THE EFFECT OF ACUTE INTERMITTENT HYPOXIA ON POSTPRANDIAL LIPID METABOLISM

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THESIS ABSTRACT

Background: Obstructive sleep apnea (OSA) consists of repeated, involuntary breathing suspension during sleep. These events induce rapid depletion/repletion of blood/tissue oxygen content, a phenomenon known as intermittent hypoxia. Aside from causing daytime sleepiness, the most important health consequence of OSA is a 2-fold increase in cardiovascular (CVD) risk. Animal studies provide evidence that intermittent hypoxia, a simulating model of OSA, causes important rise in plasma TG, especially in the postprandial state. However, the underpinning mechanisms linking intermittent hypoxia to altered postprandial TG levels remain unknown. As such, the objective of this study was to characterize the effects of acute intermittent hypoxia on postprandial TG levels in 2 distinct lipoprotein subtypes in humans: chylomicrons which are secreted by the intestine and carry dietary lipids, and denser TG carriers (mainly VLDL) which are secreted by the liver and carry endogenous lipids.

Methods: The research consisted of a randomized crossover design. In collaboration with the Sleep laboratory at Montfort Hospital, 7 individuals diagnosed with moderate sleep apnea were recruited through phone calls as well as 8 healthy individuals without OSA from the University of Ottawa. While lying on a bed, participants were given a meal after which they were

exposed for 6 hours to normoxia or intermittent hypoxia corresponding to moderate OSA, e.g. 15 hypoxic events per hour. Blood lipid levels were measured hourly.

Results: Plasma TG levels increased over time in both experimental conditions and tended to be greater under 6-h exposure to intermittent hypoxia ($p=0.093$, effect size $\eta_p^2= 0.383$.). This trend toward higher total plasma TG under intermittent hypoxia was attributable to increased levels in denser TG carrying lipoproteins such as VLDL and CM remnants ($p= 0.009$, $\eta_p^2 = 0.173$).

Conclusion: Acute intermittent hypoxia, a simulating model of obstructive sleep apnea, tends to negatively affect postprandial TG levels, which is attributable to an increase in denser TG carrying lipoprotein levels such as VLDL and CM remnants. These results lend support to the increase in blood lipid levels in animal studies observing the effect of acute hypoxia in mice.

Contribution to advancement of knowledge: This proposed research will allow a better understanding of the mechanisms by which obstructive sleep apnea may alter blood lipid profile. This information will be beneficial to the treatment of obstructive sleep apnea related dyslipidemia and contribute to reduce CVD risk in the large proportion of obstructive sleep apnea patients who are reluctant to current treatment avenues.

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J'aimerais dédier ma thèse en mémoire de mon grand frère Vincent Morin et à ma famille que j'aime tant.

PREFACE

The work presented in this thesis is my own and I take full responsibility for its content. The certificates of ethical approval for this study from the University of Ottawa Health Sciences and Montfort Research Ethics Board are included in the appendix. This study was funded by the Natural Sciences and Engineering Research Council of Canada as well as l'Institut du Savoir Montfort grants attributed to Dr. Pascal Imbeault.

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List of abbreviations

Term	Description
ANGPTL-4	Angiopoietin like-4 protein
ApoB	Apolipoprotein B
ATGL	Adipose triglyceride lipase
ATP	Adenosine triphosphate
BMI	Body mass index
BPM	Beat per minute
CHO	Carbohydrates
CVD	Cardiovascular disease
DGAT	Diacylglycerol acyltransferase
DGs	Diglycerides
DNL	*De novo* lipogenesis
FAS	Fatty acid synthase
F_iO_2	Fraction of inspired oxygen
HIF-1	Hypoxia-inducible factor 1
HL	Hepatic triglyceride lipase
HR	Heart rate
HSL	Hormone-sensitive lipase
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase

MGAT	Monoacylglycerol acyltransferase
MGs	Monoglycerides
NEFA	Non-esterified fatty acids
N_2	Nitrogen
OSA	Obstructive sleep apnea
O_2	Oxygen
PKA	Protein kinase A
PO_2	Partial pressure of oxygen
RDI	Respiratory disturbance index
SNS	Sympathetic nervous system
SREBP1	Sterol regulatory element binding protein 1
SpO_2	Oxyhemoglobin saturation
TG	Triglycerides
VLDL	Very-low density lipoprotein
Vo_2 max	Maximum volume of oxygen consumption
WAT	White adipose tissue

List of definitions

Adipose tissue: Adipose tissue, being the most variable organ in mammals when it comes to size, can vary in extremes from it being at a very low percent of total weight in the malnourished to over 50% of body weight in the morbidly obese (Trayhurn, P. 2014). It is considered the main lipid storage site.

Chylomicrons: Lipoproteins that carry dietary lipids from the intestines.

De novo lipogenesis: *De novo* lipogenesis is an enzymatic pathway that converts dietary carbohydrates (CHO) into fat; inversely, conversion of fats into CHO is not possible (Hellerstein, M. K. 1999).

Hypoxia: Condition occurring when the tissue's demand of oxygen is greater than the oxygen supply. Hypoxia can occur in response to exposure to environmental conditions such as high-altitude trekking, or by pathological conditions such as OSA.

Hypoxemia: Condition defined as abnormally low concentration of O_2 in the blood. It translates by a decrease in O_2 saturation in the hemoglobin. This may lead to hypoxia at the cellular level and/or hypoxia in tissues.

Intermittent hypoxia: Repeated cycles of hypoxia and normoxia.

Lipids: Fatty acids, sterols and triglycerides fall under the category of lipid.

Lipogenesis: Adipose tissue, energy storage organ, stores triglycerides through lipogenesis. Lipogenesis is partly driven by LPL. The LPL hydrolyzes TRLP-TG into FA for their subsequent uptake, where they can be re-

esterified into TG. Lipogenesis is a process that includes *de novo* FA synthesis and TG biosynthesis (Luo, L., & Liu, M. 2016).

Lipolysis: The catabolic process that leads to the breakdown of adipose tissue TGs into NEFAs and glycerol is called lipolysis. The products of lipolysis will be transported to the bloodstream for their subsequent uptake by other tissues.

Lipoprotein lipase: Key enzyme involved in the hydrolysis of TG. It hydrolyses the TRLP to fatty acids and glycerol for their subsequent uptake.

OSA: OSA is a condition that usually occurs by the restriction of the upper airway, inducing difficulty breathing and leading to intermittent hypoxia, excessive daytime sleepiness, and increased risk of developing cardiovascular disease (Drager, L. F et al., 2010).

Triglycerides: TG, ester composed of 3 FA and glycerol. TG are mainly stored in subcutaneous adipose tissue considering it represents about 85% of all body adipose tissue (Frayn, K.N., & Karpe, F. 2014).

Very-low density lipoproteins: Lipoproteins that carry TG synthesized by the liver. The very-low density lipoproteins are made by the liver.

CHAPTER 1: INTRODUCTION

In order to survive, complex organisms need an essential element commonly known as oxygen (O_2). This element plays the most critical role in vegetal and animal respiration (Brahimi, M. C., & Pouysségur J. 2007). Approximately 2.5 billion years ago, O_2 started accumulating in the Earth's atmosphere. Humans have adapted to an atmosphere rich in O_2, adding to the complexity of living organisms. In some varying cases, organisms have been required to adapt to variations in O_2 pressures and or O_2 concentrations in order to survive in situations such as high altitude and or hypoxia. The O_2 availability led to the evolution and development of an astonishingly efficient system of oxidative phosphorylation producing adenosine triphosphate (ATP), essential for living cells (Semenza, G. L. 2012). Mitochondria is the site of cellular oxygen sensing as they are the primary consumer of cellular oxygen (Hamanaka, R. B., & Chandel, N. S. 2007). Dysfunctional oxidative phosphorylation can lead to severe consequences, including death. Different circumstances can arise and consequently lead to a lack of O_2 supply, causing oxyhemoglobin desaturation and an insufficient supply of oxygen in various tissues, a phenomenon referred to as hypoxia (Brahimi, M. C., & Pouysségur, J. 2007). Hypoxia occurs when the tissue's demand for oxygen is greater than the oxygen supply. In hypoxemia, when the concentration of O_2 in the blood is abnormally low there is a decrease in O_2 saturation in the hemoglobin. The oxygen-loaded form of hemoglobin, also known as

oxyhemoglobin, is a marker for whole-body oxygenation. Hemoglobin, being an essential protein found in red blood cells, is capable of binding to O_2. At sea level, hemoglobin is saturated at 97-99% O_2 (Power et al. 1988). When the arterial oxyhemoglobin saturation ($\%S_aO_2$) is lower than 90%, tissues are exposed to hypoxic conditions (Semenza, G. L. 2000). Circumstances exhibiting situations of affected O_2 supply are obstructive sleep apnea (OSA), chronic obstructive pulmonary diseases (COPD), and exposure to high altitudes (Drager, L. F. et al. 2011 & Raguso, C. A. et al. 2009 & Johnson, P. L. et al. 2010). OSA is a condition that usually occurs through the restriction of the upper airway, inducing breathing difficulties. This sleep disorder affects 5-24% of men, 2-9% of women; however, in individuals with obesity, the prevalence of OSA exceeds 50% (Drager, L. F. et al. 2010). According to the Public Health Agency of Canada, 5.4 million Canadians suffer from OSA (PHAC, 2014). These individuals, affected by OSA, usually experience momentary episodes of hypopnea, characterized as partial blockage of the airway, exhibiting short periods of excessive shallow breathing and then experience intermittent hypoxia (IH) cycles. Aside from causing daytime sleepiness, intermittent as well as chronic hypoxia can lead to health consequences such as increased risk of developing cardiovascular disease (CVD), type 2 diabetes and atherosclerosis (Pack, A. I., & Gislason, T. 2009).

Obesity is widespread in Canada, and worldwide. OSA is highly prevalent in those living with obesity. This sleep disorder increases the risk for development of various cardiovascular diseases, neurocognitive dysfunction, hypertension, atherosclerosis, and metabolic disorder such as type 2 diabetes, and metabolic syndrome (Ryan et al. 2008). Typically, the hypoxic events last 10-30 seconds and result in a significant drop in oxygen concentration in the blood. Individuals with OSA will often wake up in order to resume a regular breathing pattern and restore blood O_2 concentration resulting in excessive daytime sleepiness (Resto, O. et al. 2001). A potential explanatory factor linking obesity and OSA to increased risk of developing cardiovascular diseases is the possible disturbing effect of OSA on blood lipid levels, more specifically increased triglyceride (TG) levels. In this regard, individuals with OSA usually display 30% greater TG levels individuals without OSA (Newman, et al. 2001).

It has been shown that acute hypoxia increases TG through two major mechanisms: increased hepatic secretion of VLDL and decreased triglyceride-rich lipoprotein (TRLP) clearance (Jun, et al. 2012). In mouse models, acute hypoxia exposure leads to hypertriglyceridemia as a result of decreased plasma triglyceride clearance (Jun, et al. 2012). Lipoprotein lipase (LPL) is the key enzyme that hydrolyzes TRLP. The activity of this enzyme was found to be significantly inhibited in adipose tissue of mice exposed to acute constant hypoxia. LPL is expressed in tissues that oxidize or store FA

in great quantities such as the skeletal muscle, heart, and adipose tissue (Kersten, S. 2014). The reduction of the LPL activity leads to delay of clearance of those TRLP, resulting in hypertriglyceridemia.

Considerable research has reported a detrimental effect of hypoxia on circulating TG levels (Mahat, B., 2016, Mauger, J.F. et al. 2019, Drager, L. F. et al. 2010; Drager, L. F. et al. 2012; Jun, J. C. et al. 2012). The mechanism underpinning the effect of hypoxia on TG levels alteration remains to be clarified. Therefore, the purpose of this thesis was to depict the effect of IH on circulating plasma TG levels in individuals living with OSA vs. those who are not. We hypothesized that IH would lead to reduced clearance of dietary lipids and a prolonged elevation of postprandial lipemia, this effect being more pronounced in individuals with moderate OSA than without OSA. This research will allow a better understanding of the mechanisms by which OSA may alter blood lipid profile. This information will be beneficial to the treatment of OSA related dyslipidemia and contribute to reduce CVD risk in the large proportion of OSA patients who are reluctant to current treatment avenues.

CHAPTER 2: REVIEW OF THE LITERATURE

General information on triglycerides

Triglycerides, mainly derived from food and/or produced through de-novo lipogenesis, are considered the most energy-dense substrate. TG, composed of three non-esterified fatty acids (NEFA) and glycerol, are mobilized or hydrolyzed, as required, for internal oxidation by peripheral organs. Via oxidative phosphorylation, FFA act as a nutrient for ATP production. Subcutaneous adipose tissue represents about 85% of all body adipose tissue and is thus the primary storage depot for TG (Frayn, K.N., & Karpe, F. 2014). Apart from adipose tissue, they can also be stored in the liver and skeletal muscles. TG are insoluble in water, signifying that in order to be stored and or mobilized in various tissues they require specialized transport, more specifically by TRLP. The term TRLP relates to very low-density lipoproteins (VLDL) and chylomicrons (CM) (lipoproteins will be discussed further in later sections). Postprandially, CM transport TG from the intestines while the VLDL transport the TG from the liver to the rest of the peripheral tissues via the circulatory system (**Figure 1**) (Chapman, M. J. et al. 2011). A large structural protein, moderately hydrophobic, apolipoprotein B (ApoB), plays a major role in lipoprotein biosynthesis (Shumaker, V. N. et al. 1994). ApoB is involved in the packaging and transport of TG. More specifically, a smaller version of the protein, apoB48, is used for

chylomicrons biosynthesis by the intestine while the full-length apoB100 used for VLDL biosynthesis by the liver (Shumaker, V. N. et al. 1994).

