THE EFFECT OF ACUTE HYPOXIA UNDER FED AND FASTED STATES ON CIRCULATING B-HYDROXYBUTYRATE LEVELS IN HUMANS

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THESIS ABSTRACT

Introduction: Exposure to hypoxia may alter substrate utilization through diverse mechanisms. Acute hypoxia is known to increase circulating nonesterified fatty acid (NEFA) levels and reduce systemic sensitivity to insulin. The hepatic fate of NEFA is dictated by major pathways such as esterification to triglycerides and complete/partial oxidation, the latter leading to ketogenesis. To our knowledge, the effect of hypoxia on ketogenesis, more specifically ß-hydroxybutyrate (ßOHB), remains unknown in humans. Moreover, adipose tissue is a significant site of NEFA liberation into circulation, and insulin inhibits this process. Under acute hypoxia, systemic insulin resistance develops, and the suppression of lipolysis is impeded. Therefore, the objective of this study was to determine the effect of acute hypoxia on plasma circulating ßOHB levels. Furthermore, to better understand how hypoxic and prandial conditions may modulate plasma NEFA and ketonemia, we calculated the βOHB:NEFA ratio and the adipose tissue insulin resistance index (Adipo-IR), which respectively gives indications of the partial hepatic oxidation of NEFA and the adipose tissue insulin sensitivity.

Methods: Plasma samples from 3 different randomized crossover studies were retrospectively assessed for ßOHB concentrations. In the first study, 14 healthy men (23 ± 3.5 years) were exposed to 6 hours of normoxia or intermittent hypoxia (IH) (15 hypoxic events per hour) following an isocaloric meal (IH-Fed). In the second study, 10 healthy men (26 ± 5.6 years) were exposed to 6 hours of continuous normobaric hypoxia (CH) (FiO_2= 0.12) or normoxic conditions in the fasting state (CH-Fasted). In the third study, 9 healthy men (24 ± 4.5 years) were exposed to 6 hours of CH in a constant prandial state. ßOHB, NEFA and insulin levels were measured during all sessions (CH-Fed). The adipose tissue insulin resistance index (Adipo-IR) was also calculated from NEFA and insulin levels.

Results: In study 1 (IH-Fed), ßOHB and NEFA levels tended to be greater over 6 hours of IH (condition x time interaction, p = 0.108 and p = 0.062, respectively) compared to normoxia. In study 2 (CH-Fasted), ßOHB and NEFA levels increased over time in both experimental conditions, and this effect tended to be greater under CH (condition x time interaction, p = 0.070 and p = 0.046, respectively). In study 3 (CH-Fed), ßOHB levels slightly increased up to 180 min before falling back to initial concentrations by the end of the protocol in both normoxia and CH (p = 0.062), while NEFA slightly increased under CH (p = 0.006). Adipo-IR tended to increase after 6 hours of hypoxia compared to normoxia in the first two studies (main effect of condition, p = 0.024; p = 0.097, respectively), and significantly increased over time under hypoxia in CH-Fed (condition x time interaction, p = 0.004).

Conclusion: Acute normobaric hypoxia exposure significantly increases plasma ßOHB concentrations over time in healthy men. The stimulating effect of hypoxia on plasma ßOHB levels is however attenuated during postprandial and postprandial states.

Contribution to advancement of knowledge: To our knowledge, this research provides some of the first evidence that an acute exposure to hypoxia increases plasma ßOHB levels in humans. It also reveals potential underlying mechanism that modulate ketogenesis upon hypoxia exposure. Overall, this thesis provides further insights into the homeostatic response of healthy men to oxygen deprivation.

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PREFACE

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LIST OF ABBREVIATIONS

ABBREVIATIONS	DESCRIPTION
AcAc	acetoacetate
ACC	acetyl-CoA carboxylase
Adipo-IR	adipose tissue insulin resistance index
AMP	adenosine monophosphate
AMPK	adenosine monophosphate activated protein kinase
AT	adipose tissue
ATGL	adipose triglyceride lipase
ATP	adenosine triphosphate
BDH1	ßOHB dehydrogenase
CH	continuous hypoxia
CoA	coenzyme A
CPT	carnitine palmitoyl transferase
FA	fatty acids
FAO	fatty acid oxidation
HCAR2	hydroxycarboxylic acid receptor 2
HDAC	histone deacetylase
HH	hypoxic hypometabolism
HIF	hypoxia-inducible factors
HMG	hydroxymethylglutaryl
HMGCL	hydroxymethylglutaryl-CoA lyase
HMGCS2	3-hydroxy-3-methylglutaryl-CoA synthase 2
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HPV	hypoxic pulmonary vasoconstriction
HSL	hormone-sensitive lipase
IH	intermittent hypoxia
IR	insulin resistance
KB	ketone bodies
KD	ketogenic diet

LPL	lipoprotein lipase
MGL	monoacylglycerol lipase
NAD^+	nicotinamide adenine dinucleotide
NEFA	nonesterified fatty acids
NLRP3	NOD-like receptor family, pyrin domain containing 3
OSA	obstructive sleep apnea
PO_2	pressure of oxygen
SCOT	succinyl-CoA:3-oxoacid CoA transferase
SNS	sympathetic nervous system
TG	triglycerides
TRL	triglyceride-rich lipoproteins
tHb	hemoglobin total mass
VEGF-A	vascular endothelial growth factor A
VLDL	very low-density lipoproteins
VO_2	volume of oxygen
βOHB	β-hydroxybutyrate

LIST OF DEFINITIONS

Adipose tissue: Organ composed mostly of adipocytes where the excess of energy substrate is stored as triglycerides.

Fatty acids: Carbon and hydrogen organic chains of various length, with an acid group (-COOH) at one end.

Nonesterified fatty acids: Free fatty acids, unattached to glycerol.

Triglycerides: Lipid compound composed of a glycerol bound to three fatty acids

Hypoxia: Nonspecific oxygen deficiency (Sjöberg & Singer, 2013)

Insulin: Anabolic hormone released by the pancreas

Ketogenesis: Ketone body synthesis

Ketolysis: Ketone body degradation into acetyl-CoA

Ketone bodies: Four carbons (Prins, 2008), organic molecules having both hydrophilic and lipophilic properties (Cahill, 2006). Ketone bodies are acetoacetate (AcAc) and ß-hydroxybutyrate (ßOHB) (Robinson & Williamson, 1980).

Lipogenesis: Synthesis of lipids

Chapter 1: INTRODUCTION

Rationale and statement of the problem

Oxygen is essential to sustain life since it is required by cells to release energy from foods. Through a process called oxidative phosphorylation, molecular bonds of the by-products of glucose, lipids, or proteins, are broken. These breakages release the energy required to produce an adequate level of energy turnover, the synthesis of adenosine triphosphate (ATP), the human fuel. In this metabolic route, hydrogen protons are released (Semenza, 2007). The role of oxygen is to bind with these protons and stabilise them by forming water (Bell & Chandel, 2007). When oxygen availability is limited at the cellular level, a situation known as hypoxia (Sjöberg & Singer, 2013), ATP production decreases (Wilson et al., 2012) and oxidative phosphorylation is hampered (Solaini et al., 2010).

Several environmental and pathological situations may lead to hypoxia. For instance, a decreased oxygen availability may be present under continuous form, such as an exposition to high altitude or emphysema, and an intermittent form, such as a consequence of obstructive sleep apnea. Individuals suffering from hypoxia-related pathologies are at higher risk of developing comorbidities (Kapur et al., 2017). The relation between hypoxia and the development of metabolic disorders, namely diabetes and cardiovascular diseases, is well established (Bradley & Floras, 2009; Catrina & Zheng, 2021; Doumit & Prasad, 2016; Gami et al., 2013; Javaheri et al., 2017; J. Jun & Polotsky, 2009; Liu & Wu, 2017; A. B. Newman, 2001; X. Wang et al., 2013). However, the underlying mechanisms are still under investigation.

Recently, experiments in humans studying the metabolic responses to hypoxia established that a decreased oxygen availability may lead to a dysregulation of the blood content of lipids. Several studies demonstrated that hypoxia significantly increases the blood level of fatty acids, a major component of lipids (Chopra et al., 2017; Jun et al., 2011; Mahat et al., 2018, 2016; Mauger et al., 2019; Morin et al., 2021). The main storage for fatty acids is adipose tissue (AT). Under increased energetic demand or between meals, the AT release fatty acids, so-called nonesterified fatty acids (NEFA), into the blood. This phenomenon, triggered by a drop in insulin or an increase in catecholamines, is termed AT lipolysis. In circulation, NEFA can be uptaken by most body cells to be used as a fuel. If NEFA are not uptaken and reaches the liver, they may be (1) reesterified and reincorporated into lipoproteins for reshipping into the blood, (2) used as a fuel in the oxidative phosphorylation pathway or (3) stored as lipid droplets (Donnelly et al., 2005). When NEFA enters the oxidative phosphorylation in the liver, they may branch off and enter the ketogenic pathway, which is the formation of ketone bodies.

As mentioned, under hypoxic conditions, a greater amount of NEFA is released into the blood circulation through AT lipolysis. This phenomenon was observed in several studies, notwithstanding the levels of insulin modulated through different feeding status (Chopra et al., 2017; Mahat et al., 2016, 2018; Mauger et al., 2019). The raised circulating NEFA upon hypoxic exposure may be directed towards the synthesis of triglycerides and the complete/partial utilization for energy production, the latter leading to ketogenesis in the liver. It was determined that, under hypoxic conditions, circulating triglyceride-rich lipoproteins are increased (Morin et al., 2021). Moreover, recent reports showed that exposure to hypoxia does not significantly change the relative contribution of NEFA to the total energy yield during resting (Mahat et al., 2018; Mauger et al., 2019) or exercise conditions (Griffiths et al., 2019). To the best of our knowledge, whether the stimulation of AT lipolysis observed under hypoxic conditions favors the ketogenic pathway remains to be fully characterized. We propose that the analysis of blood samples of individuals subjected to hypoxia, under various prandial states, will enable us to determine weather hypoxia leads to increased levels of circulating ketone bodies.

Objectives and hypotheses

Using retrospective analyses of three independent studies performed in our laboratory, the objectives of this thesis were as follows:

1. Assess the effects of the two main forms of hypoxia, intermittent and continuous, in fed or fasted states, on plasma concentration of βOHB, one of the main ketone bodies.
2. Evaluate how hypoxia may cause changes in blood levels of fatty acids and βOHB by using an index of adipose tissue insulin resistance previously validated (Adipo-IR) (Gastaldelli et al., 2017; Søndergaard et al., 2017).

Hypotheses:

1. βOHB concentrations will be increased under both intermittent and continuous hypoxia when compared to its normoxic condition. However, the postprandial rise in insulin levels should limit the increased in plasma concentrations of NEFA and βOHB induced with hypoxia.
2. Adipose tissue insulin resistance, estimated through the Adipo-IR index, will increase by the end of every hypoxic condition when compared to the baseline values.

Relevance of the thesis

This thesis is of interest from a fundamental physiology standpoint. The primary aim is to generate a data base that contributes to establish the kinetic of βOHB levels under hypoxia. To our knowledge, it is the first time that ketonemia is observed in humans during hypoxia. This observation may lay the grounds for future studies which could explore the biological functions of ketones during hypoxia. However, we recognize that using tracers to observe βOHB, from ketogenesis to ketolysis, would give a better view of both production and disposal rates. Still, this project offers a starting point for a thorough assessment of ketone bodies kinetic in hypoxic conditions.

