

EFFECTS OF INTERMITTENT NORMOBARIC
HYPOXIA ON SUBSTRATE UTILIZATION AND
METABOLIC RATE IN SEDENTARY MALES

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**Effects of Intermittent Normobaric Hypoxia on Substrate Utilization
and Metabolic Rate in Sedentary Males**

By

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ABSTRACT

The purpose of this study was to determine the effects of intermittent normobaric hypoxia on resting metabolic rate and substrate preference in sedentary males. After a 12-hour fast, 11 sedentary male subjects (acute) were individually exposed to 3 hours of intermittent normobaric hypoxia, achieving an isometabolic stress of ~80% oxygen saturation. A subset of 6 subjects (short term) continued with the hypoxia exposure for a further 6 sessions. Resting metabolic data was acquired immediately before and after the hypoxic exposure on day 1 for the acute exposure group and on days 1 and 7 for the short-term exposure group. Heart rate, blood pressure, oxygen saturation and Acute Mountain Sickness Score were monitored and recorded. In the acute exposure group, there was a significant increase in oxygen consumption, from 315 ml·min (± 10) to 371 ml·min (± 10). As well, there was a significant reduction of the respiratory exchange ratio, from 0.89 (± 0.1) to 0.76 (± 0.06). There was also a significant increase in oxygen consumption in the short-term exposure group, from 322 ml·min (± 10) on pre-exposure day 1, to 376 ml·min (± 7) post exposure on day 7 and a significant reduction in respiratory exchange ratio from 0.86 (± 0.09) on pre-exposure day 1 to 0.73 (± 0.05) post exposure day 7. These results

demonstrate that intermittent normobaric hypoxia, as described here, is sufficient to induce an increase in resting metabolic rate resulting in a switch from primarily carbohydrate to primarily lipid substrate as the main energy source.

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* significantly different from pre- to post-treatments, and from day 1 to day 7.

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List of Abbreviations and Symbols

VO₂ Max: Maximal Oxygen Uptake

RER: Respiratory Exchange Ratio

INH: Intermittent Normobaric Hypoxia

PAR-Q: Physical Activity Readiness Questionnaire

AMS: Acute Mountain Sickness

EPOC: Excess Post Exercise Oxygen Consumption

BMI: Body mass index

BMR: Basal metabolic rate

BP: Blood pressure

CG: Control group

CHOox: Carbohydrate oxidation

EE: Energy expenditure

FATox: Fat oxidation

HR: Heart rate

kcal: Kilocalorie

PAH: Passive acute hypoxia

PAR-Q: Physical Activity Readiness Questionnaire

PSH: Passive short-term hypoxia

SpO_2 : blood O₂ saturation

VCO_2 : Volume of carbon dioxide

VO_2 : Volume of oxygen

Co-Authorship Statement

The following information describes my role in the development, execution, and written preparation of this thesis.

1. **Design and identification of the research proposal:** This project was an original research idea as described by Dr. Fabien Basset. I met with him on several occasions to discuss and refine the research methodology.
2. **Practical aspects of research:** A research assistant and I collected the raw data for this study. Data for the sham group was provided by Dr. Basset from a previous, related study.
3. **Data analysis:** I reduced and summarized the raw data, and Dr. Basset performed the statistical analysis.
4. **Manuscript preparation:** Dr. Basset and I prepared the manuscript.

CHAPTER I THESIS OVERVIEW

1.1 Overview of the Thesis

This thesis, entitled “Effects of Intermittent Normobaric Hypoxia on Substrate Utilization and Metabolic Rate in Sedentary Males,” is presented in manuscript format.

Chapter 1 describes the background, purpose and significance of this study.

Chapter 2 consists of a review of the relevant literature in regards to the measurement of metabolic parameters using indirect calorimetry, physiological adaptations after hypoxic exposure, and the current obesity epidemic affecting much of the industrialized world.

Chapter 3 presents the results of intermittent hypoxic exposure on substrate utilization and metabolic parameters in 11 sedentary, overweight male subjects in either an acute or short-term setting.

Chapter 4 concludes the thesis with a response to the research hypothesis.

This is a brief summary of the major findings as well as the limitations of the research methodology.

1.2 Background of Study

Obesity has become an increasing health concern, affecting many industrialized nations around the world. According to World Health Organization (WHO) statistics, in 2005, approximately 1.6 billion people over the age of 15 were overweight, and at least 400 million people were obese. As well, at least 20 million children under the age of 5 were overweight (Organization, 2006). Current approaches to assist overweight and obese adults with weight loss have resulted in less than ideal long-term outcomes. Results from a number of studies have revealed that losing weight is a challenge, and maintaining weight loss presents an even further challenge. Jehn, Patt, Appel & Miller (2006) examined the long-term effectiveness of the Diet, Exercise, and Weight-loss Intervention Trial (DEW-IT) on a group of overweight, hypertensive adults. Although the intervention group lost weight in the short term (2 months) during the intervention period, 95% of the group re-gained weight by the one year follow-up. In a recent literature review focusing on the effects of exercise programs and dietary interventions, Hansen et al. (2007) found that combining dietary restrictions with an exercise program was an effective strategy for fat-mass loss. The authors emphasized the importance of an

exercise program to avoid a loss of lean mass, and that endurance training appears to assist the loss of central adiposity (Hansen, Dendale, Berger, van Loon, Meeusen, 2007).

Exercise is a common component of a weight loss intervention strategy because of the health benefits associated with increased levels of fitness, as well as the increase in daily caloric expenditure. Several studies have demonstrated the effectiveness of both endurance and strength training programs in achieving weight loss. Physiological adaptations to exercise of sufficient intensity lead to an increase in resting metabolic rate measured as caloric expenditure, an increase in lipid use as an energy substrate, an increase in aerobic enzyme cycling (Achten, Jeukendrup & Asker, 2004; McArdle, Katch, & Katch, 2007), and a decrease in several cardiovascular risk factor parameters.

However, some overweight and obese individuals meet with limited success due to their inability to fully participate in exercise programs. Barriers may include a lack of motivation, poor exercise tolerance, or pain with movement attributed to an increased body mass (Wills, 2006). For those who do attempt exercise programs, some are unable to exercise at sufficient intensity

to provide the physiological stress necessary to initiate metabolic adaptations of the skeletal muscles (Byrne & Hills, 2002).

Intermittent normobaric hypoxia (INH) has been utilized by athletes for several years in the attempt to expose the body to physiological stress above and beyond typical cardiovascular training regimens. Results have revealed that INH can induce physiological adaptations, including an increase in capillary density, hemoglobin concentration, aerobic enzyme concentration, mitochondrial number and density, and increases in basal metabolic rate, as well (Hoppeler & Vogt, 2001; Hoppeler & Fluck, 2003). These physiological changes are similar to what an individual may expect to see with consistent endurance or strength training. The study of INH has broadened to include other subject groups such as the untrained, elderly, and overweight. Similar physiological adaptations are observed in sedentary and elderly adults exposed to INH (Vogt, Puntschart, Geiser, Zuleger, Billiter, Hoppeler, 2001; Shatilo, Korkushko, Ischuk, Downey, Serebrovskaya, 2008).

1.3 Purpose of Study

The purpose of this theses study is to determine the effects of a specific passive INH protocol on resting metabolic rate and substrate utilization in sedentary males. Our hypothesis is that INH will cause an increase in the resting metabolic rate and a shift in substrate use preferentially towards lipids.

1.4 Significance of Study

This theses study will provide further insight into the role of intermittent normobaric hypoxia on oxidative metabolic systems as measured by changes in oxygen consumption and substrate selection. Although much research in the past has examined the effects of hypoxia on physiological parameters in athletes, little information is known regarding the changes that occur in sedentary, overweight individuals after hypoxic exposure. If physiological adaptations such as increased metabolic rate and substrate shift towards lipid as a preferred energy source can be induced by hypoxic exposure, it may be possible to translate this technique into a therapeutic strategy in the treatment of overweight and obesity conditions.

CHAPTER 2 REVIEW OF LITERATURE

2.1 Epidemiology of Obesity

Obesity has become a predominant health issue for many industrialized nations. Obesity is commonly defined by body mass index (BMI kg/m^2), as this provides a simple measure to categorize a complex topic. A person is defined as being overweight if their BMI is over 25, and obese if their BMI exceeds or equals 30. Obesity is further sub-divided into three levels, also according to BMI: grade I, BMI 30-35; grade II, BMI 35-40; and grade III, BMI >40 . The WHO refers to obesity as an epidemic that affects both adults and children, and continues to progress. According to WHO statistics, in 2005, approximately 1.6 billion people over the age of 15 were overweight, and at least 400 million people were obese. As well, at least 20 million children under the age of 5 were overweight (WHO, 2006). Obesity can be found in all parts of the world. In the United Kingdom, in 2003, 43% of men and 33% of women were considered overweight, and 22% of men and 23% of women were considered obese. In children aged 2 to 15 years, over 643,000 boys and 613,000 girls were overweight, while another 746,000 boys and 675,000 girls were obese. These numbers are expected to increase dramatically in the future. Figures for obese men in the

U.K. are predicted to increase from 4.3 million to 6.6 million, and, for women, from 4.7 million to 5.9 million (Zaninotto, Wardle, Stamatakis, Mindell, Head, 2006).

In the United States, the 2005 Behavioral Risk Factor Surveillance Survey (BRFSS) concluded that 60.5%, 23.9%, and 3% of the total adult population interviewed were overweight, obese, and extremely obese, respectively.

This is an increase from previous surveys, as overall age-adjusted obesity rates were 15.6% in 1995, 19.8% in 2000, and 23.7% in 2005 (Blanck et al, 2006). In Asia, overweight and obesity values are also increasing. In China, a 2002 survey revealed that 14.7% and 2.6% of the population were overweight and obese, respectively. The prevalence of obesity is relatively low compared to most western nations, however, the rate of increase has been most dramatic. Overweight rates have increased 28 times, and obese rates 4 times, in Chinese children between 1985 and 2000 (Wu, 2006). There has also been an alarming increase in the prevalence of type 2 diabetes in many Asian countries, which, in part, may be related to the increased rates of obesity (Yoon et al. 2006).

In Canada, according to the 2004 Canadian Community Health Survey, the national population average was 23% obese and 36.1% overweight. This is a significant increase from 15.2% obese in 2003 (Canada, 2006).

Provincial differences were also noted in both overweight and obese values. Newfoundland and Labrador showed the highest provincial average, at 34.5% obesity, while British Columbia had the lowest, at 19.2%. As well, persons living in urban centers had lower rates of obesity (20%) when compared to rural dwellers (29%) in most provinces.

For Canadian children, the highest rates of obesity/overweight were seen in Newfoundland and Labrador, at 36%, and the lowest rates were found in Alberta, at 22%. The National average for obese/overweight children was 26% (Canada, 2006).

Obesity in children can become evident early in life. In a study by Canning and coworkers, health records from the Preschool Health Check Program of the 1997 cohort of children born in Newfoundland and Labrador were analyzed. From this data, it was determined that 25.6% of preschool aged children (3-5 years) were either overweight or obese (Canning, Courage, Frizzell, 2004). Similar results were seen in American, Australian, and British studies (Hedley, Ogden, Johnson, Carroll, Curtin, Flegal, 2004;

Mason, Meleedy-Rey, Christoffel, Loongjohn, Garcia, Ashlaw, 2006; Zuo, Norberg, Wen, Rissel, 2006; Robbins, Khan, Lisi, Robbins, Michel, Torcato, 2007).

Several studies have examined the relationship between childhood and adult obesity. Investigators have followed groups of children and recorded their BMI as the children grew into their adult years. A study by Whitaker et al. (1997) showed that 52% of American children who were obese at age three to six were also obese at age 25. In comparison, only 12% of children who were normal-weight at age three to six years were obese at age 25 (Whitaker, Wright, Pepe, Seidel, Dietz, 1997; Anderson & Butcher, 2006).

As well, children under the age of 10 who had obese parents were more than twice as likely to become obese as adults (Whitaker, 1997).

Sun Gou designed a logistic model to predict overweight and obesity in adulthood from body mass index values during childhood and adolescence (Sun Gou, Wu, Chumlea, Roche, 2002). Application of this model concludes that, if an individual has a high BMI anytime between the ages of 3 to 20 years, then they have a high risk of being overweight or obese at age 35 years. The authors note that the risk increases with age: The older a child

is at the time of having a high BMI percentile, the higher the risk of they have of becoming overweight or obese by the age of 35.

