Is Peak FAT Oxidation within Daily Locomotor Activities?

by

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LIST OF ABBREVIATIONS

CHO	Carbohydrate
CHO_{ox}	Carbohydrate Oxidation
CNS	Central Nervous System
CUC	Caloric Unit Cost
EE	Energy Expenditure
FA	Fatty Acid
FAT_{ox}	Fat Oxidation
HIIT	High-Intensity Interval Training
MET	Metabolic Equivalent of Task
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 Production

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Chapter One: Introduction

1.1 Background of Study

Currently, obesity is a major public health challenge, affecting more than 1.9 billion adults worldwide and are rapidly increasing in developed nations (WHO, n.d.). It is undeniable that lipids have beneficial roles; for instance, serving as an energy source, forming structural components of cell membranes, and participating in signaling pathways (Fahy E. et al., 2011). However, body fat accumulation and impaired fatty acid (FA) oxidation in skeletal muscle are characteristics of being overweight and obesity (Kim J.Y. et al., 2000), and several studies have proved the prevalence of many metabolic and cardiovascular disorders caused by obesity (Borén, J. et al., 2013; Fava, M.C. et al., 2019). However, physical activity, including non-exercise activities (Levine et al., 1999) along with a proper diet, is well-recognized as a vital means of preventing morbidity and mortality due to obesity (Willett W.C. et al., 2006; Miura S. et al., 2014). The American College of Sports Medicine has encouraged the public for decades to undertake the modicum of physical activity for at least 150 min•wk^{-1}. However, this recommendation has had only modest effects on non-communicable disease so far (Matheson G.O. et al., 2013).

There have been various exercise training protocols developed as means of improving one's physical health; however, choosing and staying committed to an exercise routine has always been challenging. For instance, high-intensity interval training (HIIT) is a popular training method, well-known for being time-efficient, effective in improving cardiorespiratory and metabolic function, and, successively, boosting physical and mental health in healthy individuals and those with physical health complications (Martland R. et al., 2020). Despite the rapidly increasing interest in HIIT, it can only work with one's full compliance under ideal circumstances. Even HIIT supporters admit that this demanding protocol "may not be safe, tolerable or appealing for some individuals" (Gibala, M. J. et al., 2012). They also claimed that "given the extreme nature of the exercise, it is doubtful that the general population could safely or practically adopt the model" (Gibala, M. J. et al., 2008). In addition, there are different factors that have decisive roles in promoting physical activity. For instance, feelings of confidence and competence are psychological factors that are effective in physical activity participation. Since HIIT consists of short bouts, it may give people the confidence that they are capable of fulfilling this task; however, failing to sustain such vigorous intensities might deteriorate confidence over time.

Additionally, enjoyment is also an important factor linked to participation in physical activities. In a study by Bartlett et al. (2011), participants who took part in a HIIT protocol (6 × 3 min high-intensity interval running at 90% $\dot{V}O_{2max}$ with 6 × 3 min active recovery at 50% $\dot{V}O_{2max}$ with a 7-min warm-up and cool down at 70% $\dot{V}O_{2max}$) had a higher rating of enjoyment according to the Physical Activity Enjoyment Scale (PAES) (Kendzierski D. & DeCarlo K.J., 1991). However, such a study clearly has no public health generalizability since the sample was small (n = 8) and it recruited only young, healthy, and fit men. Moreover, these ratings were post-exercise evaluations and, more specifically, after a 7-min cool-down. Further, authors concluded that according to PAES, the higher ratings of perceived enjoyment in their study is due to the participants' feelings of challenge and accomplishment. Biddle and his colleagues believe that this pleasant feeling is unlikely to occur during high-intensity physical activity (Biddle S.J.H et al., 2015). This could be the reason why vigorous exercise has been used as a punishment by some teachers and sergeants (Rico K, 2002; United States Government US Army, 2020).

Additionally, one of the most commonly reported physical activity participation barriers is lack of time (Biddle S.J.H et al., 2015). HIIT partially addresses this issue by lowering time commitment. However, it is notable that time constraint might have an effect on psychological factors. In fact, the main issue does not reside in finding more time but in boosting people's positive feelings about exercise (Lee, H. H. et al., 2016). In doing so, they should devote more time to physical activity and value participation and its health benefits. Making physical activity harder and more painful is definitely not the proper way to achieve this goal (Biddle S.J. & Batterham A.M., 2015).

On the other side of the debate, a systematic review of 72 studies showed that more engagement in light-intensity activity could result in better acute and long-term cardiometabolic health outcomes and decrease the risk of all-cause mortality (Chastin S.F.M. et al., 2019). According to McCarthy et al. (2017), low-intensity physical activities might be more useful for metabolically impaired individuals, and since it has a lower risk of injury (Ekelund U. et al., 2019), it could be beneficial for high-risk populations such as the elderly (Tse A. C. et al., 2015).

Another significant aspect of participating in low-intensity physical activities is that it could even be a part of an individual's leisure time and a means of enjoyment and pleasure. For instance, video games, such as those using the Nintendo Wii Fit Plus™, usually consist of intensities between 1.3 and 5.6 METs, and have shown to be a safe and fun form of physical activity (Lanningham-Foster L et al., 2009). It has been shown that this video game prevents metabolic diseases or improves health condition of people with chronic diseases (Miyachi M. et al., 2010), and as the company declares, it is suitable for everyone, whether young or old. It is also a cheaper and easier alternative to traditional exercises (e.g., running, cycling, etc.), and as a home-based exercise program, it is more convenient with a higher commitment level when compared to traditional or gym-based exercises (Devereaux J. et al., 2012). Other daily life activities, either at home, work, or while travelling, could also increase low-intensity physical activity rates, with minimal obligation or inconvenience. This would keep the drop-out rates lower and make the effectiveness higher compared to moderate and high-intensity exercises.

Low-intensity exercise is typically recommended for individuals interested in weight loss and weight control (Ferguson B., 2014) because free FA are a preferential fuel source during low-intensity exercise (O'Brien et al., 1993). Of all the different types of physical activity, activities at the intensity that triggers maximal FAT_{ox} stands out as a potential regimen for body fat reduction and improving cardiorespiratory fitness in overweight and obese individuals (Romain A.J. et al., 2012; Chávez-Guevara I.A. et al., 2020). Research determined that one can achieve maximal FAT_{ox} rate at physical activity intensities between 47–53% of $\dot{V}O_{2max}$ in the general population (Achten & Jeukendrup, 2004; Venables M.C. et al., 2005).

For years specialists have mostly prescribed moderate- to vigorous-intensity physical activity to hinder the positive energy balance. However, this strategy had faced some limitations in long-term clinical trials and also when it is applied to the general population, including individuals with obesity, the elderly, and people with metabolic disorders (Department of Health UK, 2011; Hall, C. W. et al., 2012). Furthermore, over the past decades, as a result of modernization and automation (Thivel D. et al., 2018), the behaviors associated with a sedentary lifestyle have increased to the extent that it has become a new health risk factor (Biskup M. et al., 2018). Therefore, daily energy expenditure has become of interest, especially since many individuals have no/low exercise activity-related energy expenditure. Due to this fact, their physical activity-related energy expenditure mainly consists of non-exercise activity thermogenesis (NEAT), which is known to enhance lifestyle and significantly increase daily energy expenditure (Chung N. et al., 2018).

Considering all the points discussed above, one can challenge the current practice of prescribing moderate to vigorous physical activities to people with chronic diseases. One can actually argue that increasing daily light physical activities such as household chores or casual walking (Ekkekakis P., 2009) might increase physical activity adherence.

1.2 PURPOSE OF STUDY

It is undebatable that in the modern world, human energy expenditure (EE) is low. Sedentary lifestyles seem to be increasing due to the advent of motorized transport and mechanized equipment that have replaced physical activities and manual work. Leisure time is also dominated mainly by sedentary activities such as watching television or playing video games seated in front of a screen. Additionally, with an abundance of food, there is no social pressure in the current society in favor of low physical activity (Westerterp K. R., 2013). These factors have led to a worldwide rise in chronic diseases that have become the leading causes of morbidity and mortality in the developed world (Mokdad A.H. et al., 2004). Therefore, replacing sedentary time with physical activity of any intensity provides health benefits as stated by Australia's Physical Activity Guidelines (2014): "Doing any physical activity is better than doing none."

We have to incorporate more movement into our day in order to fight chronic diseases. Selecting a specific form of movement occurs in a way that will require minimum energy (Hatze H. & Buys J.D, 1977; Hagler S., 2016). In fact, it has been suggested that the central nervous system (CNS) integrates reafferences about the energy cost per distance. As such, walking represents a state known as “minimization process”, that is, movement economy (Zarrugh M. Y. et al., 1974; Minetti A. E. & Saibene F., 1992; Bertram J. E. & Ruina A., 2001; Browning R. & Kram R., 2005). Indeed, studies have shown that EE per minute in walking is lower than activities such as running in humans (Holt K. et al., 1991; Farley C. & McMahon T., 1992; Gottschall J. & Kram R., 2003; Hall C. et al., 2004). Hence, if we ask someone to travel a given distance without any time limit, that individual will most likely walk the whole distance rather than choose to run.