From a clinical standpoint, this thesis opens the way for future studies looking at methods to increase ketogenesis, and for studies looking at ways to reduce physiological impact of hypoxic exposures. More than a metabolite, βOHB has the properties to decrease AT lipolysis, increase insulin sensitivity, modulate inflammation and sympathetic activation, and reduce oxidative stress, all of which are ‘side effects’ of hypoxia. While individuals living with metabolic disorders and/or obesity are well represented in the population affected by hypoxic disorders, it is established that ketogenesis is hampered in these individuals (Balasse & Féry, 1989; Sherwin et al., 1976; Wildenhoff, 1975). Hence, it should be determined if people living with metabolic disorders and/or obesity have a reduced ketonemia under hypoxia, and if so, whether an increased ketogenesis would alleviate some of these adverse effects.

Chapter 2: LITERATURE REVIEW

OVERVIEW OF KETONE BODIES

Ketone bodies (KB), namely β-hydroxybutyrate (βOHB) and acetoacetate (AcAc), are 4 carbons (Prins, 2008) organic molecules commonly recognized as a glucose alternative metabolite used by the brain (Cahill, 2006). They are synthesised to substitute glucose in cases of decreased availability (Owen et al., 1967) and are major actors in glucose sparing as they inhibit glucose oxidation through pyruvate dehydrogenase (Hue & Taegtmeyer, 2009).

KB synthesis, termed ketogenesis, increases during fasting, adherence to low-carbohydrate diets, post-exercise, uncontrolled diabetes, neonatal period, and pregnancy (Puchalska & Crawford, 2017; Rojas-Morales et al., 2016). However, it is often underrated that KB are continuously produced. In healthy individuals, plasma concentrations of KB follow a circadian rhythm, reaching a peak around midnight and a nadir in the morning (Robinson & Williamson, 1980; Wildenhoff, 1975). The liver of a healthy adult may generate from 30 to 60 g of KB daily and up to 300 g in prolonged fasting or in pathological conditions, such as diabetic ketoacidosis and neonatal conditions (Balasse & Féry, 1989). Oxidation of KB contributes from 5% to 20% of daily energy expenditure (Puchalska & Crawford, 2017). Under non prolonged fasting and resting conditions, synthesis of KB in healthy individuals leads to a ketonemia between 0.1 to 0.2 mM (Balasse, 1986).

2.1 - SYNTHESIS OF KETONE BODIES

Ketogenic enzymatic pathway

Ketogenesis results mainly from the partial hepatic breakdown of NEFA for oxidation (Puchalska & Crawford, 2017). During β-oxidation, acetyl-CoA is freed from acyl-CoA and joins the citric acid cycle to be condensed with oxaloacetate (Laffel, 1999), derived from pyruvate during glycolysis. When acetyl-CoA exceeds citrate synthase activity and/or oxaloacetate availability, oxaloacetate is redirected towards the cytosol for gluconeogenesis. Upcoming acetyl-CoA is then converted by mThiolase, consequently activating the ketogenic pathway (Figure 1) (Salway, 2016). mThiolase condenses two acetyl-CoA into acetoacetyl-CoA (AcAc-CoA). AcAc-CoA and a third acetyl-CoA generates hydroxymethylglutaryl (HGM)-CoA through 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2). Thereafter, hydroxymethylglutaryl-CoA lyase (HMGCL) frees acetyl-CoA and AcAc. At this moment, AcAc can be decarboxylated to acetate and CO_2 or reduced to ßOHB by the action of ßOHB dehydrogenase (BDH1)

(Cotter et al., 2013; Puchalska & Crawford, 2017). In this last hepatic step, the ratio of βOHB:AcAc is directly proportional to the mitochondrial NAD^+:NADH ratio, thus usually favoring βOHB production (Puchalska & Crawford, 2017; Williamson et al., 1967). This results in βOHB to be the most abundant circulating KB in healthy individuals (J. C. Newman & Verdin, 2014a).

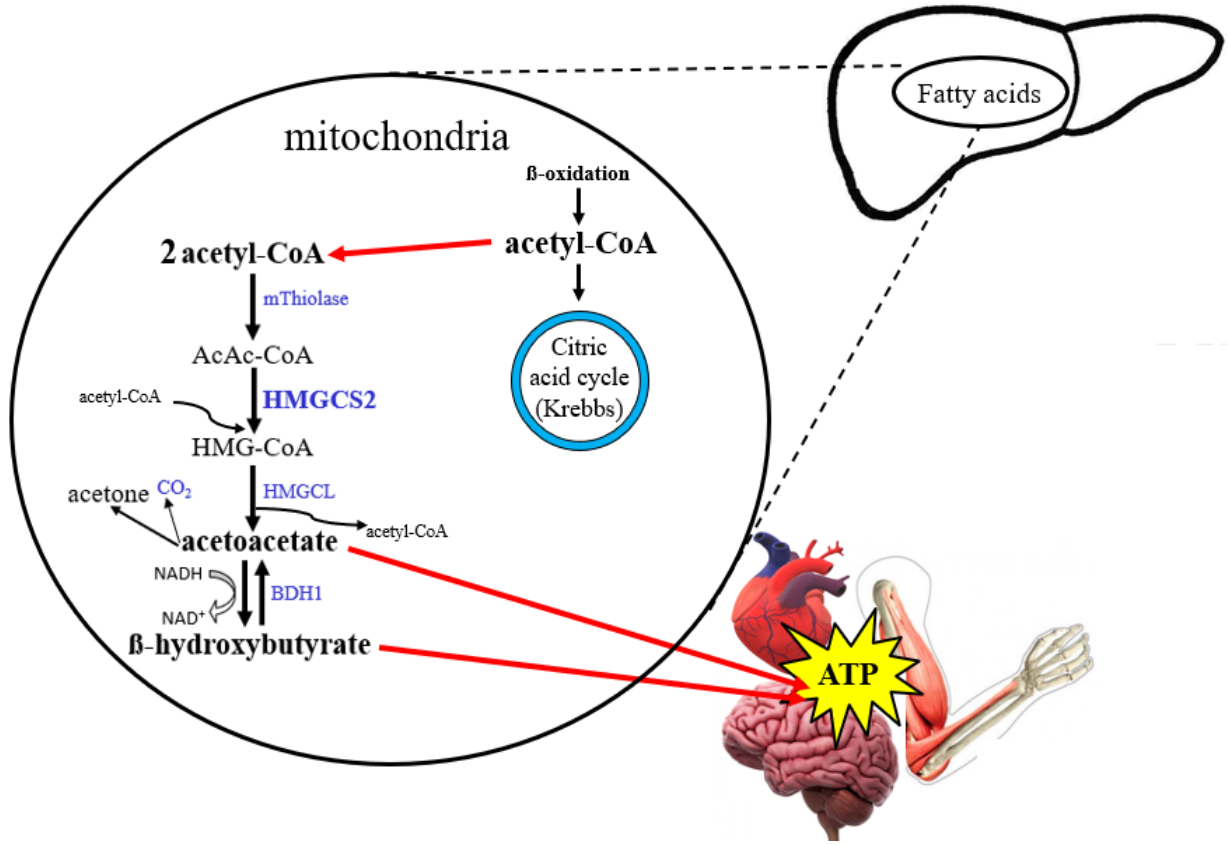

Figure 1. Hepatic ketogenesis using fatty acids as a ketogenic substrate. During beta-oxidation, acetyl-CoA is freed from acyl-CoA and joins the citric cycle (Laffel, 1999). At this moment, thiolase may condense 2 acetyl-CoA into acetoacetyl-CoA (AcAc)-CoA. AcAc-CoA and a 3rd acetyl-CoA will generate hydroxymethylglutaryl (HGM)-CoA through the rate limiting enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2). Thereafter, hydroxymethylglutaryl-CoA lyase (HMGCL) will liberate acetyl-CoA and acetoacetate (AcAc), where a portion of AcAc will finally be reduced to ßOHB by β-hydroxybutyrate dehydrogenase (BDH1) (Cotter et al., 2013; Puchalska & Crawford, 2017). During this last step, NADH is oxidized to NAD^+. The acetoacetate/ß-hydroxybutyrate ratio is directly proportional to the hepatic mitochondrial redox potential (NAD^+/NADH) (Laffel, 1999). Of note, acetoacetate may spontaneously decarboxylate into acetone and CO_2, which will be eliminate through ventilation. The remaining KB are secreted and primarily be oxidized by extrahepatic tissues.

While KB production occurs solely in the liver, the breakdown of KB for oxidation, termed ketolysis, occurs only in extrahepatic tissues, such as skeletal muscles, heart, brain, guts, and kidneys (Balasse,

1986; Laffel, 1999). To be used as metabolites, KB must be exported from hepatic mitochondria to the bloodstream via carnitine palmitoyl transferase (CPT). Extrahepatic mitochondrial BDH1 converts βOHB back into AcAc. Succinyl-CoA:3-oxoacid CoA transferase (SCOT) reconverts AcAc into acetyl-CoA, which can be used for oxidation in the citric acid cycle (Cotter et al., 2013). SCOT is found in every mammalian cells containing mitochondria, except for hepatocytes, making the liver unable to oxidize KB (Cotter et al., 2013). KB oxidation is driven by an abundant supply of AcAc and a rapid consumption of acetyl-CoA through citrate synthase. SCOT is the main rate-determining step in ketolysis and its activity is down-regulated when intracellular levels of AcAc reaches >5 mM (Laffel, 1999), making the KB uptake a saturable phenomenon (Balasse & Féry, 1989). Interestingly, the conversion of KB by SCOT into an oxidizable form does not necessitate the investment of an ATP, in comparison to hexokinase (which activates glucose) and acyl-CoA synthetase (which activates fatty acids) (Puchalska & Crawford, 2017).

Two βOHB enantiomers are known: D-βOHB and L-βOHB. The hepatic NEFA flux, the main precursor of ketogenesis, leads to the synthesis of D-βOHB, while L-βOHB is formed in extrahepatic tissues when β-oxidation-derived intermediates accumulates (Puchalska & Crawford, 2017). Given that L-βOHB is not present in the liver or the blood, only the D-βOHB enantiomer will be considered hereafter.

2.2 - NEFA: THE PRECURSORS OF KETOGENESIS

Regulation of ketogenesis remains to be fully understood and relies on intra- and extra-hepatic factors. Nevertheless, it is well established that circulating NEFA levels are major precursors of ketogenesis (Balasse, 1986; Robinson & Williamson, 1980). This was demonstrated in humans, where a lipid infusion was administered while maintaining euglycemia and hyperinsulinemia (Ferrannini et al., 1983). It resulted in a significant rise in plasma βOHB. When no lipid infusion was administered, βOHB levels declined by nearly 40%. Lipid flux, leading to increased plasma NEFA, may however arise from both exogenous and endogenous sources.

Sources of NEFA

Plasma NEFA concentrations range from 0.2 to 1.7 mM in healthy adults (Itoh et al., 2003; Spector et al., 1975). They follow a circadian rhythm with the major peak in the morning following an overnight fast, and with nadir after meals (Karpe et al., 2011). The primary source of plasma NEFA originates from AT lipolysis, where TG are hydrolyzed into FA and glycerol (Figure 2). Intracellular AT lipolysis is carried

out through the action of three lipases: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MGL) (Lafontan & Langin, 2009).

