Low-intensity Exercise Elicits Maximal Fat Oxidation in Young Healthy Men

By

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A thesis submitted to the

School of Graduate Studies

In partial fulfillment of the requirements for the degree of

Master of Science in Kinesiology

School of Human Kinetics and Recreation

Memorial University of Newfoundland

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March 2023

ACKNOWLEDGMENT

I would not have been able to complete this thesis without the support and encouragement of several special people. Therefore, I would like to express my appreciation to those who have assisted me in a variety of ways.

I would like to acknowledge and give my warmest thanks to my supervisor Dr. Fabien Basset who made this work possible. His guidance, advice, and devoted time carried me through all the stages of my project. There is no word that can adequately express how grateful I am for everything he has done for me. I admire his professional manner and scientific knowledge in completing my thesis. The start of my research coincided with the COVID-19 lockdown, which prevented me from recruiting participants in the laboratory. In those difficult times, Dr. Basset provided me with great support. I will never forget his sacrifices and assistance in helping me complete my thesis on time.

I am also thankful for all the help and support of Dr. Liam Kelly, my co-supervisor. He always kindly devoted his time to answering my questions; without his advice, I could not complete the project. I became more interested in my thesis with each research meeting we held. His approach to teaching is excellent, and he patiently explained the research aspects I was unclear about due to my background.

Last but certainly not least, I would like to extend my eternal gratitude to my beloved family for their unwavering support and unending love throughout my studies and life. This thesis as well as all my other achievements, would not have been possible without their support and encouragement.

I dedicate this thesis to my forever-beloved mom, Forouzan - I love you, Mother.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CHO – Carbohydrate

CHO_{ox} – Carbohydrate Oxidation

FA – Fatty Acid

Fat_{ox} – Fat Oxidation

HIIT – High-Intensity Interval Training

NEAT – Non-Exercise Activity Thermogenesis

RER – Respiratory Exchange Ratio

$\dot{V}O_2$ – Oxygen Uptake

$\dot{V}O_{2max}$ – Maximum Aerobic Capacity

$\dot{V}CO_2$ – Carbon Dioxide Oxidation

GXT – Graded Exercise Test

NCWS – Natural Comfortable Walking Speed

HCWS – Highest Comfortable Walking Speed

MFO – Maximal Fat Oxidation

PFO – Peak Fat Oxidation

RPE – Rating of Perceived Exertion

HR – Heat Rate

BMR – Basal Metabolic Rate

LIST OF APPENDIXES

CHAPTER ONE: INTRODUCTION

1.1 Background of Study

Most countries have begun to deal with COVID-19 and at-risk populations living with chronic diseases induced by inactivity (Ricotta et al., 2021); thus, the role of inactivity has grown more critical and disputed in public health. Inactivity has gradually increased during the last three decades. It has become a major public health issue confronting developed and developing nations (WHO news), increasing healthcare costs and negatively affecting physical and psychological health (Guh et al., 2009). However, physical activity, including non-exercise activities (Levine and Kotz, 2005), and a proper diet is well recognized as vital means of preventing morbidity and mortality due to inactivity (Miura et al., 2014; Willett et al., 2006). Accordingly, population health directives are focused on promoting a healthy lifestyle, which includes regular physical activity and choosing to eat whole foods low in sugar and saturated fats. Although several iterations of these physical activities and healthy eating guidelines have been published in Canada and worldwide, the prevalence of inactivity continues to rise (Agha, 2017).

For decades, the American College of Sports Medicine (ACSM) and Canadian Medical Association Journal (CAMJ) have urged people to engage in moderate to vigorous forms of physical activity for at least 150 minutes per week. However, these guidelines have had only a minor impact on non-communicable diseases (Matheson et al., 2013). From a physical activity point of view, researchers are currently investigating strategies to increase daily energy expenditure that are both acceptable to the general population and effective at reducing obesity and associated chronic diseases (Warburton et al., 2006). Several research groups are investigating high-intensity interval training models (i.e., exercise) (Atakan et al., 2021; Ito, 2019; Martin-Smith et al., 2020), and others are focusing on strategies to increase low-intensity non-exercise activity (e.g., changing the built environment to encourage active transport) (Chung et al., 2018).

Although both methods could increase daily energy expenditure, they could not be more different in terms of the type of energy substrates used to fuel the activity. During high-intensity interval training, energy expenditure is primarily derived from carbohydrate energy sources (Baker et al., 2010), and its effect on lipid energy stores are observed during the post-exercise recovery period (i.e., the time between exercise sessions). On the other hand, low-intensity exercise relies predominantly on lipid energy sources (Horowitz and Klein, 2000). Depending on the population studied and the target outcome, differences in substrate turnover between the two interventions may make one more effective, even when matched for total energy expenditure. For example, high-intensity interval training may be more effective for individuals with impaired glucose handling, such as those with type II diabetes (Liu et al., 2019). Therefore, interventions on increasing daily lipid oxidation may be more effective than those targeting daily energy expenditure. Furthermore, physical activity strategies focused on maximizing lipid oxidation may be acceptable to a larger population, given that it primarily includes a low-intensity activity (Romain et al., 2012).

Maximal Fat Oxidation (MFO) represents a whole-body measure of the "maximal" capacity to oxidize fat during exercise. MFO has been widely studied in order to determine its impact on substrate partitioning. However, most studies were conducted on male athletes and reported that the regulation and utilization of Fatty Acid (FA) at its maximal capacity occur at low to moderate exercise intensities; that is, between 47% and 53% of $\dot{V}O_{2max}$

(Achten & Jeukendrup, 2004; Venables et al., 2005); Still, the exercise intensity that elicits MFO remains controversial, especially in humans' natural daily ambulatory activities, such as walking and running.

MFO (also called fat_{max} or fat_{max} zone) became a critical factor in understanding the regulation and interplay of substrate utilization related to exercise intensity. Fatty acids contribute to a substantial portion of energy production, particularly during low and moderate-intensity exercise, as well as during prolonged exercise. Fat oxidation increases as a function of exercise intensity up to 45-65% $\dot{V}O_{2max}$ and has been correlated with cardiorespiratory fitness and metabolic health. The optimum fat oxidation rate plays a crucial role in preserving a limited energy source, i.e., muscle glycogen, and consequently, regulates the glucose metabolism (Achten and Jeukendrup, 2004).

There are other factors for society to favor low-intensity exercise over high-intensity interval training (HIIT). Due to modernization and automation, daily physical activity associated with a sedentary lifestyle which consists of non-exercise activity thermogenesis (NEAT), has started to become an argumentative and essential topic of scientific studies in the public health (Chung et al., 2018). Also, lack of time commitment to moderate and high-intensity exercise due to the busy lifestyle in the current modern world is a challenge that does not persuade individuals to choose and stay committed to a long-term exercise program (Biddle , Mutrie, Nanette,, Gorely, T., 2015). Furthermore, positive feelings during low-intensity exercise are reported to be higher, as psychological factors proved to affect confidence and competence, which are initial primary factors in physical activity participation, and such pleasant feelings are unlikely to occur during high-intensity exercise (Biddle , Mutrie, Nanette,, Gorely, T., 2015). This can be the reason that high intense and heavy work activities were given to enslaved people, criminals, or students as punishment in the past (Burak et al., 2013). In addition, failing to sustain vigorous intensities may deteriorate confidence over time, leading to dropping out of the exercise routine (Reljic et al., 2019). Also, demanding protocols of high-intensity interval training may not be safe. The general population may not adopt the model of practicing, as HIIT, in most cases, needs a particular environment and facilities, which can be pricy in terms of a gym membership and having training instructors. However, home-based exercise programs, which mainly include low-intensity exercise, are more convenient and cheaper compared to gym-based exercises (Devereaux et al., 2012). Thus, strategies used for HIIT have limitations in the long term when applied to the general population, which includes those who are obese, the elderly, and those who have metabolic disorders.

Considering all the points discussed above, daily life activities could also increase low-intensity physical activity rates with minimal obligation or inconvenience with desired results compared to moderate and high-intensity exercises.

Among all the different types of physical activity, activities at intensities that elicit maximal fat oxidation (Fat_{ox}) stand out as a potential regimen for reducing body fat and improving cardiorespiratory fitness in overweight individuals. Notably, less research is conducted on non-athletic populations, limiting the inference of the impact of low-intensity exercise on substrate partitioning. In addition, most metabolism studies do not include women due to the difficulty related to controlling the menstrual cycle and its impact on the substrate partitioning (Davidsen et al., 2007). Finally, the myriad of studies on high-intensity exercise training that are overrepresented in the medical sciences literature overshadows the health benefits triggered by low-intensity exercise. Therefore, examining the acute metabolic adjustments to low-intensity exercise in non-athletic young, healthy people might shed some light on substrate oxidation and metabolic flexibility as well as its impact on metabolic profile.

1.2 Purpose of Study

In the modern world, due to modernization and mechanized equipment, diet and physical activity patterns have vastly changed compared to our ancestors (Negishi, 2002). Studies have stated that tribes with higher levels of low-intensity physical activity, such as strolling, have lower rates of non-communicable diseases like hypertension and diabetes than sedentary industrialized countries (Booth et al., 2017). As evidenced by acceleratory and GPS records, running is uncommon among hunter-gatherers and subsistence farmers (Pontzer et al., 2018b). Also, according to fossil and archaeological evidence, high levels of physical activity appear to be ancient in the human lineage, which claims that the environment, not genetic, keeps individuals in small-scale civilizations healthy (Pontzer et al., 2018a). Thus, a high degree of physical activity, including the low-intensity activity of walking, is part of traditional lifestyles that protect against non-communicable diseases.

Today walking is one of the evaluation tools for clinical patients in rehabilitation because walking is a natural activity that humans engage in daily life for personal transportation, commuting, recreation, and occupational activities (Hulteen et al., 2017). Numerous elements of walking have been studied in clinical and athletic studies (Morris and Hardman, 1997). Scientists can judge the normality of targeted performance based on walking pace and manner, and when used in conjunction with other performance measures, walking speed can predict health status, which supports the assumption that walking is a natural function performed by every healthy human, making the walking speed a good predictor or even a vital sign (Fritz and Lusardi, 2009). As a result, walking is regarded as a standardized activity that can be introduced into the clinical examination and evaluation process; however, walking speed could be used as a proxy for intensity since the walking pace is different among people, representing different intensities from mild to moderate. Moreover, health benefits are dependent in part on how intense the walk is (Shephard, 2001). Although physicians may focus on various walking features, speed is frequently indicated as a measure of status and outcome (Boonstra et al., 1993).

Studies that have investigated self-selected walking pace adopted two general terminologies that were organized differently. Self-selected comfortable walking speed (CWS) and maximal comfortable walking speed (MCWS) are the two terminologies in which studies can refer to natural physical activities and exercise interchangeably. Both comfortable and maximum walking speeds have been employed as primary measures of gait quality and functional mobility (Dobkin, 2006). Maximum speed is significant for evaluating the ability to participate in daily activities such as crossing a busy street. Still, a comfortable walking pace is typically used as a measure of walking performance (Kollen et al., 2006). In such studies, subjects are asked to walk at a comfortable speed across a set walkway length to determine their comfortable walking speed. Comfortable walking speed is a responsive measure for detecting small changes in clinical status over time. To assess maximal walking speed, individuals are typically instructed to walk as quickly as they feel safe over the length of a specific path (Ng and Tsang, 2013). Thus, walking at your own pace, is considered a comfortable walking speed, whereas walking as fast as you can safely walk, is considered the maximum walking speed.