We are generally capable of walking at different speeds; however, the efficiency of walking changes with speed (Cavagna and Kaneko 1977, Hoyt and Taylor 1981). There is a speed at which the energy (or oxygen) cost to walk a unit distance is minimized, and as speed is increased or decreased away from this optimal speed, the cost of transport rises rapidly (U-shaped curve). In fact, the CNS favors walking speeds that require the least energy, and interestingly, this energetically optimal speed is known to occur when the walking speed is naturally selected (Ralston H., 1975).

Moreover, from an evolutionary perspective, humans evolved to cover long distances (12 km/day on average) at a relatively low pace (Marlowe F.W., 2010). As also mentioned before, the most efficient walking speed in terms of energy appears to be at a self-selected speed, and, likely, this is also the speed at which we are most efficient in oxidizing fat; However, the evidence supporting this hypothesis is limited. Therefore, the purpose of the current thesis is to evaluate substrate partitioning among workloads consistent with daily ambulatory activities (i.e., 3–7 METs) and determine whether the highest rate of FAT_{ox} occurs within this range and particularly at the self-selected speed in terms of efficiency (i.e., contribution per kilometer).

1.3 Significance of Study

With a constant increase in the prevalence of sedentary lifestyles and, consequently, chronic diseases, one can question the effectiveness of current physical activity guidelines and fitness trends. Therefore, to develop physical activity strategies that the vast majority of people could follow seems to be the challenge.

At present, the focus of the literature is mostly on high-intensity exercise (i.e., HIIT), which mainly favors the athlete population due to its vigorous nature (Gibala, M. J. et al., 2008). However, even though it might be of interest to other populations other than athletes, they will face some challenges after a while (Hawley, J. A., & Bishop, D. J., 2021). For instance, we perfectly know that without consistency, an individual faces a harder time responding and forming a particular habit (Gardner B. et al., 2012). One of the downsides of the HIIT protocol is the high rate of drop-outs. This causes one's inability to build a habit of exercising, which means it is not effective in the long run for keeping people fit. In this study, we shift the focus to lower exercise intensities and are mainly interested in determining what intensities work to have the potential be beneficial for the general population's metabolic health.

Although little research has investigated the benefits of low-intensity exercise (Tanaka & Shindo, 1992; Pescatello L.S. et al., 2000; van Vilsteren M.C.B.A. et al., 2005; Williams, J. et al., 2016; Liepinsh E et al., 2020), only a few studies have focused on the physical activities within the range of daily ambulatory movements, particularly those at self-selected intensities (such as self-paced walking) (Willis W.T. et al., 2005; Entin PL et al., 2010). Further insights into this physical activity pattern and its effect on substrate partitioning (e.g., FAT_{ox}) could help design alternative exercise programs encouraging more active lifestyles and hopefully decrease the rate of mortality due to sedentariness in the future.

CHAPTER TWO: LITERATURE REVIEW

2.1 The Origin of Metabolism

Before going further into detailed discussion on energy metabolism, a brief overview of initial metabolic studies will be presented.

The original recorded and more sophisticated studies of metabolism began in the closing decades of the sixteenth century. Santorio Sanctorius was born in 1561 in Capodistria, currently known known as Koper in Slovenia. He began his studies in philosophy at the age of 14 and later pursued his education in medicine, completing his degree at the age of 21. Twenty years later, he gained his reputation by publishing his first book: *Method of Combating All the Errors which Occur in the Art of Medicine* (Grmeck M.D., 1975).

Sanctorius had a tremendous impact on science through his studies on insensible perspiration and the invention of an instrument to measure body weight changes. This instrument consisted of a movable platform, attached to a steelyard, on which the subject would partake in daily activities (See Figure 1). In this self-experimentation study, he would first consume a meal until his weight reached a predetermined quantity. He then observed that if he remained on the balance for a while, his weight gradually become lighter. He realized that this drop in weight was due to a loss of matter, which he called insensible perspiration. During the course of the experiment, he would weigh the urine excreted and subtract it from the last change in weight to verify the loss attributed to insensible perspiration (Renbourn E.T., 1960).

Although the concept of insensible perspiration was not new (Eknoyan G., 1989), Sanctorius' experiment used scientific method to document his observations. He quantified these observations using his innovative instruments and assessed the validity of these measurements during repeated trials. After years of self-experimentation, he studied 10,000 subjects for 25 years and brought the data in *De Statica Medicina* (O'Malley C. D., 1970). Unfortunately, the original data is not

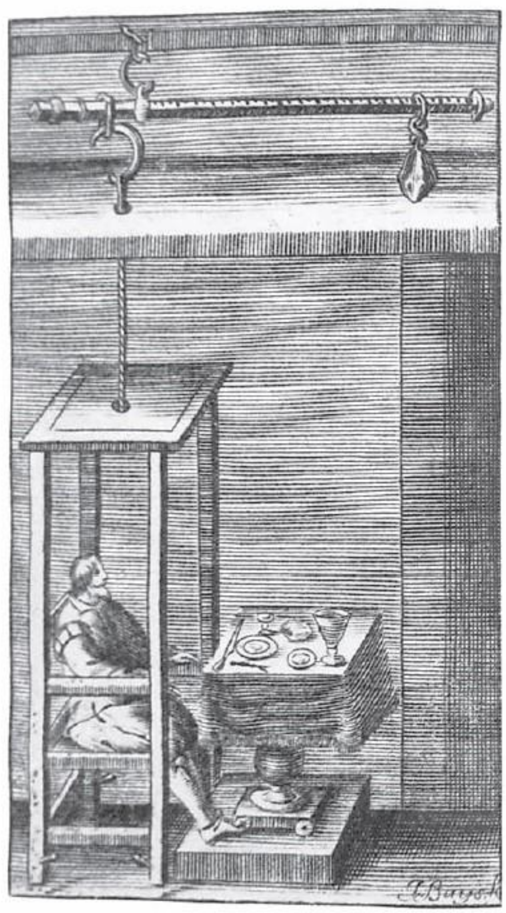

Figure 1 – The metabolic balance devised by Santorio Sanctorius for the study of insensible perspiration. From De Statica Medicina, 1690.

available today, but he summarized his findings in a series of aphorisms that clearly represent the general idea of his theories:

> In the first hours after eating a great many perspire a pound or near; and after that to the ninth two pounds; and from the ninth to the sixteenth scarce a pound. (Section III, Aphorism LXXVI) If eight pounds of meat and drink are taken in one day, the quantity that usually goes off by insensible perspiration in that time, is five pounds. (Section I, Aphorism VI) Sixteen ounces of urine is generally evacuated in the space of one night; four ounces by stool, and forty ounces upwards by perspiration. (Section 1, Aphorism XIV) Vomiting diverts urine and perspiration. (Section I, Aphorism LXXXIX) Those who piss more than they drink, little or nothing perspire. (Section I Aphorism XCIV) Robust persons discharge their food for the most part by perspiration. Those not so strong by urine, and the weak chiefly by an indigested chyle. (Section III Aphorism XIV) (London, J. Osborn and T. Longman, and J. Newton, 1728).

Sanctorius did an exceptional work in providing quantitative physiological data using precise instrumentation that became the basis of pathophysiology (Mitchell S.W., 1892). He studied insensible perspiration and documented its variations affected by different factors such as air, food, drink, rest, movement, and sexual activity. *De Statica Medicina*, published in 1612, was the outcome of such studies that started decades before, at the time prior to the scientific revolution. For that reason, his experiments were innovative and advanced for their time and formed the basis of future metabolic studies. Today in our experiments, we measure energy intake and expenditure of the body just as Sanctorius did, with a much more comprehensive chemical analysis, which Sanctorius only characterized in terms of weight and volume. His studies did not show their full potential until the 20th century, when they were revived by scientists who were capable of calculating basal and whole-body metabolism (Newburgh L.H. & Wiley F.H. & Lashmet F.H., 1931).

Indisputably, Sanctorius was a pioneer in clinical experimentation, objective physiological measurement, and medical instrumentation due to all his novel approach and seminal work. In fact, he truly merits the title of founding father of metabolic studies.

2.2 Energy Metabolism

Before discussing energy metabolism, clarification should be brought up about the distinction between physical activity and exercise. Indeed, physical activity and exercise are sometimes used interchangeably so a definition is below provided. As stated in the ACSM's guidelines for exercise testing and prescription (Ferguson B., 2014), physical activity is defined as bodily movement produced by the contraction of skeletal muscle that increases EE above the basal level and refers to daily activities such as occupational, household, leisure time, and transportation (i.e., walking, cycling, running). Exercise, on the other hand, is physical activity that is planned, structured, and repetitive for the purpose of conditioning any part of the body. It is implemented into training program to improve health, maintain fitness and is important as a means of physical rehabilitation.

Human locomotion and other physical activities in daily life and athletics are energetic events. The body needs energy to maintain cell homeostasis and life processes. Dietary macronutrients (e.g., CHOs & fats) are the sources that supply energy for such activities (Ferrannini, 1988). Conversions of the chemical energy of food to other forms of energy are required to sustain life. In the cell, respiration represents the conversion of foodstuffs' chemical energy into a useful chemical form (i.e., ATP) to power cellular needs. Almost all human energy intake is metabolized except for the portion that is excreted through the feces, urine, or perspiration (Widdowson, E.M., 1955).

