No Effect of Acute Normobaric Hypoxia on Plasma Triglyceride Levels in Fasting Healthy Men

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No Effect of Acute Normobaric Hypoxia on Plasma Triglyceride Levels in Fasting Healthy Men

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Abstract

Circulating fatty acids are a major systemic energy source in the fasting state as well as a determinant of hepatic triglyceride (TG)-rich very low-density lipoprotein (VLDL) production. Upon acute hypoxia, sympathetic arousal induces adipose tissue lipolysis, resulting in an increase in circulating non-esterified fatty acids (NEFA). Animal studies suggest that TG clearance may also be strongly reduced under hypoxia, though this effect has been shown to be dependent on temperature. Whether the hypoxia-induced rise in blood fatty acid concentrations affects fasting TG levels in humans under thermoneutral conditions remains unknown. TG, NEFA and glycerol levels, were measured in fasted healthy young men (n=10) exposed for six hours to either normoxia (ambient air) or acute hypoxia (fraction of inspired oxygen (FIO₂) = 0.12) in a randomized, crossover design. Participants were casually clothed and rested in front of a fan in an environmental chamber maintained at 28 °C during each trial. Under hypoxia, a significantly greater increase in NEFA occurred (condition x time interaction, p=0.049) and glycerol levels tended to be higher (condition x time, p=0.104), suggesting an increase in adipose tissue lipolysis. However, plasma TG levels did not change over time and did not differ between the normoxia and hypoxia conditions. In conclusion, acute exposure to normobaric hypoxia under thermoneutral condition in healthy men during fasting state increased lipolysis without affecting circulating TG.

Keywords: acute hypoxia, high altitude, plasma triglyceride, non-esterified fatty acids, fasting healthy men.
Résumé

Les acides gras circulants sont une source majeure d’énergie ainsi qu’un déterminant de la production hépatique de lipoprotéines de très faible densité (very low-density lipoprotein, VLDL) riches en triglycéride (TG). Sous hypoxie, l’activation sympathique induit la lipolyse du tissu adipeux, résultant à une hausse des concentrations d’acides gras non estérifiés (NEFA) circulants. Les études animales suggèrent que le catabolisme des TG en circulation peut aussi être fortement réduit sous hypoxie, bien que cet effet dépende de la température. Il demeure inconnu si la hausse des concentrations sanguines de NEFA induite par l’hypoxie affecte la triglycéridémie chez l’humain à jeun dans des conditions thermoneutres. Les concentrations de TG, de NEFA et de glycérol ont été mesurées chez de jeunes hommes en santé et à jeun (n=10) exposés 6 heures à de l’air ambiant (fraction inspirée en oxygène ($FIO_2$) = 0.21, normoxie) ou à de l’air appauvri en oxygène ($FIO_2$ = 0.12, hypoxie) de façon randomisée et selon un devis chassé-croisé. Les participants étaient vêtus normalement et au repos devant un ventilateur à l’intérieur d’une chambre environnementale maintenue à 28 °C. Lors de la session hypoxique, les concentrations plasmatiques de NEFA ont augmenté davantage en fonction du temps (interaction condition x temps, p=0.049) et les concentrations plasmatiques de glycérol tendaient à augmenter (condition x temps, p=0.104). Toutefois, les concentrations plasmatiques de TG n’ont pas changé au cours du temps et ne différaient pas entre les conditions normoxie et hypoxie. En conclusion, une exposition aiguë à l’hypoxie normobarique sous condition thermoneutre chez des hommes en santé en situation de jeûne augmente la lipolyse du tissu adipeux sans affecter les TG circulants.

Mots-clés: hypoxie aiguë, haute altitude, triglycéride plasmatiques, acides gras non estérifiés, hommes en santé à jeun.
Introduction

Restriction in oxygen ($O_2$) supply and/or increased $O_2$ consumption can lead to oxyhemoglobin desaturation and tissular hypoxia (Brahimi-Horn and Pouysségur 2007, Johnson et al. 2010). Recent animal studies demonstrated that chronic (Drager et al. 2012, Yao et al. 2013) and acute exposure to hypoxia (Jun et al. 2012, 2013) induces large augmentations in circulating triglyceride (TG) by increasing hepatic TG secretion in the fasted state and delaying TG clearance in the postprandial state. Proper TG metabolism is critical for global energy homeostasis. Furthermore, it is thought that impaired lipid storage and over exposition of organs to circulating lipids can lead to ectopic fat storage and lipotoxicity, which have been linked to impaired insulin secretion and reduced peripheral insulin signaling as well as the development of chronic diseases such as type 2 diabetes and cardiovascular disease (CVD) (Kalofoutis et al. 2007, Miller et al. 2011).