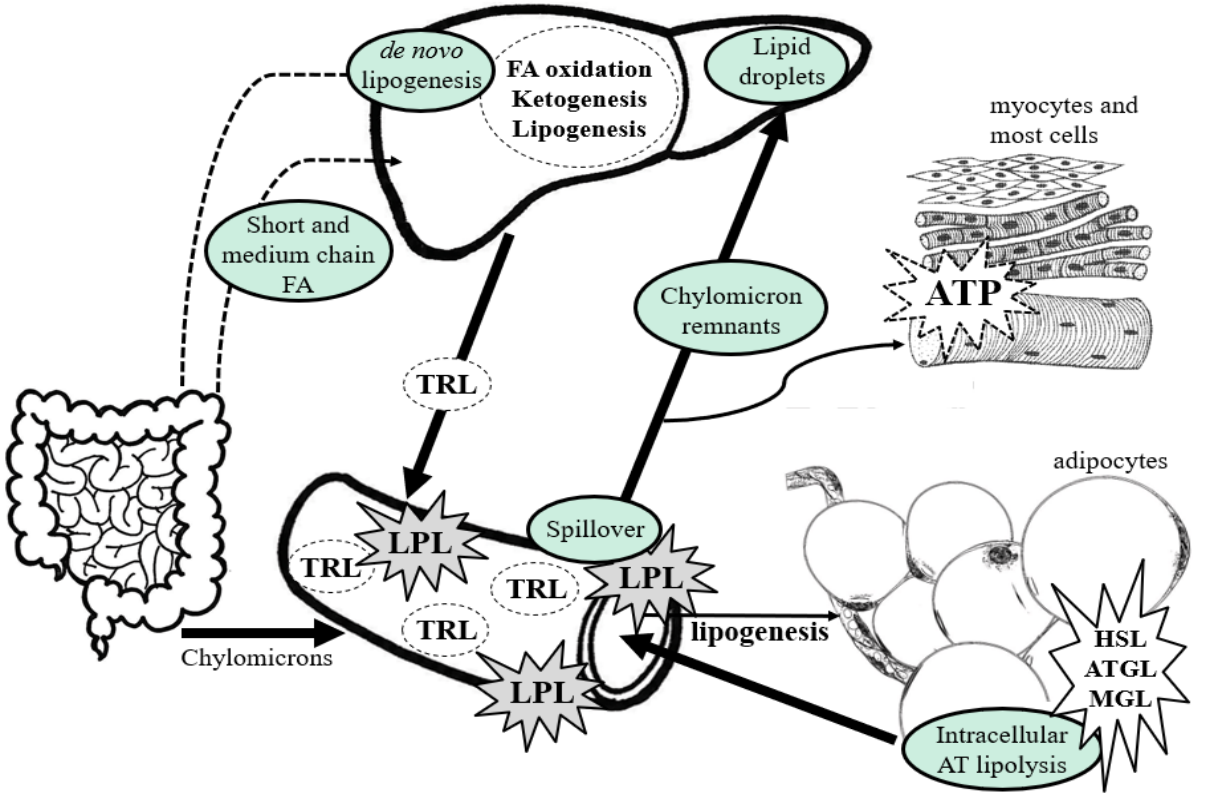

Figure 2. Hepatic fatty acids flux. The hepatic pool of FA originates from plasma NEFA, through the process of intracellular AT lipolysis through the action of three lipases: hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), and monoacylglycerol lipase (MGL), and from the spillover of NEFA during intravascular lipolysis, performed through lipoprotein lipase (LPL). Hepatic FA can also originate from the remaining triglycerides content of chylomicron remnants, the dietary intake of short and medium chain FA, the conversion of carbohydrates into FA, referred to as *de novo* lipogenesis and the hydrolysis of hepatic lipid droplets. FA may leave the liver as triglyceride-rich lipoproteins (TRL) while plasma NEFA pool may be uptaken by myocytes and other cells for ATP production.

Plasma NEFA also arise from intravascular lipolysis of TG-rich lipoproteins (TRLs), synthesised by both the liver (as very low-density lipoproteins or VLDL) and the intestine (as chylomicrons) (Karpe et al., 2011; Lafontan & Langin, 2009). Intravascular lipolysis is performed under the action of the lipoprotein lipase (LPL), expressed on the luminal surface of capillary endothelial cells. LPL rapidly hydrolyzes TG within TRLs, spilling over NEFA in the vascular milieu (Alves-Bezerra & Cohen, 2017).

In addition to the plasma pool of NEFA, the rate of appearance of NEFA to the liver can originate from the uptake of chylomicron remnants, the dietary intake, and the hydrolysis of hepatic lipid droplets (Figure 2) (Barrows & Parks, 2006; Donnelly et al., 2005). In addition, when dietary carbohydrates exceed total energy expenditure, the liver can convert them into FA, a process referred to as *de novo* lipogenesis (Alves-Bezerra & Cohen, 2017; Low et al., 2018). Hepatic FA are used for lipoprotein synthesis, local energy supply via oxidative phosphorylation, storage as cytoplasmic lipid droplets, and ketogenesis.

Although there are several sources of hepatic NEFA, AT lipolysis is the main source of NEFA pool, the primary precursor of ketogenesis. However, ketogenesis is also modulated through enzymes and hormones, and is influenced by diverse factors such as feeding, exercise, metabolic health, and biological sex, as described below.

2.3 - MODULATION OF KETONE BODIES

Key enzymes in the modulation of ketone bodies

Ketogenesis is dependent of three peripheral and hepatic enzymes: the peripheral HSL, the hepatic acetyl-CoA carboxylase (ACC) and the hepatic mitochondrial HMGCS (Laffel, 1999). All three enzymes are influenced by insulin, glucagon and catecholamines (Laffel, 1999; McGarry & Foster, 1977).

HSL is found in adipocytes and contributes to the breakdown of TG into FA and glycerol. While low insulin:glucagon stimulates the HSL activity, high insulin:glucagon dephosphorylates HSL, which in turn inhibits AT lipolysis, leading to lesser NEFA available for ketogenesis (Laffel, 1999).

ACC converts acetyl-CoA to malonyl-CoA, which blocks the transport of NEFA into the mitochondria via CPT-1 (Berg et al., 2002). High insulinemia stimulates ACC to produce malonyl-CoA, thus reducing the rate at which FA enters hepatic mitochondria for oxidation and ketogenesis. Conversely, glucagon inhibits ACC to facilitate the uptake of NEFA into the hepatic mitochondria.

Finally, HMGCS is present in the ketogenic pathway and plays a central role in the conversion of AcAc-CoA into AcAc. A high insulin:glucagon ratio will inhibit HMGCS in rodents (Puchalska & Crawford, 2017; Serra et al., 1993).

HSL, ACC and HMGCS are all three regulated through hormones, which are modulated by prandial status and stress.

Impact of hormones and feeding status in the modulation of ketone bodies

Diets containing more than 100 g of daily carbohydrate are considered non-ketotic (<0.1 mM) (Cahill, 2006). Caloric restriction can stimulate ketogenesis by shifting the dependency of energy production from glucose to lipids and its derived KB, as a result of an increased reliance on AT to release NEFA and sustain energy needs. The current literature presents heterogenous benefits and disadvantages regarding dietary regimens. For example, ketogenic diets (KD) may increase circulating TG and/or cholesterol, and induce vascular pathologies (Veech, 2004b). However, other researchers believe that KD improves plasma lipoprotein cholesterol (HDL and LDL), inflammatory markers, and decreases fasting insulin levels (Patterson et al., 2015). Notwithstanding some contradictory results, the popularity of KD keeps growing.

Several studies demonstrated that insulin decreases ketogenesis in humans. However, it should be noted that insulin does not directly control the ketogenic pathway (Keller et al., 1989; Miles et al., 1982). This was demonstrated in two studies where an insulin infusion did not modulate ketogenesis when postabsorptive concentrations of NEFA were maintained (Beylot et al., 1991; Miles et al., 1983).

Under fed and resting states, high insulin:glucagon ratio induces anabolism, which inhibits AT lipolysis, and thus refrains the release of NEFA into circulation (Gastaldelli et al., 2017). Increased insulinemia, such as following food intake, inhibits AT lipolysis through HSL dephosphorylation and stimulate ACC to inhibit FA oxidation (Jensen et al., 1989; Laffel, 1999). Hence, after a meal, the hepatic flux of FA is directed toward re-esterification for storage (Barrows & Parks, 2006; Hue & Taegtmeyer, 2009; Robinson & Williamson, 1980). High insulinemia also increases the uptake of glucose and NEFA into adipocytes and stimulates lipogenesis. Under postprandial resting conditions, NEFA concentrations decrease by nearly 50% (Barrows & Parks, 2006). Increased postprandial insulinemia may also stimulate *de novo* lipogenesis to provide newly made FAs for VLDL-TG synthesis (Barrows & Parks, 2006). Altogether, this reduces the availability of the main precursor for ketogenesis.

Conversely, in a fasted state and/or during exercise, when the insulin:glucagon ratio is low and/or when catecholamine levels are elevated, AT lipolysis increases the plasma NEFA pool (Alves-Bezerra & Cohen, 2017; Bartness et al., 2014; Gordon & Cherkes, 1956). The consequent increased load of FA to the liver stimulates β-oxidation to sustain ATP production. Increased ketogenesis was observed in fasting and exercising individuals, which further rely on ketolysis to sustain their efforts (Balasse & Féry, 1989). Hence, low insulin:glucagon stimulates the hepatic oxidative pathway to convert NEFA into acetyl-CoA

(Balasse, 1986; Robinson & Williamson, 1980), which leads to increased ketogenesis. Furthermore, under short-term fasting and resting conditions, increased FA oxidation increases the ratios of [acetyl-CoA]/[CoA] and [NADH]/[NAD^+], and both inhibits pyruvate dehydrogenase activity. This ends up inhibiting hexokinase and consequently decreases glucose metabolism in the citric acid cycle (Hue & Taegtmeyer, 2009). Moreover, when glycemia is low, oxaloacetate will preferentially be used for gluconeogenesis, also leading to increased acetyl-CoA (Laffel, 1999).

A fed state may however lead to increased ketonemia. For instance, following an oral fat loading test, a significant increase in plasma KB levels is observed in humans (C. J. M. Halkes et al., 2003). Ingestion of medium chain FA (mainly C8) also stimulates ketogenesis (St-Pierre et al., 2019). Potential mechanisms underlying the ketogenic effect of a high-fat meal might be related to an increased hepatic load of NEFA originating from short and medium-chain FA passing through the portal vein (Ramírez et al., 2001; T. Y. Wang et al., 2013), and the spillover of NEFA from the hydrolysis of circulating TG-rich lipoproteins (Karpe et al., 2011).

While insulin has great repercussion over ketogenesis, ketonemia also impacts insulin secretion. This effect may however be confusing because heterogenous results have been published (Balasse et al., 1970; Biden & Taylor, 1983; Boden & Chen, 1999; Park et al., 2011). While some observed a decreased insulin sensitivity after a rise ketonemia (Boden & Chen, 1999; Yokoo et al., 2003), Balasse (1986) demonstrated that an abrupt elevation in plasma concentrations of KB stimulates insulin secretion. In the later study, the initial increase is transient (5-15 min) and insulinemia eventually returns to its baseline value despite continuation of the KB infusion (Balasse, 1986). Later, Balasse et Féry (1989) clarified that two important conditions are required to observe an insulinotropic effect of KB in humans: first, KB concentrations must exceed 2 mM, which is considered hyperketonemia, and second, KB levels must be increased abruptly (Balasse & Féry, 1989). Hence, KB may have mitigated insulinotropic effects, but it has been shown to increase the sensitivity of AT to the antilipolytic effect of insulin (Green & Newsholme, 1979). Balasse et Féry (1989) demonstrated this phenomenon with an experimental elevations of KB concentration, induced by AcAc infusion in fasting subjects, which reduced NEFA concentration and depressed ketogenesis. In short, KB may increase insulin sensitivity, but it does not significantly stimulate insulin release.