Energy balance is the difference between energy intake and EE. There are three main components of energy balance that determine total (or daily) EE: basal metabolic rate, thermic effect of food, and physical activity thermogenesis. It is possible to measure an individual's basal metabolic rate in a standard condition (early morning after fasting overnight for 12 hours, no strenuous activity must be allowed for at least 24 hours before the test and the subject should lay down in a complete rest position during the test). Thermic effect of food is the smallest component associated with the energy demand of digestion, absorption, and storage of food. Physical activity thermogenesis, however, can further be divided into two components: Non-exercise activity thermogenesis (NEAT) – that is the energy demand of ambulation, leisure, different occupations – and exercise-related activity thermogenesis, which includes the energy demand of different exercises. Exercise-related activity thermogenesis represents a negligible part (15-30%) of daily energy demand (i.e., basal metabolic rate, thermic effect of food, NEAT) in most developed countries (Levine J.A., 2002), and thus, NEAT is the prominent determinant of activity thermogenesis. The principal factor causing total daily EE to be divergent in humans is NEAT since it is the most variable component of EE. Therefore, this factor merits further consideration in research and studies.

As discussed earlier, human energy metabolism involves energy production from the combustion of fuels (e.g., CHOs, fat, etc.). In these processes, oxygen is consumed, and carbon dioxide and heat are produced. One can indicate the metabolic rate by determining the rate of heat production (Brooks G.A. et al., 2004), measuring it directly (i.e., direct calorimetry), or by estimation using the rates of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) from expired air (Lighton, 2008). Since the rate of $\dot{V}O_2$ measured at the mouth is a direct reflection of the rate of oxygen consumption by the tissues, this method could provide an accurate way of estimating the rate of metabolism. This method is known as indirect calorimetry, a standard technique in energy balance research (Kaiyala & Ramsay, 2011).

In addition to providing an estimate of metabolic rate, indirect calorimetry provides a means of evaluating determinants of daily EE (Jéquier & Felber, 1987) and estimating the contribution of the fuels to energy production (Brooks, G.A. et al., 2004). Similarly, determining the ratio of $\dot{V}CO_2/\dot{V}O_2$ gives an indication of the type of substrate being oxidized. This ratio is usually referred to as the respiratory exchange ratio (RER) which reflects the respiratory quotient (Ferrannini, 1988; Simonson & DeFronzo, 1990), terms often used interchangeably, and varies from 0.7 (pure lipid catabolism) to 1.0 (pure CHO catabolism). They both are calculated similarly, but RER measures the air expelled with each breath and reflect lungs ventilation, while ratio quotient represents cell respiration.

2.3 Skeletal Muscle Metabolism and Effect of Physical Activity

At rest, an average person consumes about 3.5 ml O_2 per min per body weight (kg). This rate of EE is indicated as one metabolic equivalent (MET) (Ainsworth B.E. et al., 1993), which is used to express the intensity of activities (Jette M. et al., 1990). The Compendium of Physical Activities has developed a comprehensive list of EE estimations covering a wide range of physical activities [e.g., 0.9 MET (sleeping) to 18 METs (running)] (Ainsworth B.E. et al., 2011). The American College of Sports Medicine (Ferguson B., 2014) defines moderate physical activity with the range of 3–6 METs, light physical activity as < 3 METs, and vigorous physical activity as > 6 METs.

As the body shifts from rest to an active state, it requires more energy to fulfill those tasks. This can be supplied through nonoxidative (independent on oxygen O_2) and oxidative (dependent on O_2) metabolisms. The selection between oxidative and nonoxidative metabolisms depends on many factors including (but not exclusive to) the intensity (Romijn, J.A. et al., 2000), duration (Henderson G.C. et al., 2007), and type of physical activity (Knechtle B. et al., 2004).

Nonoxidative energy sources in the muscle are primarily glucose and glycogen. The terms for the breakdown processes of these two CHOs are glycolysis and glycogenolysis, respectively. Muscle tissue is rich in glycolytic and glycogenolytic enzymes, and can rapidly break down glucose and glycogen. Due to the low concentration of glucose in skeletal muscle, most of the energy comes from glycogen breakdown. Nonoxidative energy sources supply only a small fraction of the energy available through oxidative metabolism. Therefore, intense muscular activities lasting longer than approximately 30 seconds cannot be maintained without the benefit of oxidative metabolism (Brooks, G. A. et al., 2004).

Oxidative energy sources for muscle include CHOs, fats, and some amino acids. Through oxidative processes, a glucose molecule recovers more energy per liter of oxygen than nonoxidative processes. Free fatty acids are catabolized by oxidative mechanisms only, but the energy yield is very large per gram oxidized. Like fats, amino acids can be catabolized only by oxidative mechanisms. Generally, amino acids are less preferred by working muscle (Melzer K., 2011), but can come into play under extreme conditions, such as starvation (McArdle, W. & Katch, F.K.V., 2006). Thus, CHOs and fats are the primary energy sources during physical activity. It is notable that the metabolic pathways that oxidize CHOs and fats are activated simultaneously (Astrand & Rodahl, 1986). The contribution of substrates to energy production depends on the physical activity's intensity. In the transition to exercise, the body mainly switches to intramuscular sources to provide energy (van Loon et al., 2001).

Lipids store large amounts of energy, meaning it isn't surprising that they contribute to a substantial portion of energy production, particularly during low and moderate-intensity exercise, as well as during prolonged exercise (Christensen & Hansen, 1939; Krogh & Lindhard, 1920; van Loon et al., 2001). However, lipid metabolism during exercise is complex, with numerous possible sites for regulation and multiple steps to oxidation (Spriet et al., 2014).

FA oxidation provides the energy requirements at rest, following an overnight fast. For the most part, these FAs originate from visceral adipose tissue triacylglycerols and, to a lesser extent, subcutaneous adipose tissue (Arner et al., 1990). Switching from a resting condition to low-intensity exercise (25% $\dot{V}O_2$max), which is an intensity comparable to walking slowly, there is an increase in lipolysis of adipose tissue triacylglycerols and an increase in the oxidation of plasma free FAs (Klein et al., 1994; Wolfe et al., 1990; Krogh & Lindhard, 1920). Studies demonstrate that plasma FAs are the predominant contributor to overall FAT_{ox} during low-intensity exercise (25% $\dot{V}O_2$max) (Brooks G.A. & Mercier J, 1994). As the intensity increases, intramuscular triacylglycerols become a vital contributor to overall FAT_{ox} (Essen et al., 1977). As the exercise intensity rises to a moderate level (65% $\dot{V}O_2$max,) fat is not oxidized at high enough rates to supply all the energy required. As a result, about one-half of the body's total energy is derived from CHO. At some point between 45 and 65% $\dot{V}O_2$max, FA oxidation reaches a maximum (Maximal Fat Oxidation) (Romijn et al., 1993; Achten et al., 2002), also referred to as the crossover point (Brooks G.A. & Mercier J., 1994). This is the point at which FA oxidation begins to decline, and CHO_{ox} rates start to rise gradually to the point that CHOs become the predominant fuel source. The advantage of CHO metabolism during higher intensities (e.g., 85% $\dot{V}O_2$max) lies in its two times more rapid energy transfer capacity compared to fats (Peronnet F. et al., 2006).

As the exercise moves towards higher intensities, the appearance rate of plasma FA declines, which is likely due to an insufficient blood flow. In fact, blood flow deficiency impairs albumin delivery to transport FA from adipose tissue into the bloodstream. In contrast to FAs, glycerol is water-soluble, and its presence in plasma is not dependent on blood flow; therefore, higher intensities do not affect the rate of glycerol appearance. This is one of the reasons that CHO_{ox} occurs more than FA oxidation during higher intensities. Additionally, glycogenolysis increases in high intensities, resulting in lactic production, which accumulates in the muscles and blood. This glycolytic flux, in particular, seems to inhibit FA oxidation in the skeletal muscle (Hawley J., 2001).

Many studies have attempted to provide an overview of the physiological differences between genders during different exercise intensities. For instance, previous studies showed that men use more CHOs during exercise (Venables M.C. et al., 2005) but less CHOs during recovery when compared to women (Henderson G.C. et al., 2007). On the contrary, when the energy demand increases, women use more fat than CHOs to provide energy (Tarnopolsky M.A. et al., 1995). An analysis of 25 studies indicated that according to RER, relative FAT_{ox} in women is significantly greater than men during endurance exercise (Tarnopolsky M.A., 2008). Indirect calorimetry also demonstrated that during incremental tests on a treadmill, the rate of FAT_{ox} is significantly higher in women than men (Venables M.C. et al., 2005; Cheneviere X. et al., 2011).

Sex hormones are one of the factors known to be responsible for the differences in substrate metabolism during exercise between men and women (Hatta H. et al., 1988). This concludes that these differences would be less before puberty and after menopause. Previous studies also show that differences in exercise substrate metabolism are undetectable in childhood, and with puberty, they become evident (Aucouturier J. et al., 2008).

