDL assembly and subsequent release into the circulation, making the small intestine the major source of TRLs as CM (Pavlic et al., 2010). In this study, we have provided a portion ($1/12^{th}$) of liquid formula containing carbohydrates and lipids every 30 minutes to favor the inhibition of the VLDL release by the liver. Under normoxia, we have observed a steady-state in TG levels at 180 min. Based on the fact that dietary lipid absorption is not affected up to an altitude of 5 500 m (Kayser, 1994), the trend in a significant elevation in TG levels observed after 180 minutes under normobaric hypoxia may likely reflect an impairment in TG disposal rate, which is influenced by the oxidation and/or the storage of theses energy sources. As mentioned above, we found no difference in lipid oxidation rate over 6 h between hypoxia and normoxia conditions. This observation lends support to the fact that an acute exposure to normobaric hypoxia negatively affects the removal of TRLs through an impaired pathway for TG storage, which is normally and mainly occurring into adipose tissue (Koutsari, Mundi, Ali, & Jensen, 2012; McQuaid et al., 2011). This pathway refers to the hydrolysis of TRLs by LPL bound to capillary endothelium and subsequent uptake of the released fatty acids by adipose tissue. In line with this notion, Jun et al. (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012) previously demonstrated that acute hypoxia increases plasma TG levels due to decreased tissue uptake in rodents. More precisely, acute hypoxia (FiO2 = 0.1100) decreased adipose tissue LPL activity by 26% in brown adipose tissue and by 60% in white adipose tissue. We recently have reported a strong reduction (~80%) of LPL activity in differentiated human preadipocytes exposed for 24h to hypoxia (Mahat, Chassé, Mauger, & Imbeault, 2016). This finding further confirms the inhibitory effect of hypoxia on adipose tissue LPL activity. However, it remains necessary to perform future

studies in humans to determine whether acute normobaric hypoxia modulates adipose tissue LPL activity.

Following 6 h of normobaric hypoxia, we observed a significant increase in postheparin plasma lipolytic activity **(Figure 3)**. This result is contrary to our hypothesis. Administration of intravenous heparin is a common assay used to quantify whole body lipolytic activity. However, as suggested by Olivecrona (2016) (Olivecrona, 2016), administration of heparin likely affects crucial events (i.e. LPL-TRL binding) at endothelial binding lipolysis sites that are complex and not captured by postheparin lipase activity measurement. This may put into question the physiological relevance of the assay and explain why some studies reporting significant changes in LPL activity in diverse tissues following hypoxia exposure do not observe any difference in postheparin LPL activity (Jun, Shin, Yao, Bevans-Fonti, Poole, & Drager, 2012; Jun et al., 2013). The discrepancy between adipose tissue LPL activity, previously reported to decrease under acute hypoxia (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012), and plasma lipolytic activity, increased under acute hypoxia in our study, could be explained by 1) the importance of LPL-TRL binding (Olivecrona, 2016) and 2) the technique used (Basu et al., 2011). The novel technique used in this study to characterize plasmatic lipolytic activity was first developed by Basu et al. (2011) and is a valid and reproducible way to measure lipolytic activity without using radioactive compounds. Although, the fact that the postheparin plasma analysis technique is not specific to LPL and hepatic lipase (HL) could negatively mitigate the results (Connelly, 1999). Finally, in animal studies, it is reported that postheparin plasmatic LPL activity only decreases after hypoxic exposure in a cold environment, not when in thermoneutrality (Jun et al., 2013).

Irrespectively of time, postprandial NEFA levels were significantly greater during hypoxia as compared to normoxia sessions. **(Figure 2B)**. As mentioned previously, exposure to a low fraction

of oxygen leads to an increase in sympathetic tone (Hansen & Sander, 2003). The increase in sympathetic tone on adipose tissue, directly innervated by the sympathetic nervous system (Youngstrom & Bartness, 1995), causing the breakdown of TG and a net efflux of NEFA (Jun, Shin, Yao, Bevans-Fonti, Poole, Drager, et al., 2012; Samra et al., 1996). Since we report no difference in lipid oxidation rate between normoxia and hypoxia sessions, another contributing factor to the increase in plasma NEFA during the hypoxia session may include the earlier relief of lipolysis inhibition by insulin. It has indeed been reported that exposure to acute hypoxia worsens insulin sensitivity (Louis & Punjabi, 2009). Consistent with this notion, irrespective of time, plasma glucose levels in our study were significantly higher under the hypoxic condition (**Figure 2C**). Peltonen et al. (2012) identified the increased sympathetic tone under acute hypoxia as a possible reason for a transitory insulin resistance observed under hypoxia. In our study, sympathetic tone was increased, as seen by the net increase in HR under the hypoxic exposure, thus affecting plasmatic glucose levels.