Impact of insulin resistance and obesity in ketone bodies modulation

There are important differences in the metabolism of KB between metabolically healthy and insulin resistant and/or individuals living with obesity. On the one hand, in both insulin-treated and untreated individuals with diabetes, the daily mean plasma concentration of KB is about 2-3 times higher and display an abnormally high peak early in the morning when compared to healthy individuals (Wildenhoff, 1975). On the other hand, for individuals living with both obesity and insulin resistance, under fed and fasting states, βOHB plasma concentrations are lower than individuals living with obesity without insulin resistance, despite similar fasting NEFA levels (Bickerton et al., 2008). Thus, a dichotomy exists in these results while some study reported an increase and others a decrease in ketonemia.

This discrepancy may emerge from the timing of the measurements. For instance, a trial with mice specifies that increased ketogenesis is associated with mild insulin resistance (IR) while impaired ketogenesis develops as resistance to insulin worsens (Satapati et al., 2012). Consequently, ketonemia will diverge whether the data is collected at the onset of insulin resistance or when diabetes is well installed. Another source of discrepancy is whether data are expressed as raw levels or relative to plasma NEFA concentrations. Hence, in individuals with obesity, ketonemia may be higher (Wildenhoff, 1975), however KB:NEFA ratio is significantly lower when compared to lean individuals (Inokuchi et al., 1992). However, KB plasma concentrations and body mass index do not correlate.

Furthermore, an infusion of ßOHB to individuals with or without diabetes translates into a twofold greater rise in plasma levels and a 42% reduction metabolic clearance rate of ßOHB in the diabetic group. (Sherwin et al., 1976). This was later explained by Balasse and Féry (1989), who reported that regulatory mechanisms maintaining ketogenesis under maximal disposable capacity during prolonged fasting are inefficient in individuals with diabetes, suggesting it might require intact ß-cell function. One must not confuse these results with the abrupt ketogenesis rise observed in diabetic ketoacidosis, where insulin is completely absent or inefficient, leading to life threatening hyperketonemia.

These divergences between individuals with or without diabetes or obesity indicate that IR and/or obesity may alter KB metabolism. Although the mechanisms remain to be elucidated, hyperinsulinemia and IR impacts ketogenesis and ultimately contribute to hypoketonemia (Puchalska & Crawford, 2017; Soeters et al., 2009). Hence, the health status of individuals modulates the synthesis of KB, but the extra-hepatic cellular uptake of KB may also be altered through different mechanisms.

Impact of plasma levels of ketone bodies on cellular uptake of ketone bodies

Edmond and Féry (1989) demonstrated that peripheral uptake of KB is a saturable phenomenon, which is considered a crucial characteristic when assessing KB metabolism. For instance, a shift from overnight fast to a fully fasted state multiplies the production rate by a factor of 4-5 and ketonemia by a factor of 40-50. Moreover, at low concentration, muscles extract up to 50% of the KB but it drops to 2% when concentration is raised to 6 mM (Balasse, 1986). These discrepancies reveals that peripheral tissues, as a whole, have a limited removal capacity around 2.5 mM/min and the maximum clearance rate is markedly dependant on the concentrations of KB (Balasse, 1986). If production increases above 2.5 mM/min, blood concentration will increase, independently of the energy demand. Hence, in individuals fasting for various length and performing exercises, the maximum clearance rate of KB decreases as fasting ketonemia rise (Balasse & Féry, 1989). These findings suggest that muscle becomes unable to increase its capacity to utilize KB at work when saturation is already attained at rest. Therefore, an increased production reaching ~2.5 mM/min will lead to an exponential-type response of ketonemia (Balasse, 1986; Balasse & Féry, 1989; Keller et al., 1989; Reichard et al., 1974). A potential reason underlying this phenomenon may be the down-regulation of SCOT, a crucial enzyme of the ketolytic pathway (Laffel, 1999).

Impact of exercise on the modulation of ketone bodies

At low ketonemia, skeletal muscles are the main sites of KB disposal, and contracting muscles increases the capacity to extract KB from plasma and improves the clearance rate by approximately 40% (Balasse, 1986; Balasse & Féry, 1989). As previously mentioned, the ketonemic response to exercise depends on the initial degree of ketonemia. During exercise, ketogenesis and KB oxidation increases (Balasse, 1986; Egan & D'Agostino, 2016), consequent to the rise in circulating NEFA and to the increased ketogenic capacity of the liver, both of which are stimulated by the fall in insulin:glucagon ratio. Notably, for the first 30 minutes of exercise, total plasma NEFA and KB decrease transiently, as the mobilisation of AT is not yet sufficiently increased to keep up with the elevated muscular uptake (Balasse et al., 1978). Furthermore, cessation of exercise is associated with increased ketonemia.

In short, it is established that KB metabolism is modulated through circulating levels of NEFA, enzymes, hormones and prandial status, health status, plasma levels of KB and stress such as exercise. However, to our knowledge, it remains undetermined whether a stress such as hypoxia may lead to a rise in plasma concentrations of βOHB.

HYPOXIA

Hypoxia refers to a condition in which tissues of the body do not receive sufficient oxygen supply. At sea level, the usual percentage of hemoglobin saturated with O_2 in healthy individuals approximates 98% (Sjöberg & Singer, 2013). When O_2 saturation drops under 94%, tissues are exposed to hypoxic conditions (Safe et al., 2021). Reductions in oxyhemoglobin saturation are sensed by chemoreceptors in airways, carotid bodies, and individual cells, whereas the cellular volume of oxygen (VO_2) is determined predominantly by the rates of ATP synthesis, transport and utilization, the NADH supply and the citric acid cycle intermediates (Gu & Jun, 2018).

Once hypoxia is detected, complex adaptive mechanisms are triggered to facilitate cell survival. The nervous system fights back to hypoxic exposure with an increased expression of growth/trophic factors that confer neuroprotection and neuroplasticity (Dale et al., 2014). SNS will activate an immediate response and increase the respiratory rate, depth of breathing, cardiac output and blood pressure (Michiels, 2004), followed by increased anaerobic glycolysis. These mechanisms are particularly efficient in the very short-term (Solaini et al., 2010). Emergency responses are followed by an enhanced production of vasodilator and pro-inflammatory cytokine (Sjöberg & Singer, 2013), and increased erythropoiesis and angiogenesis (Catrina & Zheng, 2021). Normobaric hypoxia, as induced in the studies presented in this thesis, refers to decreased oxygen availability at sea level. Normobaric hypoxia relates to hypoxic episodes regularly observed in patient suffering from pulmonary or cardiovascular diseases, which may experience continuous hypoxia, in contrast with obstructive sleep apnea patient, which rather experience intermittent hypoxia.

Continuous and intermittent hypoxia

Hypoxia is a consequence of several environmental and pathological situations (Trayhurn, 2014). An exposition to high altitude or emphysema will lead to a continuous decreased oxygen availability for the cells. Obstructive sleep apnea, characterised by alternating episodes of complete/partial airway obstruction during sleep, will lead to intermittent hypoxia. Individuals suffering from hypoxia-related pathologies are at higher risk of developing comorbidities (Kapur et al., 2017). CH and IH both increase SNS activity, oxidative stress, and chronic low-grade inflammation (Baessler et al., 2013; Eltzschig, 2011; Ma et al., 2016; Poulain et al., 2017; Ryan et al., 2005; Sozer et al., 2018; Trzepizur et al., 2013; Ye et al., 2007; Yokoe et al., 2003). Moreover, both hypoxic modalities may decrease FA oxidation (Huss et

al., 2001) and sensitivity to insulin, all of which may affect plasma concentrations of NEFA, an important ketogenic predicator. Dyslipidemia, a consequence of hypoxia, may be linked to an increased risk of cardiometabolic diseases such as diabetes and cardiovascular diseases. However, heterogenous results are found in the literature concerning the effect of acute and chronic hypoxia towards the incidence of diabetes (Catrina & Zheng, 2021; van Hulten et al., 2021; X. Wang et al., 2013) and cardiovascular events (Ma et al., 2016; Marin et al., 2005; Michiels, 2004; Roux et al., 2000). The existing dichotomy regarding the effects of hypoxia towards these pathologies and its link to dyslipidemia leaves us with at least one certainty; acute hypoxia, under both continuous and intermittent forms, increases circulating NEFA levels (Mahat et al., 2016, 2018; Mauger et al., 2019; Morin et al., 2021)

NEFA under hypoxia

In rodents under severe hypoxia (1% O_2), an increased adipose lipolysis occurs while AT FA uptake decreases (Trayhurn, 2014). Additionally, hypoxia downregulates lipogenesis (Yin et al., 2009) and increases plasma NEFA concentrations in rodents and humans (Chopra et al., 2017; Gangwar et al., 2020; J. C. Jun et al., 2011, 2012; Poulain et al., 2017). Furthermore, studies from our team clearly established that CH and IH increase plasma NEFA levels in healthy young men (Mahat et al., 2016, 2018; Mauger et al., 2019; Morin et al., 2021). Also, we did not report significant changes in substrate oxidation measured with indirect calorimetry under CH (Mahat et al., 2018; Mauger et al., 2019). Through our observations, it seems that the increased adipocyte release of NEFA under acute hypoxic conditions at rest is not related to systemic ATP production, at least at sea level.

In summary, at rest, hypoxia-induced AT lipolysis increases circulating NEFA levels, and this increase is not directed towards ATP production, nor is it for reuptake by AT. The complete metabolic fate of the increased circulating NEFA in response to acute hypoxia exposure remains to be determined but could, at least partly, be directed towards a rise in ketogenesis.

KB under hypoxia

There is a paucity of data in the existing literature regarding the metabolism of KB during hypoxia. Whether NEFA are increasingly directed towards ketogenesis at decreased pressure of oxygen (pO_2) in humans is also unknown. What is established is that hypoxia increases ketonemia in rodents (D'Alecy et al., 1990; J. C. Jun et al., 2012; Rising & D'Alecy, 1989). The exposure of fasted rats to a simulated altitude leads to a greater survival time than fed rats, while the injection of glucose or insulin prior to

exposure inhibited the protective effect of fasting (Myles, 1976). Later on, Kirsch *et al*. (1980) increased ketonemia in rodents 30 minutes prior to hypoxic exposure (4-5% O_2), by either oral, intraperitoneal, or intravenous infusion of 1,3-butanediol, an alcohol converted to βOHB in the body. The induction of ketosis was associated with enhanced survival time up to 560% as compared to the control group (105s vs 605s of survival time) (Kirsch et al., 1980). In two other studies where rodents were exposed to severe hypoxia (4.5% O_2), ketonemia considerably increased, and the survivors had significantly higher plasma levels of βOHB than the deceased (D'Alecy et al., 1990; Rising & D'Alecy, 1989). D'Alecy et al. (1990) also observed that 100% of the ground squirrels survived hypoxic exposure compared to only 20% of the rats. Rats surviving the longest had the highest ketone levels. The suggested explanation, which remains to be validated, relies on the fact that hibernation necessitates greater ketogenic capacities. Therefore, ground squirrels, which are hibernating animals, have greater ketogenic capacities than non-hibernating rats.