In conclusion, women display a higher fat oxidation rate during exercise, showing the ability to oxidize lipids for energy production (Goodpaster, B. H., & Sparks, L. M., 2017). However, during post-exercise recovery period, lipid metabolism increases in both sexes but to a greater extent in men. Women utilize more glucose compared to men in this state and FAs seem to be re-directed toward triacylglycerol storage (Henderson G.C. et al., 2007). At the molecular level, female skeletal muscle seems to be more "primed" for lipid storage as well as oxidation, and in turn helps to keep the turnover of intramuscular triacylglycerol stores high (Lundsgaard A. & Kiens B., 2014).

2.4"WALKING" TOWARDS HEALTH

As discussed earlier, fat is the main fuel oxidized during low-intensity physical activities. Despite the advantages associated with more vigorous intensities of physical activity, low intensities are of much interest with the drastic rise in rates of obesity and cardiovascular diseases in the recent decades. The reason is that increasing fat metabolism could potentially reduce the symptoms of metabolic disorders such as heart disease, type 2 diabetes, etc. (Achten & Jeukendrup, 2004).

Walking is the most commonly reported form of low-intensity exercise (Ham S.A. et al., 2009), which is used in the course of transportation, occupation, and leisure time for most individuals (Tudor-Locke & Rowe, 2012). It is an activity that can positively alter sedentary behavior which is considered a risk factor for developing several chronic diseases such as cardiovascular disorders, diabetes, etc. (Dempsey P.C. et al., 2014). It is also related to many health benefits and can improve quality of life as it reduces the possibility of injuries or stress levels (Taylor A.H. et al., 2004; Pelssers J. et al., 2013). In fact, walking is the most preferred form of physical activity for both work and leisure (Williams D.M. et al., 2008), and a good alternative even for inactive/low-active people or individuals with maladies such as heart disease, osteoarthritis, etc. (Ogilvie D. et al., 2007). Walking is also an eco-friendly means of transport that has seen decreased use in recent decades due to vehicle usage growth. Hence, there are many persuasive reasons to encourage people to walk more, such as to improve their health and also tackle climate change (Coote A., 2006).

Although running is also a popular leisure-time physical activity with substantial health benefits (Lee, D. C., 2014), it has a greater energy cost than walking (Hall C. et al., 2004). We seem to adjust our gait in a way to minimize the metabolic energy cost of locomotion. We walk at low speeds and run to go faster, using the more economical gait at our chosen speed (McNeill Alexander R., 2002). Interestingly, compared to other mammals, the energetic cost of transport for running in humans is relatively high (Taylor C.R. et al., 1970), while walking seems economically the same in humans and quadruped mammals (Rodman & McHenry, 1980; also see Tucker V.A., 1975).

Furthermore, research suggests that in contrast to running, the energy cost of transport ($kcal \cdot meter^{-1} \cdot kg^{-1}$) for a walking human depends on speed (Cavagna & Kaneko, 1977), and as speed goes up, the metabolic rate increases (Faraji, S. et al., 2018). Previous studies (Martin P.E. et al., 1992; Ralston H., 1975) had shown a U-shaped relationship between walking speed and the energy cost of transport, with the lowest part occurring at roughly 4.5 $km \cdot h^{-1}$ (2.8 mph) when the walking speed was naturally selected (Finley & Cody, 1970; Martin P.E. et al., 1992; Cavagna & Franzetti, 1986; Cavagna & Thys, 1976; Margaria R. et al., 1963). Ralston (1958) demonstrated that humans are inclined to walk at or close to the speed that minimizes the cost of transport. The mechanisms responsible for this natural subconscious behavior is not fully known, but it is generally accepted that the CNS selects preferred walking speed to minimize EE (Ralston H., 1975). According to this hypothesis, the CNS selects the walking speed that minimizes the energy cost (or O_2 uptake) to move a unit mass a unit distance (the energy cost of transport). In other words, CNS chooses the speed that feels more comfortable for the body. In fact, CNS is guided by the perception of effort (Gandevia & Rothwell, 1987) in selecting a preferred walking speed that is correlated with minimizing dependence on CHO_{ox} rather than total energy production per se. Based on this hypothesis, at the preferred walking speed, fat should supply the majority of the energy needed for activity (Ganley K.J. et al., 2008; Willis W.T. 2005). In addition to the above-mentioned factors, self-selected exercise protocols are directly related to a decreased perceived exertion (DaSilva S.G. et al., 2009) and an improved affective experience (i.e., arousal state associated with pleasant/unpleasant feelings) (Posner J. et al., 2005, Lind E. et al., 2005), which in turn impacts adherence to exercise. Individuals engaged in an exercise training program commonly tend to deviate from the prescribed exercise intensities toward a preferred exercise intensity since the body intuitively tends to select an intensity that minimizes the energy cost of locomotion while improving and maintaining fitness (Dishman R.K. et al., 1994; Ekkekakis & Lind, 2006).

From an evolutionary point of view, locomotion must also be important in early humans' energy budgets. It seems likely that locomotion was a major cost in the energy budgets of hunter-gatherer populations such as the Bushmen of Botswana, who walk about 2,400 km per year (Lee R.B., 1979). It therefore seems reasonable to suppose that human evolution may have been strongly influenced by the selection for structures and functions of movement (symmorphosis; Taylor C.R. et al., 1996) that reduce the energy cost of locomotion. Referring to Theodosius Dobzhansky's statement (Dobzhansky T., 1973), "nothing in biology makes sense except in the light of evolution." Therefore, to acquire more knowledge and more in-depth explanation of a phenomenon's real underlying causes, we need to refer to the evolutionary theory and data. As such, we will refer to the evolution of human locomotion in the following paragraphs to gain better knowledge about humans' characteristics in developing physical activity.

The rationale behind considering evolution in many different aspects of health and disease is the Darwinian principle of natural selection and the concept of adaptation shaped by natural selection, which improves the ability to survive and reproduce in given specific condition. Adaptations promote health only if they increase reproductive success (Rose & Lauder, 1996). In this regard, the theory of energy allocation points out that in circumstances of finite food sources, we adapt the behavior of minimizing effort.

Moreover, years ago, when ancient humans practiced hunting-gathering, natural selection used to favor the best accumulators of fat. We were designed and built to walk 12 hours a day, leading a nomadic life, gathering, and hunting, running away from other predators, and always on the edge of starvation (Belcaro, G. V., 2001). In these conditions, the capability of accumulating fats in a very effective way could have been an important advantage in situations of starvation (Neel J.V., 1962). The fast and effective accumulation of fat is a very important factor when hunting, and the sources of food are either not always available or not predictable. In fact, a lot of fat acting like high energy fuel will propel hunters much more effectively towards the next prey. Therefore, individuals with a very high capability of accumulating fat and, consequently, the ability to easily use their fat body stores should be favorably selected in an evolutionary hunting ground. Also, if, for any reason, there is no food available for a while, if they have accumulated their fat stores, it will increase their chance of survival.

Furthermore, all animals, including humans, are adapted to be physically active to get food and avoid becoming prey. However, humans are unique among mammals as they evolved in a way that they are well adapted for great amount of physical activities, mainly involving endurance instead of power; in fact, humans have evolved as endurance athletes. The trait of being endurance likely began to concur with bipedalism among early hominids (Auletta G. et al., 2011). Bipedalism is believed to be driven by the principle of natural selection as it helped hominids travel and feed more effectively as forest cover was reducing and more open environments were becoming more common with the climate change in Africa (Lieberman D.E., 2015).

From an exercise standpoint, hunter-gatherers developed a lifestyle that caused a remarkable shift. This lifestyle required a great variety of unique physical activities primarily contingent on endurance, and particularly, long-distance trekking, to name the most important one. Presently, hunter-gatherers still exist in dry tropical African habitats, and investigations show that those who have a similar body mass to *Homo erectus* walk an average of 9 to 15 km per day seeking food (Marlowe F.W., 2010). This could be the evidence that most probably *Homo erectus* covered the same amount of distance per day to search for food and, therefore, could be the support for explaining many novel adaptations of the genus *Homo*, especially *Homo erectus*, for specific activities such as long-distance walking. Of these adaptations, one can name longer legs, larger joints, and a more modern pelvis (Aiello L.M. & Dean M.C., 1990; Liebenberg L., 2006). In addition, hunter-gatherers had to carry food and babies across long distances and occasionally had to throw projectiles and climb trees in between. Tropical hunter-gatherers typically spend 2 to 3 $h \cdot d^{-1}$ using sticks to dig up tubers (Marlowe F.W., 2010). All these points lead us to infer that endurance physical activities were important in the genus *Homo*.

In a nutshell, a broad range of unique adaptations resulted from hunting and gathering; many of them necessitate endurance rather than power or speed. Since endurance and power are often intuitively exchangeable (Bennett A.F., et al. 1984; Garland T., 1988), we can speculate that the process of alteration from ape-like physiology of early human ancestors, which was mostly dominated by power, to the modern human endurance-based physiology, was adopted in early *Homo*, especially *Homo erectus*. While walking was indeed the most important physical activity, running along with carrying, climbing, digging, and so forth was also considered to be of interest as forms of physical activity (Bramble, D.M. & Lieberman, D.E., 2004).