In humans, some studies examined blood TG concentrations following exposure to different hypoxia environments (normobaric vs hypobaric, altitude from 2000m up to 8800m) for various durations (from 2 hours up to 8 months) (Whitten and Janoski 1969, Férézou et al. 1988, Young et al. 1989, Leaf and Kleinman 1996, Siqués et al. 2007, Stöwhas et al. 2013). Results from these studies are conflicting, with reported increases (Whitten and Janoski 1969, Young et al. 1989, Siqués et al. 2007), decreases (Férézou et al. 1988, Stöwhas et al. 2013) or no change (Leaf and Kleinman 1996) in fasting plasma TG concentrations with hypoxia. This lack of consistency regarding the effects of hypoxia on TG concentrations in humans may be a consequence of poor control for confounding factors such as physical activity and diet. Based on recent animal studies, another important confounding factor that may have not been properly controlled for is thermal conditions. Indeed, the effects of acute hypoxia on plasma TG concentrations have been reported to be temperature-dependent and virtually absent in animals studied at thermoneutrality (Jun et al. 2013).
Fasting circulating TG concentrations reflect the balance between hepatic very low-density lipoprotein (VLDL)-TG secretion and peripheral VLDL-TG clearance (Parks et al. 1999, Barrows and Parks 2006). Hepatic VLDL-TG production, on the one hand, is thought to be a function of fatty acids availability for hepatic TG synthesis. In the fasting state, 70-80% of total liver VLDL-TG production derives from non-esterified fatty acids (NEFA) (Barrows and Parks 2006). NEFA availability, in turn, depends mainly on white adipose tissue lipolysis which is under both sympathetic and hormonal control (Desvergne et al. 2006) with catecholamines and insulin being respectively the main activator and inhibitor. The peripheral clearance of VLDL-TG, on the other hand, is catalyzed mainly by the lipoprotein lipase (LPL) and the hepatic triglyceride lipase (HL), the activity of both being assessable in post-heparin plasma (Després et al. 1999). Interestingly, we recently showed that hypoxia strongly reduces LPL activity in differentiated human preadipocytes (Mahat et al. 2016), but no study yet reported fasting post-heparin lipase activity in response to hypoxia in humans.

Despite relatively strong evidence from animal studies supporting an important deleterious impact of acute hypoxia on triglyceridemia (Jun et al. 2012, 2013), the effect of acute hypoxia on blood lipid homeostasis in fasting humans remains elusive. Therefore, the present study examined the effects of an acute 6-hour hypoxia exposure on plasma TG concentrations in resting and fasting healthy young men. Confounding factors such as physical activity and diet prior to the study, as well as ambient temperature during hypoxia were controlled. The fasting state was chosen to favor peripheral lipolysis and hepatic NEFA delivery and experimental sessions were conducted at thermoneutrality because humans, through proper clothing, usually live in such conditions. Beside plasma lipid levels, proxies of adipose tissue lipolysis and plasma lipase activity were also investigated. We hypothesized that acute hypoxia exposure in the fasting state would increase plasma TG concentrations by increasing hepatic NEFA availability for VLDL-TG production and by decreasing peripheral TG clearance.
Materials and Methods

Subjects

Thirteen healthy young men (age: 18-39 y) were recruited from the University of Ottawa population. Two participants dropped out after completing one session due to schedule conflicts and 1 participant dropped out due to altitude sickness (headache and severe vomiting during exposure to hypoxia). Body mass 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). Body fat was estimated by dual energy X-ray absorptiometry (General Electric Lunar Prodigy, Madison, Wisconsin; software version 6.10.019). On average, subjects were 26 ± 5.6 years, 177.9 ± 4.6 cm tall and weighed 79.9 ± 8.8 kg of which 22.6 ± 10.7% was fat tissue. The average time between each experimental session was 6.4 days, and participants’ weight (± 0.25 kg) did not differ between experimental sessions. Exclusion criteria included: a history of physician-diagnosed asthma or other respiratory illness, hypertension, CVD, diabetes, habitual bedtime occurring after midnight, shift work, and a current smoking habit. Study subjects provided written consent and the study protocol was approved by the Research and Ethics Board of the University of Ottawa.