2.1.1 Causation

It is clear that obesity rates have increased globally over the past several years. Additionally, many obese children are growing up to become obese adults.

Several studies have examined the potential reasons for this significant change in society. Physical inactivity, high caloric diets containing poor quality foods, genetic factors, and the biological role of adipose tissue are examples of topics currently being researched in relation to obesity. Lake and Townsend published an article describing the “Obesogenic Environment”(Lake & Townsend, 2006). This details the trends in obesity with “the sum of influences that the surroundings, opportunities, or conditions of life have on promoting obesity in individuals or populations” (Swinburn & Eggar 2002 p292). While our convenient access to food, and lessened opportunities for physical activity, are important environmental considerations, an individual’s behavior and choices are influenced both by

his environment and values. The authors acknowledge that the obesogenic environment is a very complex and dynamic issue that requires research in individual areas as well as collaboration amongst multi-disciplinary professionals (Lake & Townsend, 2006).

The aim of this area of research is to determine how an individual's environment may play a role in them becoming obese or maintaining obesity. Access to areas that facilitate physical activity is one component of the environment. There is evidence to support that physical activity levels amongst both children and adults have decreased over the past several years, as well. Statistics Canada reports that only 33% of adults meet the recommended amount of daily physical activity, and over 50% of children are not active enough. The term "active enough" refers to an energy expenditure of at least eight kilocalories per kilogram of body weight per day (Institute, 2001 p. 15). Janssen et al. conclude that physical inactivity and sedentary lifestyles have a strong relation to obesity. The authors found that physical activity levels were lower and television-viewing times were higher in overweight and obese than in normal-weight children (Janssen, Katzmarzyk, Boyce, King, Pickett, 2004). Although other studies have found less of a relation between sedentary activities like television watching

and rates of obesity, most research has found a general increase in media time for adolescents. This may include television, computer use, or video games. The *content* to which individuals are exposed during media-time may be as important as the *quantity* of time they are spending on various media activities. A study was conducted evaluating the type of advertisements being shown on three American television broadcast networks, which air specifically to a preschool audience. The results show that over half of the food advertisements were aimed specifically at children. The majority of these were for fast food chains or sugary cereals. Children, who are already spending more time watching television, are being subjected to advertising media promoting fast food and sugary snacks. The author states that the advertisements were mainly focused on building brand recognition through the use of characters, slogans, and logos that are associated with fun and happiness, or excitement and energy (Connor, 2006). Many parents feel that it is the role of the school environment to assist in promoting physical activity. Children spend considerable time at school and have access to physical education classes, sports, and playgrounds. There is conflicting information regarding the effect of physical education and recess activities at school on the physical activity levels of students. One study

found that increasing the amount of time allocated to physical education each week did not have a profound outcome on obesity rates in students (Cawley, Meyerhoefer, Newhouse, 2006). Another, however, found an increase in students' physical activity levels with the introduction of structured fitness breaks throughout the day (Scruggs, Beveridge, Watson, 2003). Researchers have also found an increase in children's physical activity levels through simple interventions such as introducing multicolor playground markings or providing game equipment during recess time (Stratton & Mullen, 2005). Researchers also note that increases in physical activity levels do not necessarily correlate to changes in obesity. This emphasizes the multi-factorial complexity of obesity.

In the case of adults, the message of the importance of physical activity has reached most of the population. Adults understand that they should eat well, participate in moderate exercise, and reduce levels of stress in their lives (Shephard & Bouchard, 1995). The most common reason provided for not participating in an exercise program among adults is lack of time. Other reasons often cited are lack of interest, confusion about what type of program to do, and concern about physical ailments. Some facilitators to exercise include corporate wellness programs, indoor walking programs, and

media information describing simple yet effective methods to achieve the minimal recommended daily activity. Canadian adults realize that poor nutrition, obesity, and sedentary behavior pose health risks comparable to smoking and excessive alcohol use (Lakka, Laaksonen, Lakka, Mannikko, Niskanen, Rauramaa, Salonen, 2003).

2.1.2 Co-morbidities and Economics

Obesity and physical inactivity have been associated with a number of co-morbid conditions, independent of each other. According to Katzmarzyk & Janssen (2004), the literature provides evidence to support a relationship between physical inactivity and coronary artery disease, stroke, hypertension, colon cancer, breast cancer, type 2 diabetes, and osteoporosis. Obesity is associated with all of the pre-mentioned conditions, except osteoporosis, and also includes gall bladder disease and osteoarthritis. Relative risk for these conditions as a result of physical inactivity ranges from 1.30 for hypertension to 1.60 for stroke. As a result of obesity, relative risk for these conditions ranges from 1.45 for colon cancer to 4.50 for hypertension. This informs us that both physical inactivity and obesity present their own health concerns, although obesity is of a higher magnitude. The authors go on to present the actual health care cost associated with these two issues. Physical inactivity costs the Canadian health care system a total of \$5.3 billion dollars annually, while costs associated with obesity total \$4.3 billion. These costs include direct health care expenses such as hospitalization as well as indirect costs such as loss of work or premature death (Katzmarzyk & Janssen, 2004).

The metabolic syndrome has a higher prevalence in obese and sedentary individuals (Lakka, 2003). According to the International Diabetes Federation (IDF) definition, for a person to be defined as having the metabolic syndrome they must have central obesity as well as any two of raised triglyceride levels, reduced HDL cholesterol, raised blood pressure (greater than 130/85), or raised fasting plasma glucose (or previously diagnosed type 2 diabetes). These constitute the major risk factors for myocardial infarction or stroke (Alberti, Zimmet, Shaw, Grundy, 2006). Sleep apnea is another common issue encountered by overweight and obese individuals. Several studies have been performed examining the health issues caused by sleep apnea, secondary to obesity (Gami, Hodge, Herges, Olson, Nykodym, Kara, Somers, 2007). Sleep apnea is a form of chronic hypoxia, which carries vastly different metabolic consequences than intermittent hypoxia and, as such, this topic will not be expanded upon in this paper. Aside from physical co-morbidities, individuals who are obese may also encounter various psychosocial problems. Depression, a poor quality of life, and emotional issues are examples of areas currently being researched (Werrij, Mulkens, Hospers, Janses, 2006; Zeller, Roehrig, Modi, Daniels, Inge, 2006).

2.1.3 Interventions and Outcomes

Obesity has garnered significant government and media attention in the past two decades. As a result, the number and types of interventions designed to reduce obesity have increased dramatically. Obesity is a complex issue involving physical, mental, social, and environmental components. Interventions have been targeted at the individual, community, national, and global levels. The scientific literature contains a plethora of articles and studies examining various aspects of obesity ranging from genetics, pharmacology, diet and nutrition, lifestyle modification, exercise and physiology, social and psychology issues, and medical/surgical approaches. These topics have been studied independently and in various combinations. It is difficult to establish a definition of effectiveness regarding obesity. For some, a reduction in an individual's body weight or BMI is considered a success. For others, reduction of co-morbid complications such as cardiovascular disease or hypertension would be considered successful. Because of the wide range of foci available in the literature regarding interventions and effectiveness for obesity management, several ideas will be briefly discussed here.

Snethen and collaborators recently completed a meta-analysis examining the effectiveness of weight loss intervention programs for children. The authors state that there is a lack of sufficient long-term follow-up studies examining the effectiveness of these programs. From the studies available, it was concluded that programs including dietary education, physical activity, behavioral change, and parental involvement could be effective in achieving weight loss in children. The specific details of each component are different for each study; therefore, the effectiveness of a single component of an intervention program could not be established (Snethen, Broome, Marion, 2006).

Avenell et al. (2004) performed a review of randomized control trials to determine the optimal combination of weight loss strategies for adults.

According to the studies reviewed by the authors, a multi-faceted approach is also required for successful weight loss in adults. This is similar to the conclusions established by Snethen et al. (2006) regarding weight loss in children. The components for successful weight loss are similar in children and adults, although Avenell et al. (2004) comments on the use of medications, which was not mentioned by Snethen. Avenell et al. (2004) concludes that a combination of medications (orlistat or sibutramine),

exercise, behavior modification, and diet advice can improve long-term weight loss in adults (Avenell et al, 2004).

In a recent literature review focusing on the effects of exercise programs and dietary interventions, Hansen et al. (2007) found that combining dietary restrictions with an exercise program was an effective strategy for fat-mass loss. This combination was more effective than an exercise program alone. It is interesting to note that combining these two approaches does not result in increased amounts of fat-mass loss compared to dietary restrictions alone. The authors emphasize the importance of an exercise program to avoid a loss of lean mass, and that endurance training appears to assist with loss of central adiposity. This point is important, as there is a correlation of central adiposity and co-morbid health risks (Hansen et al. 2007).

Meta-analysis and review articles are able to provide an excellent summary of current evidence in the literature and, in the case of the preceding studies, provide us with evidence that weight loss interventions can be successful in achieving desired weight loss in both children and adults. For the specifics of the programs, one must now delve into the individual studies to determine which methods lead to weight loss and which do not.

Jehn et al. (2006) examined the long-term effectiveness of the Diet, Exercise, and Weight-loss Intervention Trial (DEW-IT) on a group of overweight, hypertensive adults. Participants were involved in a supervised exercise program three times per week and were provided with all of their meals. The exercise program was described as of moderate intensity; however, details on the mode of exercise were not provided. As well, the term *moderate intensity* does not provide a quantifiable definition of energy expenditure or oxygen consumption. It is unclear as to whether these subjects participated in an aerobic program, strength training program, or both. Meals were a low-calorie version of the Dietary Approaches to Stop Hypertension (DASH) diet. Although the intervention group lost weight in the short-term (2 months) during the intervention period, 95% of the group re-gained weight by the one-year follow-up (Jehn et al. 2006). The pattern of weight loss followed by weight gain is a common phenomenon reported by many obese individuals. Strategies to maintain weight loss are an important consideration of any comprehensive weight loss program and have also been the topic of research in several studies.

Fogelholm (2005) conducted a review of studies examining the effects of a walking program on weight loss and maintenance of weight loss. A

prescribed daily walking program of 25 to 30 minutes per day has been shown to improve high-density lipoprotein profile, insulin sensitivity, and cardiorespiratory fitness; however, weight reduction is not always evident. The review suggests that increasing the duration to a minimum of 35-45 minutes walking per day is consistent with successful weight reduction. After weight loss is achieved, maintenance of that loss requires a further increase of exercise duration. A walking program of 70 to 80 minutes per day appears to be associated with maintaining a stable weight for 1 year after weight loss (Fogelholm, 2005). Although duration is an important consideration, the intensity of the walking program is of equal importance. Intensity parameters have not been described in the author's conclusions, however; an energy expenditure of 2200–2400 kcal/week corresponds to 70 to 80 minutes per day of walking activity. (Fogelholm, 2005).

Behavior modification and stress reduction have also been cited as important components of a weight loss program. The effects of specific strategies to achieve behavior modification and stress reduction that lead to weight loss independent of exercise and diet have been difficult to quantify. Elder and colleagues sought to determine the effects of an alternative form of mind-body therapy to assist with weight loss maintenance in a group of obese

individuals. Participants first completed a 12-week behavioral weight-loss program. This program incorporates behavioral support, social support, and motivational components into the curriculum. It is delivered in a group setting and uses group discussion and support. Sessions last approximately 90 minutes. (Elder et al. 2007) Those who met threshold weight-loss criteria were then admitted to the weight-loss maintenance study. Participants were randomized into one of two treatment groups or one control group. The control group received self-directed support in the form of written materials and group meetings to discuss progress. The treatments were either qigong or Tapas acupressure technique (TAT). Each group received ten hours of contact time for their particular intervention over the 12-week period.

Qigong is an ancient healing discipline developed in China, and is a component of Traditional Chinese Medicine. It is a discipline consisting of breathing and mental exercises and is usually combined with physical exercises. For this protocol, the specific Qigong techniques included “Shaking,” “Movements,” and “Harvest the energy method”: The qigong treatments were thirty minutes for each session.

Acupressure was developed as a system of treatment more than 5000 years ago. Acupressure points are places on the skin that are especially sensitive to

bioelectrical impulses, and these impulses are readily conducted in the body. Stimulating these points with pressure, needles, or heat produces changes in the energy flows within and around the body to promote healing, relieve pain, and alter the chemical balance within the body and mind.