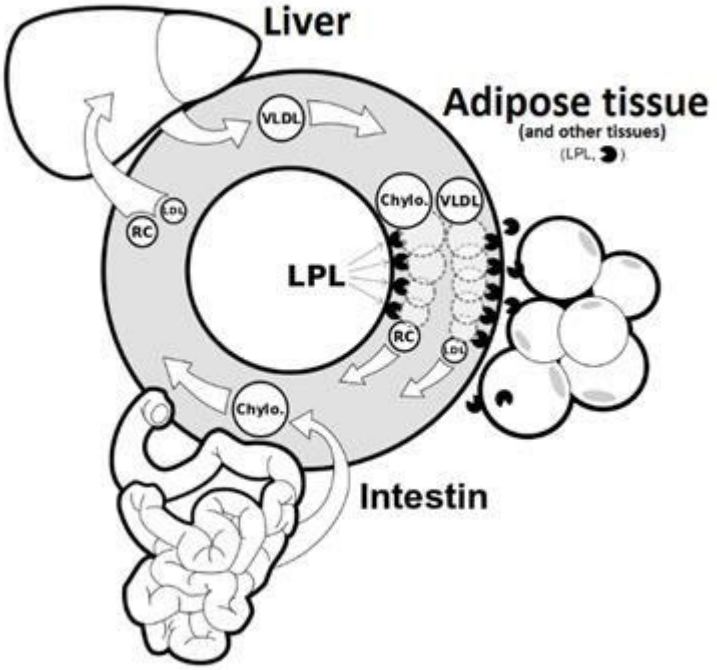

Figure 1. Triglyceride-rich lipoprotein (TRLP) metabolism. Briefly, the small intestine releases chylomicrons when dietary fats are ingested. The liver releases VLDL in a predominant manner during fasting state. Both TRLPP are mainly hydrolyzed by LPL generated by adipose tissue or other tissues. Following hydrolysis of CM and VLDL, chylomicron remnants (RC) and LDL will be metabolized by the liver to resynthesize VLDL.

Adipose tissue

Adipose tissue, being the most variable organ in mammals when it comes to size, can vary in extremes from it being at a very low percent of total weight in the malnourished to over 50% of body weight in individuals with morbid obesity (Trayhurn, P. 2014). It is considered the main lipid storage site. The concern associated with excess weight is the inflammation that develops in

adipose tissue as well as the production and release of inflammatory factors from adipocytes; factors considered to lead to the development of insulin resistance, type 2 diabetes, metabolic syndrome, and cardiovascular disease (Trayhurn, P. 2014). Studies, in both rodents and humans, elucidate the key role of adipose tissue as the energy reservoir playing a central role in the regulation of free fatty acid storage and mobilization. As illustrated in **Figure 2,** lipid metabolism and mobilization is regulated by adipose tissue. The adipocytes, hallmark cells of adipose tissue, regulate storage and release of TG and have major influence on whole energy homeostasis (Fruhbeck, G. et al. 2001). The adipose tissue is an energy storage organ as it promotes the biosynthesis of TG through lipogenesis and alternatively it induces the breakdown of TG which releases FFA and glycerol through lipolysis, depending on feeding or fasting state. Systemically, fasting stimulates the lipolytic pathway promoting FFA oxidation and/or release, while the feeding state induces the lipogenic pathway leading to the storage of TG in adipose tissue. These pathways are very sensitive to nutrition and various hormones, such as insulin and glucagon (**Figure 2**) (Luo and Liu 2016). Chronic increase in lipid availability can lead to metabolic conflict by causing enhanced *de novo* lipogenesis and FFA reesterification (Sethi, J. K., and Vidal-Puig, A. J. 2007). The aforementioned *de novo* lipogenesis is an enzymatic pathway that converts dietary carbohydrates (CHO) into fat; inversely, conversion of fats into CHO is not possible (Hellerstein, M. K.

1999). Evidence shows that adipose tissue contains lipases that break down TG and that the FA can be transported to the liver and muscles for FA oxidation. There is also evidence that these FA can be reesterified in adipocytes allowing for FFA flux to be highly regulated. In order to avoid lipotoxicity, accumulation of lipids in non-adipose tissue which may lead to metabolic disorders, there needs to be tight control between TG hydrolysis and NEFA esterification.

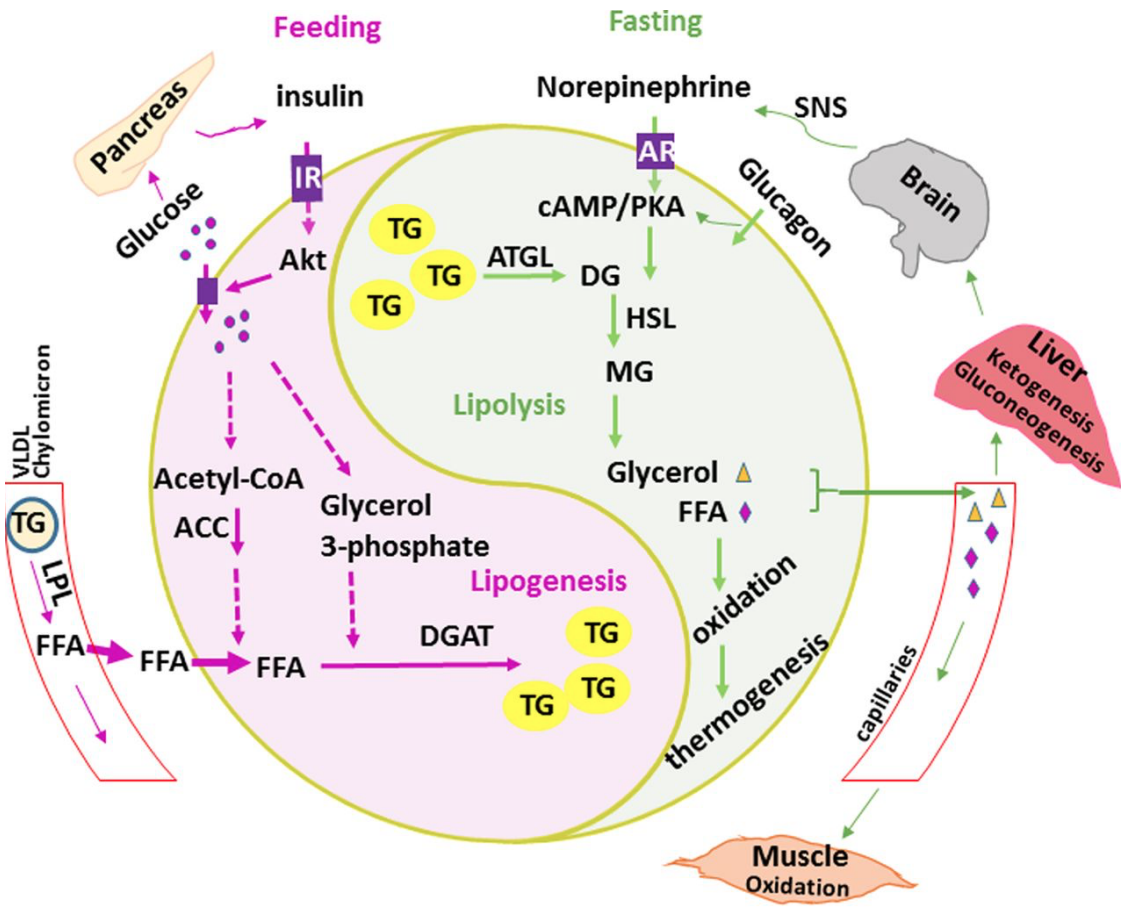

Figure 2. Adipocyte lipid metabolism. The adipose tissue is an energy storage organ as it stores TG through lipogenesis and releases FFA and glycerol through lipolysis depending on feeding or fasting state. Systemically, fasting stimulates the lipolytic pathway which promotes FFA

oxidation while the feeding state induces the lipogenic pathway leading to the storage of TG in adipose tissue. TG: triglycerides. HSL: hormone sensitive lipase. ATGL: adipose triglyceride lipase. FFA: free fatty acids. DGAT: diacylglycerol acyltransferase. DG: diglycerides. AR: adrenoceptors. IR: insulin resistance. LPL: lipoprotein lipase. MG: monoglycerides. SNS: sympathetic nervous system. cAMP: cyclic adenosine monophosphate. PKA: protein kinase A. Figure from Luo et Liu (Luo et Liu 2016).

Triglyceride-rich lipoproteins

Humans ingest approximately 30-150 g of TG per day, and these dietary fats are efficiently processed in the stomach and the small intestine in order to be absorbed and entered into the blood circulation; mainly in the form of TRLP, chylomicrons (CM) and VLDL (Dubois, C. et al. 1998). With regards to lipids, the small intestine's main roles include digestion, absorption, and secretion of dietary fats while responding rapidly and efficiently to large quantities of ingested food (Xiao, C. et al. 2011). In fact, Dubois *et al.* reported that in order to increase postprandial CM-TG significantly, there needs to be consumption of 15 g of lipids, minimally, and that the peak in blood TG concentration occurs 2-4 hours following meal ingestion (Dubois et al. 1998). Therefore, when a meal contains enough fat, there is a transient increase in TG, and a change in lipoprotein pattern occurs (Lopez-Miranda, J. et al. 2007). These postprandial changes are modulated by multiple factors and are highly variable. FFA are absorbed and converted to TG transported by CM in the intestinal epithelial cells. The TRLP apoB-48 CM enters the circulation and is then hydrolyzed by the LPL. The apoB-48 containing particles are continuously secreted from the enterocytes,

and when there is excessive TG availability, the lipid droplets, and the lipoprotein fuse together, resulting in the secretion of CM of great size (Nakajima, K. et al. 2011). According to clinical cutoffs, in a fasted state TG level should be lower than 1.7 mmol/L, and CM should be near 5.7 ± ug/ml (0.0052 ± 0.0038 g/L) (Sakai et al. 2003).

Contrary to the intestine, which does not have a capacity for persistent storage and subsequent mobilization of ingested lipids, the liver, is the master gatekeeper of lipids that are ingested, mobilized and synthesized *de novo* (Xiao, C. et al. 2011). This gatekeeper function allows for lipid homeostasis through the transition from fed to fasted state as this organ is involved in lipoprotein regulation in the whole body. The apoB-100 is the protein of VLDL particles that plays a critical role in lipoprotein assembly. TRLP production is very dependent on lipid availability; when lipid substrate is limited, apoB is degraded, and consequently, lipoprotein secretion is reduced (Xiao, C. et al. 2011). The nutritional and hormonal state of humans is important in the regulation of VLDL secretion. With that said, the production of VLDL is substrate driven with FA being the major regulatory substrate (Lewis, G. F. 1997). Lewis (1997) reported that VLDL-TG are derived from at least four sources: two involving NEFA spillover and the remaining being related to the liver. As previously mentioned, in the transition state between postabsorptive and postprandial, CM-TG are

hydrolyzed by the LPL, which preferentially acts on CM-TG in comparison to VLDL-TG. Studies observing postprandial metabolism demonstrate that a proportion of FA, derived from CM-TG, post hydrolysis, are not taken up by the adipose tissue. These FA, spillover into the systemic NEFA pool and increase postprandial NEFA concentrations (Piché, M.E et al. 2017). There are also FA derived from lipoproteins, which are eventually taken up by the liver (**Figure 3)** (Lopez-Miranda, J. et al. 2007). The other sources of NEFA related to the liver are derived from *de novo* lipogenesis and cytoplasmic TG stores. *De novo* lipogenesis is the biochemical process of synthesizing FA from subunits, produced via multiple pathways, carbohydrate catabolism being the most common (Sanders, F. W., & Griffin, J.L. 2016). Many factors, aside from the liver, will impact the cycle of circulating TG and VLDL such as neurotransmitters (epinephrine) and insulin concentrations both with opposite effect on VLDL production (Lewis, G.F. et al. 1993 & Xiao, C. et al. 2011). In fact, epinephrine plays a role in regulating lipid and lipoprotein metabolism in humans (Ward, K.D. et al. 1994). Epinephrine leads to lipolysis in adipose tissue which secretes NEFA and glycerol; main components needed for VLDL-TG production. Conversely, insulin has a powerful suppressing effect on intestinal and hepatic lipoprotein production. The fasting state, where levels of insulin are relatively low, provides a vital state for VLDL production (Xiao, C. et al. 2011). Usually, we, humans, spend the majority of the day in a postprandial state where most plasma TG are

transported in TRLP. Contrary to CM, VLDL are present in the plasma during both fed and fasting state. Following an overnight fast, when insulin concentrations are low, the lipolysis of adipose tissue leads to higher NEFA secretion. More specifically, during prolonged fasting the NEFA pool can account for almost the totality of the FA used for the production of VLDL-TG (Barrows, B.R., & Parks, E. J. 2006). In a fasting state, TG are the main energy substrate and therefore there needs to be a highly regulated homeostasis between production and disposal of TG. Without such control mechanisms, fasting and non-fasting TG levels could be unbalanced, leading to a dysregulated TG profile, a predominant factor for CVD (Mora, S. et al. 2008).