Experimental Protocol

This was a randomized crossover study consisting of two experimental sessions. Prior to each session, volunteers were counseled to sleep at least 7 hours per night, refrain from any exercise, caffeine and alcohol for at least 36 hours, and to consume the same evening dinner the day before each session. Participants wear their usual interior cloths. During each trial, an 18” diameter mechanical fan (High 117 velocity orbital air circulator, Whirlpool, Benton Harbor, MI, USA) set at an appropriate speed (maximum air velocity of ~4.0 m/s) was used to ensure the participants thermal comfort. The temperature and relative humidity were stable at 28 °C and 45% respectively during the experimental sessions. Participants were only allowed to drink water. Before each session, a catheter was inserted in the
antecubital vein for blood sampling. The line was flushed with 10 ml of physiological saline after each
blood draw to prevent coagulation and keep the catheter patent. Three milliliters of blood were discarded
before each draw to remove the saline from the sampling line and prevent any dilution of the blood
sample. Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA).
Volunteers were exposed to hypoxia (fraction of inspired oxygen (FIO₂) = 0.12) and to ambient air
(normoxia) for 6 hours on 2 different sessions in a randomized cross-over fashion. Volunteers remained in
a semi-recumbent position, 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). Blood pressure was measured upon arrival, at mid
experiment (T180) and finally at the end of the experimental session (T360) with an automatic
sphygmomanometer (American Diagnostic Corporation, E-sphyg 2, Hauppage NY, USA) following the
Canadian Society of Exercise Physiology (CSEP) standard procedures (“CSEP-PATH: Physical Activity
Training for Health” n.d.).

Normobaric Hypoxia Exposure and Altitude Sickness Symptoms

All sessions were performed in an environmental chamber at the University of Ottawa. During the
normoxia sessions, only ambient air was used (FIO₂ = 0.21). During hypoxia, O₂ extractors (CAT 12,
Altitude Control Technologies, Lafayette, Colorado, USA) connected to the environmental chamber kept
FIO₂ level stable at 12%. The CAT system uses 2 stable zirconium O₂ sensors in parallel to detect random
sensors drift. The sensors are calibrated with ambient air (assuming an ambient air O₂ concentration of
20.94%) when sensors disagree by more than 0.5% O₂. During hypoxia, O₂ concentration was also
continuously monitored by the constantly self-calibrating Vmax system used for indirect calorimetry. O₂
readings from both systems were always within 0.5%. No validated scale or questionnaire was used to
monitor altitude sickness symptoms. Participants were instead frequently asked to report any discomfort
related to altitude sickness with special attention to symptoms listed in the Lake Louise consensus scoring
system (LLS): headache, gastrointestinal upset (anorexia, nausea, or vomiting), fatigue or weakness, and
dizziness/light-headedness (Savourey et al. 1995).

**Substrate Oxidation Rate**

Substrates oxidation rates were determined by indirect calorimetry using a continuously self-calibrating
Vmax Encore 29 System metabolic cart (VIASYS Healthcare Inc, Yorba Linda, CA). \( \dot{V}O_2 \) and \( \dot{V}CO_2 \)
were measured for 30 minutes every hour and are expressed in STPD. \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were corrected to
account for protein oxidation assuming a constant oxidation rate of 60 mg of protein per minute. Total
carbohydrate (CHO), and lipid oxidation rates (g/min), were calculated using the protein-corrected \( \dot{V}O_2 \)
and \( \dot{V}CO_2 \) with the following formulas (Elia 1991):

\[
\text{CHO oxidation rate (g / min)} = 4.59 \ \dot{V}CO_2 \text{ (l / min)} - 3.23 \ \dot{V}O_2 \text{ (l / min)}
\]

\[
\text{Lipid oxidation rate (g/min)} = -1.70 \ \dot{V}CO_2 \text{ (l / min)} + 1.70 \ \dot{V}O_2 \text{ (l / min)}
\]

Total energy expenditure was calculated using the estimated oxidation rates of CHO, lipids and proteins
and the following energy equivalent: 3.896 kcal/g CHO, 9.751 kcal/g lipids, 4.708 kcal/g proteins.