There is still a knowledge gap in which intensity people can walk to be confident in maintaining and reaching health benefits from substrate partitioning and optimum lipid oxidation aspects. Substrate utilization and maximal fat oxidation (i.e., MFO, Fat_{max}, and

Fat $_{max\ zone}$) are critical factors in understanding related hormonal activity and justifying exercise intensity. Thus, considering all the points discussed above, with all the barriers to living in the modern world, why is most of the encouragement directed toward individuals to risk injuries and invest time, energy, and money in HIIT? From the evolutionary perspective of human beings, humans evolved from walking, and it has been shown that low-intensity daily physical activity, most of which involves walking, may help protect individuals from chronic diseases (Booth et al., 2017). Is this still valid to undertake high-intensity exercises rather than low-intensity activities? In fact, less research is done to adequately address these questions and investigate the potential benefits of low-intensity activities and the effect of the walking speed spectrum on substrate partitioning in daily ambulatory activities.

The purpose of this thesis is to compare substrate partitioning, particularly peak reaching fat oxidation within the range of daily ambulatory activities defined as two self-selected walking paces in different workloads of low and high conditions (e.g., Natural Comfortable walking Speed and the Highest Comfortable Walking Speed) and ascertain whether the MFO occur within this range when factors such as diet, physical activity level, and metabolic profile are controlled during participation.

After investigating the relationship between MFO and peak rates of fat oxidations in the two walking conditions, the second objective is to examine whether MFO is more related to self-selected natural non-intentional daily walking pace (non-exercise activities) or self-selected high intentionally programmed walking pace (exercise activities).

1.3 Significance of Study

A vast amount of literature focuses mostly on high-intensity exercise, which primarily favors the athlete and diabetes population (Gibala and McGee, 2008). In this thesis, the focus is directed toward low metabolic rate activities to comprehensively explore potential advantages from a defined range of ambulatory intensities, which are less studied. Also, athletes are the target population in most scientific studies in this area, whereas the general population is ignored. In addition, with the increasing rate of chronic disease triggered by obesity and lack of movement, physical activity guidelines recommendations seem to be inefficient. The possible reasons that most of the current population is unwilling to follow the recommendations are mentioned previously, as most of the young population face a more challenging time prioritizing forming a scheduled habit to increase movement engagement (Gardner et al., 2012).

Also, advertisements and social media have encouraged the young population that the HIIT favors fitness and having a good-looking body in a short period (Dunlop et al., 2016), rather than educating people about metabolic health. So, less focus is on developing simple movement routines and incorporating them into daily living to benefit from its long-term health outcomes. Alternative activity plans can create an active lifestyle by developing a more profound knowledge of low metabolic rate activities and their correlated substrate partitioning pattern.

By examining self-selected low and high walking paces, this thesis delves into the less explored substrate partitioning component of human ambulatory activities and searches for a more effective strategy to live better in the future. This study is distinguished from other similar studies in that more control variables are monitored during experiments affecting the results, such as metabolic profile, participants' reports on daily activity by wearable technology, age, BMI, sex, and diet.

CHAPTER TWO: LITERATURE REVIEW

2.1 Energy Metabolism

The term "metabolism" refers to the whole of the chemical reactions that take place inside each cell of the body and supply the organism with energy. Daily physical activity and human mobility all require energy. The body needs energy to sustain living activities and cell homeostasis. Energy is provided for such activities by dietary macronutrients (e.g., CHOs and fats) (Ferrannini, 1988). As a result, this energy is used to drive vital processes and create organic materials. By ingesting nutrients and other elements that serve as the building blocks for movement, growth, development, and reproduction, every living organism depends on its surroundings to thrive. Enzymes, proteins with specialized roles in anabolism and catabolism, mediate all the processes (Sánchez López de Nava and Raja, 2022). The chemical carrier of energy is called adenosine triphosphate (ATP), which is synthesized within mitochondria surrounded by an outer and an inner membrane. The body's internal milieu undergoes the dissociation of water into a hydrogen molecule and a hydroxyl group, which is necessary for ATP hydrolysis. ATP hydrolysis by myosin heavy chain is the main source of protons during exercise, the majority of which are carried to the mitochondria to create ATP. Using the energy liberated by the electron chain transport mechanism, these protons are carried via a series of complexes in the inner membrane of the mitochondria to create a proton gradient. The energy to create the proton gradient comes from the electron transport chain, a series of exergonic redox chemical reactions related to the electron transport from NADH and $FADH_2$. ATP is created due to the thermodynamic return of protons to the mitochondrial matrix (Chen and Lui, 2022). When ATP is broken down into ADP and Pi, a proton is released. During muscle contraction, mitochondrial respiration provides the ATP required to sustain muscle contraction, and protons are used by the mitochondria for oxidative phosphorylation and to maintain proton gradients in the intermembrane space. Once the exercise intensity exceeds steady state, a greater reliance is required on glycolysis and phosphagen for the regeneration of ATP. ATP is provided from nonmitochondrial sources and is ultimately used to fuel muscle contractions, resulting in acidosis. These conditions result in an increase in lactate production, which prevents pyruvate accumulation and supplies the NAD+ needed for glycolysis phase 2. The increase in lactate production coincides with cellular acidosis, and it remains a useful indicator of metabolic acidosis induced by cell metabolic conditions. A lack of lactate in muscle would lead to acidosis and fatigue occurring more rapidly, thereby impairing exercise performance (Robergs et al., 2004).

The rate at which energy is produced is known as the basal metabolic rate. It is influenced by gender, race, exercise, diet, and age. Most of the human energy intake is metabolized, except for the portion that is expelled through feces, urine, or sweat (Widdowson, 1955). A person's basal metabolic rate (BMR) is usually measured in the morning after an overnight fast, no exercise for 24 hours prior, free from emotional stress, familiar with the instruments, and completely rested (Henry, 2005).

In such processes, oxygen is used to create energy, but carbon dioxide and heat are produced. As a way of determining the metabolic rate, one can use a number of methods, including measuring heat production, using a direct calorimetry (Brooks, G. A. et al., 2004), or calculating it using expired air oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) (Lighton, 2008).

Due to the direct relationship between $\dot{V}O_2$ measured at the mouth and oxygen consumption in the tissues, this method could be an accurate way to measure metabolic rate. Thus, the indirect calorimetry method is a standard technique for the energy balance analyses (Kaiyala and Ramsay, 2011).

As well as providing metabolic rate estimates, indirect calorimetry can determine how fuel contributes to the energy production (Brooks, G. A. et al., 2004). The ratio of $\dot{V}CO_2/\dot{V}O_2$ indicates the type of substrate being oxidized, which is called respiratory exchange ratio (RER), and reflects the ratio quotient (Simonson and DeFronzo, 1990) and varies from 0.7 to 1.0. Ratio quotient and RER are calculated similarly; however, RER measures air expelled during breathing, whereas ratio quotient indicates cell respiration.

2.1.1 Physical Activity Metabolic Rate and Skeletal Muscle Metabolism

An activity's metabolic rate and condition prioritize the contribution of each substrate to producing energy (ATP) for the tissues in the demand (Fogelholm, 2006; Péronnet et al., 2006). The body uses intramuscular energy sources during the transition to the exercise (van Loon et al., 2001). Fat oxidation at rest provides the energy needed after an overnight fast. Triacylglycerol from visceral adipose tissue and subcutaneous adipose tissue are the main sources of these fats (Arner et al., 1990). During low-intensity exercise (25% $\dot{V}O_{2max}$), which is comparable to slowly walking, the lipolysis of adipose tissue triacylglycerol and plasma free fatty acids increases (Klein et al., 1994). During low-intensity exercise, plasma fatty acids (FA) contribute most to fat oxidation. The fact that lipids contain large amounts of energy explains why they contribute to a large portion of energy production, particularly during low and moderate intensity exercise and prolonged exercise (Volek et al., 2015). Exercise, however, can become complex as multiple sites can regulate lipid metabolism, and oxidation occurs in multiple steps (Spriet, 2014).

Intramuscular triacylglycerol is a vital contributor to fat oxidation at high intensities (Brooks and Mercier, 1994). Fat oxidation fails to supply all the energy needed when the intensity rises to a moderate level (65% $\dot{V}O_{2max}$). Fat oxidation reaches its highest value between 45 and 65% $\dot{V}O_{2max}$ (Achten et al., 2002), also known as the crossover point (Brooks and Mercier, 1994), where fat oxidation starts to decline at this point, and carbohydrate oxidation rates increase gradually until carbohydrates become the primary fuel source. The rate of plasma FA appearance declines as exercise intensifies, which is likely due to insufficient blood flow. As a result, low blood flow compromises albumin delivery to transport FA into the bloodstream from adipose tissue. Unlike FAs, glycerol is water-soluble and does not depend on blood flow (blood protein carriers, albumin) to appear in plasma; therefore, higher intensities do not affect the appearance of glycerol.

When performing high-intensity exercises [>70% VO2max], CHO becomes the predominant substrate for energy production, with 80% of energy coming from endogenous glucose (glycogen) (Péronnet et al., 2006). The switch in substrate usage occurs because the quantity of energy produced (kcal) per liter of oxygen consumed reached a greater amount of energy with CHO (~ 5 kcal/LO2) than with lipid (~4.7 kcal). Therefore, considering that oxygen delivery can be a limiting factor for the production of ATP at high exercise intensity, it seems logical that the system favors CHO oxidation in these physiological conditions. In the meantime, there exists a direct relationship between the intensity of muscle contraction and the recruitment of skeletal muscle fibers (type I, IIa, and IIx). The higher the skeletal muscle contraction, the greater the type IIx recruitment resulting in a larger amount of glycogen catabolism and a lower metabolic efficiency (Brooks, G. A., Fahey, T. D., and Baldwin, 1996). As a whole, these mechanisms explain why carbohydrate oxidation becomes the main substrate for energy production. In addition, lipid metabolism requires complex biochemical cascades that slow down substrate delivery (Simonson and DeFronzo 1990).

2.1.2 Physiological Differences Between Sexes

Women and men experience different substrate metabolism during exercise due to sex hormones (Hatta et al., 1988). It is known that women have very high metabolic flexibility and are able to adapt their metabolism to changes in metabolic demand based on the availability of nutrients (Goodpaster and Sparks, 2017). The post-exercise recovery period results in an increase in both sexes' lipid metabolism, but it is more pronounced in the men population. The female skeletal muscle is more capable of storing lipids and oxidizing them, which helps to maintain a high turnover of triacylglycerol stored in the intramuscular fat (Lundsgaard and Kiens, 2014).

Despite no sex-based differences in exercise intensity, Dasilva and colleagues found that fat oxidation contributed more to total energy expenditure (EE) in women than in men. Despite both sexes, self-selecting similar exercise intensities, women's fat oxidation contributed more to EE than men's. A striking finding was that both genders self-selected exercise intensities were within fat_{max} limits (Dasilva et al., 2011).