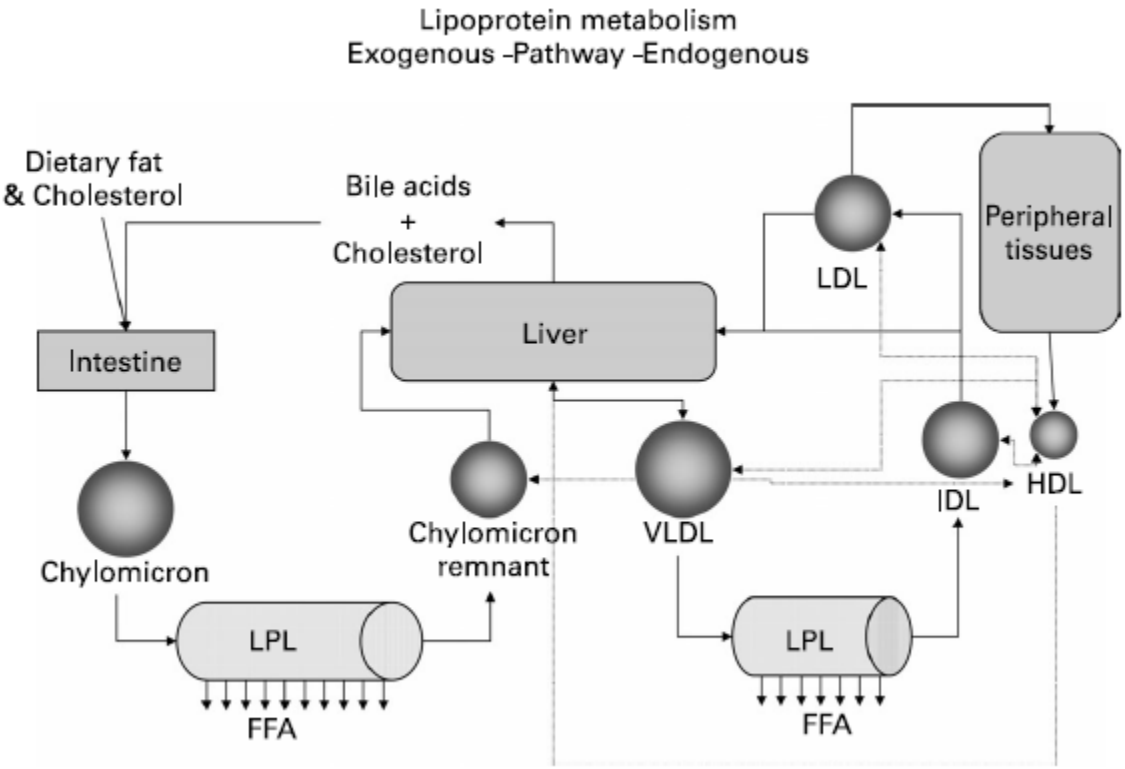

Figure 3. Lipoprotein metabolism. Dietary FFA are absorbed by the intestine and converted to TG-rich apoB-48 CM in the intestinal epithelial cells. LPL hydrolyses the TG in CM to FFA. The CM remnants exit the circulation by entering in the liver. The VLDL are TG-rich apoB-100 particles synthesized by the liver. Again, the LPL hydrolyses TRLP and the VLDL remnants are eventually taken up by the liver (borrowed, with permission, from Lopez-Miranda, J. et al. 2007).

General overview of adipose tissue lipid storage and mobilization

A primary function of adipocytes is to store and mobilize lipids (Scherer, P.E. 2006). The adipose tissue is one of the three main tissues that regulate postprandial TRLP-TG uptake. The TG stored in the adipose tissue represent the largest energy reservoir in humans. As previously discussed, postprandially, adipose tissue hydrolyses the TRLP resulting in storage of TG, in contrast, while in a fasting state, adipose tissue will release

NEFA and glycerol into the circulation for the uptake by the liver and subsequent production of VLDL. Therefore, the feeding state stimulates the lipogenic pathway, while the fasting state stimulates the lipolytic pathway (**Figure 1**) (Luo, L., & Liu, M. 2016). There needs to be a tight control between TG hydrolysis and NEFA esterification in order to maintain a concentration of NEFA in the circulation between 0.1- 0.45 mmol/L in women and 0.1- 0.60 mmol/L in men (Yahfoufi, N. et al. 2019). Without this efficient balance, there could be excessive lipid deposition in nonadipose tissues in circumstances where adipocytes cannot effectively uptake and store plasma NEFA. This imbalance can lead to lipotoxicity and increase risk of developing metabolic disorders (Yahfoufi, N. et al. 2019 & Lewis, G. F. et al. 2002). Due to the typically long duration of the postprandial state and repetition of meals during the daytime, there needs to be a remodeling of lipoproteins. Almost all dietary fat (70% of CM) ingested is absorbed and enters the circulation in the form of TRLP, the rest (30%) will be oxidized.

The following two sections will go over adipose tissue lipid storage, and mobilization in further depth and detail as both have different metabolic mechanisms, each essential to lipid homeostasis.

Adipose tissue lipid storage

Lipogenesis is a process that includes *de novo* FA synthesis and TG biosynthesis. In the feeding state, glucose will provide acetyl-coenzyme A

(acetyl-CoA), the rate-limiting molecule of lipogenesis, and will also result in the pancreas releasing insulin, which also promotes lipogenesis (Luo, L., & Liu, M. 2016) (**Figure 2**).

Adipose tissue lipid mobilization

Under period of food deprivation or excess metabolic needs, TG stored in adipose tissue can be rapidly mobilized, by hydrolytic action, in order to convert TG info FA (Lafontan, M., & Langin, D. 2009). The catabolic process that leads to the breakdown of adipose tissue TG into NEFA and glycerol is called lipolysis. The products of lipolysis will be transported to the bloodstream for their subsequent uptake by other tissues such a skeletal muscle, liver, and heart. This process mainly depends on two rate-determining enzymes responsible for TG mobilization; these hydrolases are hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) (Lafontan, M., & Langin, D. 2009). Additionally, hormones being released by the sympathetic nervous system (SNS), such as catecholamines (e.g., norepinephrine), are stimulated during fasting and exercise and markedly stimulate lipolysis (Luo, L., & Liu, M. 2016). These catecholamines activate protein kinase A (PKA) thus phosphorylating the lipid droplet-associated proteins such as HSL and perilipin, which then promotes the release of ATGL (Lafontan, M., & Langin, D. 2009). ATGL converts TG to diglycerides (DG), and HSL is instrumental for the hydrolysis of DGs to monoglycerides (MG)

(Haemmerle, G. et al. 2002). This rate-limiting enzyme, HSL, is responsible for the cellular catabolism of DG, allowing for the release of glycerol and FA, therefore, fulfilling the lipolytic pathway (Haemmerle, G. et al. 2002). Lipolysis, being under tight hormonal regulation, can be induced by various hormones in similar fashion to catecholamines, such as glucagon, and numerous autocrine/paracrine factors originating from adipocytes (Lafontan, M., & Langin, D. 2009).

Several antilipolytic pathways inhibit lipolysis, therefore, stimulate FFA uptake via the LPL on TG, increasing lipogenesis. These antilipolytic molecules can be divided into two main categories; agents acting on transmembrane domain receptors coupled to Gi proteins, and agents involved in the activation of the insulin receptor tyrosine kinase (Langin, D. 2006). Insulin receptors are the most potent antilipolytic tyrosine kinase receptors as well as the antilipolytic Gi protein coupled receptors that involve alpha2-adrenergic receptors mainly released in the fasted state (Lagin, D. 2006).

Fasting and postprandial lipid metabolism

Systematically, feeding and fasting stimulate different pathways, such as the activation of the lipogenic and the lipolytic pathways, respectively (Luo, L., & Liu, M. 2016). The following two sections will go into further detail on both fasting and postprandial lipid metabolism as the lipogenic and

lipolytic pathway play a major role in maintaining whole body energy homeostasis.

Fasting lipid metabolism

The liver and intestine are two crucial organs in charge of maintaining nutrient homeostasis. The intestine, being able to respond rapidly and efficiently to large quantities of food, digests, absorbs, assembles, and secretes TG. Although, the intestine does not have the capacity of storing and mobilizing ingested lipid for a prolonged period (Xiao, C. et al. 2011). In contrast, the liver, in the fasted state, is the master gatekeeper of ingested, mobilized, and *de novo* synthesized lipids allowing for maintained lipid homeostasis from postprandial and postabsorptive states. Upon fasting, the lipolytic pathway releases NEFA. NEFA availability depends on circulating FFA, FA derived from intracellular lipolysis of TG, TG lipid droplets in the cytoplasm, DNL and hydrolysis of phospholipids. FFA released from adipose tissue play a major role in VLDL synthesis as they are reesterified into TG in the liver and mobilized to the blood circulation. Nutrition and hormonal factors can act as main systemic activators and inhibitors of adipose tissue lipolysis, which can affect NEFA availability (Xiao, C. et al. 2011). During long fasts, nearly 100% of FA used for VLDL-TG synthesis derives from NEFA (Barrows, B. R., & Parks, E. J. 2006). Finally, two lipolytic enzymes, the LPL and hepatic triglyceride lipase (HL) catalyze the peripheral clearance of

VLDL-TG, and this lipolytic activity can be measured in post-heparin plasma (Després, J. P. et al., 1999).

Postprandial lipid metabolism

Typically, most hours in the day are spent in the fed state and the majority of plasma TG are transported by TRLP, CM and VLDL particles (Barrows, B. R., & Parks, E. J. 2006). In the fed state, there is a change in FA flux to the liver attributable to the secretion of insulin by the β-cells of the pancreas (Barrows, B. R., & Parks, E. J. 2006 & Xiao, C. et al. 2011). This postprandial elevation in insulin concentration could stimulate *DNL,* which would increase FA synthesis and subsequent VLDL-TG synthesis. Finally, the LPL, being activated by insulin, will hydrolyze plasma TG, releasing FA into the adipose tissue for their re-esterification into TG; thus, insulin increases reesterification (Ruge, T. et al. 2009). The multiple sources of dietary FA entering the plasma NEFA pool in the fed state could cause atherogenicity or postprandial lipemia as higher levels of NEFA have been associated with atherogenesis (Barrows, B. R., & Parks, E. J. 2006).

Adipose tissue blood flow and metabolism are extremely dynamic and adaptable over a 24h period. Within 24h post-meal, in a resting state, 70% of the chylomicron FA will be stored in the adipose tissue while the remaining 30% will be oxidized (Jenson, M. D. 2003). Following a meal, there are a series of physiological adaptations focused on CM-TG uptake in

adipose tissue, such as a well-timed increase in adipose tissue blood flow response, facilitating lipid storage. More specifically, the capillaries in adipose tissue vasodilate in order to increase blood flow and increase efficiency to manage TRLP (Ruge, T. et al. 2009). Several hours after consuming a meal, the LPL activity increases allowing for more efficient TG storage. After an overnight fast, when there is the ingestion of the first meal of the day, the up-regulation of LPL activity reaches its peak later than the appearance of the majority of CM. As more meals are consumed, the efficiency FA uptake increases as the LPL action also increases by twofold. The fraction of the meal directly taken up by adipose tissue increases from approximately 15-48% from the first meal to the third meal (Ruge, T. et al. 2009). The process of postprandial plasma TG clearance and adipose tissue uptake of NEFA relies mainly on the key enzyme responsible for hydrolyzing TG, the LPL. LPL, therefore, plays a key role in fuel metabolism, and its regulation is influenced by multiple factors, including the fasting and fed states, various hormones, and disease states (obesity and diabetes) (Farese Jr, R. V. et al. 1991).

Intermittent hypoxia: Obstructive sleep apnea

OSA is the recurrent obstruction of the upper airway leading to momentary cessations of breathing and sleep fragmentation (Drager et al. 2010). The repeated pharyngeal collapse leads to intermittent exposure to

short periods of hypoxia during sleep, resulting in a decrease in oxygen concentration in the blood. When the arterial oxyhemoglobin saturation (%SaO_2) is lower than 90%, tissues are exposed to hypoxic conditions. As a result, the required O_2 in order to produce energy in peripheral tissues is affected (Semenza, G. L. 2000). In one night, individuals with OSA can experience numerous cycles of IH per hour, varying from 1-30 or more events per hour; in most severe cases of OSA, hypoxia events can occur as often as 40 times per hour (**Table 1**). According to the American Academy of sleep medicine, the hypoxic events must be 10 seconds in length and associated with a decrease in blood oxygenation in order to be considered a hypoxic event (Ruehland, W. R. et al. 2009). Individuals with OSA will often wake up in order to resume a regular breathing pattern and restore blood O_2 concentration, resulting in excessive daytime sleepiness, the most salient symptom of OSA (PHAC, 2015).

Statistics reveal that over 60% of the Canadian population is dealing with excess adipose tissue (body mass index (BMI) > 35 kg/m^2), a factor that predisposes to OSA (Drager et al. 2010). A longitudinal study measured the association between weight change and variation in sleep-disordered breathing (SDB) severity. SDB is characterized by repeated episodes of apnea and hypopnea events during sleep. The results demonstrated that a 10% weight gain would predict a 32% increase in the apnea-hypopnea index (AHI), indicating a strong correlation between obesity and SDB (Peppard, P.

E. et al. 2000). As previously mentioned, obesity, a growing major health issue worldwide, is correlated with OSA and according to research is causal to SDB (Young, T. et al. 2005). According to Young *et al.*, 50-77% of individuals with SDB have OSA. Adiposity, body mass and neck girth cause the upper airway to collapse during sleep; frequency of hypoxic events and obstruction of upper airway increases as body weight increases (Young, T. et al. 2005).