**Fasting Plasma Metabolic Parameters**

Plasma was obtained by centrifugation at 3200 rpm for 12 minutes at 4 °C immediately after blood
collection. Commercially available colorimetric enzymatic assays were used to measure plasma total TG,
NEFA, glucose (Wako Chemicals USA Inc, VA, USA), lactate and glycerol (Cayman Chemical, Ann
Arbor, Michigan). Insulin was measured by enzyme-linked immunosorbent assay (EMD Millipore,
Darmstadt, Germany) as previously described (Imbeault et al. 2009, Mahat et al. 2016). Assay analyses
were completed in duplicate and the intra-assay coefficients of variation were approximately 3%.
Plasmatic lipolytic activity was measured using the EnzChek fluorescent TG-analog substrate (Basu et al.
2011) on blood samples collected 20 minutes following the injection of heparin (60 U/kg).
Statistical Analysis

All values in texts and figures are reported as mean ± standard deviation. SPSS version 12 for Windows was used for data analysis (SPSS Inc. Chicago, IL, USA). Repeated measure analyses of variance (ANOVA) were performed with condition and time as within-subject’s parameters. A level of significance of p<0.05 was considered statistically significant.

Results

Side-Effects, Oxyhemoglobin Saturation and Heart Rate Responses to Acute Hypoxia

Fasting and hypoxia were well tolerated although most participants reported drowsiness. Only 1 participant experienced severe nausea and vomiting, leading to his exclusion of the study. One participant experienced severe dizziness while standing up and headache at rest and another participant experienced dizziness. Mean heart rate was significantly increased by approximately 20% in hypoxia (p=0.001) compared to normoxia. Mean oxyhemoglobin saturation was significantly reduced by more than 15% (p=0.001) during acute hypoxia compared to normoxia. Neither systolic blood pressure nor diastolic blood pressure differed between conditions.

Substrate Oxidation Rate

CHO and lipid oxidation rates during normoxia and hypoxia are depicted in Figure 1. CHO oxidation rate decreased significantly and similarly over time in both conditions (time effect, p=0.004) (Figure 1A). Lipid oxidation rate increased significantly and similarly over time in both conditions (time effect, p=0.003) (Figure 1B). Energy expenditure remained relatively stable over time and did not differ between experimental conditions (condition x time, p=0.609) (data not shown).

Plasma Metabolic Parameters
Fasting plasma TG, NEFA, glycerol, and insulin concentrations during normoxia and hypoxia are depicted in Figure 2. Plasma TG concentrations did not change over time and did not differ between experimental conditions (condition x time, p=0.544) (Figure 2A). The over-time increase in plasma NEFA concentrations was 95% greater under hypoxia (condition x time interaction, p=0.049) (Figure 2B). Glycerol concentrations remained constant under normoxia but tended to rise under hypoxia (condition x time, p=0.104) (Figure 2C). No differences were observed in total post-heparin plasma lipolytic activity between normoxia and hypoxia (p=0.233) (data not shown). In both conditions, insulin levels significantly decreased over time (time effect, p=0.032) but tended to be higher overall during hypoxia (condition effect, p=0.061) (Figure 2D). Plasma glucose did not change over time and did not differ between experimental conditions (condition x time, p=0.461) (data not shown). Lactate levels remained relatively stable over time and were significantly higher during hypoxia (condition effect, p=0.028) (data not shown).