Several years later, Cahill (2006) asserted that any cell challenged by low oxygen availability should benefit from utilizing βOHB rather than glucose, lactate, pyruvate, or FA (Cahill, 2006). Mechanisms such as reduction of oxidative stress, mitochondrial protection, suppression of endoplasmic reticulum stress, enhanced autophagy, and inhibition of necrosis, apoptosis, and pyroptosis, may account for such protective effects of KB (Rojas-Morales et al., 2020). However, all of this remains to be demonstrated in humans under hypoxia, and further research will be required to investigate the protective mechanisms of βOHB under decreased oxygen availability. What has been further investigated is the effects of hypoxia on cellular sensitivity to insulin.

Effects of hypoxia on insulin sensitivity

Insulin is a pancreatic hormone secreted during metabolite abundance. After an overnight fast, insulin concentration should be around 7.5 µU/ml in healthy individuals (Boden et al., 1991). This hormone has pleiotropic actions, and among others, it inhibits AT lipolysis, translocates GLUT4 from the endoplasmic reticulum to the plasma membrane, and increases mitochondrial acetyl-CoA concentrations by increasing the activity of the pyruvate dehydrogenase complex (Sato et al., 1995; Veech, 2004b).

Decreased insulin sensitivity in a low O_2 environment was demonstrated in mice exposed to 6 weeks of IH (21 to 5% O_2, 60s cycle, 8 h/day) (Poulain et al., 2017). By the end of the experiment, following a glucose challenge, the IH group had similar glycemia when compared to a control group, but their insulin release and secretion index was significantly higher. This is a clear demonstration of impaired insulin

sensitivity and secretion in response to hypoxic exposure. In this line, it was also found that IH stimulated insulin secretion and increased fasting glucose levels in rats exposed to IH for 12 weeks at various concentrations of O_2 (Y. Wang et al., 2017). However, another study (Y. Wang et al., 2018), performed with obese mice with type 2 diabetes, demonstrated that a daily one-hour hypoxic exposure (15% O_2) for 4 weeks improved insulin sensitivity and increased GLUT4 translocation to cell membrane.

In humans, exposure to conditions of low oxygen has also been shown to impair insulin sensitivity (Larsen et al., 1997; Oltmanns et al., 2004). Using an hyperinsulinemic euglycemic clamp, Peltonen et al. (Peltonen et al., 2012) demonstrated an impairment of insulin sensitivity in healthy individuals exposed for 3h of normobaric hypoxia (FiO2 = 0.11), a phenomenon caused by the sympathetic nervous system activation. On the other hand, a recent review by van Hulten *et al.* (2021) indicated beneficial effects of hypoxia on glucose homeostasis. However, many of the selected studies omitted to report the participants prandial status and diet content, had no control group while some participants had an important increase in physical activity prior to data collection, such as during an altitude climb. Caution should therefore be taken prior to draw conclusions. Hence, in the study included in this thesis, a simple index estimating adipose tissue insulin resistance (Adipo-IR) (defined further below), was used to determine whether hypoxia impairs insulin sensitivity.

Adipo-IR

Adipose tissue IR (Adipo-IR) is defined as the impaired suppression of lipolysis while insulinemia is high (Gastaldelli et al., 2017). When AT becomes resistant to insulin, excess NEFA are delivered to other tissues and may contribute to ectopic fat deposition and lipotoxicity, which is associated with increased IR. Therefore, Adipo-IR may be an early step in the development of systemic IR (Søndergaard et al., 2017).

Adipo-IR index is often calculated by multiplying the fasting insulin and the fasting NEFA concentrations (Søndergaard et al., 2017). It may also be a postabsorptive quotient of the mean plasma NEFA and insulin concentrations, which can be used during an oral glucose tolerance test (Gastaldelli et al., 2017). Adipo-IR index is an analog of the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index for glucose metabolism, or more specifically, for whole-body insulin sensitivity (Matsuda & DeFronzo, 1999). An increased post-test Adipo-IR is usually associated with glucose intolerance and elevated plasma NEFA levels (Gastaldelli et al., 2017).

When using Adipo-IR index, one should be careful considering that it overestimates AT insulin sensitivity in elders and underestimates it in individuals with high physical fitness levels (Søndergaard et al., 2017). Otherwise, the index acutely reflects Adipo-IR, notwithstanding biological sex, body mass index, or fat percentage differences.

SUMMARY

Under hypoxia, AT lipolysis increases, leading to increased levels of circulating NEFA. The physiological significance of this rise in plasma NEFA is not completely understood. Given that the hepatic NEFA flux is the main precursor to ketogenesis, investigations into the effect of different hypoxic conditions on ketonemia will give a hint as to whether the increased levels of NEFA are, at least partly, directed towards ketogenesis.

This thesis is composed of an original manuscript derived from the retrospective analysis of three hypoxic protocols performed with participants under distinct feeding status. Hence, the comparison of these various feeding status combined to different hypoxic conditions helps to dissociate the impact of hypoxia from the impact of the prandial status on the circulating levels of both NEFA and ßOHB, and on the modulation of insulinemia.

Thus, the objective of this study is to examine the acute effect of intermittent and continuous hypoxia on circulating levels of ßOHB in humans, and this was performed through a retrospective analysis of plasma samples collected in three research projects (Mahat et al., 2016, 2018; Mauger et al., 2019). We hypothesized that the increase concentration of plasma NEFA, observed under acute normobaric hypoxia exposure, would lead to an increase ßOHB circulating levels.

Chapter 3: METHODS AND RESULTS

For this thesis, the methods and results chapters will be presented with the following non-published research article. The aim is to submit and hopefully publish this article in 2022.

4.1 – Thesis Article

THE EFFECT OF ACUTE INTERMITTENT AND CONTINUOUS HYPOXIA ON CIRCULATING B-HYDROXYBUTYRATE IN HUMANS

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ABSTRACT

Introduction: Acute hypoxia is known to increase circulating nonesterified fatty acid (NEFA) levels. Adipose tissue is a significant site of NEFA liberation into circulation and insulin suppresses this process when the tissue is insulin sensitive. The metabolic fate of NEFA is dictated by major pathways such as esterification to triglycerides and complete/partial oxidation, the latter leading to ketogenesis in the liver. To our knowledge, the effect of hypoxia on ketogenesis, more specifically ß-hydroxybutyrate (ßOHB), remains unknown in humans. Therefore, the objective of this study was to determine the effect of acute intermittent and continuous hypoxia on plasma circulating ßOHB levels.

Methods: Plasma samples from 3 different randomized crossover studies were assessed for ßOHB concentrations. In the first study, 14 healthy men (23 ± 3.5 years) were exposed to 6 hours of normoxia or intermittent hypoxia (IH-Fed) (15 hypoxic events per hour) following an isocaloric meal. In the second study, 10 healthy men (26 ± 5.6 years) were exposed to 6 hours of continuous normobaric hypoxia (CH-Fasted) (FiO_2= 0.12) or normoxic conditions in the fasting state. In the third study (CH-Fed), 9 healthy men (24 ± 4.5 years) were exposed to 6 hours of CH in a constant prandial state. ßOHB, NEFA and insulin levels were measured during all sessions. The adipose tissue insulin resistance index (Adipo-IR) was also calculated from NEFA and insulin levels.

Results: In study 1 (IH-Fed), ßOHB and NEFA levels tended to be greater over 6 hours of IH (condition x time interaction, p = 0.108 and p = 0.062, respectively) compared to normoxia. In study 2, ßOHB and NEFA levels increased over time in both experimental conditions, this effect being greater under CH (condition x time interaction, p = 0.070; p = 0.046, respectively). In study 3 (CH-Fed), ßOHB levels slightly increased up to 180 min before falling back to initial concentrations by the end of the protocol in both normoxia and CH (p = 0.062), while NEFA were significantly higher under CH (p = 0.006). Adipo-IR tended to increase after 6 hours of hypoxia compared to normoxia in the first two studies (main effect of condition, p = 0.024; p = 0.097, respectively), and significantly increased over time under hypoxia in CH-Fed (condition x time interaction, p = 0.004).

Conclusion: Acute normobaric hypoxia exposure significantly increases plasma ßOHB concentrations over time in healthy men. The stimulating effect of hypoxia on plasma ßOHB levels is however attenuated during postprandial and prandial states.

INTRODUCTION

Ketone bodies (KB), namely acetoacetate (AcAc) and ß-hydroxybutyrate (ßOHB) (Robinson & Williamson, 1980), are 4 carbons (Prins, 2008), organic molecules, commonly recognized as metabolites. Under non-prolonged fasting and resting conditions, in healthy individuals, plasma levels of βOHB are about twice the levels of AcAc in humans (J. C. Newman & Verdin, 2014a). Therefore, βOHB is the tracer of choice when assessing ketogenesis (Balasse & Féry, 1989; Veech, 2004a). Both KB are formed mainly in the liver from the partial oxidation of fatty acids (FA) into acetyl-CoA. Their most well-known purpose is to serve as an alternative oxidative substrate for the brain in situations of decreased glucose availability (Balasse & Féry, 1989; Owen et al., 1967), as during fasting or very low carbohydrate diets (McDonald & Cervenka, 2018). However, recent observations also attributed regulatory functions to KB (J. C. Newman & Verdin, 2014b; Shimazu et al., 2013), such as antilipolytic properties (Taggart et al., 2005) and a propensity to modulate the sympathetic nervous system activity (Kimura et al., 2011; Won et al., 2013). It is often underrated that KB are continuously produced (Balasse & Féry, 1989). Under non-prolonged fasting and resting conditions in healthy individuals, the rate of ketogenesis may contribute to 5-20% of daily energy expenditure (Balasse, 1986; Puchalska & Crawford, 2017; Robinson & Williamson, 1980). In healthy humans, ketonemia follows a circadian cycle with a peak around midnight and a nadir in the morning (Robinson & Williamson, 1980; Wildenhoff, 1975).

KB kinetics are mainly impacted by plasma NEFA (nonesterified fatty acids) concentrations and circulating hormones (particularly insulin, glucagon and catecholamines) (Cahill, 2006; Keller et al., 1989; Laffel, 1999; McGarry & Foster, 1977; Puchalska & Crawford, 2017). Circulating NEFA are mostly derived from the breakdown of triglycerides (TG) in adipose tissues (Farkas, Angel, et Avigan 1973), which is governed by the action of different hormones such as insulin, glucagon and catecholamines (Barrows & Parks, 2006). Consequently, increased ketonemia is observed both in a fasted state which lowers insulinemia, and in states of increased metabolic need, for instance under exercise and during stress (Balasse et al., 1978; Balasse & Féry, 1989; Féry & Balasse, 1983), which increases catecholamines and glucagon secretion. Under such circumstances, the increased delivery of NEFA to the liver stimulates their oxidation through β-oxidation, which produces great amounts of acetyl-CoA that feeds ketogenesis. Conversely, the fed state is generally associated with reduced ketonemia (Geisler et al., 2016; Walsh et al., 2013). The typical postprandial increase in insulinemia both inhibits adipose tissue lipolysis (Jensen et al., 1989; Laffel, 1999), which reduces the delivery of NEFA to the liver, and inhibits

the transport of long chain FA into the mitochondria (Laffel, 1999) which suppresses fatty acid oxidation. Hence, the channeling of liver lipids toward re-esterification (for storage and secretion as very-low density lipoproteins) should prevent β-oxidation and ketogenesis. It should however be noted that a significant increase in plasma KB levels was reported in men and women following an oral fat loading test (C. j. m. Halkes et al., 2003; Meijssen et al., 2000).