Humans were selected to refrain from any activity that is more than necessary. Studies of hunter-gatherer energy budgets explain the reason behind this. Evidence suggests that hunter-gatherer's homeostasis often fluctuates around the verge of energy balance. This, in turn, results in any non-essential physical activity (i.e., exercise in today's world) being maladaptive (Lieberman DE, 2015). Besides, available data indicate that females, in particular, have evolved the ability to store fat as much as possible. They have adapted this trait to have adequate energy reserves allowing them to afford several costly biological processes, such as pregnancy and feeding infants along with nursing the elderly and feeding children who are unable to forage on their own (Kelly R.L., 2007).

Studies of different populations' energy budgets found that the physical activity levels of hunter-gatherers are slightly lower than subsistence farmers but considerably higher than post-industrial Americans (James W.P.T. and Schofield E.C., 1990; Leonard W.R., 2012; Pontzer H. et al., 2012). On average, hunter-gatherer's daily EE is 30 kcal•kg$^{-1,}$ which is almost twice that of Americans with an average of 17 kcal•kg^{-1} per day (Lieberman D.E., 2015). In fact, hunter-gatherers were nearly twice as active as people in post-industrial economies, even though they were greatly physically active for only 4 to 6 hours per day doing demanding work (Lee R.B., 1979).

Being insufficiently adapted to current environmental conditions play a crucial role in surging rates of some common diseases (e.g., type 2 diabetes). Humans were rarely, if ever, physically inactive until recently. The evidence indicates that many diseases arising from physical inactivity are considerably less common in hunter-gatherers and subsistence farmers. For instance, the medical data of Kalahari Bushmen reveals no existing evidence for dyslipidemia, diabetes, hypertension, and more (Truswell & Hansen, 1976). Hence, we have to stop sudden dramatic changes in our environment. The lack of compatibility between the condition that human design evolved for and current life conditions could justify today's most frequent diseases such as atherosclerosis as well as other conditions, such as female reproductive system cancer, manic depression, etc. (Belcaro, G. V., 2001).

The quick technological changes in our environment left us basically no time to adjust, at least not in near future. Understanding our hunting nature could help combat clinical issues that influence the human race to a great extent. With respect to the fact that the human body is not yet genetically compatible with the modern world (assuming based on the fact that any biological system – including the *Homo sapiens* – tends to be economic, the probability to maintain a low physical activity is high, especially in an environment that promotes mechanical transportation and offers abundance of food), various principles have been developed to guide us to a healthier lifestyle, such as "Paleo" diet and fitness movements. This program believes since we evolved to exist in Stone Age conditions, then living like a hunter-gatherer might advance great wellbeing (Durant J., 2013).

We are very efficient hunters in a non-hunting environment. Our inherent design is suitable for a habitat in the bush or savanna. Therefore, the ideal life for what we have evolved is characterized by certain kinds of diet and physical activity. In contrast to what we are witnessing nowadays, genetically, we have evolved to demand small portions of food consisting of very little salt and sugar and perform physical activities of mostly prolonged physical effort with low energy demands (e.g., walking around, gathering, etc.) (Belcaro, G. V., 2001). Our ancestors were physically active either when necessary, such as running to hunt or escape from danger, or when it was fun and as a form of play (Lieberman D.E., 2015). Therefore, to foster exercise behavior, we are required to alter our environments and create ways to make physical activity more enjoyable.

Once we were hunters, we still are and this is not going to change at least for the foreseeable future. When we fully comprehend this fact and act as our original design required us to, extensive positive changes start to appear. Knowing our origins and tendencies will lead us to a better life, protecting us from diseases derived from the discrepancy between our original design and the present modern life we have created. Drawing to a close, fighting your biology, you always lose. If you want to live longer and better, find the "*Homo"* in yourself.

2.5 HYPOTHESIS

Referring to previous sections, we can postulate that humans have naturally evolved to be walkers. Walking is a familiar, convenient, and free form of physical activity merged into people's daily life (Morris & Hardman, 1997; Mutrie & Hannah, 2004). Among all walking speeds, Willis et al. (2005) have reported that below the preferred walking speed (4.8 km/h), which according to the latest update of the Compendium of Physical Activities (Ainsworth et al., 2011) is equal to ~3 METS, FAT_{ox} is the primary source of energy, and at the speeds above that, CHO mainly provides the fuel for energy production. Thereby, the authors concluded that the CNS selects a walking speed supported predominantly by FAT_{ox}, and most likely, it is influenced by the perception of effort (Gandevia & Rothwell, 1987).

Moreover, the quantity of energy stored as fat is about twice that stored in CHO per unit of storage (Brooks, G. A. et al., 2004). Therefore, fat stores could support very long-range locomotion, while

CHO is the fuel of choice for high-intensity activity, sudden environmental change, or predator/prey activity.

Bergman and Brooks (1999) demonstrated that the FAT_{ox} rates in trained individuals showed a peak at ~40%$\dot{V}O_2max$ whereas this value for the untrained group occurred at ~60%$\dot{V}O_2max$. Further, according to ACSM's Guidelines for Exercise Testing and Prescription, 40-60%$\dot{V}O_2max$ is considered as 3–6 METs exercise intensity.

Hence, in this study, it is hypothesized that peak FAT_{ox} for the general population occurs within the normal daily activity range (e.g., 3–7 METs) and, more specifically, during self-selected intensity activities such as self-paced walking.

CHAPTER THREE: MANUSCRIPT

3.1 ABSTRACT

The study aimed to examine substrate partitioning and metabolic efficiency to investigate whether the highest rate of fat oxidation (FAT_{ox}) occurs within the range of normal daily non-exercise physical activities. For this study, we performed a secondary data analysis on an existing set of data collected for a previous master's thesis. Forty-seven healthy people (22 men, 25 women) ranging from 20 to 64 years old participated in that study to complete a sequence of unaided exercises at different intensities on a treadmill (self-selected pace, 3 METs, 5 METs, and 7 METs). Results showed that the absolute FAT_{ox} ($g.min^{-1}$) reached a peak value at 5 METs (M = 0.18, SD = 0.12) with no significant difference from the self-pace condition (M = 0.18, SD = 0.07), while the carbohydrate oxidation (CHO_{ox}) rates increased as a function of intensity. On the other hand, analyses revealed that the relative contribution of carbohydrate (CHO) to energy production was equal to the relative contribution of FAT_{ox} ($kcal.kg^{-1}.km^{-1}$) at self-pace (M = 0.54, SD = 0.18, and M = 0.50, SD = 0.17 respectively; p = .497) and then started to significantly deflect from each other at 3 METs onwards (p = .001) [CHO at 3 METs (M = 0.64 , SD = 0.15), 5 METs (M = 0.74, SD = 0.19), and 7 METs (M = 0.90, SD = 0.19); FAT at 3 METs (M = 0.40, SD = 0.15), 5 METs (M = 0.30, SD = 0.19), and 7 METs (M = 0.14, SD = 0.18)]. Lastly, it was concluded that the peak FAT_{ox} occurs within the normal range of daily locomotor activities, and to be more specific, it happens in the course of a physical activity being done at a self-selected intensity.

Keywords: daily physical activities, non-exercise activity thermogenesis, substrate partitioning, metabolic efficiency, health

3.2 INTRODUCTION

Any body movement generated by skeletal muscles results in an increase in EE above the basal level. These movements are defined as physical activity (PA) and classified into the occupational, household, or other daily life activities. Exercise represents a specific type of PA that is planned, structured, and repetitive aiming to maintain or improve one's physical fitness (Caspersen C.J., et al., 1985). Exercise can be performed at different levels of exertion or, in other words, intensities - the so-call exercise intensity domain. To sustain such activities, energy metabolic processes should match the energy demand of the task. In that regard, CHO and lipids (e.g., fat) predominantly supply the active tissues (i.e., heart and skeletal muscle) with energy during exercise. The contribution of these fuels to energy production varies according to PA intensity and duration (Venables M.C. et al., 2005). Alteration of CHO_{ox} simultaneously impacts FAT_{ox} and, generally, the contribution of substrates to energy production changes as a function of exercise intensity (Péronnet et al., 2006; Romijn J.A. et al., 1993).

A myriad of studies has confirmed the impact of exercise intensity on energy metabolism (See Review from Spriet L.L., 2014) and cardiorespiratory response. For example, the current physical activity recommendations suggest accumulating 30 minutes or more of moderate- to vigorous-intensity physical activity on most days of the week. By doing such, an individual can expect many of the health benefits triggered by physical activity (Wassenaar, T. M. et al., 2021). Studies have also reported that engaging in HIIT improves cardiovascular health (Gibala, M. J. et al., 2012) and induces skeletal muscle morphology adjustments (Gibala, M. J. et al., 2009). Yet, this exercise protocol leads to poor adherence and high participants' attrition and, therefore, does not seems to be a feasible public health strategy. One can attribute the failure of promoting PA to its primary focus on health benefits (i.e., its positive impacts on chronic diseases) with little consideration for psychological well-being (de Souto Barreto P., 2013). Seminal works in evolutionary biology and psychology attest that humans have no inherent drive to be greatly physically active (Cordain L. et al., 1998; Eaton & Eaton, 2003; Peters J.C. et al., 2002; Saad G., 2007). Since no inborn drive pushes us to be physically active to a significant extent, any physical activity behavior consistency requires a high level of motivation. Therefore, one can question the pertinence and efficiency of high-intensity exercise protocol on exercise adherence.