Different exercise intensities have been examined in a number of studies in an attempt to provide an overview of the physiological differences between sexes. Men use more carbohydrates when exercising than women, but fewer carbohydrates when recovering (Henderson et al., 2007; Venables et al., 2005). In contrast, when energy demand increases, women burn more fat than carbohydrates in the same relative intensity (Tarnopolsky et al., 1990). According to RER, the relative fat oxidation of women during endurance exercise is significantly higher than that of men. The indirect calorimetry also showed that women oxidized fat more quickly on a treadmill than men during incremental tests (Chenevière et al., 2011).

2.2 Physical Activity Versus Exercise

Physical activity and exercise both require skeletal muscles to move in order to exert energy, but the terms physical activity and exercise refer to different concepts. The terms are often used interchangeably and are often confused with each other (H. L. Taylor, 1983). Both are measured by kilocalories and are ranging continuously from low to high and are positively correlated with physical fitness concerning the intensity, duration, and frequency of movement (Caspersen et al., 1985). Consequently, the week and day are the most common units of time used to refer to kcals spent in physical activity (H. L. Taylor et al., 1978).

The term exercise is not synonymous with physical activity. According to exercise testing and prescription guidelines published by ACSM (Ferguson, 2014), exercise is a planned, structured, repetitive, and purposeful physical activity aiming to improve or maintain components of physical fitness. For example, a common purpose of exercising is to improve or maintain physical fitness. In such instances, the majority of exercise activities are repetitive, structured, and well-planned. However, household, occupational, and many daily tasks are usually performed efficiently.

2.3 Human Evolution and the Importance of Walking

According to various research, walking is the most typical physical activity among small-scale communities. Habitual bipedalism, or walking and running on two legs, is a distinguishing trait of the human lineage, separating our forefathers from their ape-like forefathers. Since Darwin's time, the evolutionary costs and benefits of adopting a two-legged stride have been disputed (Darwin Charles, 1874).

The impact of modern technology on physical activity can be evaluated by examining a group whose lifestyle has not changed significantly in the past 150 years. One of the tribes' studies reported that adults in the Older Amish group engaged in moderate physical activity for almost eight hours per day, such as walking (Science and Arbor, 2004). In a hunter-gatherer ecology study, women walked an average of 9.5 kilometers per day, and men walked 14.1 kilometers per day (Marlowe, 2005). Tsimane and other small-scale tribes have high levels of low and moderate-intensity physical activity, according to accelerometer-based assessments (Gurven et al., 2013). According to heart rate measurements, Hadza adults also have exceptionally high amounts of daily physical activity, generally at low and moderate intensities, and engage in over 135 minutes of moderate to strenuous physical activity every day, which is many times greater than adults in the United States and Europe (Raichlen et al., 2017).

In the mentioned studies, tribal populations with a higher level of low-intensity physical activity are more likely to be physically fit and healthy than industrialized nations whose populations are sedentary and believe high-intensity exercise makes people fit and healthy. In addition to being more immune to diseases such as hypertension, obesity, diabetes, etc., tribes also showed a lower rate of non-communicable diseases.

Inactivity and sedentarism, associated with chronic conditions in industrialized communities, have yet to be investigated in small-scale groups (Booth et al., 2017). However, running is uncommon among hunter-gatherers and subsistence farmers, as evidenced by acceleratory and GPS records.

When indigenous populations renounced their traditional lifestyles and adopted western diets and exercise levels, they got the same metabolic and cardiovascular problems as modernity (O'Dea, 1991). However, because traditional lifestyles and diets are so diverse, it is challenging to draw straightforward conclusions that can be applied to industrialized cultures. In addition, hunter-gatherers have meager obesity rates and engage in many different physical activities. A high degree of physical activity is part of traditional lifestyles that protect against non-communicable diseases, and according to some fossil and archaeological evidence, physical activity and walking appear to be ancient in the human lineage.

According to some research (Garland, 1999), longer daily travel distances are associated with improved endurance. Compared to chimps and other wild animals, humans also possess greater endurance. This may lead to the conclusion that humans are born walkers, and we know that low-intensity physical activity and endurance are linked. To think of chimps as our forefathers, there is skeletal evidence for greater robusticity in the rear limbs (Bramble and Lieberman, 2004). Ecological evidence supports a shift in the diet to contain more meat (Noakes, 2011) and provides indirect proof of greater endurance. Also, in humans, increased brain size, a shift in diet to include meat, and advanced technical complexity and ecological flexibility are all indicators of high endurance (Pontzer, 2017). The economy and cost of transport for humans show that by increasing the speed, the economy rises to a point where the cost of transportation is the lowest, meaning that at a low-intensity walking activity, there is a speed at which humans may feel comfortable with optimal efficiency while departing from that point decreases locomotor efficiency. Also, the gait of humans appears to be adapted to minimize metabolic energy consumption (McNeill Alexander, 2002).

2.4 Self-selected Walking Paces in Clinical Studies

Today walking is one of the evaluation tools for clinical patients in the rehabilitation (Ogilvie et al., 2007). In fact, walking is considered a feasible activity for everyone (Hulteen et al., 2017), being a natural way of locomotion reported as a form of low-intensity exercise (Ham et al., 2009). All humans engage in daily activities for personal transportation, commuting, bathing, and recreation (Tudor-Locke, C., and Rowe, 2012).

Self-selected Comfortable Walking Speed (CWS) and Maximal Comfortable Walking Speed (MCWS) are the two speeds that studies use and can be referred to as natural physical activities and exercise accordingly. Both comfortable and maximum walking speeds have been employed as primary measures of gait quality and functional mobility (Dobkin, 2006). Maximum speed is significant for evaluating the ability to participate in daily activities such as crossing a busy street. Still, a comfortable walking pace is typically used as a measure of walking performance (Kollen et al., 2006). Subjects are asked to walk at a comfortable speed across a set walkway length to determine their comfortable walking speed. Comfortable walking speed is a responsive measure for detecting small changes in clinical status over time. To assess maximal walking speed, individuals are typically instructed to walk as quickly as they feel safe over the length of a specific path (Ng and Tsang, 2013). Walking at your own pace, as if you were walking around, is considered a comfortable walking speed, whereas walking as fast as you can safely walk is considered the maximum walking speed.

Sedentary behavior, which is associated with several chronic diseases like cardiovascular disorders and diabetes, can be positively affected by walking (A. H. Taylor et al., 2004). Traditional indoor walking speed estimations are unlikely to reflect self-selected or other guided outdoor walking paces. There is still a gap at which intensity people can walk to be confident in maintaining and reaching health benefits.

2.5 Maximal Fat Oxidation

As previously mentioned, fat is the primary fuel oxidized during low-intensity physical activity. Although vigorous physical activity has many advantages, low-intensity activity is important since obesity and cardiovascular disease rates have skyrocketed in recent decades. As a result of increased fat metabolism, metabolic disorders like heart disease, type II diabetes, etc., could potentially be alleviated (Achten and Jeukendrup, 2004). Impairments in fat metabolism seem to be important factors in the development of obesity and diabetes.

Substrate utilization and maximal fat oxidation (i.e., MFO, Fat $_{max}$, and Fat$_{max}$ zone) are critical factors in understanding related hormonal activity and justifying exercise intensity. The optimum Fat oxidation rate plays a crucial role in preserving a limited energy source, muscle glycogen, and eventually fatigue for a more extended period. In one study, both sexes self-selected the same walking intensity that falls within the fat$_{max}$ zone (Dasilva et al., 2011). There is still not enough information about the differences in walking speeds from the substrate partitioning point of view. Additionally, in most energy metabolism studies, women are underrepresented since control over the hormonal activity of women linked with their menstrual cycle is challenging and needs extra work. One study showed a need to control for metabolic changes in the plasma due to the menstrual cycle in the design of future metabolomic studies involving premenopausal women (Wallace et al., 2010). In the cross-over point where lipid oxidation decreases and glycolytic enzymes and pathways are more triggered, the MFO may occur. When the intensity of exercise increases, the oxygen uptake will increase (Brooks, 1998). Peak fat oxidation can be a marker when the cross-over concept occurs. Furthermore, the general population is less included in research studies (e.g., 20 to 45 years old), and athletes and over-aged people had the majority of the sampled population in clinical and athletic studies. Metabolic responses and their resulting health benefits, according to the information mentioned above connected to our lineage, are less investigated, covering previous studies' concepts of interest combined.

Exercise increases the mobilization of lipids from adipose tissue. As the level of work increases, the rate of lipid oxidation increases, with the maximum occurring at about

65% of maximal oxygen consumption for highly trained individuals. The breakdown of carbohydrates is primarily responsible for muscle metabolism at high workloads (Jeukendrup et al., 1998b). There is a lack of understanding of the mechanisms regulating the substrate choice of the exercising muscles. An important factor appears to be the hormonal milieu, and adrenaline (Jeukendrup et al., 1998a). The glucose-fatty acid cycle has been a classic concept since Randle's original proposal. Non-esterified fatty acids (NEFAs) are thought to be the key regulator of substrate choice in the exercising muscles because they are readily available for oxidation (Randle et al., 1963).

Literature pertaining to this cycle during exercise yields contradictory results (Dyck et al., 1993; Ferrannini et al., 1983; Ravussin et al., 1986). A study using intralipid and heparin to increase non-esterified fatty acids (NEFA) levels in the blood found that increased availability of NEFAs increases lipid oxidation during exercise (Vukovich et al., 1993). It may be possible, therefore, that adipose tissue mobilization of NEFAs limits lipid oxidation during exercise. Animal studies suggest that an increase in the relative workload of over 40% of $\dot{V}O_{2max}$ cannot increase the absolute lipid metabolism (Hoppeler and Weibel, 1998). In contrast, endurance exercises increase lipid contributions to energy production. Lipids and carbohydrates contribute in different proportions to exercise's total oxidative metabolism depending on the relative workload. According to tracer methodology, lipid metabolism reaches a maximum at 65% of $\dot{V}O_{2max}$, and at 25% of $\dot{V}O_{2max}$, peripheral lipolysis was found to be maximally stimulated (Romijn et al., 1993).