The general instructions to patients were as follows:

1. Identify the belief or action that is hindering weight loss/maintenance.
2. Focus on that negative image while holding the TAT pose: specifically, applying pressure to acupuncture points GB21, BL1, and *Yin Tang* for 30 seconds. These points are located just medial to the eyes, above the nose, and behind the head.
3. Transition the focus onto the opposite positive image while holding the same points for an additional 30 seconds.
4. Hold the TAT pose while focusing on “all the origins of this problem... healing now.”(Elder, 2007, p. 67)
5. Hold the TAT pose while resolving that “all the parts of [oneself] that got something out of having this problem are healing now.”(Elder, 2007, p. 67)
6. Forgive everyone you have blamed for the problem, and ask forgiveness of everyone who has been hurt because of the problem.

81 participants completed the study protocol. Results suggest that the acupuncture technique assisted subjects in maintaining weight loss at the 24-week follow-up period. This group showed an average of 1.2kg greater weight loss maintenance than the control group, and no weight regain (Elder, Ritenbaugh, Mist, Aicken, Schneider, Zwickey, Elmer, 2007).

For some individuals faced with serious health concerns related to obesity, surgical intervention has become an increasingly frequent option. This is included in the intervention section because of the increase in the number of surgeries performed and an increase in the number of medical centers offering the procedure.

Hansen (2006) reports on the results of bariatric surgery techniques, including gastric banding, gastroplasty, and gastric bypass. By creating a smaller gastric pouch, gastric banding limits the amount of food that the stomach will hold at any time. The inflatable ring (band) controls the flow of food from this smaller pouch to the rest of the digestive system. Individuals will feel comfortably full with a small amount of food. And because of the slow emptying, individuals will continue to feel full for several hours, reducing the urge to eat between meals. Gastroplasty achieves similar

results by dividing the stomach into two portions using a surgical stapling technique. The upper portion is very small (egg size) and therefore reduces caloric intake. Gastric bypass involves reducing the size of the stomach, and also bypassing much of the upper portion of the small intestine. The distal portion of the new smaller stomach is attached directly to the jejunum, bypassing the lower portion of the stomach and the duodenum. This causes fewer calories to be absorbed, which leads to weight loss. Gastric surgeries are successful at allowing people to achieve sustainable weight loss. As well, there are observed improvements in co-morbidities such as insulin resistance, hypertension, dyslipidemia and hepatic steatosis (Hansen, Torquati, Abumrad, 2006). Side-effects and complications of surgery do not appear to have a higher incidence than other abdominal-type procedures (Kushner & Noble, 2006).

The literature outlines the effects of diet change, exercise programs, behavioral therapy, drug therapy, surgical intervention, and combinations and permutations of these interventions. Overall, it appears that weight loss is possible in both children and adult populations. The programs need to consist of a variety of interventions specific to the needs of the individual. As well, long-term follow-up to prevent regaining weight is crucial.

Health professionals and exercise specialists who work with obese populations need to be aware of the uniqueness of this group. While sustained weight loss and improvements in co-morbid issues are important, the method and rate at which these occur is also an important factor. For example, it is recommended that weight loss occur at less than 1.5kg per week. The daily diet should continue to provide a small fat supply (10g/day); also, encourage the client to eat breakfast every day to avoid long fasting periods. These recommendations are in place, in part, to avoid the increased risk of formation of cholesterol gallstones that may occur in the obese individual with rapid weight loss (Wills, 2006). This has been linked to a super-saturation of bile with cholesterol:

This super-saturation is due to a disproportionately high rate of cholesterol secretion relative to bile acids and phospholipids, attributable to an increased synthesis and secretion of cholesterol in the liver. (Wills, 2006)

When designing a cardiovascular exercise program, heart rate is commonly used as an indicator of exercise intensity, as heart rate is easily monitored. It is understood that there is a linear increase in heart rate with oxygen consumption as work intensity increases. The American College of Sports Medicine recommends that healthy individuals exercise at intensities of 40%

to 85% of maximal oxygen consumption, which corresponds to 55% to 90% of maximal heart rate. Byrne & Hills (2002) conducted an investigation into the relationship between heart rate and oxygen consumption in obese individuals, to determine if a similar relationship between maximal oxygen consumption and maximal heart rate exists in this group. Thirty-two sedentary obese adults participated in this study—17 women and 15 men. Each subject had a BMI above 30 and did not participate in regular physical activity for the preceding 12 months. The subjects performed a graded treadmill test to determine cardiorespiratory fitness. They also underwent body anthropometry and resting metabolic rate measures. Blood samples were drawn at the end of each stage of the treadmill test in order to determine lactate threshold. Results of this study show a high degree of variability in the relationship between percentage of maximal oxygen consumption and percentage of maximal heart rate in obese individuals. The authors conclude that percentage of peak oxygen uptake and heart rate should not be used as the only method to determine activity intensity because, in this population, a specific percentage of peak oxygen uptake or heart rate is related to a non-specific exercise intensity, due to the high degree of variability observed in this population (Byrne & Hills, 2002).

Painful joints secondary to osteoarthritis or gout must be recognized and programs adjusted accordingly. Resting and exercise heart rates may be higher in obese individuals for several reasons such as a decline in parasympathetic tone, potential increases in extracellular volume, and increased blood flow to many tissues. As well, many obese individuals report altered or insufficient sleep patterns resulting in non-restorative sleep. This can also lead to changes in circadian rhythms, which in turn affect the metabolic rate (Mortola, 2007).

Obese individuals may also have reductions in total lung capacity and vital capacity, and increased residual volume. These changes may be due to altered respiratory mechanics, or may be related to a neurological impairment of central ventilatory control. Sleep apnea and hypoventilation syndrome can contribute to the altered pulmonary volumes because of the increased arterial carbon dioxide levels (hypercapnia) and reduced oxygen levels (hypoxemia). This will certainly affect their exercise tolerance. Skin breakdown in areas of skin folds or areas subjected to pressure must also be addressed and prevented (Wills, 2006).

2.2 Exercise Physiology

Endurance training is an integral component of an exercise program aimed at weight reduction. Strength training is also essential in order to avoid muscle loss while on restricted diets. The intensity, duration, frequency, and mode of exercise are also important to consider when evaluating an exercise program. Restricted energy intake does not appear to be an effective means, on its own, to induce maintainable weight loss. A significant component of the prevalence of weight gain in the population in recent years may be attributable to decreases in energy expenditure. One theory is that sedentary behavior has caused a decrease in daily energy expenditure. Endurance exercise programs may not provide enough increased energy expenditure to compensate for sedentary behavior. Calories that we would typically utilize during non-exercising activities, such as ambulation and daily activities, are now stored as adipose tissue (Kotz & Levine, 2005).

Endurance training has been shown to improve the health status of individuals. Physiological adaptations that occur include an increase in the size and number of skeletal muscle mitochondria, which improves the ability of the muscle to produce ATP aerobically. There is also an increase in

enzymatic activity associated with the mitochondrial change. This may allow an individual to maintain a higher percentage of their aerobic capacity during prolonged exercise. An increase in capillarization to the trained skeletal muscle is also evident. The ability of the muscle to use fat as a fuel during sub-maximal exercise improves with aerobic training. This allows the muscle to conserve glycogen for high intensity exercise (Achten, 2004; McArdle, 2007).

Hoppeler (2001) agrees, and adds that there is an increase of the mitochondrial volume by up to 50% in previously untrained subjects as a result of endurance training. As well, there is a shift of substrate preference as metabolism in the skeletal muscle places a higher reliance on lipids (Hoppeler, 2001; Hoppeler, 2003; Hoppeler, 2003).

Studies have been performed to determine the effects of endurance training on fat loss in obese individuals. In a review by Hansen et al. (2007) it was concluded that visceral fat depots are more sensitive to endurance training than gluteal-femoral depots. As well, increases in fat oxidation from non-plasma free fatty acids have been reported during exercise. The sources may be lipoprotein or intra-muscular triacylglycerol (Hansen, 2007). This information validates the positive health effects of endurance exercise for the

obese individual. Although the actual number of “pounds lost” may differ from one individual to the next, the effects of increased fat use as an energy source, decrease in insulin sensitivity, decrease in blood pressure, improved lipid profiles, and prevention of lean tissue mass loss should be emphasized. Also, there is an increased chance of maintaining weight loss in persons participating in exercise programs.

Malenfant (2001) stated a “reduction in adipose tissue mass accounts for the major proportion of weight loss in obese subjects...weight loss does not appear sufficient to correct the high intracellular lipid concentrations” (Malenfant, 2001, pE638).

A closer look at the skeletal muscle in an obese individual reveals some physiological changes that alter the traditional mechanism of energy use and production. Blaak (2004) has found that the uptake and oxidation of fatty acids is impaired during the post-absorptive state in abdominally obese individuals and persons with type II diabetes. Also, these individuals have an inability to increase fatty acid uptake and oxidation during beta-adrenergic stimulation and exercise. This physiological change may promote fat storage or, in the case of an inability to oxidize the fatty acid, promote increased intramuscular triacylglycerol levels. The author also

notes that these physiological disturbances continue after individuals lose excess weight. For this reason, the sequence and order of events leading to obesity and insulin resistance require further study.

In a subsequent study, Blaak (2005) explains that the disturbance in skeletal muscle lipid utilization may occur at one of several points on the metabolic pathway. These points include adipose tissue lipolysis, fatty acid transport, muscle fatty acid uptake, and fatty acid oxidation. There are key stages in each of these more general levels where impairment in the system will continue to affect the metabolic process further downstream. The altered fatty acid utilization leads to altered insulin-mediated glucose uptake. This change in glucose handling will eventually lead to changes in insulin sensitivity. The author ties together the relation between impaired lipid handling and insulin resistance, and eventual type 2 diabetes (Blaak, 2005). Bonen et al. (2004) have examined the role of fatty acid translocase, fatty acid transport 1, and fatty acid binding protein in the increased skeletal muscle triacylglycerol content seen in some obese individuals. They found in obese individuals an increased uptake of long chain fatty acids into the skeletal muscle. Although uptake is increased, oxidation of fatty acids did not change. This led to an increased storage of triacylglycerol in the muscle.

In their study, they saw a redistribution of fatty acid translocase to the surface of the sarcolemma in obese muscle. The authors feel that this may be a reason for insulin sensitivity seen in obese individuals.

While it is clear there are alterations in the skeletal muscle of obese individuals, particularly in the effective use of lipid as a substrate, the role of adipose tissue itself is less clear. Adipose tissue is now regarded “as an active participant in regulating physiologic and pathologic processes, including immunity and inflammation,” and not simply cells devoted to energy storage” (Fantuzzi, 2005, p. 917). The condition of obesity is consistent with markers of a low-grade systemic inflammatory state. It is this inflammatory state that researchers believe is linked to co-morbidities such as cardiovascular disease and type 2 diabetes. Adipose tissues release adipokines that help control “appetite, energy use, insulin sensitivity, endocrine systems, inflammation and immunity” (Fantuzzi, 2005, p. 912). Of the adipokines, leptin and adiponectin are of significant interest. Leptin is responsible for the control of appetite and also participates in immune function. High levels of leptin are associated with increased adipose tissue mass, while low levels are associated with increased susceptibility to

infection. Leptin is responsible in part to a pro-inflammatory state, while also protecting against infections.

Adiponectin has a major role in the regulation of insulin sensitivity. This adipokine appears to have low levels in the obese individual, and very low levels in individuals with type 2 diabetes. Researchers believe that adiponectin has an anti-inflammatory role. The combined effects of these and other adipokines, as well as cytokines produced by white adipose tissue, have a role in the low-grade inflammation seen in obese individuals.

Fantuzzi explains that the site of adiposity is also important, as visceral or central adiposity is associated with increased risk of cardiovascular disease and type 2 diabetes.

Bruce, Mertz, Heigenhauser and Dyck (2005) examined the effect of adiponectin on metabolism in human skeletal muscle. As well, the researchers wanted to determine if there was a difference in the role of adiponectin in lean versus obese muscle. The authors compared muscle biopsies from lean women to those from obese, non-diabetic women. The muscle strips were tested for glucose uptake, fatty acid oxidation, extraction of muscle lipids, AMPK activity and citrate synthase, and beta-hydroxy-acyl-CoA dehydrogenase activity. They found that adiponectin has a role in

fatty acid and glucose uptake in skeletal muscle in both lean and obese muscle. When exposed to insulin, there is an increased effect in lean muscle, but a 50% lower uptake effect in obese muscle. This lowered effect lead the authors to believe there is an adiponectin resistance in obese muscles, which reduces the ability of this adipokine to stimulate glucose and fatty acid uptake.