Low-intensity physical activity might address the exercise adherence issue to some extent and represents a practical way of promoting daily physical activity since it does not require much motivation, commitment, or planning as it usually incorporates into everyday life with the asset of being affordable and accessible. In fact, humans have been designed for such locomotor activity and evolved as endurance animals (Noakes T.D., 2006). As a matter of fact, through adaptative processes over generational time, humans developed a high capacity for sustaining prolonged low-intensity activity (i.e., walking/running) (Eaton S.B. et al., 2002) that provided them with the necessary survival needs (e.g., hunting, fishing, gathering, escaping, etc.).

Consequently, one can postulate that walking – low-intensity activity – should display the optimum contribution of substrates to energy production: so, the optimum cost of transport (i.e., metabolic efficiency). Indeed, research has shown that humans naturally select a walking speed that minimizes the energy cost of transport (i.e., energy required to cover a given distance) (Ralston H., 1975). However, there is still controversy regarding the relationship between metabolic rate (oxygen consumption) and caloric unit cost that might misinterpret the minimal energy hypothesis. According to the latter, the central nervous system selects the walking speed that minimizes the energy cost and, therefore, optimizes substrates' contribution to energy production.

The current study aimed to determine at which walking speed, humans reached the optimum substrate contribution. It was hypothesized that a free-chosen walking pace displays the optimum caloric unit cost

3.3 Materials and Methods

3.3.1 Participants

The study used secondary data analysis collected for the purpose of predicting physical activity types with Apple Watch and Fitbit devices. Forty-seven physically active males and females ranging from 20 to 64 years old were selected to complete a sequence of unaided activities that includes lying on a cot, sitting on a chair, walking, and running on a treadmill. Participants' characteristics are tabulated in Table 1.

3.3.2 Experimental protocol

The lab-based activity protocol consisted of bipedal locomotion activities over a 65-minute period during which participants were subjected to two 5-minute resting periods (lying on a cot and sitting on a chair) prior to partaking in four 10-minute exercise intensities (self-pace, 3, 5, and 7 METs) interspaced with 5-minute resting periods (See Figure 2). The treadmill speed for all conditions was adjusted based on individual oxygen uptake and body weight. For the sake of simplicity, henceforth, the intensities will be expressed in METs. For further information about the protocol, see Fuller et al. (2021).

3.3.3 Indirect Calorimetry

Exercise metabolic rates were recorded with an indirect calorimetry system (Oxycon Pro, Jaeger, Hochberg, Germany). $\dot{V}O_2$, $\dot{V}CO_2$, breathing frequency, and tidal volume were breath-by-breath collected via an automated open-circuit gas analysis system with O_2 fuel cell and CO_2 infrared cell in connection to a turbine flowmeter. RER and minute ventilation were calculated as the quotient of $\dot{V}CO_2$ on $\dot{V}O_2$ and as the product of breathing frequency by tidal volume, respectively. Prior to testing, volume and gas analyzers were calibrated with a 3.0 L calibration syringe and medically

Table 1 – Anthropometric Characteristics of the Participants

Parameter	Group	Mean ± SD	95% Confidence Interval	
			Lower Bound	Upper Bound
Age (years)	All (n=47)	29.6 ± 9.2	26.9	32.3
	Men (n=22)	27.3 ± 9.9	22.9	31.6
	Women (n=25)	31.7 ± 8.3	28.3	35.1
Mass (kg)	All (n=47)	70.6 ± 14.8	66.3	74.9
	Men (n=22)	80.1 ± 13.1	74.3	85.9
	Women (n=25)	62.2 ± 10.4	57.9	66.5
Height (cm)	All (n=47)	169.6 ± 10.7	166.4	172.7
	Men (n=22)	178.0 ± 7.2	174.8	181.2
	Women (n=25)	162.2 ± 7.1	159.2	165.1
Body Mass Index ($kg.m^2$)	All (n=47)	24.4 ± 3.5	23.4	25.4
	Men (n=22)	25.3 ± 3.7	23.6	26.9
	Women (n=25)	23.6 ± 3.0	22.3	24.8

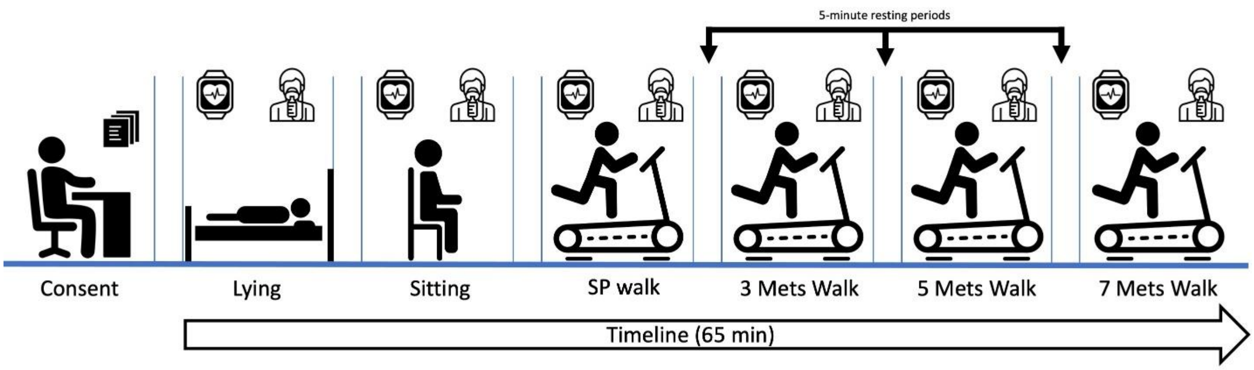

Figure 2 – Schematic of the 65-minute lab-based experimental protocol for determining peak fat oxidation within the intensity range of daily locomotor activities. Before testing, informed consent and measurement of anthropometrics (i.e., body mass, age) were obtained, and participants were familiarized with the experimental setup. The testing began with sedentary activity (i.e., lying on a cot and sitting on a chair) for 5 minutes each. Following these activities, the treadmill activity began. First, each participant was asked to select a speed that was most comfortable (SP walk). A 5-minute resting period (lying on a cot) then followed, and immediately after that, each participant walked on the treadmill at a pace of 3 METs (~3kph). These steps were repeated at 5 METs and 7 METs, respectively, including 5-minute rest between each activity.

certified O_2 and CO_2 calibration gases at 16% and 4%, respectively. The data were online digitalized from an A/D card to a computer for monitoring the metabolic rate. Exercise metabolic rates were used to monitor treadmill speeds for determining METs of each participant and calculate substrates partitioning and caloric unit cost (CUC).

3.3.4 DATA REDUCTION

The data were processed through an R code that contains four main scripts related to adjusting timestamp, imputing missing data, applying a band-pass filter, and a spline curve fitting function with the degree of freedom of 50 on metabolic data and developed according to Robergs and Burnett (2003) proposed methodology. For further information on the data reduction techniques, refer to

https://github.com/walkabillylab/jaeger_analysis/blob/master/JaegerDataPrepValidity.R.

3.3.5 CALCULATION

Oxygen uptake and carbon dioxide output were computed to estimate rates of CHO_{ox} and FAT_{ox} as well as EE through the following formulae (Kelly & Basset, 2017).

$$CHO_{ox} = 4.210\,\dot{V}CO_2 - 2.962\,\dot{V}O_2 \quad \text{Eq.1}$$

$$FAT_{ox} = 1.695\,\dot{V}O_2 - 1.701\,\dot{V}CO_2 \quad \text{Eq.2}$$

$$EE = 4.07\,CHO_{ox} + 9.75\,FAT_{ox} \quad \text{Eq.3}$$

Protein oxidation rate was estimated at 0.066 $g \cdot min^{-1}$ as reported by Haman et al. (2004) in 12 h post-absorptive state.

The energy cost of transport was expressed in caloric unit cost ($kcal \cdot kg^{-1} \cdot km^{-1}$) for a better indicator of energy use as suggested by Fletcher et al. (2009) and computed as follows;

$$CHO\ caloric\ unit\ cost = 4.07 \bullet CHO_{ox} \bullet BW^{-1} \bullet speed^{-1} \quad \text{Eq.4}$$

$$FAT\ caloric\ unit\ cost = 9.75 \bullet FAT_{ox} \bullet BW^{-1} \bullet speed^{-1} \quad \text{Eq.5}$$

3.3.6 STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS software (version 26). The Kolmogorov-Smirnov test, Levene's test, and Mauchly's test were run for testing the normality of values, homogeneity of variance, and the equality of variances between the differences, respectively. A one-way repeated measures ANOVA was run to test for differences in substrate oxidation between different intensities and a two-way repeated-measures ANOVA to reveal any interactions between CUC and intensities. Bonferroni adjusted *post hoc* analysis was applied to detect any differences between conditions and to decompose any significant interaction. Data were reported as means ± standard deviations (SD) unless otherwise specified. Statistical significance was set at $p < 0.05$.