The present work supports previously observed findings in animal that acute normobaric hypoxia negatively affects postprandial triglyceride levels. Our results demonstrate that higher levels of postprandial triglyceride observed during an acute exposure to reduced oxygen availability while resting is not caused by a shift toward decreased dependence of lipid utilization. Future studies in humans are required to further our understanding of the regulatory cascades leading to changes in triglyceride levels upon hypoxia exposure, which may occur in response to high altitude exposure as well as pathological conditions of oxygen deprivation such as obstructive sleep apnea.

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**Table 1**. Characteristics of our healthy young participants (n=7)

| Characteristics of participants | Mean (st.dev) |
|---|---|
| Age (years) | 24.9 (4.6) |
| Height (cm) | 180.6 (1.6) |
| Weight (kg) | 79.2 (8.5) |
| Lean Mass (kg) | 68.2 (4.9) |
| Fat Mass (kg) | 9.9 (4.2) |
| Fat Mass (%) | 12.4 (4.4) |

Data are expressed as mean (Standard deviation).

**Table 2.** Heart rate and oxyhemoglobin saturation.

| | Mean (St.dev) | |
|---|---|---|
| | Hypoxia | Normoxia |
| HR (bpm) | 73.7 (8.0) | 59.9* (7.0) |
| SaO2 (%) | 82.3 (2.6) | 97.9* (1.0) |

Dara are expressed as means (standard deviation). * Significant difference between experimental conditions, $p < .01$.

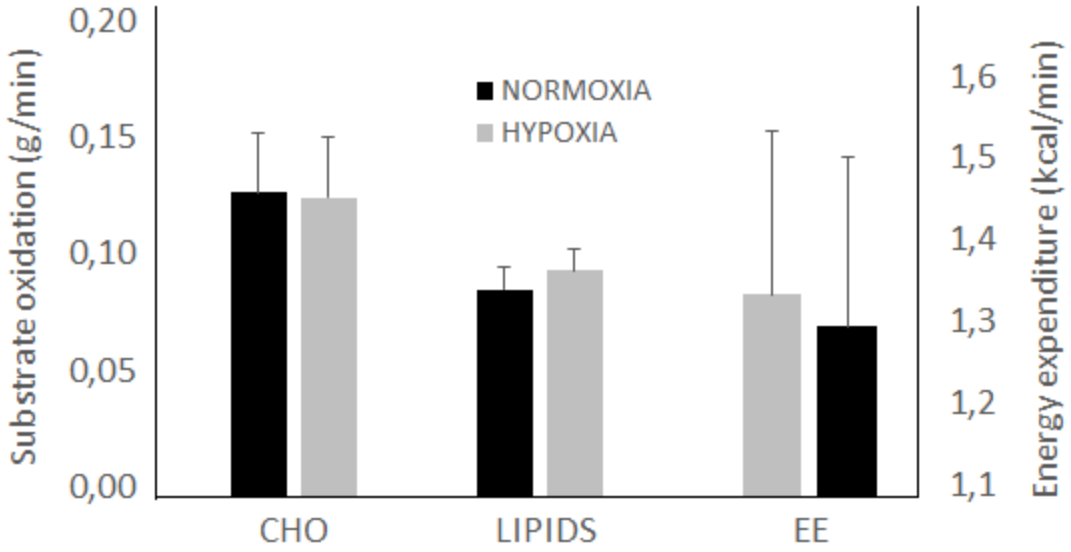


**Figure 1.** Average rate of utilization of carbohydrates (CHO) and lipids and energy expenditure (EE) (kcal/min) measured for 6 h during normoxia and hypoxia in young healthy men in postprandial state. No significant difference was observed between experimental conditions.