2.6 MFO Testing Protocol

Scientists are particularly interested in determining the exercise intensity and maximum fat oxidation because these are integral components of metabolic flexibility that affect both endurance performance and metabolic health (Goodpaster and Sparks, 2017; Maunder et al., 2018). It was Achten and his colleagues who validated a graded exercise protocol based on 3-min stages and 35-W workload increments in 2002 to determine MFO and its relative intensity (Achten et al., 2002). As time went on, other graded exercise protocols were developed to determine MFO and the relative intensity based on participants' sex, age, training status, and weight status. The graded exercise protocol has traditionally been modified on two points: 1) the duration of the stages (e.g., from 1 to 10 minutes) and 2) the work increment (e.g., from 10 to 50W) (Amaro-Gahete et al., 2018). Gradual exercise protocols for determining MFO and its related intensity are not agreed upon as far as stage duration is concerned (Amaro-Gahete, Sanchez-Delgado, Alcantara et al., 2019), however, reaching a steady state seems essential (Macfarlane, 2017; Shephard and Aoyagi, 2012). In moderately trained men, Achten found no differences in MFO and the intensity between 3 and 5-minute stage protocols (Achten et al., 2002). Others claim that a 3-min stage duration is not enough to achieve a steady state in obese and diabetic patients with very low maximal oxygen uptake levels and recommend a 6-min stage duration in sedentary individuals (Bordenave et al., 2007). Like stage duration, workload increment also varies across studies and is adapted to a participant's biological characteristics (Amaro-Gahete, Sanchez-Delgado, Alcantara et al., 2019; Maunder et al., 2018). It is possible to accurately determine MFO and its related intensity by using relatively small workload increments, regardless of the biological characteristics of the participant. For sedentary patients with low $\dot{V}O_{2max}$, a 6-min stage duration would be appropriate to reach the steady state based on the above-mentioned factors. Moreover, a graded exercise protocol should be applied to determine MFO and its related intensity in a more accurate manner by selecting small workload increments (e.g., 10-W increments). As a consequence, tests can last for a long time, which can negatively impact MFO and intensity determinations due to peripheral and central fatigue (Hultman and Greenhaff, 1991). It is, therefore, clinically relevant to develop strategies that reduce the overall duration of a graded exercise protocol while maintaining adequate stage durations and relatively small workload increments.

The respiratory exchange ratio (RER) of 1.0 is the standard criterion for stopping the MFO and Fat_{max} tests despite the heterogeneity of protocols. Achten first applied this criterion, and all subsequent studies followed it (Achten et al., 2002). However, it remains unclear whether reaching a RER of 1 in both sedentary and trained individuals is necessary to quantify MFO in terms of reliability and validity. The answer to this question was sought by Amaro and his colleagues, as they have recruited a total of 248 sedentary and trained healthy adults aged between 18 and 65 years (124 young sedentary adults aged 22.1 ± 2.2 years; body mass index: 25.0 ± 4.8 kg/m^2; maximal oxygen uptake: 41.2 ± 7.8 ml.min^{-1}.kg^{-1}; 83 women/41 men and 42 middle-aged sedentary adults aged 52.1 ± 4.6 years; body mass index: 27.8 ± 3.6 kg/m^2; maximal oxygen uptake: 30.4 ± 5.6 ml.min^{-1}.kg^{-1}; 23 women/19 men), and a total of 52 young trained adults (age: 22.6 ± 2.2 years; body mass index: 24.3 ± 5.1 kg/m^2; maximal oxygen uptake: 44.3 ± 9.5 ml.min^{-1}.kg^{-1}; 35 women/17 men) and 30 middle-aged trained adults (age: 52.4 ± 4.6 years; body mass index: 27.1 ± 3.9 kg/m^2; maximal oxygen uptake: 34.8 ± 6.3 $ml.min^{-1}.kg^{-1}$; 15 women/15 men) participated in Amaro's study (Amaro-Gahete, Sanchez-Delgado, Helge, et al., 2019). It has been found that a graded exercise protocol aimed at determining MFO and its related intensity would end when a RER of 0.93 is reached in healthy sedentary adults and when a RER of 0.90 is reached in trained adults, regardless of gender, age, weight, or Fat_{max} data analysis methodology. To ensure that MFO is reached in outliers, they recommend lowering the RER from 1.0 to 0.95. As a result of this methodological consideration, smaller workload increments can be applied, and stage durations can be extended in order to reach a steady state without extending test durations.

2.7 Hypothesis

Taking into consideration the previous sections, we can postulate that humans evolved naturally to walk, and people walk as a form of the most common form of physical activity because it is familiar, convenient, and free (Morris and Hardman, 1997; Mutrie and Hannah, 2004). At various walking speeds, Willis et al. reported that fat is the primary source of energy below the self-selected walking speed (4.8 km/h), and at higher speeds, CHO is the major source of energy (Willis et al., 2005). Therefore, the authors concluded that the central nervous system selects a walking speed that is primarily based on fat metabolism (Gandevia and Rothwell, 1987). Furthermore, another master's thesis study from a former master's student conducted under the supervision of Dr. Basset at physiology lab at Memorial University of Newfoundland found MFO to be achieved when walking at the self-selected speed (Behnamfar, 2021).

The hypothesis presented in this thesis is that MFO occurs within the daily physical activity of the general population of young males. More specifically, that it is related to self-selected maximum comfortable walking speed.

CHAPTER THREE: MANUSCRIPT

3.1 Abstract

Maximal Fat Oxidation (MFO) is a marker of skeletal muscle oxidative capacity measured through submaximal exercise testing. However, the intensity at which it occurs remains to be evaluated in nonathletic populations. The study investigated the effect of the lowest and highest range of self-selected comfortable walking paces on whole-body fat oxidation. Fourteen young healthy men (age = 28.5 ± 5.0 years, weight = 76.9 ± 10.9 kg, height = 176.9 ± 5.9 cm, BMI= 24.5 ± 2.8, $\dot{V}O_{2max}$ = 3.8 ± 0.6 ml/min) performed a running incremental test (GXT) prior to partaking in two 30-min self-selected walking pace (low and high) trials. Walking paces, $\dot{V}O_2$, $\dot{V}CO_2$, HR, and RPE, were recorded, and substrate oxidation rates were calculated through stoichiometry equations. The MFO rate determined during the GXT was 473 ± 201 mg/min and occurred at 39.6 ± 11.8 % of $\dot{V}O2_{max}$. Peak fat oxidation (PFO) rates at the lowest (3.5 ± 0.7 km/h) and highest (5.7 ± 0.8 km/h) walking paces were significantly different (*p*-value=0.001; 254 ± 11 vs. 436 ± 11 mg/min, respectively). The lowest and highest self-selected walking paces corresponded to 21.2 ± 3.8 and 35.8 ± 5.9 % $\dot{V}O2_{max}$, respectively. At the highest walking pace, no statistical difference was observed between MFO and PFO.

Keywords: Daily physical activity, exercise, substrate partitioning, male, maximal fat oxidation.

3.2 Introduction

Physical Activity (PA) does not equate to exercise as it involves a wide range of behaviors, including exercise. On the one hand, exercise is a type of planned, structured, and repetitive movement with the intention to improve and maintain fitness characteristics that may constitute a small part of daily activity. On the other hand, PA encompasses any movement produced by skeletal muscles that exceed resting energy expenditure, which includes occupational, household, or other regular daily life activities with little regard to the physical fitness (Caspersen et al., 1985). Physical Activity can be performed over a wide range of metabolic rates, in other words, intensities. Therefore to match the energy demand of functional tissues, the cardiorespiratory system adjusts oxygen and substrates delivery which impacts the substrate utilization (Spriet, 2014). The term “substrate utilization” indicates the contribution of lipids (e.g., fat), carbohydrates (CHO), and proteins (e.g., amino acids) to Energy Production (EP) and mirrors fuel sources used during activity. However, CHO and lipid substrates constitute the primary sources of energy during any form of physical exertion (Spriet and Watt, 2003). Protein can also serve as an energy source, but amino acid oxidation correlates strongly with amino acid intake, and their contribution to total energy production represents a small part of the whole (Gibala, 2001). An activity's metabolic rate and condition prioritize the contribution of each substrate to producing energy (ATP) for the tissues in the demand (Fogelholm, 2006; Péronnet et al., 2006).

Exercise intensity, expressed as a percentage of the maximal oxygen consumption ($\dot{V}O_{2max}$), can be characterized as the level of physical power generated by the body while performing physical activity. During low-intensity activities, most of the energy requirements are met with the oxidation of fatty acids (< 35% of $\dot{V}O_{2max}$). At moderate exercise intensities (40-55% $\dot{V}O_{2max}$), fat oxidation tends to be the highest (Lanzi et al., 2014). However, at higher exercise metabolic rates, the absolute rate of fat oxidation decreases, while the oxidation of carbohydrates contributes to more than two-thirds of the energy required (Brooks, 1998; Kriketos et al., 2000). This transition from fat oxidation to carbohydrate oxidation during high-intensity exercise emerges from glucose capacity to produce energy at a faster rate with a lower volume of oxygen compared to the free fatty acids (Pérez-Martin et al., 2001). During low-intensity activity, fat is the predominant energy substrate due to its higher abundance in the body and its greater potential energy per gram of substrate (9.46 kcal per gram of fat compared to 3.74 kcal per gram of glucose) (Horowitz and Klein, 2000). In contrast, during high-intensity exercise, glucose becomes the major contributor to the energy requirements (Baker et al., 2010).

Depending on the studies' target demographics, there has been debate over the potential health benefits of various intensities of physical activity. There is evidence to suggest that high-intensity exercise is beneficial for cardiovascular health (Gibala et al., 2012). However, this approach might have low commitment and motivation, followed by large dropout rates, making it impractical as a means to tackle chronic diseases (Biddle , Mutrie, Nanette, Gorely, T., 2015; Reljic et al., 2019). Also, physical activity guidelines have urged people to engage in moderate to vigorous forms of physical activity for at least 150 minutes per week. However, these guidelines have had only a minor impact on the non-communicable diseases (Matheson et al., 2013). In contrast, low-intensity physical activity is thought to be the point at which fat oxidation reaches its nadir (Liepinsh et al., 2020). In support of low-intensity PA as an efficient means of burning fat, studies in human evolutionary biology show that humans evolved to be endurance animals able to sustain low-intensity activities such as walking and running in order to meet daily survival needs (Eaton et al., 2002; Peters et al., 2002; Pontzer et al., 2018a). In this regard, low-intensity physical activity such as walking can effectively impact daily energy expenditure since it does not require much motivation or commitment and can be incorporated into everyday life. Thus, physical activity strategies focused on maximizing lipid oxidation can be acceptable to a larger population, given that it primarily includes a low-intensity activity, including walking (Romain et al., 2012).

Walking is regarded as a standardized activity used in clinical examination and evaluation; however, walking speed could be used as a proxy for intensity since the walking pace is different among people, representing different intensities from mild to moderate. Also, health benefits may depend on walking intensity or pace (Shephard, 2001). Self-selected comfortable walking speed (CWS) and the highest comfortable walking speed (HCWS) are the two terminologies in which studies can refer to natural physical activities and exercise accordingly. Despite the fact that walking is a natural survival activity inherited from ancestors, it is still unclear at what intensity people can walk confidently to maintain and attain health benefits by reaching optimum substrate partitioning, particularly lipid oxidation patterns.

A myriad of studies have been conducted using different protocols to determine MFO in the general population as well as in the athletic population, using the treadmill and bicycle as two different modes of exercise. However, the majority of MFO measurement tests have been conducted on athletic populations using bicycles (Paton and Hopkins, 2001). Furthermore, there are still ambiguities regarding MFO, such as whether it occurs within the lowest or highest range of daily ambulatory activities. And if MFO can be achieved at the ambulatory intensities of the average non-athletic population. Furthermore, it has not been established what the relationship is between maximal fat oxidation (MFO) as determined by the incremental test and peak fat oxidation (PFO) during steady-state activity.