3.4 RESULTS

The summary of the participants' characteristics is presented in Table 1. The mean difference for age between men and women was 4.4 ± 1.6, and on average, women were older than men. In addition, women were leaner (95%CI = 57.9 – 66.5 kg) than men (95%CI = 74.3 – 85.9 kg). Among men, 59% and among women, 72% of participants were in the normal range for BMI according to the American College of Sports Medicine guidelines (Ferguson B., 2014). There were one woman with a BMI of 15.8 and two men with a BMI of over 30.0.

According to Kolmogorov-Smirnov and Levene's tests, the data were normally distributed, and homogeneity of variance was met. Mauchly's test indicated that the assumption of sphericity had been violated in all conditions and for all the variables; therefore, degrees of freedom were corrected accordingly.

Table 2 displays, as expected, a significant increase in $\dot{V}O_2$ ($F_{(1.63,74.89)}$ = 615.66, p = .001), $\dot{V}CO_2$ ($F_{(1.64,75.69)}$ = 565.13, p = .001), and RER ($F_{(2.47,113.56)}$ = 94.15, p = .001) as a function of activity intensity. In addition, the metabolic responses of those variables to the intensities (METs) did not fluctuate much when intensity increased. In fact, the coefficient of variation for $\dot{V}O_2$, $\dot{V}CO_2$, and RER went from 19 – 24%, 19 – 25%, and from 4 – 6%, respectively, which showed that the dispersion was minimal and therefore, the measurement valid and reliable. *Post hoc* comparisons revealed that the self-pace condition had the lowest oxygen uptake value and was different from all other conditions except for the 3 METs condition.

As displayed in Figure 3, there was a main effect of intensity on absolute CHO_{ox} ($F_{(1.69,77.61)}$ = 324.45, p = .001) and FATox ($F_{(1.69,77.61)}$ = 4.77, p = .015). Further analysis indicated that absolute CHO_{ox} did progressively increased from self-pace to 7 METs while absolute FAT_{ox} followed a different trend reaching a peak at 5 METs. In fact, there was no significant difference for FATox between self-pace (M = 0.18, SD = 0.07) and 5 METs (M = 0.18, SD = 0.12) but significant differences between these and the two other conditions [3 METs (M = 0.15, SD = 0.06) and 7 METs (M = 0.13, SD = 0.17)]. Note that the lowest absolute FAT_{ox} occurred at 7 METs.

Furthermore, as displayed in Figure 4, two-way ANOVA revealed a significant interaction between substrates CUC and four intensities of activity ($F_{(2.57,\ 118.33)}$ = 125.13, p = 0.001), the result of a significant increase in CHO CUC and decrease in fat CUC as a function of intensity. Since

Table 2 – Metabolic Outcomes According to the Experimental Conditions

Parameter	Condition	Group	Mean ± SD	95% Confidence Interval	
				Lower Bound	Upper Bound
METs	**Self-Pace**	All	3.03 ± 0.59	2.85	3.20
		Men	2.88 ± 0.55	2.64	3.13
		Women	3.15 ± 0.60	2.90	3.40
	3 METs	All	3.21 ± 0.41	3.09	3.33
		Men	3.12 ± 0.43	2.92	3.31
		Women	3.29 ± 0.37	3.14	3.44
	5 METs	All	5.09 ± 0.61	4.92	5.27
		Men	4.93 ± 0.71	4.61	5.24
		Women	5.24 ± 0.47	5.05	5.43
	7 METs	All	7.53 ± 0.79	7.29	7.76
		Men	7.38 ± 0.85	7.00	7.76
		Women	7.66 ± 0.72	7.36	7.96
VO_2 (L.min^{-1})	**Self-Pace**	All	0.74 ± 0.18	0.68	0.79
		Men	0.80 ± 0.17	0.72	0.87
		Women	0.68 ± 0.16	0.61	0.75
	3 METs	All	0.78 ± 0.16	0.73	0.82
		Men	0.86 ± 0.16	0.79	0.93
		Women	0.70 ± 0.11	0.66	0.75
	5 METs	All	1.24 ± 0.26	1.16	1.31
		Men	1.37 ± 0.26	1.25	1.48
		Women	1.13 ± 0.19	1.05	1.20
	7 METs	All	1.84 ± 0.36	1.74	1.95
		Men	2.05 ± 0.35	1.89	2.20
		Women	1.66 ± 0.27	1.55	1.77
VCO_2 (L.min^{-1})	**Self-Pace**	All	0.63 ± 0.16	0.58	0.68
		Men	0.68 ± 0.15	0.61	0.74
		Women	0.59 ± 0.16	0.52	0.65
	3 METs	All	0.69 ± 0.14	0.64	0.73
		Men	0.76 ± 0.15	0.69	0.82
		Women	0.62 ± 0.10	0.58	0.66
	5 METs	All	1.13 ± 0.26	1.05	1.21
		Men	1.25 ± 0.27	1.13	1.36
		Women	1.03 ± 0.20	0.94	1.11
	7 METs	All	1.76 ± 0.35	1.65	1.86
		Men	1.94 ± 0.36	1.78	2.10
		Women	1.60 ± 0.27	1.49	1.71
RER	**Self-Pace**	All	0.85 ± 0.05	0.84	0.87
		Men	0.84 ± 0.05	0.82	0.87
		Women	0.86 ± 0.47	0.84	0.88
	3 METs	All	0.89 ± 0.04	0.87	0.90
		Men	0.89 ± 0.04	0.87	0.90
		Women	0.89 ± 0.05	0.87	0.91
	5 METs	All	0.91 ± 0.05	0.89	0.93
		Men	0.91 ± 0.06	0.89	0.94
		Women	0.91 ± 0.05	0.89	0.93
	7 METs	All	0.96 ± 0.06	0.94	0.98
		Men	0.96 ± 0.07	0.92	0.99
		Women	0.96 ± 0.05	0.94	0.98

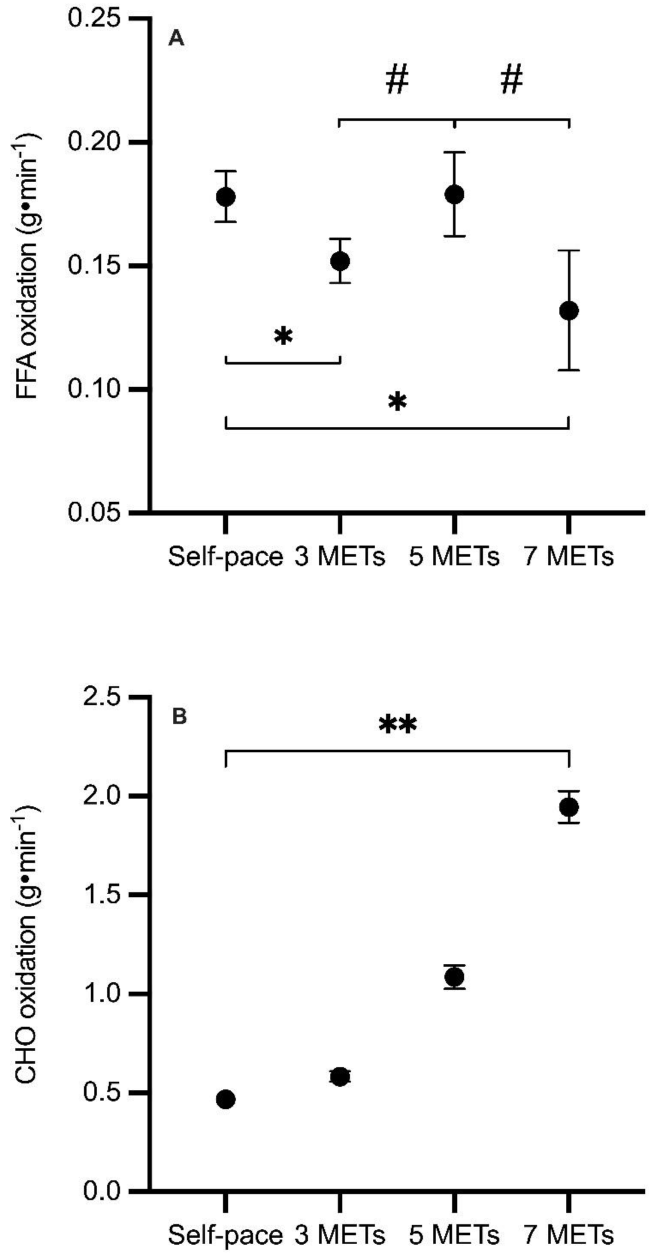

Figure 3 – Substrate oxidation during four experimental conditions. Figure 3-A shows FAT_{ox} and Figure 3-B demonstrates CHO_{ox}. The values in these figures are presented as Mean ± SEM. * and $^{\#}$ denote significant differences between SP, 5 METs, and the two other conditions, respectively ($p < .05$). ** denotes the main effect of intensity ($p < .05$).