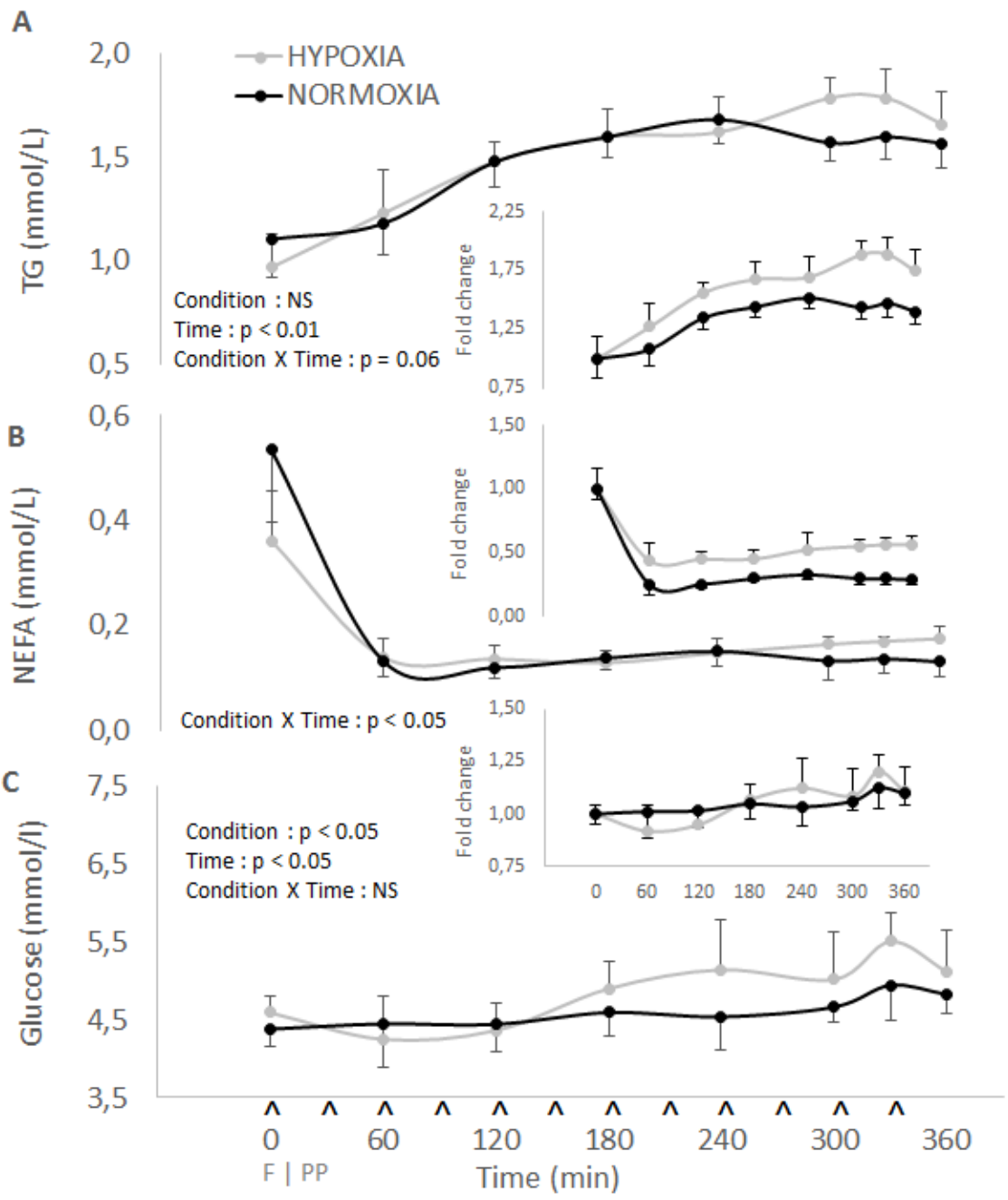


**Figure 2.** Plasmatic (A) TG (mmol/L), (B) NEFA (mmol/L) and (C) glucose (mmol/L) levels over time (min) under acute exposure to hypoxia (grey) or normoxia (black) measured before (fasting, F) and after (postprandial, PP) repeated serving (^) in young healthy men. Inserts represent fold change for TG, NEFA and Glucose over time (min).