MFO is hypothesized to occur in the daily range of physical activities in the general young population. In particular, it is hypothesized to be related to the higher range of self-selected Highest Comfortable Walking Speed. Therefore, encouraging people to promote environments that encourage physical activity, as did our ancestors, can increase physical activity engagement and, as a result, decrease morbidity associated with noncommunicable diseases caused by inactivity.

3.3 Materials and Methods

3.3.1 Study Participants

Fourteen male participants ranging from 21 to 35 years old were selected for the study. All participants are considered to be healthy with no known medical conditions or musculoskeletal injuries, they did not participate in any serious sports activities and were engaged in less than 300 minutes of weekly moderate to vigorous exercise. A set of experiments, including resting on a bed, walking, and running on a treadmill, were made in order to examine the substrate partitioning pattern during a range of self-selected walking paces. Participants' characteristics are aggregated in Table 1. Based on participants' three-day dilatory log reports, the daily energy intake of calories and food compounds (i.e., carbohydrates, fat, proteins) in grams were calculated through the Total Coaching website, reported in Table 1The study was approved by the provincial Health Research Ethics Board (HREB) (project no. 20222100).

3.3.2 Experimental Protocol

Participants partook in two consecutive experimental sessions on separate days that lasted 4.5 hours total (figure 1) with a one-week washout period in between. Given the objectives of the current study and the influence of prior meal composition on whole-body energy metabolism (Kelly and Basset, 2017), participants were instructed to only consume water four hours prior to experimental session one and to fast 12 hours prior to experimental session two.

3.3.2.1 Session One

During the first experimental session, participants' anthropometrics and the consent form were collected, and their daily physical activity was reported through the "Get Active Questionnaire" form designed by the Canadian Society for Exercise Physiology (CSEP). Next, participants were asked to do the Graded Exercise Test (GXT) on a treadmill. During this test, maximal oxygen uptake ($\dot{V}O_{2max}$), absolute Maximal Fat Oxidation (MFO), and Heart Rate (HR) were measured. The main parameters recorded include changes in oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) with the increasing workload during the GXT. The incremental test includes three-minute stages (i.e., step test) starting at 2km/h and increasing by 1km/h every 3 minutes. After reaching 8 km/h, the speed was increased by 1km/h every 30 seconds until participants were exhausted. After five minutes of a recovery period, participants were asked to run at 105% of their maximal running speed achieved during the GXT to verify whether the true $\dot{V}O2_{max}$ was completed.

3.3.2.2 One-week Diet and Physical Activity Control

During the week between sessions, participants' activity levels were recorded using a wrist-worn activity monitor (FITBIT Inspire 2 Activity Tracker). Participants were also asked to report their dietary habits through a diet-log form for three days before coming for the second session in order to control for abnormal dietary behavior that affects energy metabolism and to calculate the average daily calorie intake.

3.3.2.3 Session Two

The second session consisted of a Basal Metabolic Rate (BMR) test and two experimental walking conditions. Participants came to the lab early in the morning after fasting for 12 hours. First, participants were instructed to lay on a bed while the canopy connected to the metabolic cart was placed over the head for 45 minutes. This procedure measured the expired gases for the determination of substrate partitioning. Later, participants walked on a treadmill at self-chosen walking conditions (i.e., NCWS and the HCWS) in a randomized order, each for 35 minutes with a 30-minute washout period in between. The experimental tests were designed to measure Peak Fat Oxidation (PFO), HR, and Rating of Perceived Exertion (RPE) in each walking condition.

For the NCWS condition, participants were asked to walk close to their usual preferred walking speed. To help participants better self-select their NCWS, an imagined situation was presented to the participants. The situation as presented to participants is as follows: You choose to walk a distance to a meeting location, and you have enough time to get there before your companions arrive. You prefer to enjoy your surroundings and feel comfortable covering a one-hour distance without rush or intent. What speed do you choose to walk? Participants can imagine themselves in the situation and self-choose the walking pace during the first five minutes while the speed monitor on the treadmill is hidden from the participants. The following 30 minutes of self-selected walking speed on the treadmill was constant. The same procedure was conducted for the HCWS experimental condition; however, the situation presented to participants was slightly different: You choose to walk a distance to a meeting location, and you ran out of time. You still prefer to enjoy your surroundings and feel comfortable covering a one-hour, but this time with the intent of walking fast. What speed do you choose to walk? In the HCWS condition, participants were asked to keep a continuous comfortable brisk walk for the purpose of fulfilling the imaginary scenario, which covered the same distance as the NCWS condition but in less time.

3.3.3 Indirect Calorimetry

An indirect calorimetry system (Sable Systems International, Las Vegas, NV) was used to measure oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) during non-exercise (BMR) and exercise (incremental and steady-state) conditions. The system was set to record the fractional amount of oxygen and carbon dioxide, mixing chamber temperature, water vapor pressure, barometric pressure, subsample flow rate, and mass flow rate in a negative pressure design. The mass flow generator and controller (FK-500) was set at a rate of 75 L min^{-1} for non-exercise metabolic rate and at a rate of 400 L min^{-1} for exercise metabolic rate. A subsample of that flow (sub-sampler, SS4) was then pulled at 150 ml min^{-1} through a water vapor analyzer (RH-300), a dual infrared carbon dioxide analyzer, and a paramagnetic oxygen analyzer (CA-10 Carbon Dioxide and PA-10 Oxygen Analyzers). Fractions of gases in the room were recorded before and after each measurement for baseline references. Prior to testing, the oxygen and carbon dioxide analyzers were calibrated with room air and reference gases (100% nitrogen and 1% carbon dioxide). Water vapor pressure was zeroed after drying samples gases by passing through a column of magnesium perchlorate and the sub-sampler pump was calibrated using a flow meter (Gilmont Rotameter). Gas volumes included in metabolic calculations are expressed at standard conditions of temperature, pressure, and dry from water (STPD). Additionally, to ensure accurate performance of both indirect calorimetry systems, a propane gas verification was performed with a gas mass flow meter set at 200, 300, and 400 ml.min^{-1} and measured $\dot{V}O_2$ and $\dot{V}CO_2$ were compared against theoretical stoichiometric values as described by Ismail et al.

3.3.4 Data Reduction

Basal metabolic rate measurements were truncated by 10 min out of 45 min of data collection. This procedure discarded the first 5 min and the last 5 min to nullify any signal instability due to familiarization with the ventilated hood and the expected termination of data collection. Respirometry data were then integrated, normalized over time, and used for substrate oxidation calculations and ultimately, EP. Data collected during the MFO exercise test were integrated over time to determine absolute $\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{V}E$, BF, HR, and substrate oxidation.

All metabolic parameters of interest collected during the incremental test were smoothed using polynomial regression models (absolute $\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{V}E$, BF, HR). Ventilatory threshold was determined by plotting the ventilatory equivalent of O_2 and CO_2 over absolute $\dot{V}O_2$ in order to determine the ventilatory threshold. MFO was determined by computing substrate oxidation from equations 2 to 4 and plotting L_{ox} over time to detect its nadir. In addition, the latter time point was used to determine the speed at MFO. Data from the incremental test were used to determine the speed at which the participant exercised during the maximal fat oxidation exercise.

3.3.5 Calculation

Oxygen uptake and carbon dioxide output were computed to estimate carbohydrate oxidation (CHO_{ox}) and fat oxidation (FAT_{ox}) using the following formulae (Kelly & Basset, 2017). Protein oxidation rate (PROox) was estimated at 0.066 $g.min^{-1}$ based on previously published urinary urea excretion measurements made on 12-hour post-absorptive men with normal CHO reserves (Haman et al., 2004).

Resting Stoichiometric Equations:

$$CHOox = -3.226\ \dot{V}O_2 + 4.585\ \dot{V}CO_2 - 0.461\ PROox \quad \text{Eq.1}$$

$$FATox = 1.695\ \dot{V}O_2 - 1.701\ \dot{V}CO_2 - 0.319\ PROox \quad \text{Eq. 2}$$

Exercise Stoichiometric Equations:

$$CHOox = 4.210\ \dot{V}CO_2 - 2.962\ \dot{V}O_2 - 0.461\ PROox \quad \text{Eq. 3}$$

$$FATox = 1.695\ \dot{V}O_2 - 1.701\ \dot{V}CO_2 - 0.319\ PROox \quad \text{Eq. 4}$$

3.3.6 Statistical Analysis

A power analysis was performed with G*Power software prior to the study. The analysis was performed by computing the alpha and beta error probability, the effect size (0.75) for 1 group, and two measurements. The sample size of 8 participants in the group was set with a λ of 18.0, a critical F of 5.59 for a power of 0.95 (G*Power software). All statistical analyses were performed using open-source RStudio (version 2022.02.2+485) software. Shapiro Wilk and Mauchly's tests were run to test the normality of values and homogeneity of variance (sphericity), respectively.

Paired-sample t-test was used to identify differences in MFO and peak fat oxidation (PFO) between incremental test (Fat_{max}) and the HCWS condition.

One-way ANOVA was used to test for differences in substrate oxidation as a function of speed during the Fat_{max} test, followed by Tukey's HSD test. A two-way repeated-measures ANOVA was performed to detect any interactions in PFO rates between NCWS and the HCWS conditions, and Tukey's HSD was applied to detect any differences between conditions and to decompose any significant interaction. Another two-way repeated-measures ANOVA was run to detect HR and RPE changes over time in both experimental conditions.

Data were reported as means ± standard deviations (SD), and statistical significance was set at $p < 0.05$.

3.4 Results

According to the Shapiro-Wilk test, the data was normally distributed, and the homogeneity of variance was met. Mauchly's test indicated that the assumption of sphericity had been violated in all conditions and variables; therefore, the degree of freedom was corrected accordingly.

3.4.1 Anthropometrics

The summary of participants' characteristics is presented in Table 1. 92.8% of the participants were in the normal range for BMI (minimum = 20.5, maximum = 31.5), according to the American College of Sports Medicine Guidelines (Ferguson, 2014), and there was one participant with a BMI of over 30.0. 57.1% of participants ranging from 21 to 35 were above the average age of 28.5 years who were engaged in less than 300 minutes of weekly moderate to vigorous exercise (i.e., Mean = 112.8 ± 57.7 minutes) based on their response in the Get Active Questionnaire developed by the Canadian Society for Exercise Physiology (CSEP). Also, the average daily steps and energy expenditure recorded by wristband physical activity trackers were less than 9000 steps and 3600 calories, respectively. Based on three-day reports of daily food ingestion, the participants' daily energy intake was less than 2500 calories, from which carbohydrates, on average, constituted 52.7%, and protein and fat 27.8 % and 19.3%, respectively. Furthermore, the average maximum oxygen uptake was less than 40 $ml.min^{-1}.kg^{-1}$ corresponding to MFO.

3.4.2 Fat_{max} Test

As mentioned in the methodology, there was one portion of the incremental test dedicated to MFO determination at 6 different speeds from 2 to 7 km/h and a ramp test to determine maximum oxygen uptake. The one-way ANOVA analysis indicated that by increasing the speed, $\dot{V}O_2$, $\dot{V}CO_2$, whole-body fat, and carbohydrate oxidation as a function of intensity ($p < 0.0001$), and MFO was reached at the speed of 6.3 ± 1.2 km/h (i.e., 0.473 ± 0.201 g/min). The average relative maximal oxygen uptake for participants derived from the ramp test was 49.54 ml/min/kg.