To summarize, we understand that visceral adipose tissue plays a role in developing co-morbid health conditions. Also, we understand that the obese skeletal muscle may function in a different metabolic manner than non-obese skeletal muscle. Endurance exercise causes physiological adaptations to improve the efficiency of the skeletal muscle and to improve the health status of obese individuals. Exercise appears to have a significant effect on visceral adipose tissue. We will next examine how hypoxia may play a role in the physiological adaptations seen in skeletal muscle.

2.2.1 Hypoxia

Hypoxia is defined as a lower than atmospheric concentration of oxygen. The air we breathe consists of 20.93% oxygen; hypoxia would include any concentration less than this amount. Researchers are interested in the amount of oxygen that reaches body tissues for use in metabolism, and how lower concentrations of oxygen affect the physiology of the body. The effects of hypoxia on human physiology have been an area of interest for many years. Since mountaineers were observed to have decreased body-mass after ascents to altitude, researchers have been interested in determining how the environmental changes of hypoxia and hypobaria affect metabolism.

Hamad & Travis (2006) present a paper discussing several components that may be involved in weight loss at altitude. These include energy balance, basal metabolic rate changes, exercise demands, intestinal function, body composition, and body water changes. There is generally a decrease in appetite at altitude, and this is a common symptom of Acute Mountain Sickness. The underlying reason for this has not been determined; however,

leptin may play a role. Decreases in appetite appear to worsen as altitude increases, or as the duration at altitude lengthens. Increases in basal metabolic rate are also observed at altitude. Hypotheses as to why this occurs include increased thyroid activity, as well as a result of exposure to cold and hypoxia. The authors state that further studies isolating the effects of cold versus hypoxia would help determine which factor was responsible for the increased basal metabolic rate. Activity at altitude is much more demanding as compared to the same activity at sea level. The cardiorespiratory system is faced with the stress of exercise, as well as the added stress of hypoxia. While at altitude, any work performed demands the same oxygen consumption that it would at sea level. However, an individual's VO_2max will decrease as altitude increases due to the decreased availability of oxygen (decreased partial pressure). Therefore, working at altitude causes individuals to perform much closer to their $\text{VO}_2\text{ max}$, compared to performing the same intensity of task at sea level. The authors conclude that climbers to altitude will lose weight because of a negative energy balance. This negative balance occurs as a result of decreased energy intake due to decreased appetite and an increase in basal metabolic rate.

However, the exact mechanism for decreased appetite and increased basal metabolic rate has not been established.

Physiology researchers have long been involved with comparisons of native high-altitude inhabitants and low-land dwellers. Examining how the highlanders have adapted to their hypobaric, hypoxic environment over the generations has created a wealth of biological and physiological data.

Hochachka & Somero (2002) summarized and described acute responses as well as longer term adaptations in lowlanders who have spent time at altitude. The acute response to high altitude includes the hypoxic ventilatory response, which attempts to compensate for low oxygen levels. The hypoxic pulmonary vasoconstrictor response adjusts ventilation-perfusion matching to optimize oxygen loading of the blood in response to low levels of oxygen. Vascular endothelial growth factor 1 (VEGF1) is activated, which initiates angiogenesis. Erythropoietin is activated to increase red blood cell volume, thereby increasing oxygen-carrying capacity. Metabolic alterations are also observed in the acute response to hypoxia. These responses continue with prolonged exposure to the hypoxic environment, and eventually lead to more permanent adaptations. Hochachka & Somero (2002) go on to state that

hypoxia inducible factor 1 (HIF1) plays an integral role in initiating the cascade of responses to the presence of hypoxia. Various oxygen-sensing structures throughout the body will cause an increase in the concentration of HIF1 in response to low levels of oxygen. Once the HIF1 concentration has been increased, the acute responses begin. The observation and initial understanding of these responses and adaptations led researchers to attempt to isolate independent stimuli and measure their associated effects. For example, exposing subjects to a simulated hypoxic environment at sea level allowed researchers to evaluate the effects of hypoxia independent of hypobaria. Research has also examined differing intensities and durations of hypoxia, as well as various combinations of simulated and natural environments. The approach of living at altitude and training at altitude (Living High, Training High) does demonstrate hematological changes that would suggest an improvement in endurance performance; however, the negative effects of a prolonged stay at altitude seem to detract from the benefits. Both “living high, training low” and “living low, training high” show beneficial physiological adaptations without the negative effects of prolonged stay at altitude. The “living high” design is an example of passive hypoxia. This is a condition where the subject is exposed to the hypoxic

environment while at rest. Overall, exposure to hypoxic environments of various intensities and durations have shown metabolic and physiologic changes, as described previously by Hoppeler (2001, 2003). These physiological changes include an increase in capillary density, size and number of mitochondria, red blood cell count, and oxidative enzymes.

Mazzeo (2008) summarizes the factors involved in considering how an individual adapts to altitude:

Included among these are (i) the degree of hypoxia; (ii) the duration of exposure to hypoxic conditions; (iii) the exercise intensity (absolute vs relative workload); and (iv) the inter-individual variability in adapting to hypoxic environments ('responders' vs 'non-responders')(Mazzeo 2008, p.

1)

There have been several studies that examine the effects of hypoxic or altitude training on aerobic performance measures such as oxygen consumption and respiratory rate. (Bernardi, 2001; Rusko, Tikkanen, Peltonen, 2004; Morton & Cable, 2005). These studies agree that hypoxia can increase the physiological adaptations in the muscular system; however,

performance improvements will only be seen at altitude. The improvements will not necessarily be observed when tested at sea level (Morton & Cable, 2005).

Basset et al. (2006) completed a study examining the effects of short-term normobaric hypoxia on highly trained athletes. Eight cross-country skiers and 4 long-track speed skaters were randomly assigned to group A or B. Group A spent eight hours each night over two consecutive nights in a week, over a three-week period, in short-term normobaric hypoxia. Group B spent the same period in normobaric normoxia. The hypoxic environment was created in a tent where the oxygen percentage was controlled to reach 12–14%. After a three-week washout period, the groups switched treatments. Subjects were tested for VO₂ max and time to exhaustion, as well as anaerobic capacity using the Wingate test. These tests were performed before and after each test environment. Blood samples and muscle biopsies were also taken before and after each test environment. Results showed an increase in red blood cell count, haematocrit, haemoglobin, platelet number, and erythropoietin concentration after short-term normobaric hypoxia. Other physiological and hematological parameters such as muscle enzyme activity,

buffer capacity, and capillary density and morphology did not show any change as a result of the treatment. There were also no significant changes in the aerobic or anaerobic performance of the subjects. This study demonstrates the physiological impact of hypoxia, although the adaptations did not translate into increases in athletic performance. This is partially explained by the fact that these athletes had already achieved a high level of fitness and performance prior to the short-term hypoxia treatment.

Vogt et al (2001) have studied the adaptations that occur in muscle while training in a simulated normobaric hypoxic environment. Thirty untrained males were assigned randomly to one of four groups. The groups trained on a cycle ergometer for thirty minutes five times per week for six weeks. Group 1 was high intensity in normoxia. Group 2 was high intensity in a hypoxic environment simulating 3,850 m. Group 3 was low intensity in normoxia. Group 4 was low intensity in hypoxia. High intensity was measured as a training blood lactate level of 4–6 mM, while the low intensity group was 2–3mM blood lactate. The researchers found increases in hypoxia inducible factor-1 (HIF-1) after training in the hypoxic environment, both at low and high intensity. Also, there were increase in mRNA contents of

myoglobin and vascular endothelial growth factor. The authors conclude that HIF-1 is involved in the regulation of muscle adaptations after hypoxic training. Again, physiological adaptations were observed; however, no performance data was collected.

Shatilo, Korkushko, Ischuk, Downey & Serebrovskaya (2008) examined the effects of intermittent hypoxia training on exercise performance in healthy senior men and also evaluated changes in hemodynamics and ventilation. In this study, subjects were divided into two groups. Group 1 included men who exercised on a routine basis, and group 2 consisted of men who avoided exercise. The participants were subjected to normobaric, isocapnic hypoxia for five minutes, four times per day for ten days. The hypoxic periods were separated by five-minute periods of room-air inspiration. The hypoxic periods consisted of a fraction of inspired oxygen (FiO_2) of 12%, which corresponded to an arterial saturation level of 85-86%. Partial pressure of expired carbon dioxide was maintained at 38-40mmHg. The subjects were evaluated on exercise performance both before and after the hypoxia protocol. This included anaerobic threshold and physical work capacity. The exercise protocol was carried out on a cycle ergometer. Anaerobic

threshold was estimated from ventilatory gas exchange indexes, while physical work capacity was determined by peak VO_2 at volitional exhaustion. ECG, blood pressure and ventilation were monitored during the testing protocol. Also, blood tests were performed examining erythrocyte and leukocyte count, hemoglobin, blood glucose, content of alanine aminotransferase, aspartate aminotransferase, bilirubin, creatinine, urea, albumin, and cholesterol. Forearm cutaneous perfusion was also assessed to examine microvascular reactivity. The results show no changes in hemodynamic measures or work capacity in the first group. In group 2, there was a decrease in blood pressure and an increase in submaximal work capacity and anaerobic threshold. The authors conclude that intermittent hypoxia training had greater positive effects in untrained senior men compared to trained men. This study supports the idea that hypoxic stress alone is sufficient to cause improvements in exercise capacity in untrained individuals. This is a similar improvement that we would expect to observe as a result of a cardiovascular exercise program, although of a different magnitude.

Hypoxia and exercise in combination provide two separate stressors for the skeletal muscle that may have compounding effects. Gustafsson et al. (2007) examined the effect of exercise on the angiopoietin and VEGF-A systems in skeletal muscle to help unravel the process of angiogenesis. Eleven male subjects were instructed to perform one-legged exercise four times per week for a five-week period. Each subject exercised one leg for 45 minutes and then the other leg for 45 minutes, with a 10-minute rest between each session. One leg was exercised under normal, unrestricted conditions, whereas the other leg was exercised under restricted blood flow conditions. The blood flow restriction was established by placing the subject in a supine position with both legs in a pressure chamber. The chamber pressure was elevated to 50 mmHg above atmospheric. This caused a reduction of blood flow of about 15-20% to the exercising leg. The subjects were randomly divided into two study groups, where one group exercised with their right leg in a restricted blood flow environment, and the other with their left leg restricted. Muscle biopsies of the vastus lateralis from both legs were taken on three different occasions: at rest before the first exercise session, after ten days of the protocol, and at the end of the five-week session. The authors observed that the exercise protocol on the cycle ergometer caused a change

in the angiopoietin system such that endothelial cell activation occurred. This is a result of changes in the transcriptional factors, and ratios of VEGF-A and VEGFR-2 mRNA. The authors conclude:

“... in response to exercise, the angiopoietin and the VEGF-A systems are transcriptional activated in a temporal fashion known to stimulate angiogenesis and that hypoxia/ischemic-related metabolic perturbation is likely to be involved as stimuli in this process” (Gustafsson 2007, p1019).

Aside from physiological adaptations and changes to athletic performance, research has also been conducted to evaluate the impact of hypoxia interventions on measures of health. Bailey, Davies & Baker (2000) used hypoxic training to determine if such interventions were beneficial in reducing cardiovascular risk factors. They trained healthy men in either normoxic or hypoxic environments for four weeks on cycle ergometers. Subjects trained three times per week for 20-30 minutes at 70–85% of maximum heart rate. The maximal heart rate was determined in normoxia or hypoxia as applicable. The hypoxic environment was maintained at FIO₂ of approximately 16%. Both groups demonstrated improvements in cardiovascular risk factors such as cholesterol, HDL, LDL and nonesterified

fatty acids. Hypoxic training also reduced systolic blood pressure by 10mmHg and the rate pressure product by 14 mmHg X beats per minute.

The authors conclude that both environments reduce cardiovascular risk factors; however, there was “an additive cardio protective effect” (Bailey 2000, P 1058) with the hypoxic environment.