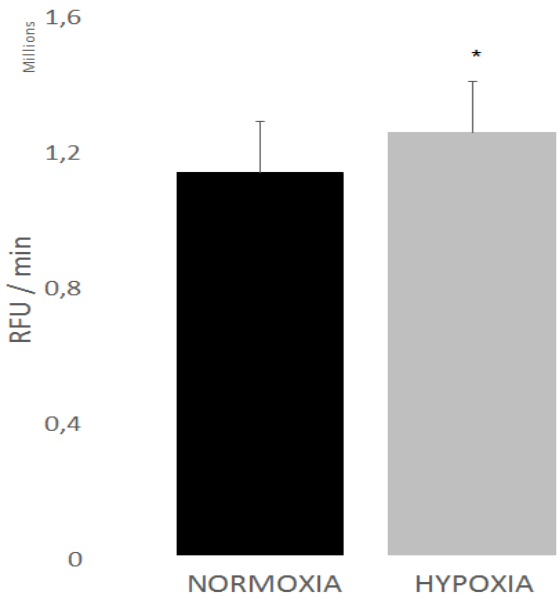


**Figure 3.** Postheparin plasma lipolytic activity following 6 hours of normoxic and hypoxic conditions in young healthy men in postprandial state. * Significant difference between experimental conditions, $p < .05$.

## CHAPTER 4. PERSPECTIVES

In this thesis, adipose tissue's crucial role in lipid metabolism upon hypoxic exposure was discussed. Mainly, adipose tissue has a dual function, being the primary tissue storing excess dietary TG in postprandial state and releasing NEFA in the fasted state. Adipose tissue has therefore a major role in the modulation of plasma TG levels.

Hypoxia is reflected by an insuffient supply of oxygen as compared to cellular consumption. As previously explained, a systemic marker for hypoxia is the oxyhemoglobin saturation. As observed in the protocol used for this thesis, healthy individuals acutely exposed to reduced fraction of oxygen have a $SaO_2$ reduced which translates to a systemic hypoxia.

Hypoxia also occurs in a chronic condition called obstructive sleep apnea. Obstructive sleep apnea is mainly a breathing disorder that happens when at sleep. When suffering of obstructive apnea, patients suffer of a collapse of the upper airway of the throat causing an impossibility to breathe leading to rapid depletion/repletion of $S_aO_2$. These pauses in breathing induce a temporary hypoxemia that can go as low as 60%. The Public Health Agency of Canada estimated that 850 000 people in Canada are affected by this condition. A part of the problem is the incapacity to have restorative sleep and thus, daytime sleepiness, impaired cognitive function and, reduced quality of life and common symptoms (PHAC, 2013). However, as described in the current thesis, chronic exposure to hypoxia could have a negative impact on TG metabolism and could explain the increased risk for cardiovascular diseases in this population (Yaggi et al., 2005). Individuals affected with sleep apnea commonly have higher than normal adiposity level. It is accepted that a high adiposity level is generally associated with insulin resistance (Amati et al., 2012) and a greater postprandial lipemia due to a higher endogenous hepatic lipoproteins secretion (Lewis et al., 1990). Future studies remained to be performed to evaluate whether sleep apnea alters lipid metabolism in individuals with high adiposity level.

A previous project on TG metabolism and hypoxia inspired me for the current thesis. Briefly, we showed the marked difference in TG metabolism between human differentiated preadipocytes exposed to hypoxia and healthy individuals exposed to acute intermittent hypoxia. This two-phase study was about the similarities and differences from in vivo and in vitro exposure to hypoxia. In terms of the in vivo experiment, we observed that 6 h of intermittent hypoxia, at a rate of 17

events per hour, had no significant effect on postprandial TG level nor adipocyte lipolytic activity. In terms of the in vitro experiment, we observed that 24 h of acute continuous hypoxia at 3% $O_2$ on differentiated human preadipocytes induced a significant decrease on LPL activity and a significant increase on ANGPTL-4 level. Therefore, we concluded that there is a diverging effect of hypoxia on differentiated preadipocytes and healthy individuals. This study was recently published in the Journal of Translational Medicine (Mahat et al., 2016). For this thesis, we wanted to further investigate the effects of acute hypoxia on TG metabolism. With our innovative protocol that stabilized postprandial TG levels, we have found that acute exposure to normobaric hypoxia tends to increase postprandial plasmatic TG levels in healthy individuals.