There exist several treatment avenues for OSA with continuous positive airway pressure (CPAP) being the most generally prescribed and effective (Pelleteri-Fleury N. et al. 2001). Some theorists hypothesized that thermoregulatory, metabolic and biochemical mechanisms could be effective treatment avenues, although clinical trials have not supported these hypotheses (Sengul, Y.S. et al. 2011). CPAP also reduces cortical arousals (associated with apneic events) and AHI while normalizing oxyhemoglobin saturation (Sawyer, A. M. et al. 2011). While CPAP is a highly effective treatment for OSA, its main limitation is related to a problem of adherence. Indeed, failure to comply with treatment has been reported to be as high as 25-50% (Zozula, R., & Rosen, R. 2001). A systematic review of CPAP adherence across age groups identified various factors impacting CPAP use such as disease and patient characteristics, treatment titration procedures, technical device side effects and factors, as well as psychological and social factors (Sawyer, A. M. et al. 2011). Data illustrate a lower adherence rate to

individuals >60 years of age, with only 55.92% of CPAP use compared to a 73.52% adherence to CPAP with a population of >60 years of age (Pelletier-Fleury, N. et al. 2001). Considering the prevalence with OSA and obesity, there is reason to evaluate and assess the potential effectiveness of lifestyle interventions for OSA in order to see if appreciable weight loss and or exercise regimes could act as potential prevention and or treatment strategies for these individuals reluctant to the current treatment avenues such as CPAP.

Aside from causing daytime sleepiness, the most important health consequence of OSA is a 2-fold increase in cardiovascular disease (CVD) risk (Drager, L. F. et al. 2018). A potential explanation linking OSA with increased risk of developing CVD could be a disturbance in lipid metabolism, leading to a deteriorated blood lipid profile. It has been shown that individuals with OSA display increased triglyceridemia (by ~30 %), independent of age and body mass index, compared to individuals without OSA (Newman et al. 2001).

OSA: an independent risk factor for CVD

Epidemiological and observational studies have demonstrated that OSA is independently associated with increased risk of CVD after adjusting for cofounding variables such as age, sex, race, smoking status, alcohol consumption, use of lipid lowering drugs, body mass index, and traditional

CVD risk factors such as; type 2 diabetes (glucose intolerance), hypertension, and hypercholesterolemia (Hu, F. et al. 2002; Mannarino, M.R. et al. 2012; Scha, H. et al. 2002; Yaggi, H. K. 2005). Additionally, a multisite cross-sectional study by Trzepizur *et al.* tested the hypothesis that there is an independent relationship between OSA and dyslipidemia; the latter being an independent risk factor for CVD. Regardless of the effects of traditional CVD risk factors including abdominal obesity (measure by waist circumference) on lipid profile, this study was able to demonstrate that nocturnal IH and OSA severity were independently associated with dyslipidemia manifested through elevated TG levels and lower HDL levels (Trzepizur, W. et al. 2013). To further support the independent relationship between OSA and CVD, a study by Marin, M *et al.* showed that CPAP therapy was associated with lower risk of incident hypertension in OSA participants after adjusting for confounding factors, including changes in BMI. The lack of change in body weight and concomitant reduction in risk of cardiovascular events following an adequate CPAP treatment regimen supports the independent relationship established between OSA and CVD (Marin, J. M. et al. 2012).

Table 1. Degree of sleep apnea severity according to apnea-hypopnea index

Degree of sleep apnea severity	Apnea-hypopnea index (AHI)
Mild	5-14 events/hour
Moderate	15-29 events/hour
Severe	30 + events/hour

*AHI events are characterized by SaO_2 ≤90%

Effects of hypoxia on cardiovascular system, substrate utilization, and nervous system

Cardiovascular system

Exposure to low O_2 activates the sympathoadrenal system, a system that influences both physiologic and metabolic adaptations such as blood pressure, heart rate, vascular resistance as well as lactate, glucose, and lipid metabolism (Mazzeo, R. S. et al. 1994). During low O_2 exposure, the reduction in the systemic partial pressure of O_2 leads to an increase in heart rate. Despite inter-individual differences in responses to hypoxic exposure, the increase in heart rate is generally 10-15 beats per minute (BPM) faster when exposed to 12.5% O_2 (400 m above sea level) in comparison to sea level with 20.93% O_2 (Hooper, T., & Mellor, A. 2011). The increase in sympathetic drive causes an increase in both resting and exercising heart rate, the resting heart rate will generally return to sea level values once acclimatized (Hooper, T., & Mellor, A. 2011). With acute exposure to

hypoxia, in order to maintain mean arterial pressure and blood redistribution among the limbs, heart rate increases, and stroke volume decreases without affecting the blood pressure (Sagawa, S. et al. 1993). However, when exposed to low oxygenation for 2-6 days, blood pressure increases (Hooper, T., & Mellor, A. 2011).

Nervous system

The sympathetic nervous system (SNS) is responsible for producing adaptive responses to stressful stimuli (Mesarwi, O. A. et al. 2015). In response to certain stressors such as exercise, bleeding, and hypoxia, circulatory adjustments are made by the activation of the SNS. In humans, hypoxia stimulates chemoreceptors that are oxygen-sensitive and increases the efferent sympathetic outflow (Hansen, J., & Sander, M. 2003). The activation of the sympathetic nervous system (SNS) is demonstrated using direct microneurography recordings of sympathetic discharge (Hansen, J., & Sander, M. 2003). The microneurography technique measures SNS activity by measuring the nervous system output by bursts per minutes directly in a tissue (e.g. muscle). Studies on individuals exposed to high altitude demonstrated a 3-fold increase in sympathetic nerve activity; based on this evidence, hypoxic exposure is associated with a shift in sympathovagal balance toward heightened SNS activity (Hansen, J., & Sander, M. 2003). The SNS causes a variety of reflexes and signals acting upon adipose tissue,

liver, and skeletal muscles. The activation of the SNS also leads to an increase in the secretion of catecholamines into the plasma by the adrenal medulla (Mesarwi, O. A. et al. 2015). Of note, in response to prolonged hypoxia exposure, the SNS demonstrates a degree of overactivity in healthy humans not only acutely, but persistent for 3 days even after returning to sea level (Hansen, J., & Sander, M. 2003).

IH stimulates chemoreflexes, which in turn stimulate the SNS. The functional consequences of IH-induced SNS activation are insulin resistance, glucose intolerance, an increase in blood pressure, and as previously mentioned, and increased secretion of catecholamines (Mesarwi, O. A. et al. 2015). These cathecholamines stimulate glucagon secretion, trigger glycogenolysis and gluconeogenesis in the liver, and will lead to the breakdown of muscle glycogen and TG of adipose tissue (Mesarwi, O. A. et al. 2015). It has become clear that OSA is an independent risk factor for the development of metabolic syndrome; the associated oxidative stress further worsens the metabolic dysfunction and impairment in glucose and TG metabolism. More specifically, a study by Phillips, A.C. 2011, highlights the importance of the potential adverse effects of persistent and/or prolonged SNS activation in the development of metabolic disorders and blunted cardiovascular response (Philips, A.C. 2011). These said adverse effects being increase in blood pressure and heart rate, during sleep and awake.

The following section elaborates on how oxygen is sensed and the responses of lipid metabolism to reduced oxygen tension.

Oxygen sensing system: Hypoxia-inducible transcription factor-1

The ability to maintain and regulate O_2 homeostasis has evolved with the development of physiological systems that ensure optimal oxygenation of all cells in an organism (Semenza, G. L. 2000). O_2 is essential for ATP production as it serves as the electron acceptor during oxidative phosphorylation. Humans must have a highly effective regulation system to sense fluctuations in O_2 and react accordingly to maintain homeostasis, sustain to energy demand, and reduce the risk of developing hypoxia-induced pathogenesis: including cancer, ischemia and heart and liver diseases (Semenza, G. L. 2000).

In response to hypoxia, changes in gene expression are mediated by a class of transcriptional factors linked to O_2 sensing called hypoxia-inducible factors (HIF). There are 3 major HIF isoforms: HIF-1, HIF-2 and HIF-3 with different actions regarding O_2 sensing (Semenza, G. L. 2003). HIF-1, being the most studied, is a heterodimer that consists of HIF-1 alpha (α) and HIF-1 beta (β) subunits (Trayhurn, P. 2014). HIF-1α protein levels increase and become the main transcription factor sensing O_2 at cellular level when O_2 concentration is reduced. In response to hypoxia, more than 1000 genes are directly transactivated by HIF-1 (Semenza, G. L. 2014). At the cellular level, HIF-1

signals low oxygenation and controls oxygen-related gene expression. HIF-1, also known as master regulator of cellular responses to hypoxia, activates the transcription of genes encoding enzymes, transporters, and mitochondrial proteins that decrease O_2 utilization (Semenza, G. L. 2014). The main HIF-1 targets are genes that will increase O_2 delivery and those that decrease O_2 consumption, for example: HIF-1 will activate glycolytic enzymes, such as lactate dehydrogenase A and pyruvate dehydrogenase kinase 1, which allows for cells to switch from oxidative to glycolytic metabolism (Semenza, G. L. 2014). As previously mentioned, HIF-1, is composed of subunits α and β, both essential in allowing HIF-1 to act upon gene expression, however, the expression and activity of subunit α is responsible for the biological activity of HIF-1 (Semenza, G. L. 2000). (**Figure 4**)

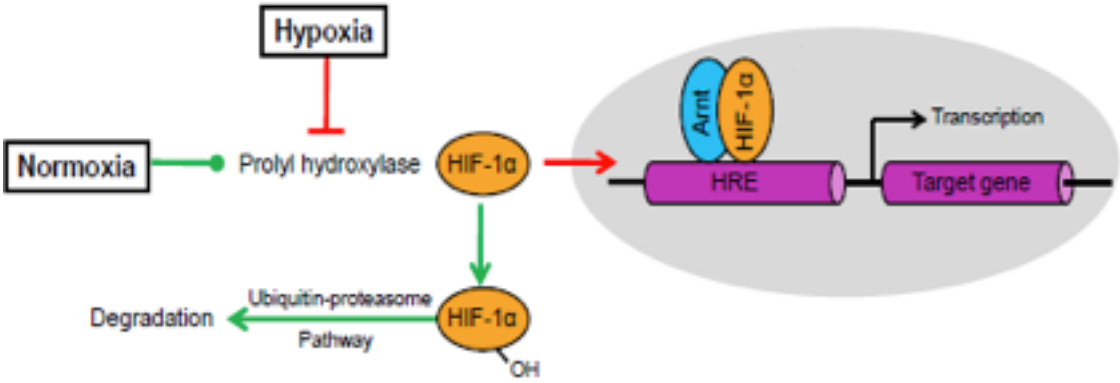

Figure 4. HIF-1α regulation under normoxia or hypoxia. (Adapted from Myre and Imbeault (2014). Under normoxic conditions, HIF-1α is hydroxylated through prolyl hydroxylase-domaine (PHD) enzymes, which initiate the ubiquitin-proteasomal pathway which eventually leads to the proteasomal degradation of HIF-1α. Under hypoxic conditions, PHDs are inhibited and consequently HIF-1α is not hydroxylated. HIF-1α translocates to the nucleus where it interacts with its cofactor HIF-1β; there is dimerization of HIF-1α with HIF-1β (Myre, M., & Imbeault, P. 2014).

Hypoxia and triglyceride mobilization and storage

A precise control between TG mobilization and NEFA esterification (storage) for the maintenance of appropriate circulatory NEFA concentration must be exerted. Evidence for this control process is provided through the observation of excessive lipid deposition in nonadipose tissues when fat cells are unable to effectively uptake and store plasma NEFA or display an

increased TG mobilization capacity, a phenomenon linked to lipotoxicity and a greater prevalence of metabolic disorders (Defronzo, R. A., 2004; Lelliott, C., & Vidal-Puig, A. 2004). Evidence shows that this fine balance between fat mobilization and storage is influenced by hypoxia. We have recently demonstrated that acute hypoxia had a concentration-dependent stimulating effect on basal lipolysis of differentiated human subcutaneous adipocytes (Mahat, B. et al. 2019). This finding is consistent with those of O'Rourke et al. who reported that there is an increase basal lipolysis in human visceral and subcutaneous adipocytes after a 24h exposure to 1% O_2 (O'Rourke, R. W. et al. 2013). It was proposed that this pattern of lipolytic response to acute hypoxia could be attributable to the inhibition of hexosamine biosynthesis (HBS). Hypoxia inhibits HBS, which attenuates lipogenesis and induces lipolysis. These data provide evidence that hypoxia shifts adipocyte lipid metabolism towards a pro-lipolytic and anti-lipogenic phenotype, this can impair lipid buffering capacity and predispose to systemic lipotoxicity (O'Rourke, R. W. et al. 2013).

Hypoxia and very low-density lipoprotein metabolism

As previously mentioned, impaired adipocyte buffering capacity and over exposition of circulating lipids leading to ectopic fat storage contributes to systemic lipotoxicity. Fasting circulating TG levels reflect the balance between hepatic VLDL-TG secretion and peripheral VLDL-TG clearance. In

the fasted state, the primary role of NEFA pool is used for 70-80% of VLDL-TG synthesis (Barrows, B.R., & Parks, E. J. 2006). Drager and colleagues suggest that IH increases hepatic TG and VLDL secretion due to the activation of the SNS under hypoxia (Drager, L. F. et al. 2010). The increase in TRLP is almost exclusively VLDL particles. Chromatographic separation, a laboratory technique for the separation of a mixture, confirmed that hypoxia led to a TG increase mostly within VLDL fractions (Drager, L. F. et al. 2011 & Jun, J.C et al. 2012). Therefore, Drager suggests that dyslipidemia under hypoxia may be a consequence of the up-regulation of lipid biosynthetic pathways in the liver (Drager, L. F. et al. 2010). Moreover, the activation of the SNS under hypoxia increases lipolysis leading to an increase in plasma NEFA levels. This accumulation of NEFA induces ANGPTL-4 gene expression (Drager, L. F. et al. 2013). The peripheral clearance of VLDL-TG is catalyzed by the LPL; several animal studies demonstrated a reduction in adipose tissue LPL activity in response to hypoxia also contributing to the increase of NEFA delivery to the liver and increase plasma TG concentrations. Drager and colleagues quantified a 0.5-fold reduction in LPL activity under hypoxia leading to a delay in TG clearance, contributing to dyslipidemia (Drager, L. F. et al. 2011).