Discussion

This study aimed at determining the effect of an acute 6-hour bout of hypoxia on plasma TG concentrations in fasting healthy young males. Plasma TG are important risk factor in the development of chronic diseases such as type 2 diabetes and cardiovascular diseases (Kalofoutis et al. 2007, Miller et al. 2011). Recent evidence from animal studies suggest that O₂ deprivation as experienced during journeys at altitude or in the context of diseases such as chronic obstructive pulmonary disease and sleep apnea, can substantially raise plasma TG concentrations (Drager et al. 2012, Jun et al. 2012, 2013, Yao et al. 2013). If such a response occurs in humans, individuals frequently exposed to hypoxia could be vulnerable to cardiometabolic complications. Some studies have reported conflicting results regarding the effect of hypoxia on plasma TG concentrations in humans (Whitten and Janoski 1969, Férézou et al. 1988, Young et al. 1989, Leaf and Kleinman 1996, Siqués et al. 2007), which could be due to a poor level of control for
confounding factors such as physical activity, diet and environmental conditions, namely temperature. To
our knowledge, this is the first well-controlled study to report the effects of acute normobaric hypoxia on
fasting blood lipid profile in humans. We hypothesized that the combined effects of fasting (low
insulinemia) and hypoxia (sympathetic arousal) would increase NEFA delivery to the liver and increase
plasma TG concentrations. We show that acute hypoxia progressively increases fasting NEFA (95%
greater increase) and glycerol (33% increase) levels, suggesting an increased in adipose tissue lipolysis,
but do not alter post-heparin plasma lipolytic activity nor circulating TG concentrations in young men
with normal adiposity level.

Our findings corroborate observations by Leaf and Kleinman (Leaf and Kleinman 1996) who reported no
change in plasma TG levels in humans exposed to simulated altitude, although they used less severe
hypoxia conditions for a significantly shorter duration (FiO₂ = 16%, equivalent to 2200m altitude for 2
hours). Altogether, these observations seem conflicting with emerging evidence from animal studies
showing a strong and rapid deleterious impact of hypoxia on lipid metabolism (Muratsubaki et al. 2003,
Jun et al. 2012, 2013). Discrepancies in TG response to hypoxia may be related to two important factors,
namely the thermal conditions and the nutritional status during which hypoxia occurs. In terms of thermal
conditions, Jun et al. (Jun et al. 2013) have shown that, in mice, elevations in TG levels in response to
hypoxia occurs in cold conditions (22 °C) but not at thermoneutrality (30 °C). They showed that cold up-
regulates TG uptake in several tissues, namely brown adipose tissue, favoring sustained low TG levels in
cold exposed rodents. At thermoneutrality, they demonstrate that mice TG levels are considerably higher
than those of counterparts kept at 22 °C and that hypoxia no further increased plasma TG in these
conditions. Whether a similar cold-hypoxia interaction is species-specific or occurs also in humans is
unknown and warrant further research. However, recent experiments done on cold-acclimated humans
showed no effect of a 5-hour cold exposure both on postprandial TG levels and dietary TG clearance rate
(Blondin et al. 2017), suggesting that the lipid response to cold exposure is not as strong in humans as in
rodents.
Regarding the influence of the nutritional status on the lipid response to hypoxia, experiments conducted in rodents by Muratsubaki et al. (Muratsubaki et al. 2003) showed that fasted rats, contrary to sated rats, show no increase in plasma TG levels when exposed for 5h to hypoxia (9.45% O$_2$). Fasting is recognized to decrease circulating TG concentrations by stimulating skeletal muscle LPL activity (Lithell et al. 1978) and whole-body fatty acid oxidation rates (Koutsari et al. 2011). Our observations suggest that the TG-lowering effects of fasting are not significantly altered acutely by hypoxia. To determine whether the nutritional status affects the lipid response to hypoxia in humans, a study examining the effect of hypoxia on lipid metabolism in the constantly fed state is currently being undertaken in our laboratory.