Multiple interrogations remain however unanswered after the completion of the present study. Due to technical limitations in isolating triglycedide-rich lipoproteins, we could not answer whether the increase in TG levels observed after hypoxic exposure is derived from an increase in endogeneous VLDL production and/or a decrease in chylomicron breakdown. We are currently performing a protocol investigating the effect of the same hypoxic insult (6h) while individuals are fasted to better understand the effect of hypoxia on VLDL metabolism. Since the liver usually does not respond as well as it should to insulin in individuals characterized with obesity, it remains unknown whether individuals with adiposity would respond similarly than our sample, which was characterized by individuals with normal adiposity level.

To address some limits of our study, future experimental protocol could include innovative methodology to further our understanding of the effect of hypoxia on lipid metabolism. First, adipose tissue biopsies could be assessed in order to characterize adipose tissue lipolysis and LPL quantification (Mahat et al., 2016). Since adipose tissue is one of the main tissue regulating circulating TG, its kinetics under normobaric hypoxia would be great information. Second, TRL kinetics could be assessed by using a primed-constant infusion of l-[5,5,5-d3]leucine for 12 h in the fed state. Real-time polymerase chain reaction quantification was performed on duodenal biopsy samples taken at the end of each phase of supplementation (Tremblay et al., 2014).

Third, a novel imagery system like the positron emission tomography (PET) scans could be used to analyze the whole body metabolism under acute normobaric hypoxia (Hames, Vella, Kemp, & Jensen, 2014). These three potential projects would add information in order to explain the mechanism responsible for the elevation in postprandial TG levels seen in this thesis.

## CHAPTER 5. REFERENCES

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Appendix: Notice of ethics approval

**File Number:** H05-13-13B **Date (mm/dd/yyyy):** 04/02/2015

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

## Ethics Approval Notice

### Health Sciences and Science REB

**Principal Investigator / Supervisor / Co-investigator(s) / Student(s)**

| First Name | Last Name | Affiliation | Role |
|---|---|---|---|
| Pascal | Imbeault | Health Sciences / Human Kinetics | Principal Investigator |
| Bimit | Mahat | Health Sciences / Human Kinetics | Co-investigator |
| Jean-François | Mauger | Health Sciences / Human Kinetics | Co-investigator |
| Etienne | Chassé | Health Sciences / Human Kinetics | Research Assistant |

**File Number:** H05-13-13B

**Type of Project:** Professor

**Title:** The effects of acute hypoxia on postprandial metabolism

| Approval Date (mm/dd/yyyy) | Expiry Date (mm/dd/yyyy) | Approval Type |
|---|---|---|
| 04/02/2015 | 04/01/2016 | Ia |

**(Ia: Approval, Ib: Approval for initial stage only)**

**Special Conditions / Comments:**
N/A



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**File Number:** H05-13-13B **Date (mm/dd/yyyy):** 04/02/2015

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement (2010) and other applicable laws and regulations in Ontario, has examined and approved the ethics application for the above named research project. Ethics approval is valid for the period indicated above and subject to the conditions listed in the section entitled "Special Conditions / Comments".

During the course of the project, the protocol may not be modified without prior written approval from the REB except when necessary to remove participants from immediate endangerment or when the modification(s) pertain to only administrative or logistical components of the project (e.g., change of telephone number). Investigators must also promptly alert the REB of any changes which increase the risk to participant(s), any changes which considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project and safety of the participant(s). Modifications to the project, including consent and recruitment documentation, should be submitted to the Ethics Office for approval using the "Modification to research project" form available at: http://research.uottawa.ca/ethics/submissions-and-reviews.

Please submit an annual report to the Ethics Office four weeks before the above-referenced expiry date to request a renewal of this ethics approval. To close the file, a final report must be submitted. These documents can be found at: http://research.uottawa.ca/ethics/submissions-and-reviews.

If you have any questions, please do not hesitate to contact the Ethics Office at extension 5387 or by e-mail at: ethics@uOttawa.ca.

**Signature:**

Riana Marcotte
Protocol Officer for Ethics in Research
For Daniel Lagarec, Chair of the Health Sciences and Sciences REB



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