Hypoxia, circulating triglyceride levels and lipoprotein metabolism

In mouse models, acute and chronic intermittent hypoxia led to hypertriglyceridemia due to the decrease in plasma TG clearance in postprandial state and increased hepatic TG secretion in the fasted state (Drager, L. F. et al. 2011 & Jun, J.C et al. 2012) (**Table 2**). The TG-raising effect was apparent after 2 hours of hypoxia exposure. On the other hand, the severity of the increase in TG levels was proportional to the severity of hypoxia with a peak observed when the fraction of inspired O_2 (FIO_2) was 10% (Drager, L. F. et al., 2010). These changes were mainly due to an increase of lipid influx to the liver caused by an increase in adipose tissue lipolysis and by the hypoxia-induced suppression of LPL activity. More specifically, there was a 5-fold decrease in LPL activity in the epididymal fat of mice (Drager, L. F. et al. 2011). This LPL activity suppression was caused by the upregulation of a critical post-translational repressor of LPL: AGNTPL-4 (Drager, L. F. et al. 2011). The potentially detrimental effect of intermittent hypoxia on circulating TG levels is not as clear in humans. Recent findings from our lab indicated that acute IH (as examined in the current study) does not increase postprandial TG levels in young, healthy men (Mahat et al. 2016). One limitation of our previous study is that it relied solely on plasma TG concentrations, which provide limited information regarding TG and lipoprotein dynamics. Using a method to fractionate

lipoproteins to better assess/measure distinct lipoprotein subtypes (CM and denser lipoproteins such as VLDL and CM remnants), Mauger et al. recently reported that the prandial denser lipoprotein fraction levels are increased under 6h of continuous hypoxia (Mauger, J. F. et al. 2019). To our knowledge, whether an increase in denser lipoproteins levels such as VLDL and CM remnants are observed under intermittent hypoxia remains unknown.

Table 2. Animal studies reporting the effect of acute/chronic hypoxia exposure on TG levels.

Authors	Year	IH/CH	Exposition Time	Results on AT LPL activity	Results on fasting blood TG	Animal model
Li et al.	2005	IH	5 days	N/A	Increase by 40%	Lean C57BL/6J
Drager et al.	2011	IH	4 weeks	Decrease by 80%	Increase by 200%	Lean C57BL/6J
Jun et al.	2012	CH	6 hours	Decrease by 60%	Increase by 240%	Lean C57BL/6J
Drager et al	2013	IH	4 weeks	N/A	Increase by 50%	Lean C57BL/6J
Yao et al.	2013	IH	4 weeks	Decrease by 50%	Increase by 30%	Lean C57BL/6J

Note. IH: intermittent hypoxia; CH: continuous hypoxia.

SUMMARY

Individuals with OSA experience short periods of hypopnea (blockage of the airway), inducing intermittent hypoxia cycles. Studies on animal models show that restriction of O_2 has a deleterious effect on adipose tissue metabolism leading to the downregulation of LPL by up to 5-fold, a key enzyme involved in hydrolysis of TRLP, promoting increased circulating TRLP such as CM and VLDL (**Table 2**).

Important health consequences of individuals with OSA are: increased risk of developing metabolic disorders such as Type 2 diabetes and a 50% increase risk of cardiovascular disease. A potential explanation for the underlying increased cardiovascular disease risk lies in the detrimental effect of hypoxia on lipid storage and mobilization functions, ultimately leading to dyslipidemia. To our knowledge, the effects of acute exposure to hypoxia on postprandial plasma TG levels remain to be investigated as the effect on human lipid metabolism and the mechanisms involved are still unknown.

Specific Problem

Obstructive sleep apnea (OSA), a sleep disorder frequently observed in (but not restricted to) individuals living with obesity, consists of repeated, involuntary breathing suspension during sleep. These events induce rapid depletion/repletion of blood/tissue oxygen content, a phenomenon known as intermittent hypoxia (IH). Studies report that hypoxic exposure in animals

negatively affects postprandial TG levels due to inhibition of lipoprotein lipase (LPL), a key enzyme that hydrolyses TRLP (Drager, L. F. et al. 2011 & Jun, J.C et al. 2012). Aside from causing daytime sleepiness, the most important health consequence of OSA is a 2-fold increase in cardiovascular (CVD) risk.

Objective

The objective of this study was to characterize the effects of acute intermittent hypoxia on postprandial TG-rich lipoprotein levels in individuals with and without OSA.

Hypotheses

Acute intermittent hypoxia was predicted to prolong the elevation of postprandial triglyceride levels, likely due to a reduced clearance of dietary lipids. Increased postprandial triglyceride levels in response to intermittent hypoxia was expected to be greater in individuals with OSA as compared to individuals without OSA.

Significance

This proposed research furthered our understanding of the mechanisms by which intermittent hypoxia, a simulated model of OSA, may alter blood lipid profile. This information will be beneficial to the treatment of

OSA-related dyslipidemia and may contribute to reduce cardiovascular risk in the large proportion of OSA patients who are reluctant to current treatment avenues.

Chapter 3: Materials and Methods

Research program: Overview

This research consists of a randomized crossover design. Our sample was composed of 7 individuals diagnosed with moderate sleep apnea (aged 44-65) recruited from the Sleep Laboratory at Montfort Hospital as well as 8 healthy individuals (aged 21-24) without OSA from the University of Ottawa. Participants provided written consent and the study protocol was approved by the Research Ethics Board of the University of Ottawa and from Montfort Hospital.

Study samples:

Table 3 presents the inclusion/exclusion criteria for this study as well as pre-experiment session criteria. Upon arrival at the sleep clinic, these individuals were asked if they want to be contacted to participate in our study. Upon consent, they were contacted by telephone to arrange for a meeting. They needed to be non-smokers, as smoking may alter blood oxygen saturation due to the destruction of alveoli. Participants needed not to take medication on a regulation basis and not having travelled to high altitude (>2000 m) in the month before the experimental sessions. Exclusion criteria included: hypertension, cardiovascular diseases, diabetes, habitual

sleep duration of less than 7 hours per night, allergies to lactose, and current smoking stats.

Table 3. Inclusion and exclusion criteria and pre-test conditions.

Inclusion/Exclusion criteria	Pre-experiment session criteria
Male/Female	No exercise 36h prior to experiment
18-65 years old	No alcohol 24h prior to experiment
no metabolic disorder	No caffeine 24h prior to experiment
no medication	Standard dinner prior to experiment
non-smokers	12h fast prior to experiment
	food journal for 24h prior to experiment
	good night of sleep (≥ 7 hours)

Anthropometric and metabolic measurements

Body weight and height were measured using a standard beam scale (HR-100, BWB-800AS; Tanita, Arlington Heights, IL) and a standard stadiometer (Perspective Enterprises, Portage, Michigan, USA) respectively. Body composition was measured via percentage of fat mass (%FM), total fat mass (FM) and fat-free mass (FFM) using dual energy X-ray (DXA) (General Electric Lunar Prodigy, Madison, Wisconsin; software version 6.10.019). Resting energy expenditure (REE) was measured in a thermo-neutral dark

room for 30 minutes 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 crossover study consisting of two experimental sessions. In order to reduce variability, a randomized cross-over protocol was used. Before each experimental session, volunteers were expected to follow the pre-experiment criteria; 7 hours sleep the night before an experimental session, restrain from moderate-intense physical activity, caffeine, and alcohol for 36 hours, and to consume a provided standardized evening dinner between 7-8:00 PM (650 kcal; 54% from carbohydrates, 22% from fat, and 24% from protein). Each participant underwent three sessions; 1 preliminary session and 2 experimental sessions.

Much of our knowledge about the relationship between lipid metabolism and CVD are based on measurements in the fasted state (Lopez-Miranda, J. et al. 2007). However, it is important to consider that humans spend a significant amount of time in the postprandial state both during the day and at night. Studies monitoring TG responses overnight following a fat-dense evening meal showed elevated TG values for up to 8 hours with fasting values only returning between 4-6 hours in the morning (William, C. M. et al. 1992). This dynamic of circulating TG levels justifies the reason for

studying postprandial lipids under intermittent hypoxia and its relevance to the risk of CVD.

Preliminary sessions

The preliminary session was the first time meeting the participant in person. The participant was asked to visit the Behavioural and Metabolic Research Unit (BMRU) at Lees campus of the University of Ottawa. The participant was given an in-depth explanation of the protocol and the experimental sessions. Informed consent was then collected. The preliminary session also consisted of taking anthropometric measurements (height, weight and body composition). A dual-energy x-ray absorptiometry (DXA) scan was performed to quantify FM and FFM. We then continued by measuring REE by indirect calorimetry. Standard procedures such as 12h fast, thermoneutral room, and supine posture were followed for both tests. The participant was then exposed to hypoxia for 20 minutes (by using our experimental setup and exposing the participant to medical nitrogen (N_2)) in order to detect any symptoms related to altitude sickness (i.e., headache, nausea, dizziness). When exposing the participant to IH cycles, he or she was resting in a reclining seated position. A participant was considered tolerant to hypoxia if no symptoms occurred (dizziness, nausea, extreme fatigue). The researcher then reminded the participant of pre-experimental session criteria and provided the participant with the standardized meal to

be consumed the night before the first experimental session. The preliminary session was then completed.

Experimental session

For the experimental session, once the participant arrived, they completed a questionnaire that ensured that all the pre-experiment criteria had been respected. Therefore; no exercise 36h before the experimental session, only water can be consumed afterwards (no caffeine, no alcohol). We expected the participant to have adhered to exact same diet for the second experimental session, thus the participant was asked to complete a food journal the day before the first experimental session. It was crucial that the pre-experiment criteria were respected to standardize the baseline values for our experimental variables. Both experimental sessions had the same environmental conditions (temperature and humidity) in order to avoid differences in effect on lipid metabolism (Jun, J.C et al. 2013).

Body mass was measured and compared with the measurements taken during the preliminary session. A phlebotomy-certified researcher or nurse collected a 20 ml blood sample after inserting an IV catheter in the forearm. Blood was drawn every 30 minutes for the first two hours, and every hour for the remainder of the experimental session; resulting in a total of 9 samples (9* 20 ml = 180 ml). The participant consumed a fat-dense meal (60% fat), representing 33% of the estimated daily energy expenditure

multiplied by a sedentary factor of 1.375 (Harris & Benedict, 1918) and entered the experimental chamber. The oxygen concentration in the room was set at 20.93%. Once the participant finished the meal, the stopwatch would start. The participant was monitored with an oximeter and heart-rate monitor. The duration of the protocol was a total of 6 hours during which the participant remained seated in the hypoxic chamber and had the choice of watching a movie or reading a book; sleeping was not allowed. In order to induce the simulation of IH the subjects wore an oro-nasal mask with a two-way Hans Rudolph non-rebreathing valve that was connected to an inspiratory line (Louis, M., & Punjabi, N. M. 2009). In the normoxia condition, the subject only inspired ambient air (20.93%) oxygen). In the IH condition, medical pure N_2 was inspired by the participant through the inspiratory line. The subject inspired N_2 until oxyhemoglobin saturation (S_pO_2) drops to 85%. Once oxyhemoglobin saturation reached 85%, N_2 flow was stopped, and the subject proceeded to inspire ambient air containing oxygen. We induced 15.5 (3.8) hypoxic events/hours (simulating moderate OSA).

Pulse rate (HR) and S_pO_2 were recorded in 2-second intervals during both the normoxia and hypoxia sessions using a Masimo, radical 7 unit (Masimo, Irvine, Ca, USA). A mean average was calculated with all the values for each experimental session. Blood pressure (BP) was also measured at baseline and every 30 minutes of the experimental session for the first two hours and

every hour for the reminder of the session (T0, T30, T60, T90, T120, T180, T240, T300, 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).

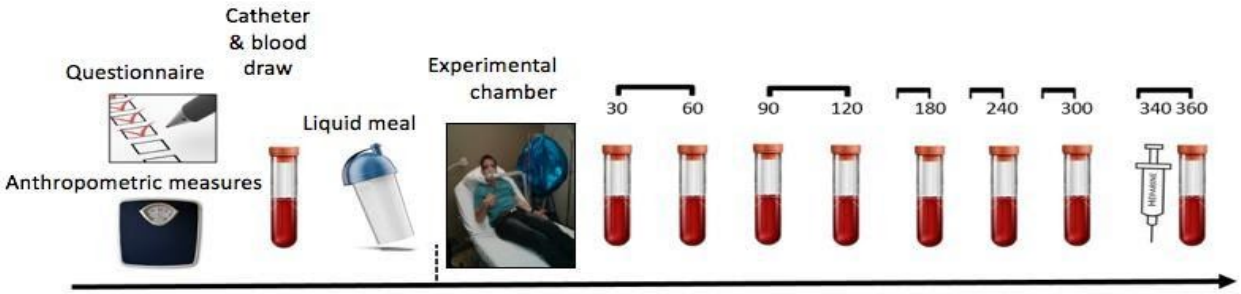

Figure 5. Timeline for both experimental sessions.

Fasting and postprandial plasma metabolic parameters

Plasma was separated immediately after blood collection by centrifugation at 3000 rpm for 10 minutes at 4°C. Plasma total TG concentrations were measured by a colorimetric enzymatic assay (Walko Diagnostics). Relative changes in intestinal plasma TRLP over time was determined by using the retinyl ester technique. Plasma levels of glucose, insulin, and NEFA were measured at baseline (pre-test meal) and 30, 60, 90, 120, 180, 240, 300, and 360 minutes after test meal ingestion. Concentrations were assayed

using enzymatic spectrophotometric analysis (glucose and NEFA) or commercially available enzyme-linked immunosorbent assay (ELISA) kits (insulin) (Imbeault, P. et al., 2009 Metabolism).