Plasma NEFA fluctuate according to several physiological conditions, notably an activation of the sympathetic nervous system, as observed upon hypoxia exposure (Eichhorn et al., 2017; Somers et al., 1989). Hypoxia is a state of oxygen deficiency (Sjöberg & Singer, 2013) that can manifest intermittently such as during obstructive sleep apnea (Drager et al., 2010) or continuously such as during high altitude exposure or emphysema (Vanier et al., 1963). Under hypoxic conditions, oxygen availability becomes limited and oxidative phosphorylation is hampered (Solaini et al., 2010). Recently, experiments in humans studying the metabolic responses to hypoxia in continuous and intermittent forms, and under fed or fasted state, showed that hypoxia significantly increases plasma NEFA concentrations (Chopra et al., 2017; J. C. Jun et al., 2011; Mahat et al., 2016, 2018; Mauger et al., 2019; Morin et al., 2021), which should translate into an increased ketogenesis. In this regard, limited studies conducted in rodents have reported that circulating βOHB levels significantly increase in response to acute hypoxia exposure (D'Alecy et al., 1990; J. C. Jun et al., 2012; Rising & D'Alecy, 1989). Nevertheless, to our knowledge, it remains undetermined whether hypoxia increases ketonemia in humans.

Thus, to examine the effect of hypoxia on circulating levels of KB in humans, we measured ßOHB concentrations in plasma samples from three previous studies. The first study assessed healthy individuals acutely subjected to normobaric intermittent hypoxia following a high fat liquid meal (referred to as IH-Fed) (Mahat et al., 2016). In the second study, healthy individuals were acutely exposed to continuous normobaric hypoxia in the fasted sate (referred to as CH-Fasted) (Mahat et al., 2018). In the third study, healthy individuals were subjected to continuous hypoxia in a constantly fed state (referred to as CH-Fed) (Mauger et al., 2019). We retrospectively analyzed those studies to assess the acute effects of two main forms of hypoxia, intermittent and continuous, under different feeding status, on ketonemia. We hypothesized that the rise in circulating NEFA levels under acute normobaric hypoxia exposure would increase plasma ßOHB concentrations.

MATERIALS AND METHODS

Participants

Healthy male volunteers (n=14, for IH-Fed study; n=10, for CH-Fasted study; n=9, for CH-Fed) were recruited for the study and provided informed consent prior to data collection. Participant characteristics are summarized in **Table 1**. All methodologies were approved by the Research and Ethics Board of the University of Ottawa. Individuals with a medical history of asthma or other respiratory illness, hypertension, cardiovascular disease, diabetes, usual sleep duration of less than 7 h per night, habitual bedtime occurring after midnight, shift work and/or current smoking habit were excluded. Body weight was determined with a standard beam scale (HR-100, BWB-800AS; Tanita, Arlington Heights, IL) and height was measured using a standard stadiometer (Perspective Enterprises, Portage, Michigan, USA). The percentage of fat mass, total fat mass and lean mass were determined using dual-energy X-ray absorptiometry (DXA) (General Electric Lunar Prodigy, Madison, Wisconsin; software version 6.10.019). Resting energy expenditure (REE) was measured by indirect calorimetry using a Vmax Encore 29 System metabolic cart (VIASYS Healthcare Inc, Yorba Linda, California, USA).

Experimental studies

This is a retrospective analysis of plasma sample collected in three different randomized crossover studies (Mahat et al., 2016, 2018; Mauger et al., 2019). For the IH-Fed study, it should be noted that 4 participants were added to the sample used in Mahat *et al*. (Mahat et al. 2016). In each study, participants performed two experimental sessions. Prior to each session, participants were counseled to sleep at least 7 h per night, refrain from any exercise, caffeine, and alcohol for at least 36 h, and to consume the same evening dinner the day before each session (lasagna of 3220 kJ or 770 kcal; 42 % from carbohydrates, 28 % from fat, and 30 % from protein).

Each study consisted of 2 different sessions of 6 hours: a hypoxic session and an ambient air session. Volunteers remained in a semi-recumbent position for the duration of the experimental session and occupied themselves by watching television. Sleep was not allowed. Oxyhemoglobin saturation and heart rates were continuously monitored by pulsed oximetry using a Masimo, Radical 7 unit (Masimo, Irvine, CA, USA). In each study, an intravenous line was inserted in the antecubital vein for blood sampling and kept patent with a continuous infusion of 0.9 % saline.

***Intermittent hypoxia-fed experimental study (IH-Fed)*:**

On study days, volunteers arrived at the laboratory at 7:30 AM after a 12-h overnight fast. Volunteers were thereafter asked to consume a fat-rich liquid meal (59 % of calories from fat, 28 % from carbohydrates and 13 % from protein), providing one-third of their estimated daily energy expenditure times a physical activity factor of 1.375 (Harris & Benedict, 1918). Participants were wearing an oro-nasal mask with a two-way Hans Rudolph non-rebreathing valve. During normoxia session, ambient air only was provided through the mask. During IH-Fed sessions, pressurized medical N_2 was intermittently administered until oxyhemoglobin saturation (SpO2) dropped to 85 %. At this point, the flow of N_2 was stopped and ambient air was re-administered until SpO_2 returned to the patient's normal value (between 95~100 %). Intermittent hypoxia was well-tolerated and presented no adverse effects. This experimental setup allowed us to produce 17.3 ± 3.8 hypoxic events per hour, which is comparable to moderate OSA.

***Continuous hypoxia-fasted experimental study (CH-Fasted)*:**

All sessions were performed in an environmental chamber at the University of Ottawa. Volunteers arrived at the laboratory at 7:30 AM after a 12-h overnight fast and remained fasted for the duration of each experimental session. Drinking water was allowed. During normoxia, only ambient air was used (FIO_2 = 0.21). During hypoxia, O_2 extractors (CAT 12; Altitude Control Technologies, Lafayette, Colo., USA) connected to the environmental chamber kept the FIO_2 level stable at 12%. The CAT system uses 2 stable zirconium O_2 sensors in parallel to detect random sensors drift. The sensors are calibrated with ambient air (assuming an ambient air O_2 concentration of 20.94%) when sensors disagree by more than 0.5% O_2. During hypoxia, O_2 concentration was also continuously monitored by the constantly self-calibrating Vmax system used for indirect calorimetry. O_2 readings from both systems were always within 0.5%. To ensure the participants thermal comfort, temperature and relative humidity were stable at 28 °C and 45%, respectively, and a mechanical fan was used if needed.

***Continuous hypoxia-fed experimental study (CH-Fed)*:**

As for CH-Fasted, all sessions were performed in an environmental chamber at the University of Ottawa. Volunteers arrived at the laboratory at 7:30 AM after a 12-h overnight fast. Drinking water was allowed. Volunteers were thereafter asked to consume the first of twelve liquid meals (35% of calories from fat, 55% from carbohydrates, and 10% from protein), providing a total of 40% of their estimated daily energy expenditure. Liquid meal servings were provided every 30 min. Participants were exposed to either hypoxia (FIO2 = 0.12) or ambient air (normoxia) for 6 h. During normoxia, only ambient air was used

(FIO_2 = 0.21) while during hypoxia, FIO_2 level was kept stable at 12%. Calibration and thermal settings were the same as for CH-Fasted.

Plasma parameters

Blood samples were collected hourly, in tubes containing EDTA. Immediately after collection, plasma was obtained by centrifugation at 3000 rpm, for 10-12 min, at 4 °C. Plasma samples were kept frozen at -80°C until further analyses. Commercially available colorimetric enzymatic assays were used to measure plasma total NEFA (Wako Chemicals USA Inc, VA, USA). Insulin was measured by enzyme-linked immunosorbent assay kits (EMD Millipore, MA, USA), from which the Adipo-IR was calculated, as previously reported, by multiplying the fasting NEFA concentration (mmol/L) by the fasting insulin concentration (pmol/L) (Gastaldelli et al., 2017; Søndergaard et al., 2017). Adipo-IR has been shown to be a reliable and reproducible index of adipose tissue insulin resistance in both fasting and postprandial conditions in individuals with normal glucose tolerance (Gastaldelli et al., 2017). βOHB concentration was measured using a commercial enzymatic colorimetric assay kit (Cayman Chemical, Ann Arbor, Mich., USA). Assay analyses were completed in duplicate and the intra-assay coefficients of variation were approximately < 5%.

Statistical analysis

Student T-test were used to compare anthropometric and fasting plasma parameters between studies. Repeated measures analyse of variance (ANOVA) were performed with condition and time as within-subject's parameters. When data from each study were combined, study was added as a between-subject factor in the model. Identification of significant interactions led to further analysis of simple main effects for hypoxia. The Greenhouse-Geisser correction was used whenever the sphericity assumption was violated. Partial eta squared are provided as an estimate of effect size. For IH-Fed and CH-Fed, time 0, which corresponds to fasting state, were excluded to account for prandial and postprandial effects only. Error bars in Figures 1A-I were adjusted to eliminate between subjects' variability and better reflect the statistical power of the study crossover design (Cousineau et al., 2005). A level of significance of $p < 0.05$ was considered statistically significant. Jamovi version 1.2.27.0 for Windows was used for data analysis (The Jamovi project, Sydney, Australia).

RESULTS

Characteristics of participants

No statistical differences were observed between the participants of each study (Mahat et al., 2016, 2018; Mauger et al., 2019) as reported in **Table 1**. Fasting plasma parameters of participants are outlined in **Table 2** and were comparable between normoxia and hypoxia sessions for the IH-Fed, CH-Fasted and CH-Fed studies.

Effects of hypoxia on plasma parameters

Plasma ßOHB, NEFA, and Adipo-IR levels during 6h of normoxia or hypoxia are shown in **Figures 1A-I**.

In the IH-Fed study, which was conducted in the postprandial state, ßOHB levels (**Figure 1A)** tended to evolve in a different manner over time between normoxia and hypoxia (condition x time interaction, $p = 0.108$, $\eta_p^2 = 0.171$). More specifically, ßOHB levels transiently increased after the meal and returned to initial levels after 6 hours in normoxia but remained elevated during hypoxia. Average ßOHB concentrations were increased by 34% compared to fasting levels after 6 hours of IH-Fed whereas a marginal increase of only 3% was observed under normoxia. A trend toward greater increase in plasma NEFA levels over time was observed under IH-Fed compared to normoxia (condition x time interaction, $p = 0.062$, $\eta_p^2 = 0.168$) (**Figure 1B**). The Adipo-IR was significantly higher under IH-Fed than normoxia regardless of time (main effect of condition, $p = 0.024$, $\eta_p^2 = 0.333$) (**Figure 1C**). Irrespective of experimental conditions, the Adipo-IR increases over 360 min of exposure, but this fell short of statistical significance (effect of time, $p = 0.090$, $\eta_p^2 = 0.206$).