Despite no changes in plasma TG concentrations, the increase in plasma NEFA concentrations from baseline to 360 minutes was 95% greater under hypoxia compared to normoxia (Figure 2). It is worth noting that NEFA concentrations showed no evidence of stabilization after 6 hours, which suggest that higher plasma NEFA concentrations could be reached given a longer exposure. Exposure to reduced partial pressure of O$_2$ is well recognized to increase sympathetic activation (Hansen and Sander 2003, Prabhakar and Kumar 2010), which is an important activator of adipose tissue lipolysis. Consistently, the 24% increase in heart rate and the 33% increase in glycerolemia after 360 minutes of hypoxia exposure (Figure 2) are strong indicators that our experimental hypoxia exposure induced sympathetic arousal and stimulated lipolysis. Sympathetic activation is also well recognized to impair insulin sensitivity (Lambert et al. 2015). In this regard, Peltonen et al. (Peltonen et al. 2012) have elegantly demonstrated that the sympathetic nervous system activation induced by hypoxia disrupts insulin sensitivity in humans. Consistent with this observation, fasting insulin levels in our study were 66% greater after 360 minutes of hypoxia exposure (Figure 2) despite similar glucose levels (data not shown). This apparent reduction in global insulin sensitivity, if present at the adipose tissue level, may also have contributed to a hypoxia-induced increase in lipolysis by lifting the inhibitory effect of insulin. Importantly, the major increase in plasma NEFA observed in the present study had no apparent effects on fatty acid oxidation according to indirect calorimetry measurements (Figure 1), which is concordant with previous studies suggesting that
acute exposure to hypoxia has no significant effects of lipid oxidation rate (Brooks et al. 1991, Roberts et al. 1996). Since the oxidative disposal of fatty acids was seemingly not altered by hypoxia, it remains possible that the more abundant circulating NEFA under acute hypoxia exposure could eventually serve for hepatic VLDL synthesis. Nonetheless, we observed no significant changes in plasma TG concentrations. It appears unlikely that the higher insulinemia under hypoxia may have inhibited VLDL-TG secretion. Indeed, the suppressing effect of insulin on VLDL production has only been demonstrated under hyperinsulinemic conditions (Lewis and Steiner 1996) whereas in the present study, while insulin concentrations were higher under hypoxia after 6-hours, values were still below baseline (fasting) levels. Another possible explanation for the absence of shift in plasma TG despite an increase in NEFA availability is that NEFA are not utilized directly as an energy substrate in organs or for VLDL assembly in the liver, but first enter a temporary and probably expendable intracellular TG pool (Gibbons and Burnham 1991). This buffering capacity of the liver and/or peripheral organs could delay an increase in hepatic TG output in response to a rise in plasma NEFA and mitigate the effects of acute hypoxia on TG metabolism in humans. On the other hand, one could speculate that if tissue TG accumulation is not, in the longer term, compensated by an increase output in hepatic VLDL-TG or an increase in lipid oxidation, hypoxia could lead to ectopic fat storage and favor the development of metabolic abnormalities such as insulin resistance.

The present study has some limitations. First, the main endpoint, plasma TG concentrations, does not provide all the information regarding TG metabolism. The use of stably-labelled tracer infusions (Adiels et al. 2015) could allow to better estimate lipid and lipoprotein production and clearance rates and provide a more detailed picture of the effect of hypoxia on blood lipid homeostasis. Second, the duration of the hypoxia exposure was restrained to 6 hours to limit the burden, fasting time and potential side-effects on the hypoxia naïve participants. Whether a prolonged exposure could induce significant changes in plasma TG concentrations remains to be tested. A third limitation regards the homogeneity of our study sample, which consisted exclusively of healthy young men. This prevents the generalisation of our observations to
women and/or metabolically deteriorated individuals. Whether individuals characterized by greater body fat % or by an adversely altered lipid metabolism could be affected differently by hypoxia will have to be addressed.

Conclusions

The current study supports the hypothesis that acute exposure to normobaric hypoxia increases adipose tissue lipolysis but the resulting increase in fatty acid availability does not translate into elevated circulating TG concentrations in fasting healthy men.

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Competing Interests

The authors declare that they have no competing financial interest.
References


Figure Legends:

Figure 1. (A) Carbohydrate (CHO) oxidation rate, and (B) Lipid oxidation rate measured for 6h during normoxia and acute hypoxia sessions in young healthy men in fasting state. Values are mean ± standard deviation.

Figure 2. Effect of normoxia or acute hypoxia on fasting plasma (A) Triglyceride, (B) Non-esterified fatty acids (NEFA), (C) Glycerol, and (D) Insulin levels in healthy men. Values are mean ± standard deviation.
Figure 1.

**A)**

- **Normoxia**
- **Acute hypoxia**

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