Data analysis

Data were analyzed using a two-way repeated measure analysis of variance (ANOVA) with time x condition as within subject's parameters. Time (by 60-minute interval) e.g., 0, 60, 120, 180, 240, 300, 360 min) and the independent variable of condition (either hypoxia or normoxia) was done in order to determine if there was a difference in postprandial response to hypoxia. In order to correct for any violations of sphericity we used the Greenhouse-Geisser corrected degrees of freedom. Partial eta squared (η_p^2) was used to estimate the proportion of the variance attributed to the tested factor. Furthermore, where differences were identified, a Bonferonni correction post-hoc test was applied to determine the locus of the differences. Errors bars in Figures were adjusted to account for between subjects variability, which better reflects the statistical power of the study crossover design (Cousineau, 2005: Cousineau, D. (2005). Confidence intervals in within-subject designs: a simpler solution to Loftus and Masson's method. Tutor. Quant. Methods Psychol. 1,

42–45. doi: 10.20982/tqmp.01.1.p042).All data analyses were performed using SPSS version 12 for windows (IBM, Inc., Armon, NY, USA). Alpha was set at 0.05 to establish significance.

Chapter 4. Results, Discussion & Conclusion

Characteristics of participants

Participant characteristics are summarized in **Table 4.** During the experimental procedures, participants from both groups remained weight stable (SD: 0.55 OSA and 0.88 control). Both experiment sessions were separated by 9 (3) days for the OSA group and 14 (7) days for the control group. There was a statistical difference for age (years), height (cm) and fat mass (%), as identified in the **Table 4**(*). There was a trend towards significant statistical difference for fat mass (kg) (p= 0.07).

Table 4. Characteristics of participants (OSA group, n=7), (control group, n=8).

Characteristics of participants	OSA group (men: N=6 women: N=1)	Control group (men: N=8)
Age (years)	54.4 (6.4)	22.0 (1.1) *
Height (cm)	174.8 (5.8)	183.0 (8.8) *
Weight (kg)	90.5 (22.6)	86.6 (11.2)
BMI (kg/m^2)	29.47 (6.6)	25.8 (4.8)
Lean Mass (kg)	57.7 (12.4)	64.9 (2.8)
Fat Mass (kg)	30.2 (14.6)	19.1 (11.7)
Fat Mass (%)	33.0 (8.7)	23.0 (10.1) *

Data are expressed as mean (standard deviation). * Significantly different compared to OSA group, $p<0.05$.

Cardiorespiratory Responses to intermittent hypoxia

Table 5 shows the variations in heart rate, blood pressure and oxyhemoglobin saturation during both experimental sessions. During IH, an average of 15.5 (4.8) hypoxic cycles were induced per hour, simulating moderate OSA. Although mean heart rate increased by ~7 bpm during hypoxia exposure, it did not reach significance level.

Table 5. Mean hypoxia counts, blood pressure, heart rate oxyhemoglobin saturation during normoxia and intermittent hypoxia in the OSA and control group.

		OSA		Control	
		Hypoxia	Normoxia	Hypoxia	Normoxia
Frequency/hour		15 (3.8)	0 (0.0)	16 (5.7)	0 (0.0)
Systolic blood pressure (mmHg)		135.4 (19.9)	133.7 (19.8)	128.4 (8.6)	124.6 (19.9)
Diastolic blood pressure (mmHg)		88 (8.8)	78 (10.8)	68.8 (5.4)	65.1 (6.6)
Heart rate (BPM)	Mean	79 (7.2)	76 (10.4)	68 (12.9)	63 (11.3)
	Maximum	107 (12.9)	106 (19.4)	105 (7.2)	95 (16.2)
SpO2 (%)	Mean	0.92* (0.0)	0.95 (0.0)	0.93 (0.0)*	0.97 (0.0)
	Maximum	1 (0.0)	0.99 (0.0)	0.99 (0.0)	1 (0.0)
	Minimum	0.58 (0.0)	0.85 (0.1)	0.6 (0.1)	0.9 (0.0)
Time SpO2 (%)	≤90%	15.1 (11.9)	5.8 (12.8)	9.7 (4.6)	0 (0.0)
	≤85%	3.71 (0.9)	0.1 (0.1)	4.3 (2.8)	0 (0.0)
	≤80%	4.1 (1.1)	0 (0.0)	3.5 (2.0)	0 (0.0)

Data are expressed as mean (standard deviation). * Statistical difference between normoxia and intermittent hypoxia, $p<0.05$.

Plasma glucose and insulinemia

The effects of IH on plasma glucose and insulin concentrations are summarized in **Figure 6 & 7**. There was no status x condition x time interaction for glucose levels. However, there was a condition x status interaction ($F(1,12) = 16.905$, $p = 0.001$, $\eta_p^2 = 0.585$), meaning that irrespective of time, glucose levels significantly differed between control and OSA groups under both conditions (normoxia and hypoxia). More specifically, the OSA group's glucose levels were generally higher across every time point in either condition in comparison to the control group. The control and the OSA groups' glucose levels tended to be higher during hypoxia compared to normoxia, ($p = 0.1$ and $p = 0.09$), respectively. There was no condition x status x time interaction for insulin levels. There was a statistically significant increase in postprandial blood insulin levels over time, regardless of the status or condition (main effect of time, $p<0.01$). There was a significant status x time interaction, ($F(8, 104) = 3.641$, $p< 0.001$, $\eta_p^2 = 0.219$), indicating that insulin's peak occurred later in OSA and remained higher over time as compared to control. Condition x time, and condition x status interactions were not statistically significant, $p> 0.05$. A trend was observed for condition x status interaction ($p= 0.084$).

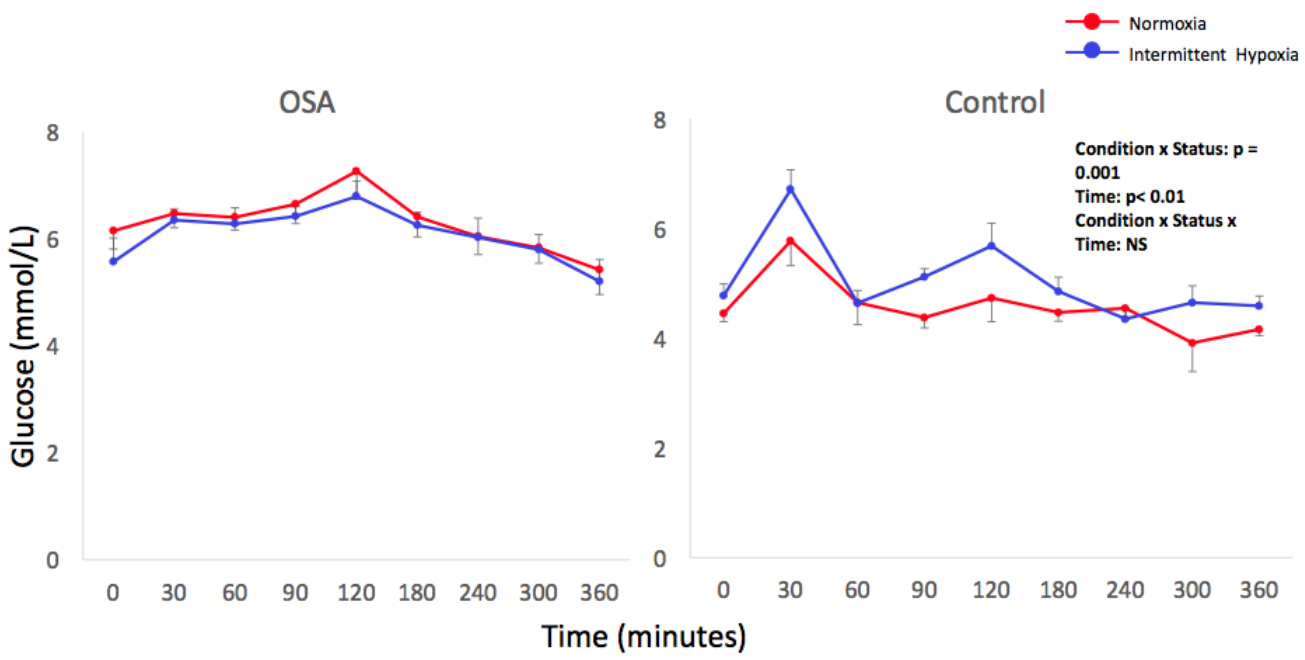

Figure 6. Fasting and postprandial plasma glucose levels measured during normoxia and IH. Values are means ± standard errors adjusted for between-subject's variability.

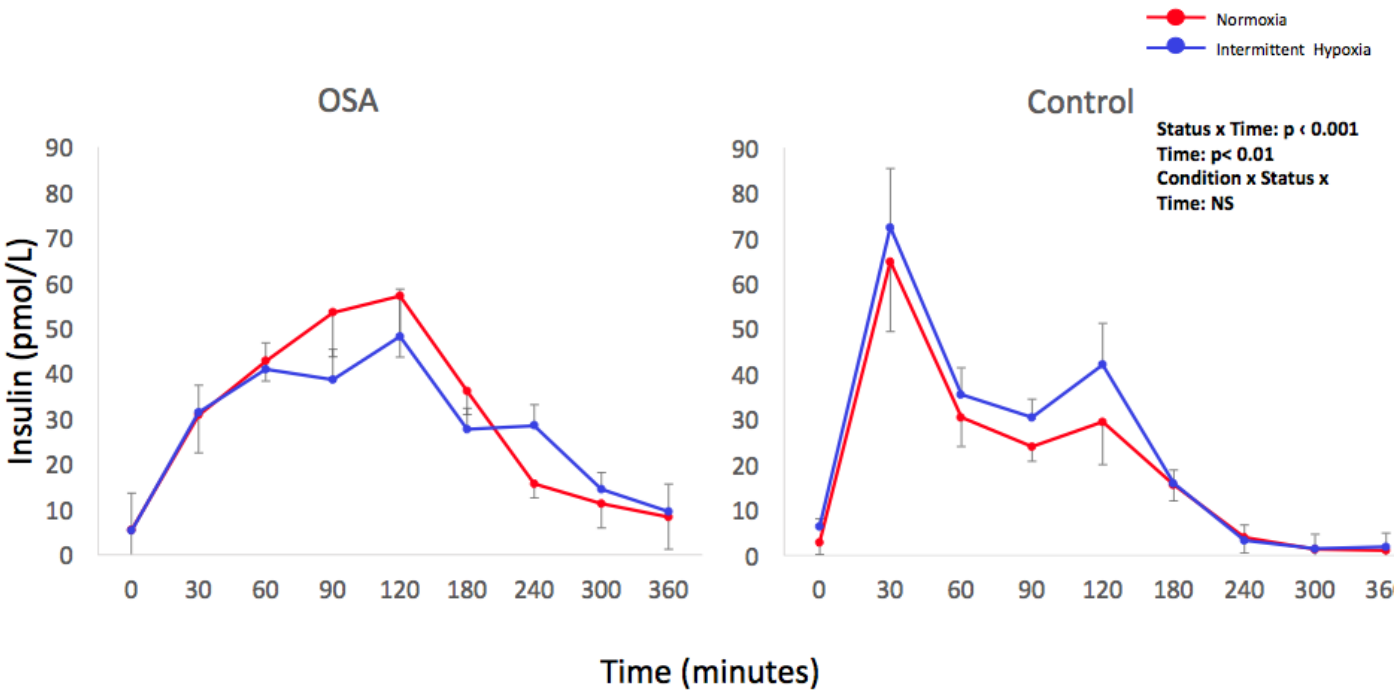

Figure 7. Fasting and postprandial plasma insulin levels measured during normoxia and IH. Values are means ± standard errors adjusted for between-subject's variability.

Plasma lipid levels

Postprandial plasma NEFA, TG, chylomicron TG and denser lipoprotein TG levels during normoxia and intermittent hypoxia sessions are shown in **Figures 8-11**.

NEFA

As shown in **Figure 8**, there was no significant three-way interaction between status x condition x time for NEFA levels. However, there was a significant status x time interaction, ($F(8,96) = 2.32$, $p = 0.026$, $\eta_p^2 = 0.162$. Data demonstrates that NEFA levels changed differently across time

between groups; the control group's NEFA levels dropped more rapidly following the meal than the OSA group's levels initially and then increased gradually to the highest level at the final hour. Condition x time, and condition x status were not statistically significant ($p > 0.05$) although there was a trend observed for condition x time (p= 0.133).

Total plasma TG

There was no status x condition x time interaction for plasma TG levels (**Figure 9**). However, plasma TG levels evolved differently across time between groups, $p<0.05$. Specifically, TG plasma levels for the control group decreased with time, while they did not significantly change for the OSA group, (status x time interaction, ($F (8, 96) = 7.463$, p= 0.004, $\eta_p^2 = 0.383$). Condition x time, and condition x status were not statistically significant ($p > 0.05$) although there was a trend towards significance observed for condition x time (p= 0.093).

The TG levels, discussed in the following sections, were measured in two lipoprotein subtypes: buoyant TG-rich lipoproteins comprising mostly chylomicrons (CM), and denser lipoproteins TG comprising CM remnants and VLDL).

Chylomicrons TG

There was no status x condition x time interaction for chylomicron TG levels (**Figure 10**). The repeated measures ANOVA for postprandial chylomicron TG revealed a main effect of time ($F (8,96) = 13.04$, p< 0.001, $\eta_p^2 = 0.521$

and a trend was observed for condition x status, p= 0.095). The data demonstrated that the TG-CM levels were significantly different over time. The main effects for condition and status were not statistically significant nor were interactions between condition x time, condition x status, and status x time failed.