In the CH-Fasted study, ßOHB levels increased over time in both experimental conditions and this increase tended to be greater under CH-Fasted (condition x time interaction, $p = 0.070$, $\eta_p^2 = 0.307$) (**Figure 1D**). The mean average increase in ßOHB was of 133% and 38% by the end of the 6-hour session under CH-Fasted and normoxia, respectively. Similarly, NEFA concentrations significantly increased over 6 hours in both experimental conditions with a greater increase observed under continuous hypoxia (condition x time interaction, $p = 0.046$, $\eta_p^2 = 0.254$) (**Figure 1E**). The Adipo-IR tended to be greater under CH-Fasted than under normoxia, regardless of time (main effect of condition, $p = 0.097$, $\eta_p^2 = 0.276$) (**Figure 1F**).

In the CH-Fed study, conducted in a continuously fed state, ßOHB levels slightly increased up to 180 min before falling back to initial concentrations by the end of the protocol in both normoxia and CH (main effect of time, $p = 0.062$, $\eta_p^2 = 0.294$) (**Figure 1G**). Mean plasma NEFA levels were significantly higher under CH than normoxia, regardless of the effect of time (main effect of condition, $p = 0.006$, $\eta_p^2 = 0.633$) (**Figure 1H**). Adipo-IR significantly increased over time in both normoxic and hypoxic conditions, and this increase was greater under CH-Fed (condition x time interaction, $p = 0.004$, $\eta_p^2 = 0.671$) (**Figure 1I**).

DISCUSSION

This study evaluated the hypothesis that acute normobaric hypoxia exposure, which elevates circulating NEFA levels, would augment plasma ßOHB concentrations. We corroborated that acute normobaric hypoxia, conducted in controlled environment, elevates circulating NEFA levels and we confirmed the hypothesis that this is accompanied by a significant increase in plasma ßOHB concentrations in healthy men. The hypoxic ßOHB rise is however repressed by prandial and postprandial status. We also determined that upon hypoxic exposure, Adipo-IR, a surrogate of adipose tissue resistance to insulin, is increased.

To our knowledge, this is the first study examining the impact of acute hypoxic exposure on ketonemia in humans. Using different modalities of normobaric hypoxia exposure (intermittent or continuous) and feeding status (fasting, postprandial or prandial), we report that hypoxia, either intermittent or continuous, tends to elicit greater elevations in ßOHB levels during postprandial state (IH-Fed) and fasting state (CH-Fasted). The increased ketogenic response observed upon hypoxia is however abrogated for the first two to three postprandial hours (IH-Fed) and under a constantly fed state (CH-Fed). These results are consistent with previous observations in rodent studies showing that hypoxia increases blood ketone levels. Indeed, in a series of experiments aiming at understanding the physiological responses associated with survival time under hypoxia, Rising *et al.* (Rising & D'Alecy, 1989) reported a significant 267% increase in ßOHB levels 30 min following short exposures (90 to 150 seconds) to severe hypoxic conditions ($FiO_2 = 0.046$). The same group also reported dose-dependent increases in ßOHB levels in rats and ground squirrels following 5, 10 and 60 min exposures to hypoxic conditions ($FiO_2 = 0.045$) (D'Alecy et al., 1990). Ground squirrel showed greater increases in blood ßOHB than rats (517% vs 378%, respectively) after the 60 min exposure, which suggests important inter-species differences in ketone metabolism adaptation to hypoxia. More recently, Jun *et al.* (2012) reported a significant 3-fold increase in ketonemia in mice exposed for 6 hours at $FiO_2 = 0.07$ while no changes in ketonemia were

noted in less severe hypoxic conditions (FiO_2 = 0.10, 0.14 and 0.17). Together, these observations and ours demonstrate that hypoxia leads to an increase in ketonemia.

The physiological mechanisms responsible for the increase in ketonemia under hypoxic conditions in rodents and in humans have yet to be elucidated. However, one certainty is that the observed increase in ketonemia is the result of a rate of ketone production exceeding the rate of ketone utilization. In that regard, several physiological responses to hypoxia are likely to disturb the balance between ketone production and utilization. First, there is the increase in sympathetic tone in response to hypoxia (Somers et al., 1989), which is well recognized to stimulate adipose tissue lipolysis (Rayner, 2001) and thus, lead to a rise in NEFA levels, as observed in the current and other human studies(Chopra et al., 2017; J. C. Jun et al., 2011; Mahat et al., 2016, 2018; Mauger et al., 2019; Morin et al., 2021). Since plasma NEFA are the main substrate for ketogenesis (Robinson et Williamson 1980), the hypoxia-induced increase in sympathetic tone and its stimulating effect on NEFA levels is likely to stimulate ketogenesis in the liver. While our experiments did not include direct measurement of sympathetic tone, our hypoxia experimental sessions, especially the ones under continuous hypoxia, was reported to significantly increase heart rate by 20% (Mahat et al., 2018; Mauger et al., 2019), which would most likely reflect a sympathetic activation .

The reduction of adipose tissue insulin sensitivity, as estimated by the Adipo-IR index, observed in response to hypoxia in the current study could also favor the release of NEFA into circulation by attenuating the insulin-suppressive effect on adipose tissue lipolysis (Lafontan & Langin, 2009; Young & Zechner, 2013). It is interesting to note that when systemic insulin sensitivity surrogate indexes such as the homeostasis model assessment (Matthews et al., 1985) and the Matsuda index (Matsuda & DeFronzo, 1999), which are based on fasting or postprandial plasma insulin and glucose concentrations, no change of insulin sensitivity was observed in response to hypoxia (data not shown). This latter observation is not in line with other studies in humans which reported that insulin sensitivity is attenuated in response to continuous (Peltonen et al., 2012) or intermittent hypoxia (Louis & Punjabi, 2009) protocols comparable to the ones we conducted. However, the methods used for quantifying insulin sensitivity (e.g., hypersinsulinemic euglycemic clamp and intravenous glucose tolerance test) in these previous studies were more direct, which likely explains the divergence with our insulin sensitivity surrogate indexes. Nonetheless, the fact that hypoxia significantly affected Adipo-IR index in the current study could be interpreted as an indication that adipose tissue insulin sensitivity may precede systemic insulin sensitivity in response to hypoxia. Such a hypothesis however needs to be formally tested.

In hypoxic conditions, the increased energy requirement may benefit the partial hepatic fatty acid oxidation, forcing the channeling of β-oxidation products towards ketogenesis. The subsequent flow of acetyl-CoA into the ketogenic pathway would then stimulate ketogenesis under hypoxia. The hypoxic rise is however invisible in the first three postprandial hours (IH-Fed) and in the prandial state (CH-Fed), which suggest ketonemia is further driven by the meal. As expected, the postprandial insulinemic surge decreased circulating NEFA in both studies (Figure 1A and 1G), but surprisingly, ketonemia increased simultaneously. Hence, the proportion of NEFA converted to ßOHB may be driven by the prandial and postprandial status of participants. It should however be noted that insulin does not directly control the ketogenic pathway (Keller et al., 1989; Miles et al., 1982). This was demonstrated in two studies where an insulin infusion did not modulate ketogenesis when postabsorptive concentrations of NEFA were maintained (Beylot et al., 1991; Miles et al., 1983). Hence, the possible postprandial increased conversion of NEFA into ßOHB may be directed toward lowering the surge of dietary NEFA and/or maintain a minimal level of ßOHB to sustain ketogenic functions.

Another factor that could contribute to the rise in NEFA and ketonemia observed under hypoxic conditions is a reduction in peripheral NEFA and KB disposal. Acute hypoxia is usually recognized to favor glucose utilization over lipids to sustain ATP production. Such a shift in substrate oxidation is likely to decrease peripheral NEFA utilization and uptake, leading to an increase in liver lipid influx. It is also likely that KB peripheral oxidation be limited under reduced oxygen availability. Indirect calorimetry data previously published from our CH-Fasted and CH-Fed studies does not suggest a significant whole-body shift toward glucose oxidation under hypoxia (Mahat et al., 2018; Mauger et al., 2019). The present results raise the possibility that acute hypoxia exposure may have affected substrate partitioning, including KB oxidation, in a more significant manner than we first estimated. Balasse et Féry (Balasse & Féry, 1989) demonstrated that the fractional clearance rate of KB is strongly decreased with only slight elevations in ketonemia in humans, suggesting that KB clearance is saturable and that factors still unknown limit ketone utilization by human tissues. Whether acute hypoxia may further limit the utilization of KB in humans remains unknown and warrant further studies.

Some limitations and strengths of this study warrant discussion. First, only plasma ßOHB concentrations were measured which forbids us from inferring about a possible increase in ketogenesis. However, current knowledge of KB kinetics suggests that the changes we observed in ketonemia are likely explained by an upregulation of ketogenesis rather than a reduced uptake capacity from peripheral tissues (Balasse & Féry, 1989). Additionally, our samples consisted solely of healthy young men, which prevents us from

extending our conclusions to women, older adults, and less healthy individuals. The main strength of the present study lies on the statistical strength of the crossover design, that counterbalance the relatively small sample size of each study so that changes in plasma levels of ßOHB were detected. The comparison of three different prandial status could also be considered as a proof of the robustness of the hypoxia-induced raise in ketonemia since the difference between normoxia and hypoxia is visible thenceforth the postprandial decreasing effect over plasma NEFA declines.

CONCLUSION

We tested whether acute normobaric hypoxia would raise circulating ßOHB levels in response to the anticipated elevations in NEFA levels. Although no direct link or mechanism can be inferred from our observations, we found that acute normobaric hypoxia tends to elevate circulating NEFA and ßOHB concentrations in healthy men. The ketonemic effect of hypoxia is however abrogated in the hours following the ingestion of a meal while the effect upon NEFA levels is considerably reduced. Ketone bodies are important metabolic and signaling mediators (Puchalska 2017). Hence, further understanding of the regulatory and metabolic cascade leading to changes in ketone bodies production can provide further insights into the homeostatic responses of humans to oxygen deprivation.

RESOURCE IDENTIFICATION INITIATIVE

N/A

LIFE SCIENCE IDENTIFIERS

N/A

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

RM, JF-M, and PI conceived and designed the experiments. RM and JF-M performed the experiments. CM, JF-M, and PI analysed the data. CM, RM, JF-M and PI interpreted the data. All authors edited, revised, and approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