3.4.3 NCWS and the HCWS

According to the two-way repeated measures ANOVA, there was no significant interaction over the three 10-minute time phases among all the parameters in both the HCWS and NCWS, however as table 2 indicates, there was a significant effect of walking condition on parameters such as $\dot{V}O_2$ ($M_{NCWS} = 0.79$, $M_{HCWS} = 1.34$, SE = 0.07, $p < 0.0001$), CHO ($M_{NCWS} = 0.29$, $M_{HCWS} = 0.54$, SE = 0.05, $p < 0.0008$), and Fat ($M_{NCWS} = 0.25$, $M_{HCWS} = 0.43$, SE = 0.03, $p < 0.0001$). There was no walking condition effect on RER.

There was a slight increase in whole-body CHO utilization rate, through time in the NCWS condition ($M_{phase1} = 0.23$, $M_{phase\ 2} = 0.31$, $M_{phase\ 3} = 0.32$, SE = 0.06); however, this pattern was reversed in the HCWS condition ($M_{phase\ 1} = 0.57$, $M_{phase\ 2} = 0.54$, $M_{phase\ 3} = 0.52$, SE = 0.06). Also, the whole-body fat oxidation rate showed a slightly decreasing pattern in NCWS condition (M_{phase} 1 = 0.28, $M_{phase\ 2} = 0.24$, $M_{phase\ 3} = 0.23$, SE = 0.03) and a slightly increasing pattern in the HCWS condition (M_{phase} 1 = 0.42, $M_{phase\ 2} = 0.43$, $M_{phase\ 3} = 0.44$, SE = 0.03). Notably, no effect of time, but the effect of walking condition was detected for the intensity, or the metabolic rate reported in the percentage of $\dot{V}O_{2max}$ ($M_{NCWS} = 21.2$, $M_{HCWS} = 35.5$, SE = 1.3, $p < 0.0001$).

3.4.4 MFO and PFO

According to the t-test results, there were no significant differences in maximal fat oxidation rate from the MFO determination test and peak fat oxidation rate from the HCWS condition. Results indicated that the MFO derived from the Fat_{max} determination test and PFO from the HCWS are roughly equal (MFO; mean = 0.47 ± 0.2 g/min, and PFO; mean = 0.43 ± 0.13 in g/min, $p = 0.88$), meaning that the Highest Comfortable Walking Speed coincides with the highest rate of oxidizing lipid energy substrates. However, scrutinizing the results show a variance in the speed ($Speed_{MFO}$ = 6.3 ± 1.2, $speed_{PFO}$ = 5.7 ± 0.7 km/h, $p = 0.08$) and the intensity ($intensity_{MFO}$ = 39.58 ± 11.8%, $intensity_{PFO}$ =35.46 ± 5.8% of $\dot{V}O_{2max}$, $p = 0.30$) corresponding to MFO and FPO.

3.4.5 HR and RPE

The two-way ANOVA (condition*time) analysis indicated a significant condition effect in HR and RPE values, as revealed in figure 2. The two minutes averaged HR values did not change through time but were different between conditions (M_{NCWS} = 90, M_{HCWS} = 109. SE = 3.7, $p < 0.0001$). No time effect was detected for the RPE scores reported by participants; however, the scores varied significantly by changing the condition (M_{NCWS} = 8.4, M_{HCWS} = 12.3, SE = 0.3, $p < 0.0001$).

Table 1 – Anthropometrics, Diet, and Activity Characteristics of the Participants

Parameter	**Median**	**Mean ± SD**	**%95 Confidence Interval of Difference**	
			Lower Quartile	**Upper Quartile**
Age (years)	30.00	28.5 ± 5.0	24.0	32.0
Mass (kg)	78.25	76.9 ± 10.9	73.2	80.8
Height (cm)	177.0	176.9 ± 5.9	175.0	179.4
BMI (kg/m^2)	24.85	24.53 ± 2.8	23.0	25.5
Daily Diet (Cal) – Diet log	1825	1863.9 ± 606.3	1326	2257
Daily Fat (g) – Diet log	68.0	73.2 ± 31.8	47.3	88.8
Daily Protein (g) – Diet log	103.03	105.6 ± 37.0	78.4	130.7
Daily Carbohydrate (g) – Diet log	177.9	200.0 ± 94.3	119.4	262.2
Daily EE (Cal) - Tracker	2252	2306.7 ± 388.3	2196	2343
Daily Steps - Tracker	5319	5487.3 ± 1513.0	4482	6366
Weekly Exercise (min) – Get Active Questionnaire	120.0	112.8 ± 57.7	60.0	131.2
$\dot{V}$**O2**$_{max}$(L/min)	3.71	3.81 ± 0.69	3.35	4.40
MFO (g/min)	0.477	0.473 ± 0.20	0.344	0.60

Mean ± Standard Deviation

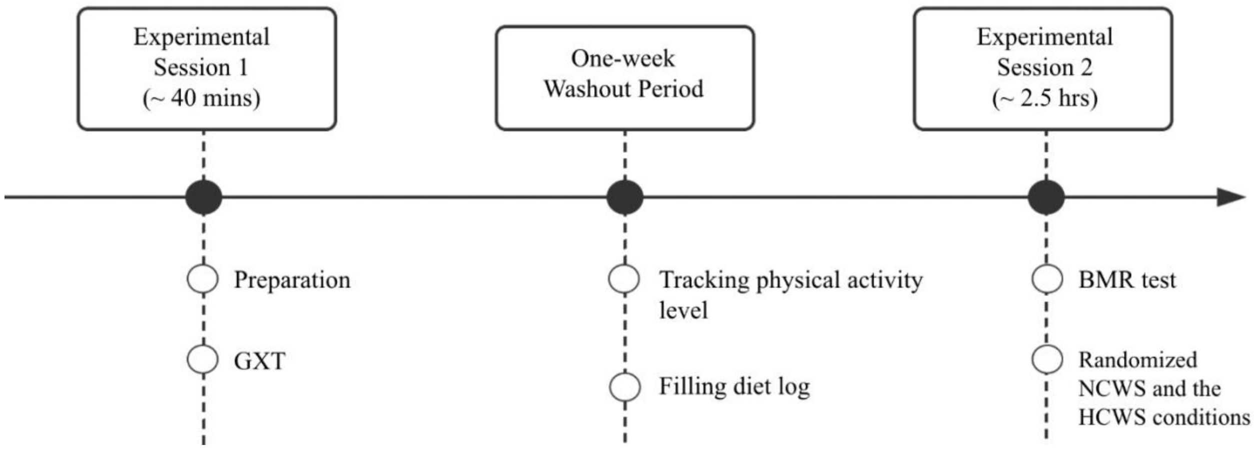

Fig. 1. The block diagram of the experimental design, including two experimental sessions and one week washout period consisting of GXT, BMR, and walking conditions at two different paces. Diet and the level of physical activity were monitored and recorded via a three-day diet log and seven-day wristband-worn physical activity tracker.
Preparation: Includes verbal explanations, instrumentation, and filling out the consent form.
GXT: The incremental test of a graded exercise on the treadmill was estimated to take 25 minutes.
BMR: The basal metabolic rate test was conducted while being at rest for 45 minutes under a canopy machine.
NCWS: The test of walking at a natural comfortable walking pace on the treadmill for 35 minutes.
HCWS: The test of walking at the highest comfortable walking pace on the treadmill for 35 minutes.

Table 2 – Metabolic Outcomes According to the Experimental Conditions

Parameter	Condition	Mean ± SD	%95 Confidence Interval of Difference	
			Lower Bound	Upper Bound
Self-selected Pace (km/h)	NCWS	3.50 ± 0.72	2.77	4.07
	HCWS	5.73 ± 0.76	5.00	6.30
$\dot{V}O_2$(L/min)	NCWS	0.794 ± 0.25	0.65	0.938
	HCWS	1.348 ± 0.25**	1.20	1.492
$\dot{V}CO_2$	NCWS	0.629 ± 0.21	0.512	0.746
	HCWS	1.075 ± 0.21**	0.958	1.192
RER	NCWS	0.796 ± 0.04	0.771	0.820
	HCWS	0.797 ± 0.04	0.772	0.821
CHO mg	NCWS	0.293 ± 0.22	0.168	0.418
	HCWS	0.548 ± 0.22**	0.424	0.673
FAT mg *	NCWS	0.254 ± 0.11	0.191	0.318
	HCWS	0.436 ± 0.11**	0.372	0.499
% OF $\dot{V}O2_{max}$	NCWS	21.2 ± 5.08	18.3	24.0
	HCWS	35.5 ± 5.08**	32.6	38.3
% CHO	NCWS	32.5 ± 16.68	23.3	41.8
	HCWS	32.9 ± 16.68	23.6	42.2
% Fat	NCWS	67.5 ± 16.68	58.2	76.7
	HCWS	67.1 ± 16.68	57.8	76.4

Mean ± Standard Deviation
* Denotes peak fat oxidation. ** Denotes significant differences between the conditions ($p < .05$).

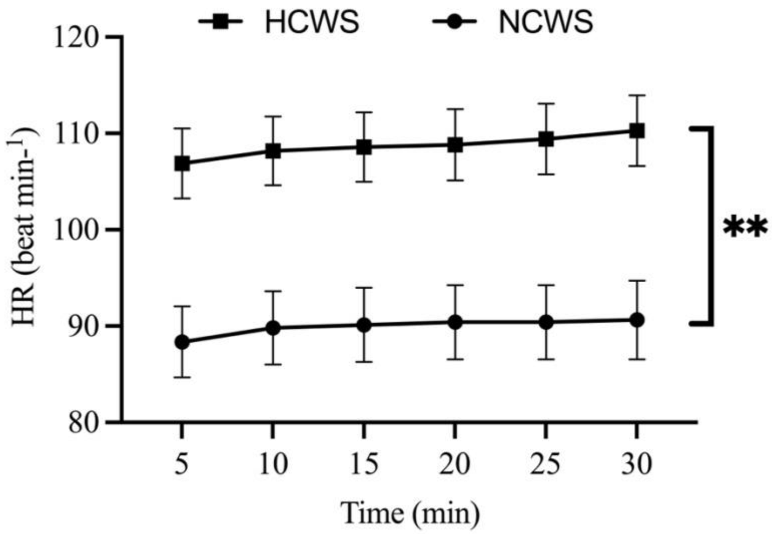

Fig. 2-A

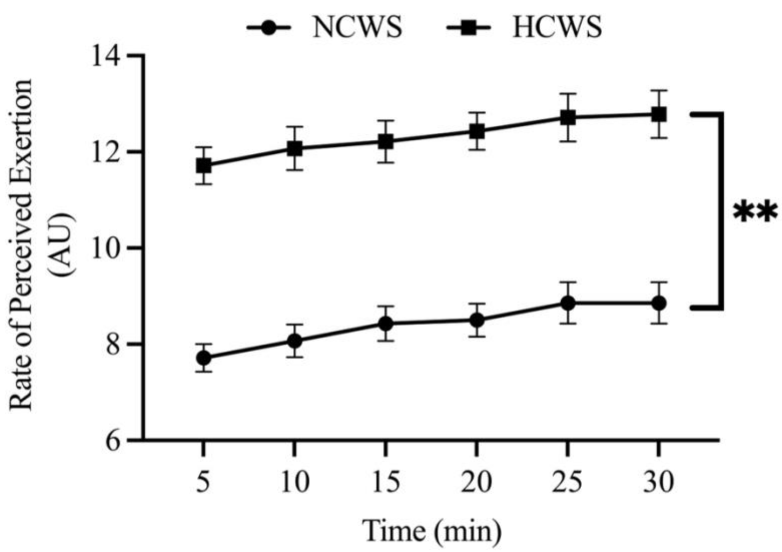

Fig. 2-B

Fig. 2. HR and RPE during both experimental conditions. Figure 2-A belongs to heart rate, and figure 2-B presents RPE. ** Denotes significant differences between the conditions ($p < .05$).