To summarize, endurance training will cause specific adaptations to the skeletal muscle. Intermittent hypoxia seems to augment the adaptations observed with training in a variety of subject populations. Hypoxia inducible factor 1 is an important component in regulating the adaptations of metabolism, capillarization, and mitochondrial volume.

2.2.2 Substrate Partitioning

The human body has available to it 3 main sources of energy: Carbohydrate, lipid, and protein. At rest, it is generally agreed that approximately one third of the body's energy is derived from the metabolism of carbohydrates, while two thirds derive from lipid. Protein is certainly metabolized, but it makes up a small proportion of substrate used for energy production at rest. During low intensity exercise, the body will continue to rely on lipid as a primary substrate for energy production; however, the proportion of lipid to carbohydrate use begins to change as the intensity of exercise increases. During high intensity exercise, most of the energy for activity is provided by the metabolism of carbohydrate. Intensity of exercise, overall fitness of an individual, and the duration of exercise will each contribute to determine at what point in an activity that metabolism switches from carbohydrate substrate to lipid, and what percentage of each fuel will be utilized for energy production. Although there is always individual variation, the trend reflects an overall shift in substrate utilization with higher intensity exercise.

Haman et al. (2004) have examined the effects of carbohydrate availability during sustained shivering to determine the contribution of oxidation of plasma glucose, muscle glycogen, and proteins. In this study, subjects were exposed to 2 hours of cold that produced shivering. Prior to cold exposure, the first group of men had low levels of glycogen reserves, while the second group had high reserves. It was concluded that the size of carbohydrate reserve prior to cold exposure did not have an impact on heat production, but did significant impact fuel selection before and during shivering. The group with low glycogen reserves utilized lipid as the major substrate for heat production, while the group with high glycogen reserves used mainly carbohydrate as their substrate of choice. The authors conclude that the human body has great flexibility in oxidative substrate selection to maintain heat production when exposed to a prolonged cold environment.

In a further study, Haman et al. (2005) examined the effects of changing the intensity of shivering on substrate selection. Subjects were divided into 2 groups, the first undergoing low intensity shivering and the second undergoing moderate intensity shivering. The study was designed to determine whether the pattern of substrate selection would be similar to that observed in increasing intensity of exercise. That is, as intensity increases,

would substrate oxidization switch from lipid at lower intensity to carbohydrate at a higher intensity? The results of the study demonstrated that substrate selection during shivering of low or moderate intensity does not follow the same type of pattern as typically found during increasing intensity of exercise. During low and moderate intensity shivering, muscle glycogen is the dominant substrate, and it is noted that the oxidation rate of muscle glycogen doubles as shivering intensity increases from low to moderate. As well, the rate of lipid oxidation remains constant as shivering intensity increases from low to moderate levels. It should also be noted that plasma glucose levels increase slightly as shivering intensity increases, reflecting the increase in overall metabolic rate. It is thought that there are several other homeostatic mechanisms involved in this finding, possibly with the end result of preventing hypoglycemia during cold exposure.

It is apparent from these studies that the body has the ability to alter the oxidation rates of lipid, carbohydrate and protein substrates depending on metabolic demand and stressors such as shivering, or exercise. As well, the change in substrate selection can be different depending on the type of stressor and not merely the intensity of metabolic demand.

The question then becomes, will substrate selection change during exposure to acute hypoxic stimuli, and if so, will the change be significant. How will the body alter the oxidation rates of lipid and carbohydrate substrates in response to an isometabolic stress combined with sedentary activity? To answer this, precise measurement of metabolic activity will be required, in our study, using indirect calorimetry.

2.2.3 Indirect Calorimetry

Exercise, as well as exposure to a hypoxic stimulus, has been shown to produce changes in a variety of physiological parameters as previously discussed, as well as changes in metabolic rate and substrate utilization. Metabolic rate and substrate utilization can be determined using indirect calorimetry techniques. Basal metabolic rate (BMR) is defined as “the metabolic rate of an adult animal at rest in a thermoneutral environment and postabsorptive state.” (Hulbert & Else, 2004, p.870). The metabolic rate can be determined by analyzing factors such as oxygen consumption, carbon dioxide production, heat production, or calculating the difference between energy consumed and waste products excreted. Indirect calorimetry estimates metabolic rate from measurements of oxygen consumption and carbon dioxide production. Direct calorimetry, on the other hand, measures total heat loss from the body. Indirect calorimetry works on the assumption that all oxygen consumed is used to oxidize substrates, and that carbon dioxide collected represents all carbon dioxide produced from oxidation. By realizing this relation, one is able to calculate the amount of energy produced (Ferrannini, 1988). Indirect calorimetry is a useful technique because of its non-invasive nature and ease of use. Although the technique is similar, there

are different types of equipment available to collect and analyze oxygen consumption and carbon dioxide production.

The Douglas bag technique has been in use for decades and is considered a classic method for the collection of expired gases. Some authors consider this to be the gold standard of sampling expired gases (Haugen, Chan & Li, 2007). This technique involves collecting a large amount of expired air into the Douglas bag while the total sampling time is recorded. The volume of the air is measured in a spirometer, while the temperature and pressure are also recorded. A sample of the air is analyzed for its fraction of oxygen and carbon dioxide. The oxygen uptake is calculated from these fractions using either assumed or measured concentrations of oxygen and carbon dioxide of the inspired air. This is a precise method, but it is also time consuming and cumbersome.

Equipment and techniques have evolved that now include the use of electronic oxygen and carbon dioxide analyzers. A metabolic cart is another method of indirect calorimetry whereby a subject breathes into a mask or mouthpiece connected to the cart. The cart typically has a mixing chamber where gas samples are obtained and analyzed, or in some instances, breath-by-breath analysis can be performed. Use of a metabolic cart is accurate,

however, requires some technical expertise to maintain. Hand-held indirect calorimeters are small and portable, and usually easy to operate. A subject breathes into a mask or mouthpiece connected to the device. Electronic sensors in the device can measure humidity, barometric pressure and ambient temperature. Breath-by-breath analysis is recorded (Haugen, 2007). Manufacturers of such devices include Vmax, Cosmed, and Deltatrac. Stewart, Goody & Branson (2005) compared two systems of measuring energy expenditure using indirect calorimetry. They compared the DeltaTrac to the Med-Gem RMR analyzer (Healthetech, Golden, CO). DeltaTrac (Datex-Ohmeda, Madison, WI) is a validated, traditional open-circuit indirect calorimeter that utilizes the dilution principle (Weissman, Sadar & Kemper, 1990). The authors tested 18 healthy subjects with the 2 devices simultaneously. This was accomplished by placing the subject under a canopy hood, which was attached to the DeltaTrac metabolic cart. The Med-Gem RMR was also placed under the hood so the subject could breathe directly in to the mouthpiece of this device. The subject's nose was clipped, ensuring that all expired gas first entered the Med-Gem, and then was collected by the DeltaTrac. The subjects were tested in a quiet room, in a reclined position, after fasting for 12 hours and refraining from exercise for

the previous 24 hours. The authors found no statistically significant difference between the devices for measuring oxygen consumption and RMR in their healthy, spontaneous breathing subjects (Stewart et al, 2005).

Medbo, Mamen, Welde, Von Meimburg & Stocke (2002) compared the Metamax I and II oxygen analyzers to the Douglas bag technique and the Vmax 29 instrument. Oxygen uptake and respiratory exchange ratio were the two parameters being evaluated in this study. This comparison was made on healthy subjects during a cycling or treadmill protocol. This comprehensive study utilized different combinations of the devices in series, to allow simultaneous analysis of the same expired air by the different devices. As well, different groups of subjects performed different tasks such as stationary cycling or treadmill running, at different intensities, and different incrementally progressive protocols. These combinations allowed the authors to evaluate each indirect calorimetry device in different situations and different ranges of oxygen consumption. The Douglas bag technique and the Vmax 29 were used as the control devices to which the Metamax I and II would be compared. The authors conclude that the Metamaxes are fairly reliable in terms of reporting oxygen uptake, however, the respiratory exchange ratio (R) is not accurate. In the range of 0.9–1.0, the Metamaxes

show good agreement with the control methods, however, when the R value is higher than 1.0, the Metamax underestimates the true value. And when the R value is below 0.8, the Metamax overestimates the true value.

Interestingly, the authors state that the Metamax reported R-value can be improved by utilizing the collected raw data and applying a mathematical equation. This equation can be found in the original article.

Different indirect calorimetry protocols may also influence the resting metabolic rate (RMR) results. Compher, Frankenfield, Keim & Roth-Youssey (2006) performed a review of literature to determine the ideal subject condition and test methodology to obtain the most reliable measure of RMR with indirect calorimetry. Best practice recommendations were provided based on differing levels of evidence in the literature.

Recommendations can be summarized to include the following factors: fasted for at least 6 hours, and abstained from caffeine overnight, nicotine and alcohol for at least 2 hours, moderate physical activity for at least 2 hours, and vigorous physical activity for at least 14 hours. Testing should be done with the subject in a supine or reclined position. Ambient temperatures should be comfortable for the subject, and testing performed in a quiet, private space. RMR can be obtained from 10 to 15 minutes of testing, and

the examiner should discard the first 5 minutes of data. The author states that this information is best practice guidelines from available literature (Compher, 2006).

It is clear that intermittent hypoxia exposure will cause physiological adaptations similar to the effects of endurance training. As well, endurance training and energy restriction is able to induce weight loss, particularly from adipose tissue mass (Malenfant, 2001).

The question then becomes, will hypoxia exposure induce weight loss in obese individuals? To answer this, one must understand the physiological processes that occur for an individual to become obese. This will include the balance of energy intake and expenditure, the role of hormones such as insulin, the metabolism and storage of lipids, and the substrate usage during rest and activity. Secondly, one must understand how these processes change to ultimately result in a loss of weight. Thirdly, one must determine how hypoxia exposure will affect these processes, either independently or in some form of combination, to induce weight loss.

Few studies examine the effects of a hypoxic environment on physiological parameters that do not include a training component. The effects of short-

term, intermittent hypoxia on metabolism, in a sedentary population independent of exercise induced changes, needs to be addressed. Using a passive hypoxic exposure under normobaric conditions will achieve this goal.

The purpose of the present study, entitled "Effects of Intermittent Normobaric Hypoxia on Metabolic Rate in Sedentary Males," is to determine the magnitude of change in metabolic rate after exposure to one week of intermittent hypoxia (SPO_2 approximately 80%), three hours per day. The hypothesis is that there will be a decrease in the respiratory exchange ratio, representing a shift in substrate utilization at rest to a higher use of lipid. This is a pilot study to examine the feasibility of the hypothesis. If the hypothesis is proven true, further studies will be undertaken to examine the hematological and cellular adaptations occurring as a result of the hypoxia protocol.

CHAPTER 3 MANUSCRIPT

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POST-METABOLIC RESPONSE TO PASSIVE NORMOBARIC
HYPOXIC EXPOSURE IN SEDENTARY OVERWEIGHT MALES:

A PILOT STUDY

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ABSTRACT

Background: The present pilot study was designed to test the impact of acute (PAH) and short-term (PSH) normobaric hypoxic exposures on energy expenditure (EE) and substrates utilisation (glucose and lipid oxidation).

Methods: Eleven participants have completed the PAH session while the control group (CG) underwent a simulated experimental condition in normobaric normoxic condition.

A subset of 6 participants underwent an additional six 3-hour sessions on consecutive days. Metabolic rates were obtained pre- and post-treatments on the morning following an overnight (12 hours) fast in PAH, PSH, and CG groups.

Results: The statistical outcomes showed a significant increase in EE for PAH, control, and PSH while a shift in substrate utilization towards lipid sources was only detected for PAH and PSH, respectively.

Conclusion: this pilot study showed that passive acute normobaric hypoxic exposure did affect EE and fuel utilization in sedentary overweight males and that further passive normobaric hypoxic exposures (PSH) magnified these metabolic adjustments. These

outcomes provide valuable information for further research in the area of hypoxia as a new therapeutic strategy to improve the management of weight loss.