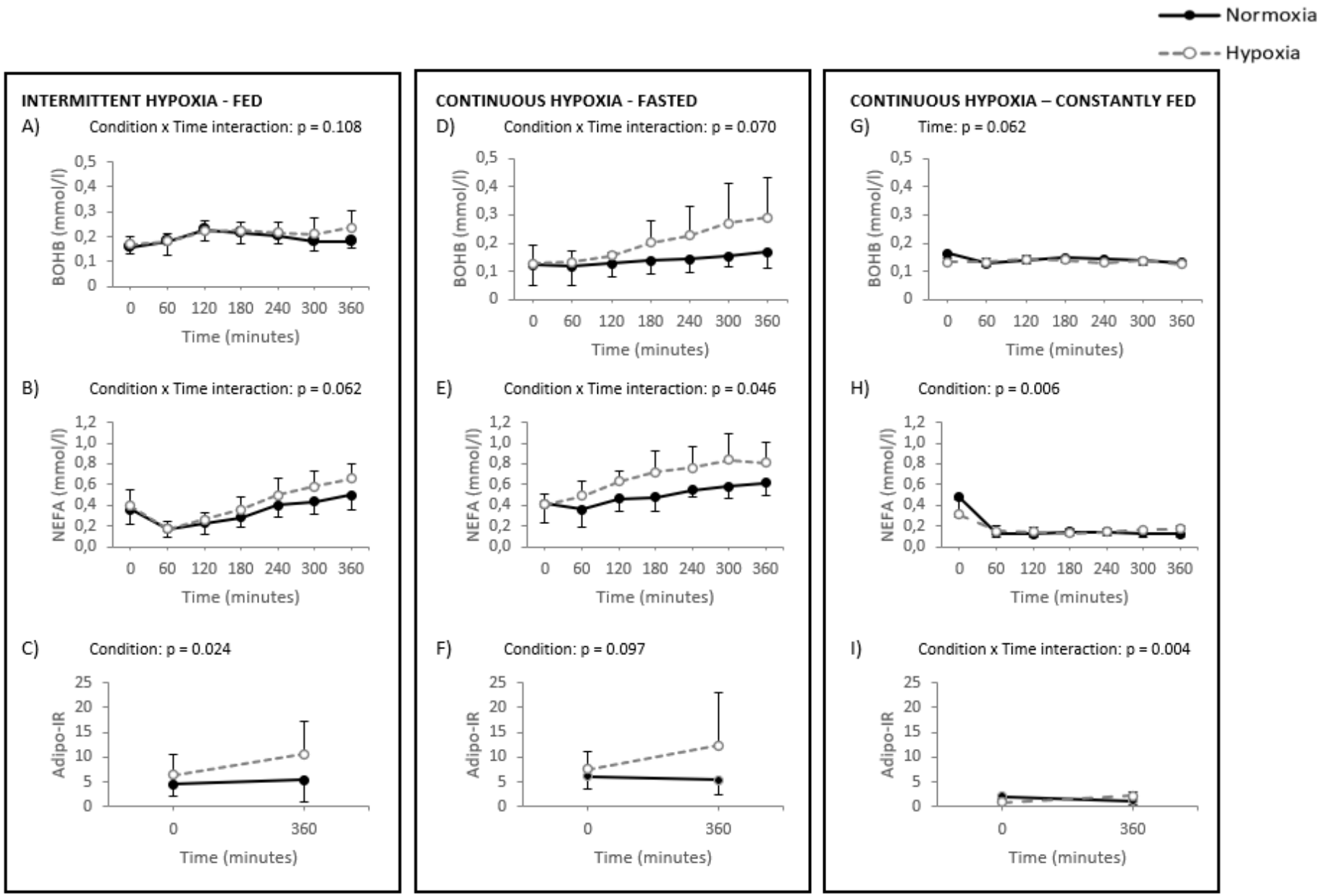

FIGURE 1. Plasma ß-hydroxybutyrate (ßOHB), nonesterified fatty acids (NEFA), and adipose tissue insulin resistance index (Adipo-IR), measured during 6 hours of normoxia or intermittent hypoxia following an isocaloric high-fat meal (Panels A, B and C), during 6 hours of normoxia or continuous hypoxia under fasted stat (Panels D, E, F) and during 6 hours of normoxia or continuous hypoxia in a constantly fed stat (Panels G, H, I), in healthy men. P values exclude time 0 in IH-Fed and CH-Fed trials. Values are means ± standard errors

TABLE 1. Characteristics of participants in the intermittent hypoxia (IH-Fed) and continuous hypoxia (CH-Fasted and CH-Fed) studies

	IH-Fed	**CH-Fasted**	**CH-Fed**
Participants	n = 14	n = 10	n = 9
Age (y)	23 ± 3.5	26 ± 5.6	24 ± 4.5
Height (cm)	180.5 ± 6.6	177.9 ± 4.7	178.9 ± 3.6
Weight (kg)	85.5 ± 11.8	79.9 ± 8.9	77.8 ± 8.0
Body mass index (kg/m^2)	26.2 ± 3.5	25.2 ± 2.5	24.3 ± 2.6
Lean mass (kg)	66.2 ± 8.3	58.6 ± 6.7	65.9 ± 5.7
Fat mass (kg)	16.3 ± 9.1	17.8 ± 9.6	8.8 ± 3.7
Fat mass (%)	18.9 ± 8.1	22.6 ± 10.7	11.5 ± 3.8

Data are expressed as mean ± standard deviation.

TABLE 2. Fasting plasma parameters of participants measured during normoxia, hypoxia intermittent hypoxia (IH-Fed) or continuous hypoxia (CH-Fasted and CH-Fed) studies.

	IH-Fed		**CH-Fasted**		**CH-Fed**	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
ßOHB (mmol/l)	0.16 ± 0.03	0.17 ± 0.04	0.12 ± 0.02	0.13 ± 0.02	0.16 ± 0.05	0.13 ± 0.03
NEFA (mmol/l)	0.36 ± 0.18	0.40 ± 0.21	0.41 ± 0.16	0.41 ± 0.12	0.46 ± 0.22	0.31 ± 0.17
Glucose (mmol/l)	4.45 ± 0.67	4.70 ± 0.61	4.54 ± 0.41	4.53 ± 0.61	4.40 ± 0.18	4.61 ± 0.29
Insulin (pmol/l)	13.29 ± 8.70	16.82 ± 8.99	14.48 ± 8.24	16.59 ± 15.50	36.04 ± 32.78	24.93 ± 17.64
Adipo-IR	4.57 ± 4.42	6.48 ± 5.90	6.17 ± 4.83	7.63 ± 8.34	2.11 ± 1.64	0.99 ± 0.82

Data are expressed as mean ± standard deviation.

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Chapter 4: PERSPECTIVES

Circulating NEFA levels are major precursors of ketogenesis (Balasse, 1986; Robinson & Williamson, 1980). Hence, the rise in NEFA plasma concentration under hypoxia, which increases the hepatic NEFA flux, should lead to an increased synthesis of KB. Furthermore, the current knowledge of KB kinetics, setting the maximal disposal rate around 2.5 mmol/min (Balasse & Féry, 1989), suggests that the observed changes in ketonemia are likely explained by an upregulation of ketogenesis rather than a reduced disposal capacity from peripheral tissues. Thus, the complete physiological significance of increased NEFA levels during acute hypoxia remains to be elucidated, and we hypothesize that it could partly be directed towards an increase in ketogenesis.

The purpose for the increased synthesis of βOHB may be directed towards extra-hepatic oxidation. However, βOHB is not only a metabolite, but also acts as a signaling molecule (J. C. Newman & Verdin, 2014a, 2014b). There are currently two known receptors for βOHB: hydroxycarboxylic acid receptor 2 (HCAR2) and the NOD-like receptor family, pyrin domain containing 3 (NLRP3). HCAR2, also named GPR109a (or PUMA-G in mice), is expressed in AT and immune cells (Graff et al., 2016), and its activation by βOHB inhibits AT lipolysis (Ahmed et al., 2009; Taggart et al., 2005). Therefore, the increase in βOHB levels observed during acute exposure to hypoxia could be aimed at lowering circulating NEFA levels, thereby creating a negative feedback loop depriving βOHB from its main precursor (Rojas-Morales et al., 2016; Taggart et al., 2005).

A rise in plasma concentration of βOHB in response to acute hypoxia may also lead to an increased insulin sensitivity and glucose uptake (Green & Newsholme, 1979; Park et al., 2011). For instance, in diabetic rodents, an infusion of βOHB improved glucose disposal rate without reducing insulin secretion (Park et al., 2011). Another study in mice (Plaisance et al., 2009) demonstrated that the binding of βOHB to HCAR2 induces a significant rise in plasma concentration of adiponectin and a significant decrease in plasma NEFA. Adiponectin is an insulin-sensitizing, anti-atherogenic, and anti-inflammatory hormone secreted by adipocytes (Achari & Jain, 2017)

The increased plasma concentration of βOHB in hypoxic conditions may be useful for its anti-inflammatory properties. Hence, still through the activation of HCAR2, βOHB acts as an anti-inflammatory messenger (Graff et al., 2016). It was suggested that the activation of HCAR2 by βOHB in

physiologically stressful situations, such as during nutrient deprivation, prevents immune system activation to spare metabolite reserves through the reduction of energy expenditure (Rojas-Morales et al., 2016).

Moreover, under ketoacidosis, βOHB modulates the SNS by decreasing β-adrenoceptor affinity, which inhibits catecholamine-stimulated lipolysis (Pergola et al., 1990). This decreased β-adrenoceptor affinity seems to be specific to human fat cells and takes places through the inhibition of NLRP3 (also named GPR41) (Kimura et al., 2011). This phenomenon might represent another negative feedback loop that prevents uncontrolled lipolysis and indirectly regulates βOHBs' own production rate.

Additionally, increasing interests are oriented toward the ability of KB to inhibit histone deacetylase (HDAC). This promotes hyperacetylation of histone proteins, which leads to an open chromatin environment and activates transcription, inducting a stress response (Rojas-Morales et al., 2016; Shimazu et al., 2013). Some of the genes known to be affected by KB are associated with resistance to oxidative stress (Shimazu et al., 2013), which is observed under hypoxic conditions. NAD^+, an acceptor of hydrogen ions, is a marker for mitochondrial and cellular health. The action of βOHB against oxidative stress lies on its capacity to promote a high ratio NAD^+:NADH (Elamin et al., 2017; Rojas-Morales et al., 2020; Shimazu et al., 2013; Veech et al., 2019; Xin et al., 2018) and to facilitate NADH oxidation.

Altogether, the antilipolytic and insulinotropic actions, and the inhibitory effects towards the immune system, the sympathetic stimulation, and histone deacetylation are major functions of βOHB. Interestingly, these are all aspects, such as dyslipidemia (Gangwar et al., 2020; Yin et al., 2009), modulation of insulin sensitivity (Poulain et al., 2017; van Hulten et al., 2021; Y. Wang et al., 2017), inflammation (Eltzschig, 2011; Sozer et al., 2018), sympathetic activation (Eichhorn et al., 2017), and oxidative stress (Guzy & Schumacker, 2006), impacted by hypoxia. Considering the overlap of these information, it might be very instructive to define whether βOHB modulate some of these effects under hypoxia. Hypothesizing an affirmative response, the induction of a rise in ketonemia through ketone ester drinks may be an avenue to explore whether KB ca mitigate some adverse effects related to acute hypoxia exposure.

Moreover, heterogenous results, both adverse and beneficial, were reported regarding the impact of hypoxic exposure on insulin sensitivity (Oltmanns et al., 2004; Peltonen et al., 2012; Poulain et al., 2017; van Hulten et al., 2021; Y. Wang et al., 2017). In addition to the heterogeneity of protocols, the variety of the participants health status have an incidence on the physiological responses to hypoxia. Under hypoxia,

Morin *et al.* (2021) reported an increased glycemia, increased insulinemia and lower plasma levels of NEFA in participants living with OSA, when compared to healthy volunteers. Additionally, some studies reported that ketogenesis is hampered in humans living with metabolic disorders and/or obesity (Balasse & Féry, 1989; Bickerton et al., 2008; Inokuchi et al., 1992; Sherwin et al., 1976; Wildenhoff, 1975), and both conditions are well represented in the population affected by hypoxic disorders such as OSA patients (Catrina & Zheng, 2021; Ferreira-Santos & Pereira Rodrigues, 2018; Ye et al., 2007). Hence, it might be revealing to determine if people suffering from metabolic disorders and/or obesity have a reduced ketogenic capacity under hypoxia. If βOHB can modulate some adverse effects of hypoxia, while ketogenesis is reduced in individuals living with obesity and metabolic disorders, could an increased ketonemia, through the intake of exogenous ketones, alleviate some of the adverse effects of hypoxia? Further studies are required to answer these questions.

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