3.5 Discussion

The current study aims to assess whether MFO falls within the spectrum of self-selected ambulatory intensities in the general male population and to investigate the intensity and speed corresponding to the maximum fat oxidation rate. Accordingly, fat oxidation rates are examined to gain an understanding of substrate contribution during intensities of self-selected natural and highest comfortable daily walking paces. The findings of this study indicate that MFO corresponds to daily ambulatory domain intensities. As well, statistical outcomes showed that the peak fat oxidation rate at the self-chosen HCWS and maximal fat oxidation rate at the standardized MFO determination test were roughly identical. These findings confirmed our primary hypothesis that MFO occurs within the range of daily ambulatory activities and particularly corresponds to the HCWS.

3.5.1 Population Characteristics

As aggregated in Table 1, the population in this study consisted of fourteen male participants ranging from 21 to 35 years old selected from a non-athlete general population. The data indicated that the participants' BMI range was normal according to American College of Sports Medicine guidelines (Ferguson, 2014), and only one participant was considered obese. Thus, the outcomes represent a good cross-section of the general male population with no known medical conditions or musculoskeletal injuries, who did not participate in any serious sports activities and were engaged in less than 300 minutes of weekly moderate to vigorous exercise based on their response in the Get Active Questionnaire developed by Canadian Society for Exercise Physiology (CSEP). Also, the average daily steps and energy expenditure recorded by wristband physical activity trackers supported the assumption that all the participants were not highly physically active (Tudor-Locke et al., 2011). Furthermore, the average maximum oxygen uptake was less than 40 $ml.min^{-1}.kg^{-1}$, supporting that they are not considered highly trained athletes (Shvartz and Reibold, 1990).

3.5.2 Steady-State Walking Conditions

The steady-state walking conditions were well-standardized, as no cardiovascular drift was observed during each condition, as previously demonstrated by Basset and Boulay (2000). HR values did not change within each condition's time frame but were different in terms of condition ($p < 0.05$). The RPE scores reported by participants every five minutes in each experimental condition did not significantly vary. However, the scores varied significantly by changing the condition ($p < 0.05$). Thus, based on steady-state HR and RPE values, participants did not experience fatigue during self-selected Natural Comfortable and the Highest Comfortable walking conditions.

In our study, NCWS and the HCWS do not differ significantly in RER ($p = 0.95$). Following that, CHO oxidation was significantly different in terms of condition ($p = 0.0008$). Within the conditions, the whole-body CHO utilization rate indicates a slightly increasing pattern within the three 10-minute time phases of the NCWS condition. However, this pattern was reversed in the HCWS. The whole-body fat oxidation rate was also significantly different between the two walking conditions ($p < 0.0001$), with a slightly decreasing pattern in NCWS and an increasing pattern in the HCWS. Notably, the intensity or the metabolic rate reported in the percentage of $\dot{V}O_{2max}$ was significantly different between the conditions ($p < 0.0001$); however, the value remained the same among the three 10-minute time phases of each condition.

3.5.3 MFO and PFO

On the basis of the accepted methodology for the GXT and the experimental walking conditions, MFO can be investigated among the ambulatory intensities. Results indicated that the MFO derived from the Fat_{max} determination test and PFO from the HCWS are roughly equal, meaning that the Highest Comfortable Walking Speed coincides with the highest rate of oxidizing lipid energy substrates. However, by scrutinizing the results, it was shown that PFO was reached at a lower speed and intensity compared to MFO, but the difference was not significant. However, we are confident that during the HCWS, the PFO represents what is occurring at the cellular site at the respiratory chain, whereas during the incremental test, no steady-state condition was seen because of the change in $\dot{V}O_2$, HR, and RER when the speed was increased from 2 to 7 km/h based on the GXT protocol. Thus, by walking at the HCWS, Maximal Fat Oxidation was approached at a lower speed and workload compared to the standardized MFO test. It is well-documented that the exercise intensity at which maximal fat oxidation occurs varies according to training status, sex, and mode of exercise, and announced maximal fat oxidation rates of ~ 0.4 – 0.5 g/min and the intensities at which it occurs (47-52% of $\dot{V}O_{2max}$) are reached in moderately trained individuals (Melanson et al., 2009). Nariman AL Mulla et al. recruited 6 young, healthy men and measured the whole body fat oxidation (e.g., 0.58 ± 0.05 g/min) during 90 minutes of exercise at 40% of $\dot{V}O_{2max}$ on cycle ergometer (Mulla et al., 2000), which both correspond to our data.

In most studies investigating PFO during walking (Achten et al., 2003; Venables et al., 2005; Venables and Jeukendrup, 2008), the walking speed was imposed and relatively fast at 6.5 to 7.5 km/h, which is close to the transition speed from walking to running and even faster than the highest range of self-selected comfortable walking speed we examined in the current study (i.e., HCWS = 5.73 ± 0.76 km/h).

Similar to the present study but with few subtle discrepancies, Nordby et al. (2006) used the same graded exercise tests, including the MFO test protocol. They recruited a group of eight healthy untrained individuals with a BMI of 23 and $\dot{V}O_{2max}$ 3.5 ± 0.1 L/min and had a 3-hour walking test at 58% of their $\dot{V}O_{2max}$. As a result, a higher PFO than MFO was reached (PFO: 0.462 ± 0.03 g/min, MFO: 0.384 ± 0.049 ± g/min), which is maybe due to cardiovascular drift leading to higher PFO due to glycogen depletion.

If the current study had been conducted under prolonged conditions, probably the same amount of fat oxidation would have been reached, as the evaluation characteristics are roughly the same between the two studies. This raises a question on the validity of the MFO determination test since higher fat oxidation was reached after prolonged exercise, supported by the fact that scientists believe maximal fat oxidation should be reached when the muscle glycogen is at its lowest (Achten and Jeukendrup, 2004).

We have reasons to question the validity of the MFO determination protocols used in the literature. According to Nordby (2006) reports, further studies are needed as not only the PFO during the steady-state condition do not resemble the MFO value from Fat_{max}, but also PFO was higher than MFO, which violates the MFO determination test validity. However, our results indicated that MFO and PFO are roughly the same. Still, the intensity and speed at which they were achieved were different, making a question mark again if the common MFO determination test is a reliable procedure.

The results indicate that walking paces involving ambulatory movement contribute to a higher percentage of fat contribution to energy production in accordance with what the MFO determination test indicates (Maunder et al., 2018). The impairment of fat metabolism appears to play a significant role in the development of obesity and diabetes. There has been evidence that reduced fat oxidation rates (e.g., increased resting respiratory quotient; RQ) are associated with weight gain (Houmard, 2008). Exercise acutely increases fat oxidation, and endurance training increases the capacity to oxidize fat (Jeukendrup, 2002), suggesting that regular physical activity could induce fat mass loss by increasing fat oxidation. Physical activity, including ambulatory activities, is often prescribed to improve health and fitness (Bensimhon et al., 2006). To combat chronic diseases, walking is a popular activity for people of all ages with a low risk of injury (Hardman, 1999).

Malenson et al. have found that the oxidation of fat over 24 hours is little affected by exercise. There is, therefore, an apparent paradox between chronic and acute physical activity in the fat metabolism (Melanson et al., 2009). Clearly, high metabolic rate intensities do not affect fat balance if intake remains unchanged, and participation in moderate amounts of physical activity can help to maximize fat oxidation capacity (Melanson et al., 2009). It is well established that as exercise intensity increases up to approximately 55%–65% of $\dot{V}O_{2max}$, whole-body fat oxidation increases but decreases at higher levels (Achten and Jeukendrup, 2004; Romijn et al., 1993). There is no clear explanation for why fat oxidation decreases at high exercise intensities, but evidence suggests a decrease in FFA availability as a result of reduced blood flow to adipose tissue and a limited capacity to oxidize plasma FFA to generate adenosine triphosphate (Achten and Jeukendrup, 2004).

During exercise, skeletal muscle is provided with lipids by circulating very low-density lipoprotein-triglycerides (VLDL-TG), intramuscular triglycerides (IMTG), and long-chain FA (LCFA) derived from lipolysis of subcutaneous and visceral adipocytes (Melanson et al., 2009). There is mounting evidence that the relative contributions of carbohydrates and lipids to oxidative metabolism during exercise vary with the workload (Jeukendrup et al., 1998b; Mul et al., 2015; Spriet and Watt, 2003). The maximum lipid metabolism was found to occur at a power output of about 65% of $\dot{V}O_{2max}$ in highly trained individuals using tracer methodology (Romijn et al., 1993). In that study, peripheral lipolysis was stimulated maximally at 25% of maximal $\dot{V}O_{2max}$ power. Although the mechanism is unclear, the fat oxidation rate is higher in female subjects at a given exercise intensity, and maximal fat oxidation occurs at higher intensities. Female subjects may use more fat during exercise due to differences in hormone and catecholamine levels, an increase in the oxidative distribution of muscle fiber types, increased sensitivity to catecholamine-stimulated lipolysis, or an increase in hormone-sensitive lipase activity (Achten and Jeukendrup, 2004). Walking and running seem to result in greater fat oxidation rates than cycling, perhaps due to the recruitment of smaller muscle mass and lower catecholamine responses (Achten and Jeukendrup, 2004).

3.6 Methodological Considerations

As previously mentioned in the results of the current study, PFO and MFO were indicated to be the same, but the intensity, speed, and $\dot{V}O_2$ at which they were achieved were different. This is while exercise intensity is repeatedly used as one of the most fundamental determinants of whole-body fat oxidation rate in many studies (Venables et al., 2005). The validated MFO determination test developed by Achten et al. (2002) and Venables et al. (2005) could be valid in terms of MFO determination but can be debatable in terms of at which percentage of $\dot{V}O_{2max}$, MFO may occur.

Although this graded exercise testing with 3-minute stages is the gold standard and the validated method to discover MFO, based on our data, there may be other circumstances under which speed and metabolic rate associated with MFO will be lower than we observed during the Graded Exercise Testing. Many determinants of MFO have been identified as of the development of the original protocol (Achten et al., 2002). However, considering the practical utility of this protocol, further research, including glycogen depletion and fasting, must be taken into account to understand the characteristics better while assessing MFO.