Denser lipoproteins TG

There was no significant three-way interaction between status x condition x time for denser lipoproteins TG (**Figure 11**). However, there were status x time and condition x time interactions for this variable, (F (8, 104) = 9.196, p< 0.001, η_p^2 = 0.414 and (F (8, 104) = 2.726, p= 0.009, η_p^2 = 0.173 respectively. The status x time interaction revealed that regardless of the condition, the denser lipoproteins TG evolved differently over time between the control and the OSA group. More specifically, the OSA group's VLDL and CM remnants levels were higher than the control group's values across time and progressively increased from baseline up to the end of the experiment. In the control group, VLDL and CM remnants levels increased up to 120 min and then returned to the baseline value by the final hour. Irrespective of the status, plasma denser lipoprotein TG levels were higher across time during hypoxia in comparison to normoxia. Condition x status, and condition x status x time were not statistically significant ($p > 0.05$).

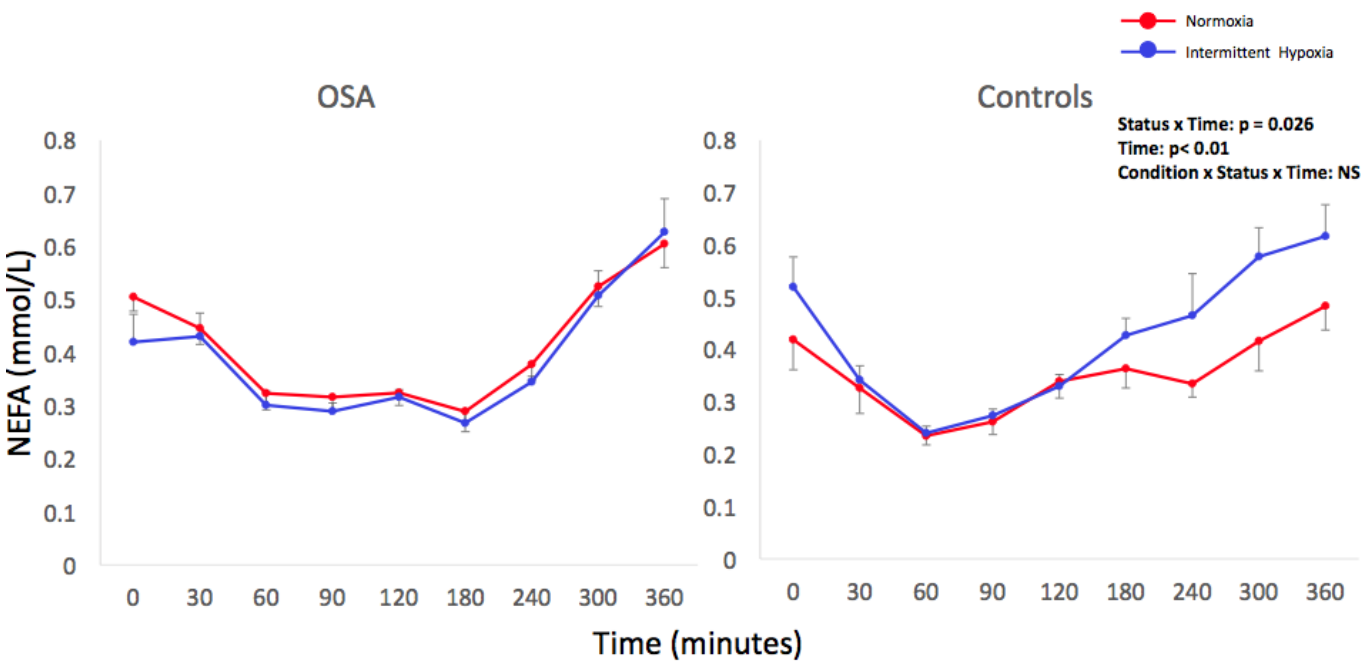

Figure 8. Fasting and postprandial plasma NEFA levels measured during normoxia and IH. Values are means ± standard errors adjusted for between-subject's variability.

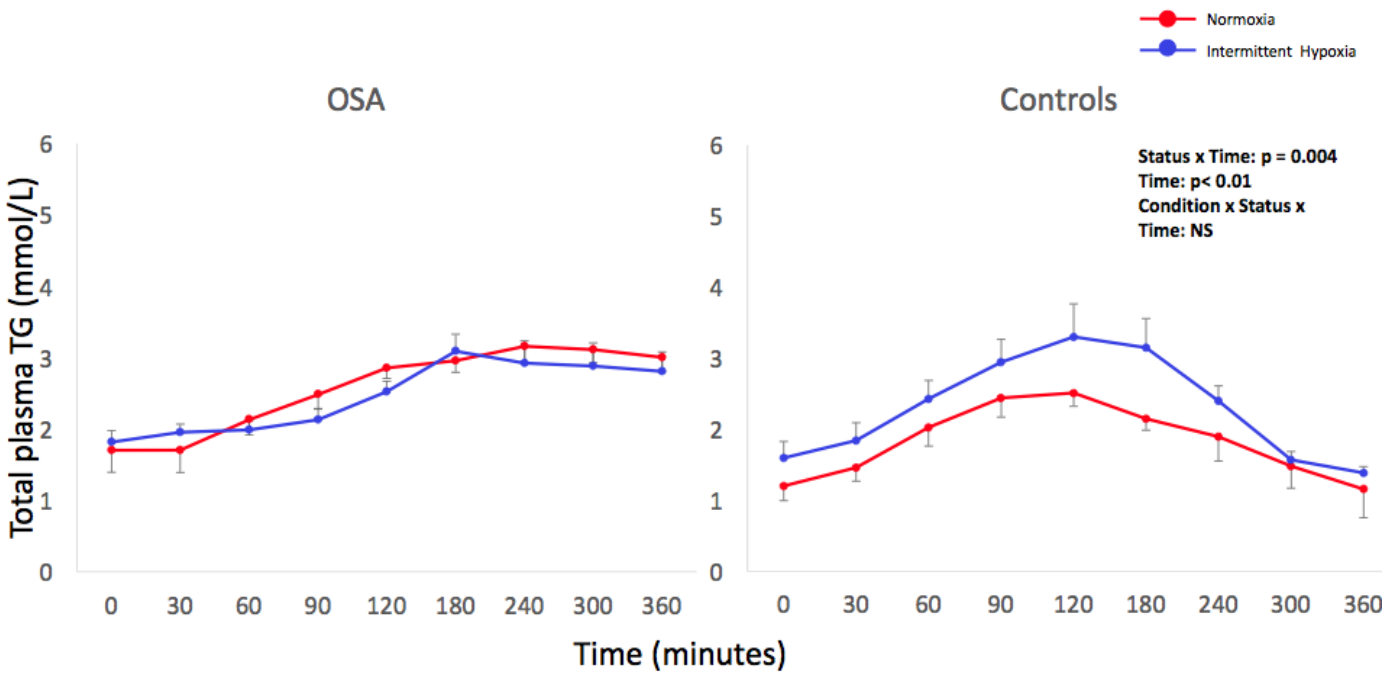

Figure 9. Fasting and postprandial total plasma TG levels measured during normoxia and IH. Values are means ± standard errors adjusted for between-subject's variability.

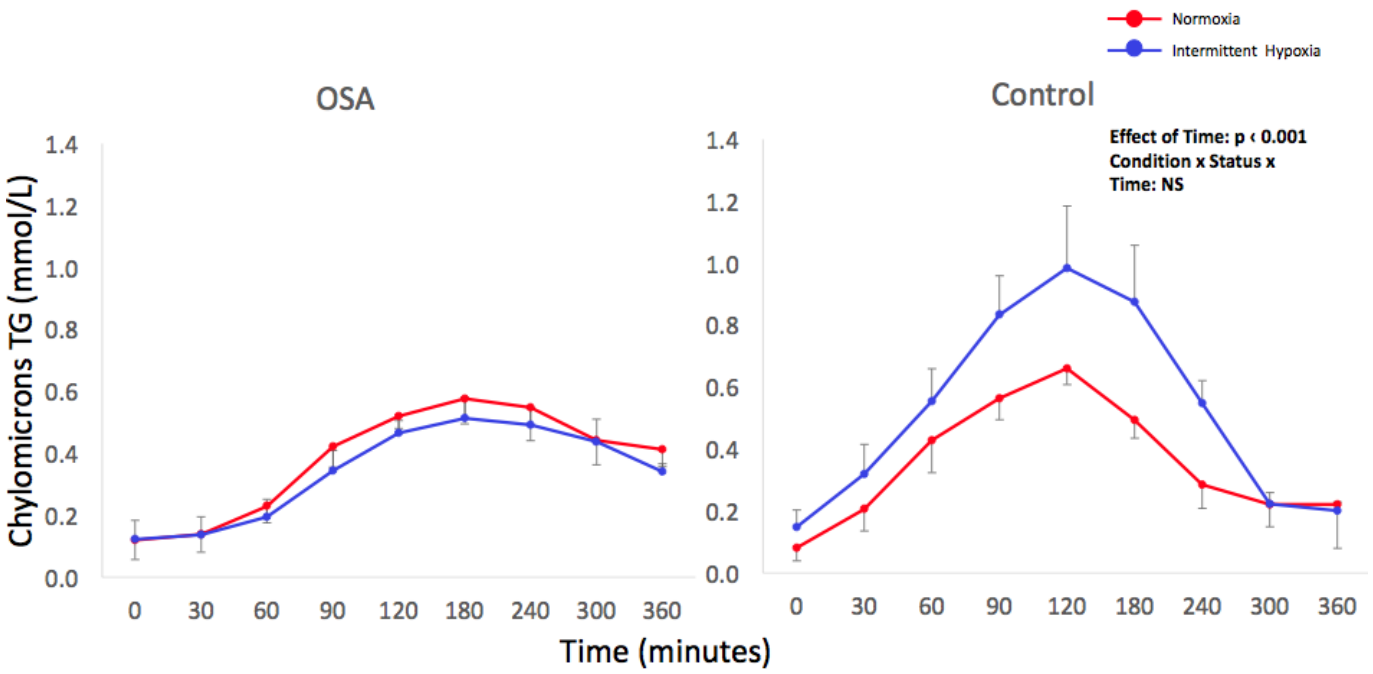

Figure 10. Fasting and postprandial plasma chylomicrons TG levels measured during normoxia and IH. Values are means ± standard errors adjusted for between-subject's variability.

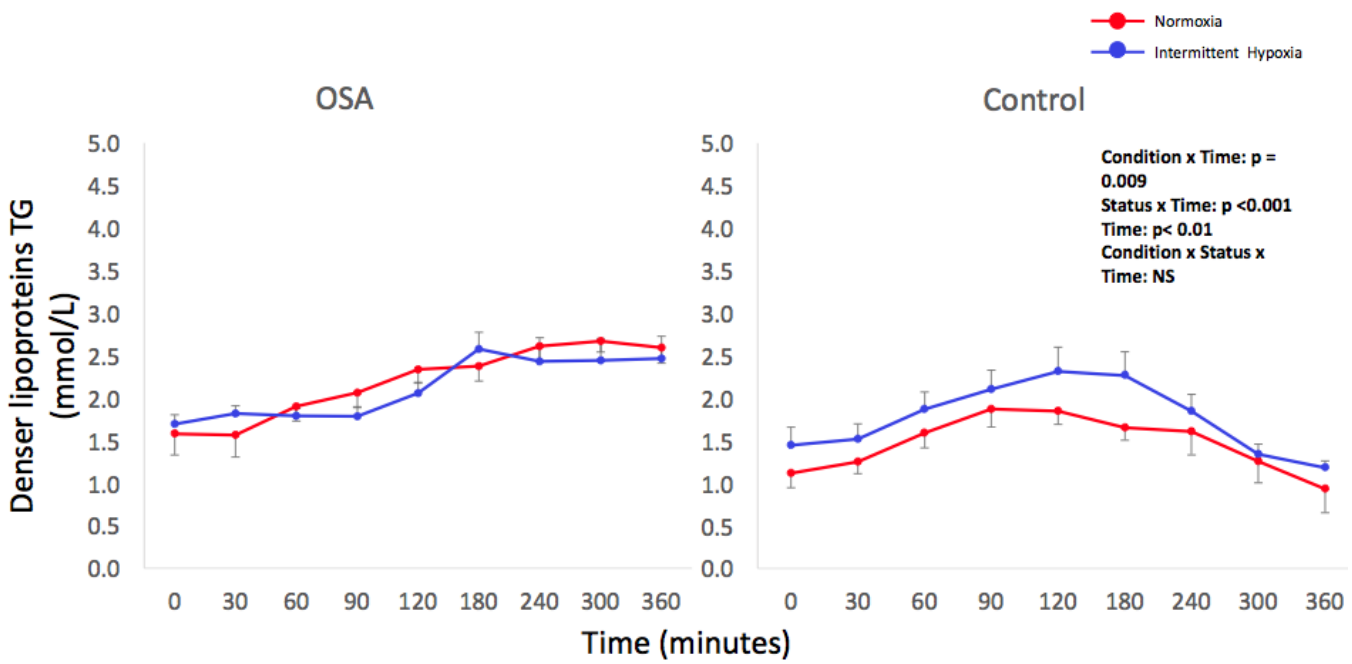

Figure 11. Fasting and postprandial plasma denser lipoprotein TG levels measured during normoxia and IH. Values are means ± standard errors adjusted for between-subject's variability.