Key Words: Metabolic Rate, Indirect Calorimetry, Substrate Partitioning, Hypoxia

INTRODUCTION

Obesity seriously threatens the public health in the westernized world (Azagury & Lautz, 2011). Even though efforts have been made to reduce obesity, efficient solutions proposed from biological and behavioural sciences often do not appeal to all members of society as an effective means to reduce body fat. Obesity impairs physical performance and leads to an unfitness status that negatively affects whole body metabolism and daily energy expenditure (Menshikova et al., 2005). Even though many scientific reports confirm the beneficial effects of regular physical activity on reduced mortality from all causes, including obesity, a mix of personal (e.g., past experience with exercise, health status), behavioural (e.g., skills), and environmental (e.g., access to facilities, type of program) factors influence both uptake and maintenance of exercise. In fact, most of the obese population in North America has a sedentary lifestyle, and approximately 60% of individuals who initiate an exercise program drop out within 3 to 6 months, well before any significant health benefits (Basset, 2008). New perspectives have, however, emerged from studies on human hypoxia tolerance showing that some effects might be potentially beneficial in specific physiologic or

pathologic conditions and could be an effective means to reduce body fat (Terao, Miyakawa, & Yamanami, 2003). In fact, according to the most recent studies on the topic, moderately obese subjects did significantly lose weight after intermittent hypoxic exposures (Terao, 2003). Hypoxia has also been associated with an augmented metabolic rate and an increase in energy expenditure (Nair, Malhotra, & Gopinath, 1971), a general metabolic perturbation that might affect fuel utilization (Shatilo et al., 2008). Previous studies have reported that the majority of weight loss in lean fit subjects was attributed to fat mass reduction, possibly due to increased fat oxidation (Westerterp & Kayser, 2006; Westerterp et al., 2000; Westerterp-Plantenga, 1999). Yet, what effect low O₂ concentration has on post-hypoxic substrate metabolism is still not fully understood? One can postulate that hypoxia exposure triggers metabolic responses similar to, but not identical with, exercise-induced metabolic disruption (Hochachka, Gunga, & Kirsch, 1998). If so, post hypoxic exposures (acute and short-term) might result in shifting substrate utilization towards lipid sources, due to the greater dependency on glucose under hypoxia (Braun, 2008); a metabolic pattern that slightly differ from the excess post-exercise oxygen consumption concept and its related mechanisms (LaForgia, Withers, & Gore, 2006). To date, however, no study

has examined the effect of passive acute and short-term hypoxic exposure on post-metabolic responses as related to substrate partitioning and energy expenditure. The present pilot study was, therefore, designed to test the impact of acute and short-term normobaric hypoxic exposure on substrate partitioning and energy expenditure. It was hypothesized that (a) acute normobaric hypoxic exposure would shift the fuel utilization towards lipid sources and would increase basal metabolic rate; (b) short-term normobaric hypoxic exposure would result in a cumulative effect on the above-mentioned metabolic responses.

MATERIAL AND METHODS

Subjects

Eleven sedentary overweight males – (BMI: 28 ± 5 kg•m²; height: 179 ± 8 cm; weight: 88 ± 5 kg) aged 28 ± 3 years old – participated in this study after having a medical examination. In addition, four sedentary overweight males – (BMI: 27 ± 2 kg•m²; height: 173 ± 12 cm; weight: 82.2 ± 11.5 kg) aged 36 ± 5 years old – served as a control group. Participants filled in a Physical Activity Readiness Questionnaire (PAR-Q) to determine level of activity, and to screen for a history of any risk factor and health condition including smoking history, hypertension, cardio-respiratory disease, diabetes, musculoskeletal injuries or family history of any of the above-mentioned conditions in addition to known previous mountain sickness or altitude symptoms. They were excluded from the study if they took prescribed medication of any kind, were smokers or diagnosed as having; respiratory problems, heart disease, hypertension, chronic or acute illness, anxiety disorders, and drug or alcohol abuse. They were also excluded from the study if they were involved in any form of sports or structured exercise programs during the previous 12 months. The selected participants, then, attended an orientation session in which they were given information about

the equipment and the experimental design, in addition to undergoing anthropometrics measurement. Finally, each participant signed a written informed consent in compliance with the declaration of Helsinki and with Memorial University's ethics committee regulations.

Experimental design

Three sets of data were obtained from which two distinct groups underwent either a passive acute normobaric hypoxic exposure (PAH) consisting of a 3-hour normobaric hypoxic exposure or a passive short-term normobaric hypoxic exposure (PSH) consisting of 7 days of a single 3-hour normobaric hypoxic exposure session. The third set of data was obtained from the control group (CG). Basal and post-treatment metabolic rates were measured on the first (day 1) and last day (day 7) of the experiment. During the treatment the oxygen concentration was maintained ~ 80% blood O₂ saturation (SpO₂) as monitored by pulse oximetry. Food intake and physical activity were obtained from daily logs to estimate the total daily energy expenditure. All experimental sessions were conducted at the same time of the day. Eleven participants have completed PAH session while CG (n=4) underwent a simulated experimental condition in normobaric normoxic condition.

A subset of 6 participants (BMI: 26 ± 7 kg•m²; height: 177 ± 9 cm; weight: 83 ± 12 kg) underwent an additional six 3-hour sessions (PSH) on consecutive days.

Metabolic rate determination

Participants were first subjected to a basal metabolic rate (BMR) and were, therefore, requested to comply with the following criteria prior to undergoing BMR: (1) to engage in no exercise during the preceding 36-hours; (2) to ingest no caffeine or alcohol during the preceding 24-hours; (3) to consume a last meal before 20:00 on the preceding evening and drink only water afterwards; (4) to travel to the laboratory by car or public transportation; (5) to rest for thirty minutes on a bed in a quiet environment prior to commencing recording metabolic data. Upon arrival to the laboratory, participants were placed in a comfortable, supine position in a quiet environment in preparation for metabolic rate measurement via indirect calorimetry technique. The participants were instructed to remain quiet and relaxed during data collection, but to stay awake. Upon the completion of treatment (experimental and control), participants underwent anew a metabolic rate measurement following the procedures described above.

Experimental Condition

A modified generator, equipped with a semi permeable filtration membrane (nitrogen filter technique), continuously pumping air at a flow rate of 20 l•min⁻¹ into a facial mask lowered atmospheric O₂ concentration to expose

participants to an isometabolic stress, that is, the same relative SpO₂ (~80%)

during treatments (GO2 Altitude, Biomedtech, Melbourne, Australia). Gas concentrations were monitored by oxygen sensor (Cambridge Sensotec, Cambs, UK). SpO₂ and heart rate (HR) were recorded online with a pulse oximeter (GO2 Altitude, Biomedtech, Melbourne, Australia). In addition, blood pressure and acute mountain sickness inventory questionnaire were collected every 30-min over the course of exposure. During treatment and control sessions participants were allowed to perform sedentary tasks such as reading, writing, or television viewing. The session was stopped if blood pressure rose more than 30 systolic points from baseline, heart rate increase

or decrease of more than 20 beats per minute from baseline or an increase in AMS score above 3 points.

Cardio-respiratory Measurements

Oxygen uptake ($\dot{V}\text{O}_2$, $\text{l}\cdot\text{min}^{-1}$), carbon dioxide output ($\dot{V}\text{CO}_2$, $\text{l}\cdot\text{min}^{-1}$), breathing frequency (Bf) and tidal volume (VT) were continuously collected with an automated breath-by-breath system (Sensor Medics® version Vmax ST 1.0) using a nafion filter tube and a turbine flow meter (opto-electric).

Minute ventilation and respiratory exchange ratio were calculated from Bf and VT, and from $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$, respectively.

Heart rate values were transmitted with a Polar heart rate monitor (PolarElectro, Kempele, Finland). The signal was transmitted to and recorded via the metabolic cart. Prior to testing, gas analyzers and volume were calibrated with medically certified calibration gases (15% O₂ and 5% CO₂) and with a 3-liter calibration syringe, respectively. In addition, to insure accurate calibration of the cart, the propane gas calibration was performed to assess the sensitivity of the oxygen and carbon dioxide analysers.

Fuel selection

Oxidation rates ($\text{g} \cdot \text{min}^{-1}$) of carbohydrate (CHO_{ox}) and lipid (FAT_{ox}) were calculated according to the following equations [14]:

$$(\text{Eq.1}) \text{ CHO}_{\text{ox}} (\text{g} \cdot \text{min}^{-1}) = 4.59V \cdot \text{CO}_2 (\text{l} \cdot \text{min}^{-1}) - 3.23V \cdot \text{O}_2 (\text{l} \cdot \text{min}^{-1})$$

$$(\text{Eq.2}) \text{ FAT}_{\text{ox}} (\text{g} \cdot \text{min}^{-1}) = -1.70V \cdot \text{CO}_2 (\text{l} \cdot \text{min}^{-1}) + 1.70V \cdot \text{O}_2 (\text{l} \cdot \text{min}^{-1})$$

where $V \cdot \text{CO}_2 (\text{l} \cdot \text{min}^{-1})$ and $V \cdot \text{O}_2 (\text{l} \cdot \text{min}^{-1})$ were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and $0.843 \text{ l} \cdot \text{g}^{-1}$, respectively). For relative contribution of substrates protein oxidation rate was estimated at $66 \text{ mg} \cdot \text{min}^{-1}$ based on previously published urinary urea excretion measurements made on 12-h post-absorptive men with normal CHO reserves[15, 16]. Atmospheric conditions (atmospheric pressure, humidity, and temperature) were collected over the course of the study.

Data Reduction and Analyses

Pre- and post-exposure metabolic rates were truncated by 10-min out of 30-min of data collection. The procedure discarded the first and last 5-min in order to nullify any metabolic rate fluctuation due to familiarisation with the facemask and the expected termination of data collection. The remaining 20-minute segment was, then, integrated, normalized over time, converted in and expressed as energy expenditure (EE) in kilocalories (Kcal) and as

oxidation of glucose and fat ($\text{mg}\cdot\text{min}^{-1}$). The same truncation and integration were applied to HR, SpO₂ pre- and post-exposure as well as during exposure.

Statistical Analyses

All data are presented as mean and standard deviation unless otherwise specified. First, paired t-test was performed on EE and substrate partitioning to detect any significant effect of PAH, and on SpO₂ to insure an isometabolic stress during treatment. Second, a two-way ANOVA [2 periods (Pre and Post) x 6 epochs (30, 60, 90, 120, 150, and 180-min)] with repeated measures on HR, and BP was computed to assess the effect of PAH.

Third, a two-way ANOVA [2 periods (Pre and Post) x 2 time (day 1 and day 7)] with repeated measures on EE and substrate partitioning was run to detect the effect of PSH, and on SpO₂ as for the previous condition. Finally, a three-way ANOVA [2 periods (Pre and Post) x 6 epochs (30, 60, 90, 120, 150, and 180-min) x 2 time (day 1 and day 7)] with repeated measures on HR, and BP was computed to assess effect of treatment. Prior to running the statistical plans data sets were verified for normality (Wilk-Shapiro, Lilliefors, and Kolmogorov-Smirnov tests). As well, the assumption of

sphericity was tested. When statistical significance was reached (alpha level of $p \leq 0.05$), post-hoc analyses were run to identify where significant mean differences occurred. The statistical Package for Social Sciences (SPSS, version 19) was used for all statistical analyses (SPSS Inc., Chicago, USA).

RESULTS

Environmental parameters

Room temperature was maintained between 22 and 24°C throughout the experiment, while atmospheric conditions averaged between 99.5 ± 0.6 kPa and 86.0 ± 10.7 Rh% at 7:30 and 99.6 ± 0.5 kPa and 73.4 ± 15.0 at 12:30 for barometric pressure and humidity, respectively.

Health related parameters

Based on the visual inspection of the acute mountain sickness score no statistical analysis was run. AMS scores ranged from 0 to 1 – with a minimum possible score of 0 and a maximum of 15 – confirming that participants did not experience any symptom of AMS. In addition, no participant has been removed from the experiment based on the criteria set by the experimenters such as blood pressure rising more than 30 systolic points from baseline, and/or a change of more than 20 beats·min⁻¹ in heart rate or an increase in AMS score above 3 points. Although participants were

required to record a daily diet and physical activity log, data obtained were insufficient for further analyses. However, from the questionnaire, the self-reported physical activity level of all applicants was below the Canadian guidelines for the general population.