The recruitment of motor units may be quite different between self-selected walking speeds and treadmill incremental exercise. No difference in RER between walking conditions clarifies that both conditions are within the oxidative fiber type recruitment intensity. Moritani et al. (1984) provided evidence of EMG showing that type II fibers are recruited over increasing intensity. Although not completely elucidated, motor units recruitment during incremental treadmill exercise may be predicted by Henneman's size principle (Conwit et al., 1999). The size principle states that as more force is needed, motor units are recruited in a precise order according to the magnitude of their force output, with small oxidative motor units being recruited first and large glycolytic and less oxidative motor units being increasingly recruited along with load increment. On the other hand, in self-pacing activity, the fiber type recruitment pattern is not so obvious to predict or easy to investigate.

Also, there are several limitations to the current study: we have examined the daily ambulatory activities on a treadmill, but further studies are needed to determine whether the treadmill relates to real living conditions. However, the current study investigated the workload approaching MFO, and the outcome is not invalidated by testing on a treadmill or on the ground.

In addition, women are not included in this study due to the constraints of monitoring menstruation. The female sex-steroid hormones significantly influence metabolic acute and chronic responses to exercise and therefore demand stringent control of phases of hormonal variations. These sex-steroid hormones fluctuate in a timely manner throughout the menstrual cycle to modulate the energy homeostasis (Davidsen et al., 2007).

3.7 Conclusion

MFO (also called fat_{max} or fat_{max} zone) is a critical factor in understanding the regulation and interplay of substrate utilization related to exercise intensity. Among all the different types of physical activity, activities at intensities that elicit maximal fat oxidation (fat_{ox}) stand out as a potential regimen for reducing body fat and improving cardiorespiratory fitness in overweight individuals. The primary form of human physical activity that can be investigated for fat oxidation is walking. In order to determine the maximum rate of fat oxidation during walking, we investigated a range of walking speeds (e.g., NCWS and HCWS).

In conclusion, as hypothesized, Maximal Fat Oxidation (MFO) falls within the range of daily physical activity intensities for the general population, in particular, at the highest range of ambulatory activities (e.g., HCWS).

There is also a key practical message from this study that competitive advertisements for fitness and high-intensity training should be modified to promote a more active work-life balance and make the surrounding environment more physically active. Thus, society can achieve its objective of reducing the number of non-communicable diseases.

Future research can examine the metabolic differences between treadmills and tracks, as well as those between men and women, with further investigation through the validity of the MFO determination test, as using the MFO test as a valid means of determining maximal fat oxidation may be questionable.

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CHAPTER FOUR: OVERALL SUMMARY OF STUDY

For decades, social advertisements and physical activity guidelines such as from the American College of Sports Medicine (ACSM) and the Canadian Medical Association Journal (CAMJ), have urged people to engage in moderate to vigorous forms of exercise for at least 150 minutes per week (Yang, 2019). However, these guidelines have failed to decrease chronic non-communicable diseases (Matheson et al., 2013) as high-intensity protocols such as HIIT have one of the biggest shortcomings: ineffective adherence.

Scientists are particularly interested in determining the exercise intensity and maximum fat oxidation because these are integral components of metabolic flexibility that affect endurance performance and metabolic health (Goodpaster & Sparks, 2017; Maunder et al., 2018), and fat is the primary fuel oxidized during low-intensity physical activity. Also, an increased fat metabolism, and metabolic disorders like heart disease, type II diabetes, etc., could potentially alleviate (Achten and Jeukendrup, 2004).

Thus, bringing more non-exercise activities to our daily routine could be an easy solution for the lack of commitment to exercise as one can increase daily light physical activities. These activities could vary from household chores to going for a walk every day. Following this, researchers are currently investigating strategies to increase daily energy expenditure that are both acceptable to the general population and effective at reducing associated chronic diseases by changing the built environment and encouraging active transport (Chung et al., 2018).

In this study, an investigation of whole-body fat oxidation among healthy young men was conducted using the lowest and highest ranges of self-selected comfortable walking paces aimed primarily to examine whether Maximal Fat Oxidation (MFO) is met during daily ambulatory activities and, if so, whether MFO is in the scope of the intentional type of everyday activities (exercise) or the non-intentional activities of daily living. First, it was hypothesized that MFO is within the self-selected walking pace range. Second, MFO occurrence is more related to the highest range (e.g., HCWS).

In the present study, a number of variables were controlled, including diet, sex, duration, and type of weekly physical activity. The study consisted of fourteen young, healthy non-athletic men ranging from 21 to 35 years old who completed two lab-based experimental sessions and a one-week washout period. In the first experimental session, participants came for four hours of fasting in the morning to do the graded exercise test (GXT) for about 25 minutes. Following the first session, participants were asked to wear physical activity trackers for a week and fill out the designed diet recall form for three days before coming for the second experimental session. On the day of the second experimental session, participants were asked to lie on a bed under the canopy system for forty-five minutes and, immediately after, to do two different walking activities on a treadmill with thirty minutes of rest in between (walking at self-pace, NCWS, and the HCWS) while being on a twelve-hour overnight fast.

During the test, oxygen uptake and carbon dioxide output were collected from each participant by an indirect calorimetry system (Sables Systems International, Las Vegas, NV, USA). Later, daily fat oxidation and carbohydrate oxidation in absolute (g/min) and relative (kcal/kg/km) terms were calculated.

After applying proper statistical analysis, the evidence showed that the absolute MFO determined by GXT and Peak Fat Oxidation (PFO) at the HCWS are roughly the same (PFO = 436 ± 0.1 mg.min-1, MFO = 473 ± 0.20 mg.min^{-1}). These findings confirmed our primary hypothesis that MFO occurs within the normal range of daily activities, particularly at the self-selected Highest Comfortable Walking Speed. However, the intensity and speed at MFO were higher than the PFO ($Speed_{MFO}$ = 6.3 ± 1.2, $speed_{PFO}$ = 5.7 ± 0.7 km/h, p = 0.08, $intensity_{MFO}$ = 39.58 ± 11.8%, $intensity_{PFO}$ =35.46 ± 5.8% of $\dot{V}O_{2max}$, p = 0.30). Exercise intensity is repeatedly used as one of the most fundamental determinants of whole-body fat oxidation rate in many studies (Venables et al., 2005). The validated MFO determination test developed by Achten and Venable (Achten et al., 2002; Venables et al., 2005) could be valid in terms of MFO determination but can be debatable in terms of at which percentage of $\dot{V}O_{2max}$ MFO may occur. Although we accept this graded exercise testing with 3-minute stages as the gold standard and the validated method to discover MFO, based on our data, there may be other circumstances under which speed and metabolic rate associated with MFO will be lower than we observed during the Graded Exercise Testing. Many determinants of MFO have been identified as of the development of the original protocol (Achten et al., 2002). However, considering the practical utility of this protocol, further research must be taken into account while assessing MFO.

A key practical message from this study is that competitive advertisements for fitness and high-intensity training should be changed to encourage people to create their work-life balance in an active way and make their surrounding environment more physically active. Therefore, society can accomplish its objective of reducing the number of non-communicable diseases.

Future research can examine the metabolic differences between treadmills and tracks, as well as those between men and women, with further investigation through the validity of the MFO determination test.

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APPENDIX A – FULL ETHICS CLEARANCE – HREB APPROVAL

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

Research Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

February 08, 2022

22 Byron St, St. John's, NL, Canada.

Dear Mrs Zebarjad:

Researcher Portal File # 20222100
Reference # 2021.206

RE: Exploring Physical Activity Paradigm to Maximize the Utilization of lipid Energy Substrates.

Your application was reviewed by subcommittee under the direction of the HREB and your response was reviewed by the Chair and the following decision was rendered:

X	Approval
	Approval subject to changes
	Rejection

Ethics approval is granted for one year effective February 7, 2022. This ethics approval will be reported to the board at the next scheduled HREB meeting.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- Research proposal, approved
- Recruitment poster, approved
- Introductory Session Outline, approved
- 3-day diet recall form, approved
- Email Script for communication, approved
- Consent form, approved
- Get Active Questionnaire, approved

Please note the following:

- This ethics approval will lapse on February 7 2023. It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date.
- This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approvals.
- Modifications of the study are not permitted without prior approval from the HREB. Request for modification to the study must be outlined on the relevant Event Form available on the Researcher Portal website.
- Though this research has received HREB approval, you are responsible for the ethical conduct of this research.
- If you have any questions please contact info@hrea.ca or 709 777 6974.

The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), ICH Guidance E6: Good Clinical Practice Guidelines (GCP), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

We wish you every success with your study.

Sincerely,

Dr Fern Brunger, Chair Non-Clinical Trials Committee
Health Research Ethics Board

APPENDIX B – DIET RECALL FORM

Use this table to help you record your eating meals. Please see the other side to learn how to use this table.

Meal	Day 1: ------	Day 2: ------	Day 3: ------
Breakfast (1st meal)			
Snack			
Activity			
Lunch (2nd meal)			
Snack			
Activity			
Dinner (3rd meal)			
Snack			
Activity			

Modified version of 3-day food and activity journal (Alberta Health Services)
Modified by Niloofar Zebarjad
Date: 01/07/2022

How to fill this table:

- Write down everything you eat and drink three days before making the second experimental session.
- Include:
    - How much food you eat. See the suggestion below to estimate portion sizes. If the food comes in a package, just write down the package size. Example: 175 ml container of yogurt.
    - How the food is cooked (for example: fried, baked, boiled, barbecued).
    - Anything you add to food, during or after cooking. Example: cream, sugar, oil, butter, jam, syrup, ketchup or other sources, dressing or condiments.
    - Details about restaurant foods, fast foods, or packaged foods (for example: McDonald's Big Mac or KFC chicken).
- Measure the food you eat for a day or two to help you understand how much you eat and drink. Use measuring cups and spoons.
- Write down all your activities for the day. Include planned activities (going for a walk or swim) and activities of the daily life (housework or grocery shopping). Comments may include where you ate, your mood, or stress level.
- Use more paper if you need to, or photocopy the other side of this handout.

To estimate portion sizes, use the guidelines below:

This amount of food:	…is about the same size as:
2½ oz (75g) of meat	a hockey puck
1½ oz (50 g) of cheese	2 white erasers
1 cup (250 mL)	a baseball or fist
½ cup (125 mL)	a hockey puck
1 medium piece of fruit	a tennis ball
2 Tbsp (30 mL)	1 golf ball
¼ cup (60 mL)	2 golf balls
1 tsp (5 mL) – use for butter, margarine, mayonnaise	a thumb tip or one die

Example of how to fill in your food table:

Meal	Day 1: ---	Day 2: ---
Breakfast (1st meal)	1 cup Bran Flakes with 1 tsp sugar and 1/2 cup 1% milk. One cup coffee black Slice whole wheat toast with 2 tsp soft margarine.	1 egg fried in 1 tsp butter with 3 stripes of bacon. Cups tea (chamomile).
Snack	1 carrot muffin – Tim Hortones. 1 medium black coffee – Tim Hortones.	1 medium apple
Activity	Stressful day at work	30 min walk

Modified version of 3-day food and activity journal (Alberta Health Services)
Modified by Niloofar Zebarjad
Date: 01/07/2022