Discussion

This study is, to our knowledge, the first to determine the effect of acute IH on postprandial TG-rich lipoprotein levels in young, healthy adults and in individuals with OSA. Our results show that acute IH induces a significant rise in denser TG containing lipoproteins like chylomicron remnants and VLDL. Overall, these results give insight on the mechanism by which IH, a simulating model of OSA, negatively impacts postprandial TG levels. These results partially support our hypothesis that acute intermittent hypoxia negatively impacts postprandial TG metabolism. However, this effect was not more pronounced in individuals with moderate OSA than without OSA.

Oxyhemoglobin saturation and heart rate response to intermittent hypoxia

Over a 6-hr exposure to IH we observed a significant decrease in oxyhemoglobin saturation (S_aO_2) and a trend towards an increased HR. Oxyhemoglobin saturation was decreased intermittently by introducing participants to medical nitrogen in order to simulate moderate OSA (e.g., apnea hypopnea index ≥ 15, but < 30 per hour). Using the same design, Louis *et al*. (Journal of Applied Physiology 2009) previously demonstrated that IH is associated with a shift in sympathovagal balance toward an increase in sympathetic nervous system activity.

Effects of intermittent hypoxia on NEFA levels

The elevation of postprandial NEFA levels during intermittent (Mahat et al. 2016) or constant (Mauger, J.F. et al. 2019) hypoxia exposure was observed in previous human studies from our laboratory. In the current study, postprandial NEFA levels decreased gradually following the meal in both groups and then significantly increased at the end of IH session in the control group only. The increase in plasma NEFA levels towards the end of the IH session is likely due to an earlier relief of lipolysis inhibition by insulin as compared to the OSA group composed of middle-aged individuals with greater adiposity levels.

Plasma metabolic parameters

Recent animal studies using chronic (IH or continuous) or acute IH have shown that mice exposed to hypoxia develop elevated plasma TG levels (up to 40% increase in TG) (Drager, L. F. et al. 2010; Drager, L. F. et al 2012; Jun, J. C., et al. 2012). It has been shown that chronic IH increased TG through two major mechanisms: increased hepatic secretion (up-regulation of lipid biosynthesis in the liver), and a decrease in lipoprotein clearance (Drager, L. F. et al 2012; Jun, J. C., et al. 2012). Recent findings from our group indicated that acute IH (as examined in the current study) does not increase postprandial TG levels in healthy young men (Mahat et al. 2016). However, the methodology used in this study did not allow us to quantify the

2 distinct TRLP subtypes comprising plasma TG levels. Briefly, the small intestine releases CM when dietary fats are ingested. The liver release VLDL in a predominant manner during fasting state. Both TRLP are mainly hydrolyzed by the LPL generated by adipose tissue or other tissues. Following the hydrolysis of CM and VLDL, CM remnants and LDL will be metabolized by the liver to resynthesize VLDL (Chapman, M. J. et al. 2011). The novelty of the current study is that we were able to fractionate plasma lipoproteins enabling us to characterize the effects of acute IH on postprandial TG levels in 2 distinct lipoprotein subtypes: CM TG and denser lipoproteins TG. The method used to fractionate plasma lipoproteins produces a buoyant fraction (d< 1g/ml) highly enriched in intestine-derived CM and a denser fraction (d>1g/ml) likely composed of CM remnants and intestine-derived VLDL, as recently reported (Mauger, J. F. et al. 2019). We report that plasma TG levels increased over time in both experimental conditions and tended to be greater over the 6-h of IH ($p=0.093$, effect size $n^2= 0.383$, **Figure 9**). This trend toward higher total plasma TG during exposure to intermittent hypoxia is attributable to increased levels in denser TG carrying lipoproteins such as, VLDL and CM remnants. This new finding provides novel mechanistic insight on the trend observed for total plasma TG being driven by the changes in the metabolism of denser TG carrying lipoproteins. These results lend support and extend our recent observation that acute continuous hypoxia in healthy men increase prandial VLDL-TG

levels (Mauger, J.F et al. 2019). Potential mechanisms that could be attributed to the hypoxia-induced increase of VLDL TG are either a decrease in tissue uptake of VLDL and CM remnants or an increase in VLDL output from the liver as a consequence of hypoxia. Our current understanding of the regulation of VLDL TG production reports that the regulation of liver-derived VLDL involves the interaction between systemic FFA delivery and hormonal factors such as insulin (Nielson, S., & Karpe, F. 2012). Although recent studies state that regulation of short-term and long-term VLDL TG is still poorly understood, it has been demonstrated that, in the postprandial state, insulin will have a temporary suppressing effect on VLDL TG production as it acts as an adipose tissue lipolysis inhibitor (Nielson, S., & Karpe, F. 2012). Consequently, the antilipolytic effect of insulin reduces the hepatic NEFA delivery directly by decreasing the availability of the substrate essential for VLDL TG production (Nielson, S., & Karple, F. 2012). In one of our previous studies conducted in the fasted state, a significant increase in plasma NEFA was displayed during hypoxia, but not in total TG levels. This finding suggests that the increase in NEFA alone may not acutely affect hepatic VLDL production (Mahat et al. 2016 & Mauger, J.F. et al. 2019). In our present study, the trend observed towards elevated total plasma TG and increased VLDL TG levels, under acute intermittent hypoxia, could be a result of increased hepatic VLDL production due to a potential increase in CM remnants delivery combined with increased NEFA levels. It is important to

mention that, as opposed to previous studies from our laboratory, we only observed a trend towards significance when looking at NEFA levels, which could be due to the lack of change in NEFA levels for the OSA group under both normoxia and hypoxia (**Figure 8**). The proposed mechanisms explaining an increase in hepatic VLDL production are based on the assumption that hypoxia could promote lipid recycling and/or a compensatory change meant to retain a balance in circulating TG and their secretion (O' Brien, K. A. et al. 2019).

To summarize, the main finding of this work is that the trend toward higher total plasma TG levels during IH is attributable to an increase in denser TG carrying lipoproteins levels such as VLDL and CM remnants. This work also lends support to previous findings that an increase in NEFA levels and lipoprotein production under hypoxia is likely caused by an increase in sympathetic tone and an impairment in TG disposal rate, subjective to the mobilization or hydrolysis of these energy sources. Future studies in humans need to further elucidate the precise mechanisms involved in the hypoxia-related regulatory cascades that lead to a deteriorated lipid profile and that significantly increases the risk of developing CVD in individuals living OSA.

Limitations of the study

There are limitations to this study. The main weakness being the size and composition of the cohort. The cohort of this study is only composed of 7

individuals with OSA and 8 control subjects that are not weight-matched or matched for body composition, making it difficult to isolate the effect of OSA *per se* on postprandial lipoprotein metabolism. The sample being principally composed of men also limits the generalization of our findings to the overall population. It is also important to mention that the duration of the hypoxia session, being of 6 hours, is not mimicking the repetitive IH that may occur in undiagnosed and/or non-treated individuals living with sleep apnea.

Conclusion

Our results indicate that acute intermittent hypoxia, a simulating model of obstructive sleep apnea, tends to negatively affect postprandial TG levels, which is attributable to an increase in denser TG carrying lipoprotein levels such as, VLDL and CM remnants. These findings provide novel mechanistic insight and will help further investigate and better our knowledge of the cascade of mechanisms leading to changes in lipid metabolism under hypoxia.

CHAPTER 5. Perspectives

For this thesis, we wanted to further investigate the effects of acute IH, a simulating model of OSA, on TG metabolism. We were able to characterize the effects of acute IH on postprandial TG levels in 2 distinct lipoprotein subtypes: CM and denser TG carriers (mainly VLDL and CM remnants). We report that 6-hours of IH, at a rate of 15.5 events per hour caused a trend toward higher total plasma TG. This trend is attributable to increased levels in denser TG carrying lipoproteins such as VLDL and CM remnants.

Since we discovered that acute IH significantly increased VLDL and CM remnants levels, further studies should be conducted for developing strategies that could alleviate or counter the effects of IH on TRLP denser lipoprotein levels and therefore concomitantly reduce the associated cardiovascular risk associated with OSA. With this further understanding of the physiological processes associated with adverse changes in TG levels caused by hypoxia, future studies could include several innovative methodologies to help develop specific pharmacology and/or interventions such as new statin drugs and/or implement a physical activity regime in order to help lower TG levels. In relevance to this, a study by Gahfouri and colleagues explored the effects of exercise on affinity of CM and VLDL for LPL mediated TG hydrolysis and discovered that a 90-minute treadmill walk at 50% VO_2max increased affinity of VLDL for LPL-mediated TG hydrolysis in

both fasting and postprandial conditions (Gahfouri, K. et al. 2015). These findings demonstrate that acute exercise increases the affinity of VLDL for LPL leading to increased VLDL clearance from the circulation; this data interpretation gives mechanistic insight on how exercise could increase VLDL-TG clearance without any modification in LPL activity (Gahfouri, K. et al. 2015). Additionally, they demonstrated that exercise substantially reduced both fasting and postprandial LDL cholesterol concentrations, a critical risk factor associated with high atherogenicity thus, exercise-induced reduction in VLDL and LDL levels has clinically relevant implications for CVD risk and should be further tested with individuals living with OSA. Knowing this, the next step in our research could be to use this protocol in a real-life environment for individuals with OSA. In other words, participants with OSA could spend the night with or without the use of CPAP during which TRLP could be measured. The participants would be asked to perform an exercise protocol similar to that of Gahfouri *et al.* in order to demonstrate that it is a real phenomenon and that acute exercise could help manage VLDL levels in apneic individuals.

Not only could acute exercise reduce VLDL levels, but chronic/prolonged exercise regimes could help protect against the negative effects of hypoxia as well as diminish the severity of OSA in patients (Netzer, N et al. 1997 & Araghi, M. et al. 2013). A study by Netzer and colleagues investigated the effect that physical activity could have on decreasing the symptoms and

severity of OSA in patients (Netzer, N. et al. 1997). Following a 6-month period, there was no significant change in body weight, but a significant decrease in severity of the OSA and therefore a reduction in the associated risk of developing cardiovascular disease (Araghi, M. et al. 2013). These positive outcomes could be attributed to the possible stabilization in muscle tone in the upper airway and an increased strength/endurance of the ventilatory muscles: increasing airflow (O'Donnel et al. 1998 & Norman, F. et al. 2000). The exercise regimes have shown to reduce apneic activity, therefore demonstrating that exercise should be assessed as a primary treatment in the role of managing OSA as opposed to exercise solely being adjunct to other treatments (Giebehlhaus, V. et al. 2000). Engaging in a weekly exercise routine can improve OSA by generally strengthening and increasing fatigue resistance of the ventilator and upper airway dilator muscles, reduction of nasal resistance, prevention of fluid accumulation and overall increase in respiratory stability and reduced sleep fragmentation (Kline, E. et al. 2011).

In summary, it can be hypothesized that acute and prolonged exercise could be used as a method/strategy to help manage and protect against the OSA related symptoms and potential risk of dyslipidemia and CVD. With this knowledge, we need to further explore the possibility of combining physical

activity with drugs designed to 1: ensure respiratory stability and 2: inhibit lipolytic processes (Markus, H. B., & Ball, E. G. (1969).

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Appendix: Notice of ethics approval

25/06/2018

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE I CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-06-18-837
Titre du projet / Project Title	THE EFFECTS OF ACUTE HYPOXIA ON POSTPRANDIAL LIPID METABOLISM
Type de projet / Project Type	Recherche de professeur / Professor's research project
Statut du projet / Project Status	Approuvé / Approved
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	25/06/2018
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	24/06/2019

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
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Conditions spéciales ou commentaires / Special conditions or comments

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25/06/2018

Université d'Ottawa

Bureau d'éthique et d'intégrité de la recherche

University of Ottawa

Office of Research Ethics and Integrity

Le Comité d'éthique de la recherche (CÉR) de l'Université d'Ottawa, opérant conformément à l'*Énoncé de politique des Trois conseils* (2014) et toutes autres lois et tous règlements applicables, a examiné et approuvé la demande d'éthique du projet de recherche ci-nommé.

L'approbation est valide pour la durée indiquée plus haut et est sujette aux conditions énumérées dans la section intitulée "Conditions Spéciales ou Commentaires". Le formulaire « Renouvellement ou Fermeture de Projet » doit être complété quatre semaines avant la date d'échéance indiquée ci-haut afin de demander un renouvellement de cette approbation éthique ou afin de fermer le dossier.

Toutes modifications apportées au projet doivent être approuvées par le CÉR avant leur mise en place, sauf si le participant doit être retiré en raison d'un danger immédiat ou s'il s'agit d'un changement ayant trait à des éléments administratifs ou logistiques du projet. Les chercheurs doivent aviser le CÉR dans les plus brefs délais de tout changement pouvant augmenter le niveau de risque aux participants ou pouvant affecter considérablement le déroulement du projet, rapporter tout évènement imprévu ou indésirable et soumettre toute nouvelle information pouvant nuire à la conduite du projet ou à la sécurité des participants.

The University of Ottawa Research Ethics Board, which operates in accordance with the *Tri-Council Policy Statement* (2014) and other applicable laws and regulations, has examined and approved the ethics application for the above-named research project.

Ethics approval is valid for the period indicated above and is subject to the conditions listed in the section entitled "Special Conditions or Comments". The "Renewal/Project Closure" form must be completedfour weeks before the above-referenced expiry date to request a renewal of this ethics approval or closure of the file.

Any changes made to the project must be approved by the REB before being implemented, except when necessary to remove participants from immediate endangerment or when the modification(s) only pertain to administrative or logistical components of the project. Investigators must also promptly alert the REB of any changes that increase the risk to participant(s), any changes that 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 or the safety of the participant(s).

Kim THOMPSON
Responsable d'éthique en recherche / Protocol Officer
Pour/For **Daniel LAGAREC** Président(e) du/ Chair of the **Comité d'éthique de la recherche en sciences sociales et humanités / Social Sciences and Humanities Research Ethics Board**

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