Cardiovascular parameters

Descriptive statistics for SpO₂, HR, and BP (systolic and diastolic) are presented in Table 1. None of the above-listed parameters did reached significance neither in PAH nor in PSH. As expected SpO₂ did not vary much – because it was monitored and controlled during treatment – as reflected by the coefficient of variation [CV= 3.5%, 3%, and 2.9%] in PAH and PSH, respectively. Although a large within-condition variability (on average: SD±11.2) was observed due to individual biological variance, the average HR in PAH and PSH did not vary much – by 6% at most. The systolic and diastolic pressures were affected neither in PAH nor in PSH, the greatest variation reaching only 3% and 4% for SBP and DBP, respectively. For CG the average HR was 72±7; unfortunately, the SpO₂ and BP were not recorded for this group.

Table 1: Cardiorespiratory parameters for PAH and PSH.

PAH (n=11)			PSH (n=6)			
S _p O ₂ (%)	81±2		82±2		82±2	

	3-hour exposure		Day 1		Day 7	
	Pre	Post	Pre	Post	Pre	Post
HR (beat•min ⁻¹)	76±7	83±6	81±6	89±6	80±5	85±11
SBP (mmHg)	116±8	116±9	114±9	117±10	114±9	113±8
DBP (mmHg)	80±8	78±8	80±9	80±9	76±9	79±6

Mean±SD

Energy Expenditure

Figure 1 depicts the pre- to post-treatment variation in EE (PAH, CG, and PSH groups on the upper and lower panel, respectively). The paired t-test outcomes revealed that EE significantly increased by 16% ($p=0.002$) and by 12.5% ($p=0.029$) for PAH and CG, respectively. The analysis of variance revealed that EE for PSH significantly differed from day 1 to day 7 increasing by 12% ($p=0.037$). However, it is worth noting that EE did increase by 18% on day 1 and continued to increase by 3% on day 7.

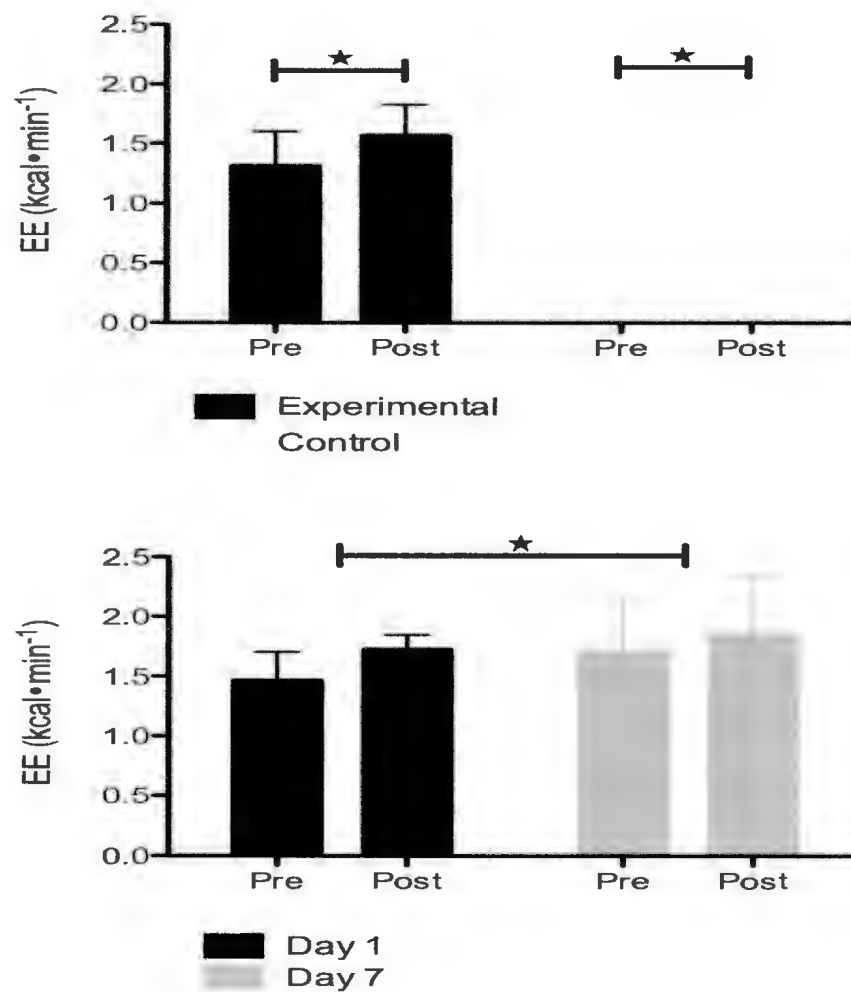


Figure 1: Energy expenditure (EE) in $\text{Kcal} \cdot \text{min}^{-1}$ pre- and post-treatments for PAH($n=11$) and control ($n=4$) [upper panel], and pre- and post-treatments in day 1 and day 7, respectively for PSH ($n=6$) [lower panel].

significantly different from pre- to post treatments, and from day 1 to day 7.

Fuel utilization

Figure 2 depicts the substrate oxidation for PAH and CG, respectively. From the upper panel one can see that glucose oxidation significantly decreased by 31% ($p=0.034$) and fat oxidation significantly increased by 44% ($p=0.001$) from pre- to post-exposure. In comparison on lower panel CG increased glucose oxidation and slightly decreased fat oxidation by 35% and by 4%, respectively but none of those scores did significantly differ from each other. Figure 3 (upper panel) showed that in PSH glucose oxidation, although not statistically significant, decreased from pre- to post-exposure by 34% ($p=0.057$), both days combined. Glucose oxidation did decrease from pre- to post exposure on day 1 by 26%, and continued to decrease from pre- to post-exposure on day 7 by 49%. In parallel and as displayed on figure 3 (lower panel), fat oxidation significantly increased by 44% ($p=0.006$) from pre- to post-exposure and by

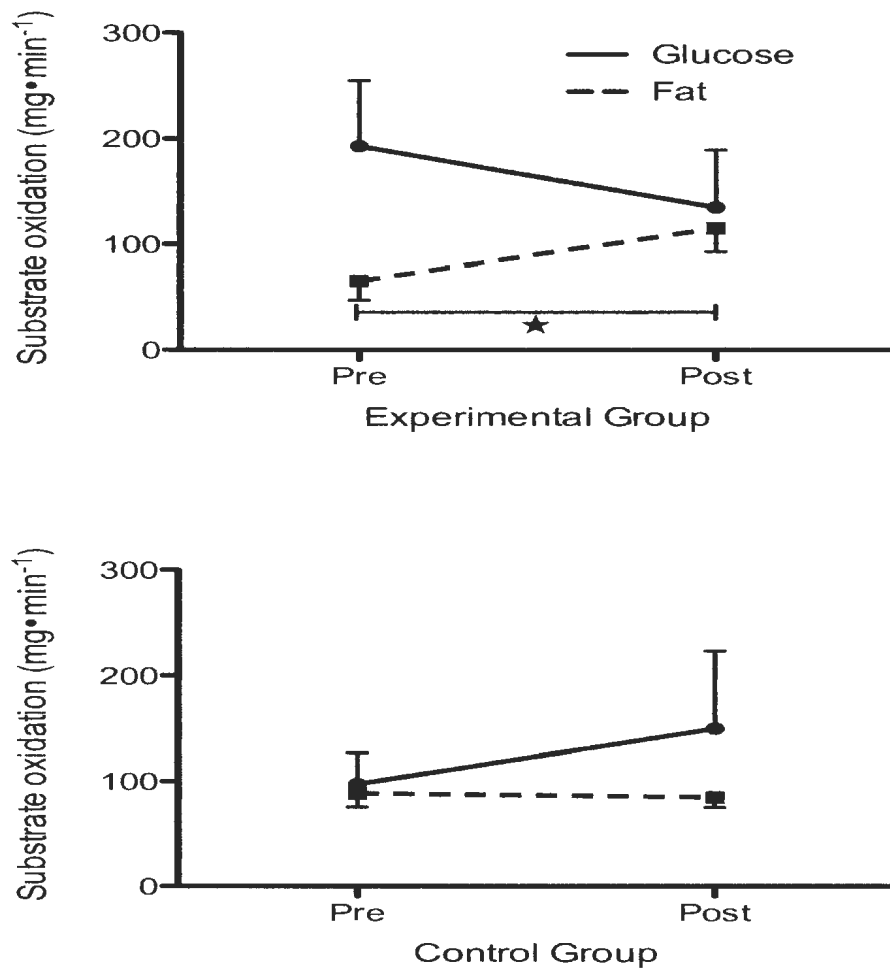
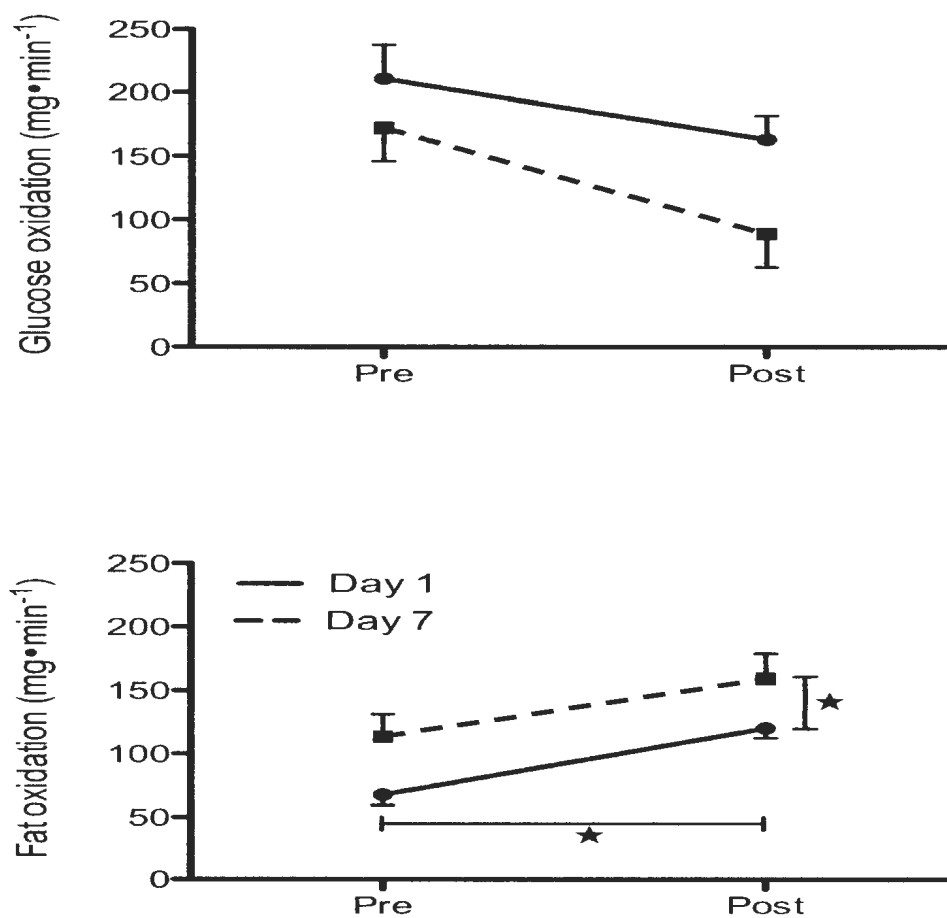


Figure 2: Substrate oxidation (glucose and fat) in $\text{mg}\cdot\text{min}^{-1}$ pre- and post-treatments for PAH ($n=11$) [upper panel] and control ($n=4$) [lower panel], respectively. ★ significantly different from pre- to post-treatments.



% ($p=0.05$) from day 1 to day 7.

Figure 3: Fat oxidation [upper panel] and glucose oxidation [lower panel] in $\text{mg}\cdot\text{min}^{-1}$ pre- and post-treatments in day 1 and day 7, respectively for PSH ($n=6$). ★ significantly different from pre- to post-treatments, and from day 1 to day 7.

Table 2: Relative contribution of substrates

	Acute				Short-term			
	Experimental (n=11)		Control (n=4)		Day one (n=6)		Day Seven (n=6)	
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>
Glucose	58±2	45±2	38±2	45±3	54±4	51±3	44±2	25±3
Lipid	21±1	34±1	35±2	31±2	25±4	31±2	31±2	48±3
Protein	21±1	21±1	27±1	23±2	21±1	18±1	17±1	18±1

Means±SEM

Protein oxidation rate was estimated at 66 mg·min⁻¹

DISCUSSION

The purpose of this pilot study was to examine the effect of acute and short-term normobaric hypoxic exposure on post-metabolic responses. The novelty of the study resides in its experimental design. In fact, to the authors' best knowledge, it is the first time that a study was designed to record post-metabolic responses in normoxia immediately after being exposed to acute and short-term passive hypoxia. As postulated the outcomes showed a shift in substrate partitioning and an increase in energy expenditure in both acute and short-term conditions. Indeed, acute and short-term normobaric hypoxic exposures did lead to an increase in lipid oxidation and a decrease in glucose oxidation along with an augmented basal metabolic rate. The main outcomes of the investigation confirmed prior research reporting short-term hypoxic exposures to be of sufficient amplitude to initiate positive adaptive responses (Bernardi, 2001; Clanton & Klawitter, 2001).

Cardiovascular parameters and health population characteristics

As mentioned by Lippl et al. (2010), most published studies have involved fit individuals or normal-weight participants rendering the interpretation quite difficult since confounding variable such as exercise, fitness and cold exposure might have interfered with altitude / hypoxic exposure. It has also

been pointed out that acclimatory responses to hypoxia depend on the inter-individual variability, and training profile (Basset et al., 2006). For instance, in highly trained athletes the extent to which performance improves could be too little to reach statistical significance (Hopkins, Hawley, & Burke, 1999) owing to optimization of their physiological systems (e.g., respiratory, cardiovascular, muscular). The data obtained, therefore, could barely be applied to unfit / sedentary obese subjects. In the current study, caution was made on selecting individuals with a BMI > 25 kg•m² who were not involved in any form of sports or structured exercise program during the previous 12 months. Monitoring these parameters strengthened the effect of hypoxic exposure. In addition, the participants were submitted to a isometabolic stress, that is, SpO₂, was monitor and controlled during treatment to maintain oxygen desaturation around 82% at 2% of the initial target (80%). The rationale underlying the isometabolic stress was to standardize as much as possible the environmental stimuli between subjects and to minimize the inter-subject variability. This may explain why none of the participants experienced acute mountain sickness event. No cardiovascular parameter was affected by the treatment neither in acute or short-term exposure. However, although none significant, heart rate was

higher post compared to pre-exposure values, a result that matched the higher significant EE observed in the present study as a consequence of the hypoxic treatment. It sounds then logical to observe an augmented cardiopulmonary function along with an increase metabolic rate since the cardiopulmonary system delivers the oxygen and substrate for replenishing stores (Laforgia et al., 2006).

Energy expenditure

Relatively brief periods of hypoxic exposure via a hypobaric chamber or inhalation of a normobaric hypoxic gas mixture stimulates erythropoietin (Wilber, 2001) resulting in improved reticulocyte count, haemoglobin, and haematocrit. These studies suggest that there is a good correlation between circulatory O₂ transport and higher basal metabolic rate. Some authors have reported a significant increase in energy expenditure at least transiently at altitude or under hypobaric / normobaric hypoxia. For instance, Butterfield et al. (1992) reported a significantly elevated BMR above sea level at the initial stage of a 21-day altitude stay whereas Mawson et al. (2000) showed an increase in resting metabolic rate after 3 days of a 12-day altitude stay. Lastly, Lippl et al. (2010) did show a significant increase in BMR after an altitude stay (~2650 m) in sedentary obese people. Contrary to previous

studies on the topic, the authors carefully controlled for the confounding factors, such as cold and physical activity. In the present study all groups did significantly increase EE from pre- to post-hypoxic exposure at rest and in a thermo-neutral condition.

This result was partly explained by the transition from supine to seated position. However, there was a greater increase in EE in PAH compared to CG (16% vs. 12.5%) in addition to a carry-over effect from day one to day seven in PSH confirming that acute and short-term hypoxic exposure have had a greater effect than energy requirement of the seated position. The cumulative effect observed over the course of 7 days (12.5% increase) corroborated previous studies showing an increase in basal metabolic rate following altitude stay (Lippl et al., 2010; Mawson et al., 1992; Picon-Reategui, 1961; Picon-Reategui, Lozano, & Valdivieso, 1961; Nair, Malhotra, Tiwari, & Gopinath, 1971). The mechanisms behind the increase in EE at altitude / hypoxic exposure still remain debated. However, Mawson et al. (2000) and Louis et al. (2009) have suggested that an increase in sympathetic nervous system activity might play a role.

Fuel utilisation

The main outcome of this pilot study showed that hypoxic exposures (acute and short term) did affect substrate partitioning. The rationale underlying the current study was, based on the mechanics contributing to the elevated post-exercise metabolism, that hypoxic exposure results in a general metabolic perturbation of which the repayment of the oxygen deficit may only contribute partially. LaForgia et al. (2006) reported that the post-exercise metabolic rate appears to be associated with higher lipid usage which is partially stimulated by increased catecholamine concentrations. The oxidation of lipid is known to contribute significantly to whole body energy turnover both at rest and during exercise (Kiens & Richter, 1998; Sahlin & Harris, 2008) and it is also greatly influenced by hypoxia. As recently reported, plasma levels of malondialdehydes (MDA), as determined by thiobarbituric acid reactive substances (TBARS), increased by 56% during exercise in hypoxia compared to normoxia, suggesting a shift towards lipid substrate (Pialoux et al., 2006). This may reflect a greater autonomic neuroendocrine stimulation of lipolysis during hypoxia (Barnholt et al., 2006; Kjaer, Bangsbo, Lortie, & Galbo, 1988). In fact, plasma epinephrine and norepinephrine levels significantly increased during hypoxic exercise

compared to normoxic exercise (Kjaer et al., 1988; Strobel, Neureither, & Bartsch, 1996) and still elevated post-exposure (Strobel et al., 1996), indicating an additive effect of hypoxia on exercise. Our outcomes revealed that passive hypoxic exposure led to a significant shift in substrate utilization towards lipid sources in PAH and PSH, respectively. Therefore passive hypoxic exposure is of sufficient amplitude to initiate acclimatory responses in sedentary people that may result in reduced body weight as previously shown in recent literature on the topic (Bernardi, 2001; Terao et al., 2003). What effect low oxygen concentration has on post-hypoxic exposure metabolic systems related to weight loss is still not fully understood? Adaptive thermogenesis might be modified under hypobaric or normobaric hypoxia and could therefore be partially responsible for an impaired energy balance (Tschop & Morrison, 2001) and weight loss. However, the current results confirm that hypoxia modifies substrate metabolism in sedentary people and that might occur through alteration of the neuroendocrine system. For instance, adrenocorticotrophic hormone (ACTH) induced steroidogenesis observed at rest and during exercise in hypoxia suggests that adrenal sensitivity for ACTH may be altered. In turn, it increases lipolytic responsiveness of adipocytes to catecholamines.

The effects of hypoxic exposure on substrate partitioning are still debated mainly because experimental designs used in the literature differ from one study to another (Basset et al., 2006). Most of the studies that showed hypoxia induced insulin resistance and diminished glucose uptake (Louis & Punjabi, 2009; O'Donnell, 2007; Polotsky et al., 2003) was undergone with severe hypoxic gas mixture ($\sim 10\%$ [O₂]). However, under more moderate hypoxic exposure ($\sim 12\%$ [O₂]), a partial pressure of oxygen corresponding to 4 000 metres of altitude and less, the metabolic response differs from the well-studied obstructive sleep apnea syndrome (OSAS) that leads to metabolic disruption (O'Donnell, 2007). For instance, Tonini et al. (2011) observed in healthy participants a shift towards fat oxidation following 14 consecutive nights of intermittent hypoxia at 13% [O₂]. This somewhat surprising outcome was interpreted as a secondary consequence of reduced glucose uptake. According to these authors the availability of glucose or diminished glucose uptake induced by hypoxia causes the shift towards fat oxidation (Sidossis & Wolfe, 1996). An alternative explanation could be that the increased sympathetic activity observed by Tamisier et al. (2011)—same participants and same experimental design—might also explain the increased fat utilization. Repetitive severe intermittent hypoxic

exposure as experienced by OSAS patients certainly leads to reduced insulin sensitivity over long periods of time (O'Donnell, 2007). However, short sessions of moderate hypoxic exposure as in the current study transiently affect fuel utilization, a positive metabolic response that resembles exercise-induced metabolic acclimation.

Limitation of the study.

We acknowledge that the small sample size for the short-term condition might have affected the statistical power. However, we are very confident that the difference observed between the two conditions, which are at the heart of our discussion, is a true difference and not a type II error. In fact, the observed statistical power for glucose and lipid in PSH are 0.512 and 0.946, respectively. Another limitation relates to the sample size of the control group consisting of only four individuals. However, this group has been implemented to monitor for any fasting effect on the dependent variables (Poehlman, Arciero, Melby, & Badylak, 1988; Zinker, Britz, & Brooks, 1990) rather than a true control group as above-mentioned. Although the lack of biological markers related to the substrate metabolism can be viewed as a possible shortcoming, the indirect calorimetry technique has been used extensively to measure energy expenditure and substrate partitioning from

respiratory gas exchanges (Ferrannini, 1988; Tappy & Schneider, 1997; Simonson & DeFronzo, 1990) and has been proven to be a reliable and valid technique of metabolic measurement, especially at rest (Lighton, 2008). In addition, in this study particular caution was taken on calibration technique. Indeed, we did use the propane gas technique to calibrate the metabolic cart for very low metabolic responses.

Potential clinical applications

If the present results are confirmed, it will lead to a new non-pharmacological strategy for the treatment of obesity. In fact, the dramatic increase in obesity worldwide being a serious threat to public health hypoxic exposure might offer a new therapeutic strategy to improve the management of weight loss. Higher aerobic pathway efficiency will lead to an increase in basal metabolic rate and daily energy expenditure. Hypoxia could, then, reverse the inefficient oxidative capacity of the obese muscle and cause weight loss.

Conclusion

The present experiment aimed at examining of the role of short-term normobaric hypoxia exposure on oxidative processes. The experimental design did evaluate and compare the efficacy of hypoxic exposures that

brings global systemic physiological changes. The main results of the study confirmed that in overweight people acute and short-term normobaric hypoxia increase metabolic rate and shift substrate utilization towards lipid sources.

List of Abbreviations:

BMI: Body mass index

BMR: Basal metabolic rate

BP: Blood pressure

CG: Control group

CHO_{ox}: Glucose oxidation

EE: Energy expenditure

FAT_{ox}: Fat oxidation

HR: Heart rate

Kcal: Kilocalorie

PAH: Passive acute hypoxia

PAR-Q: Physical Activity Readiness Questionnaire

PSH: Passive short-term hypoxia

S_pO₂: blood O₂ saturation

V̇·CO₂: Volume of carbon dioxide

V̇·O₂: Volume of oxygen

Conflict of Interest

The authors insure that they have no conflict of interest of any kind.

Authors' Contributions

Both authors have equally contributed to the design of the study, the data collection and analysis, data interpretation and manuscript writing.

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CHAPTER 4 CONCLUSION

4.1 Responses to the Research Hypothesis

The present hypothesis stated that there would be an increase in oxygen consumption after exposure to intermittent normobaric hypoxia in sedentary overweight male subjects. This effect would be observed during a single acute exposure and would be magnified by a prolonged short-term exposure. The results of this pilot study prove the hypothesis true. As well as an increase in oxygen consumption, a shift in substrate utilization was observed in this group of subjects. Indeed, a switch from glucose utilization pre-exposure to lipid utilization post-exposure was observed in both the acute and short-term groups.

4.2 Summary

Previous studies have confirmed that physiological adaptations do occur after exposure to a hypoxic stimulus of various intensities and durations. However, to date, no study has examined the effects of intermittent normobaric hypoxia on physiological parameters, specifically oxygen consumption and substrate utilization in sedentary overweight males. The purpose of this thesis was to examine the effects of an intermittent normobaric hypoxic protocol on sedentary overweight males.

4.3 Limitations of Study

The small sample size of this pilot study certainly affects the overall power of our results. However, the significance of the results would also lend support to continue this protocol with a larger group of subjects. As well, indirect calorimetry is able to provide a considerable amount of physiological data relating to metabolic parameters, however, further biochemical data could have provided more information and possibly evidence to support the shift in substrate utilization and possibly explain a mechanism responsible for the metabolic changes observed.

CHAPTER 5 OVERALL REFERENCES

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