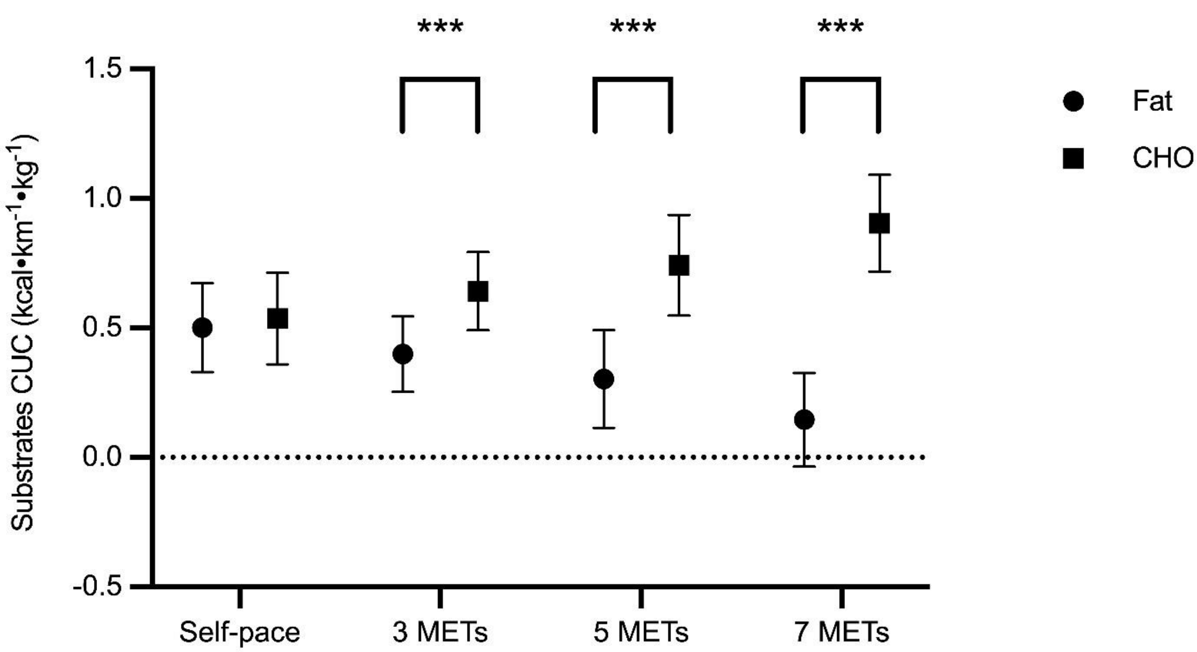

Figure 4 – Substrates caloric unit cost during four experimental conditions. Caloric unit cost of a substrate (e.g., CHO and fat) is defined as calories utilized per body weight to cover a given distance. CHO_{ox} values are represented by squares and FAT_{ox} values are represented by circles. The values in this figure are presented as a Mean ± SEM. *** denote significant differences between fat CUC and CHO CUC ($p < .05$).

substrates oxidation mirror each other, *post hoc* analysis revealed that the CHO CUC was equal to the fat CUC at the self-pace condition (*M* = 0.54, *SD* = 0.18, and *M* = 0.50, *SD* = 0.17 respectively; *p* = .497) and then started to significantly depart from each other at 3 METs onwards (*p* = .001) [CHO at 3 METs (*M* = 0.64 , *SD* = 0.15), 5 METs (*M* = 0.74, *SD* = 0.19), and 7 METs (*M* = 0.90, *SD* = 0.19); fat at 3 METs (*M* = 0.40, *SD* = 0.15), 5 METs (*M* = 0.30, *SD* = 0.19), and 7 METs (*M* = 0.14, *SD* = 0.18)]. Results demonstrated that fat and CHO caloric unit cost in all other conditions were significantly different from one another (*p* = .001).

Moreover, as illustrated in Table 3, based on the repeated measures ANOVA, there was a main effect of intensity on EE ($F_{(1.63, 74.97)}$ = 614.50, *p* = 0.001). Alongside the intensity increment, the EE also increased progressively with the lowest level showed in the self-pace condition (*M* = 3.63, *SD* = 0.87). All comparisons were significantly different except between self-pace and 3 METs (*M* = 3.85, *SD* = 0.77) (*p* = .07).

3.5 DISCUSSION

The current study aimed to assess FAT_{ox} rates in the general population and both men and women across the spectrum of daily physical activity intensities and determine at which intensity we are more efficient at oxidizing fat. As such, we examined FATox and CHO_{ox} in both absolute (g.min^{-1}) and relative (kcal.kg^{-1}.km^{-1}) terms and then evaluated daily EE to gain an understanding of substrate contribution during different intensities of daily locomotor activities. The findings of this study indicate that although the highest value of absolute FAT_{ox} occurred at 5 METs, the optimum substrate contribution to energy production would be at self-pace (when expressed in terms of body weight and distance covered). However, evidence showed that absolute FAT_{ox} rates at self-pace and 5 METs are roughly the same. These findings confirmed our primary hypothesis of peak FAT_{ox} occurs within the normal range of daily activities and particularly at the self-selected intensity.

Table 3 – Energy Expenditure (kcal•min^{-1})
According to the Experimental Conditions

Condition	**Mean**	**SD**
Self-Pace	3.63	0.87
3 METs	3.85	0.77
5 METs	6.16	1.28
7 METs	9.20	1.79

3.5.1 POPULATION CHARACTERISTICS

The population in this study consisted of 53% women and 47% men (Table 1) among which 56% of women were above, and 68% of men were below the average age. Male participants, on average, were taller and heavier in comparison to women, as expected. The data indicated that men's BMI ranged from normal to obese (Minimum = 19.4, Maximum = 34.3), whilst women ranged from underweight to overweight (Minimum = 15.8, Maximum = 29.7) according to American College of Sports Medicine guidelines (Ferguson B., 2014). As a whole, those data represent a good cluster of the western population and indicates no major health issues.

3.5.2 METABOLIC OUTCOMES

The rate of energy spent during different tasks of physical activity, termed 'metabolic equivalent of tasks' (abbreviated to 'METs'), is a useful, convenient, and standardized way to characterize a variety of physical activities (light, <3.0 METs; moderate, 3.0–5.9 METs; vigorous ≥6.0 METs; Physical Activity Guidelines for American, 2008). In the current study, we were interested in examining metabolic rates and efficiency through a continuum of intensities corresponding to daily activities, the so-called "non-exercise activity thermogenesis." Therefore, the 'metabolic equivalent of tasks' was chosen because it has been shown to be a valid and reliable way of reporting the continuum of physical activity intensities. In fact, the experimental protocol consisted of three intensities set at 3, 5, and 7 METs, at imposed speeds, and a freely chosen pace at which participants felt the most comfortable. While the statistical analysis showed a clear effect of intensity as expected, the value at self-pace was 3.03 ± 0.59, which is seemingly the same as 3 METs (Table 2).

Moreover, because the body's rate of energy transfer from macronutrients is greatly contingent on the breakdown of energy substrates in the presence of an adequate amount of O_2, proper determination of $\dot{V}O_2$ and $\dot{V}CO_2$ has been shown to be an accurate way of estimating the rate of substrate oxidation and subsequently the EE. To ensure the accuracy of indirect calorimetry (IC) systems, proper calibration techniques should be performed before testing participants. In regard to our data, two methods were used to calibrate and to assess validity and reliability of the IC system: 1) Infusion of pure gaseous of CO_2 and nitrogen (N_2) (Schadewaldt P. et al.,

2013) and 2) burning propane (Lighton J. R. B., 2008). The system was also calibrated according to the manufacturer recommendations and the values were checked regularly during the data collection. Our statistical outcomes showed that oxygen uptake increased linearly with increasing work rate, as notably shown by Basset and Boulay (2000). Brooks G.A. et al., (2004) also found that this linear increment results from elevated substrate catabolism and oxygen requirement of working skeletal muscles. However, it is worth mentioning that in our study, the amount of oxygen uptake at self-pace and 3 METs were roughly the same, with the lowest amount occurring at self-pace.

RER is one of the well-established fitness indicators that indirectly mirrors the muscle's substrate use. The RER (CO_2 production/O_2 uptake) increases with exercise intensity and indirectly reflects the relative contribution of CHO and fat to overall energy production (Simonson & DeFronzo, 1990; Romjin J.A. et al., 1993). Our results for RER are consistent with previous reports. Although RER represents a good indirect marker of substrate partitioning, from here on, only the stoichiometric equation outcomes will be reported. Accordingly, EE increased as a function of intensity as expected from the literature (Hunter G. et al., 1988).

Physiological and anthropometric differences between the sexes as well as divergent lifestyles have hindered comparisons of FAT_{ox} among men and women. As emphasized by Tarnopolsky et al. (1990), male-female comparisons necessitate matching subjects' training status, standardizing diet, and testing females' menstrual cycle (e.g., mid-follicular). In addition, in a crossover design study conducted by O’Sullivan et al. (1998), FAT_{ox} was reduced in postmenopausal women. Therefore, since we did not control for the above factors, we did not explore sex differences in the present study, and it should be the focus of future investigations. Hence, any sex-related outcome is going to be a crude estimate and should be interpreted with caution.

3.5.3 SUBSTRATE PARTITIONING

Many studies have shown the interplay of substrates to match metabolic energy demand (See the reviews by Ferrannini (1988) and Simonson and DeFronzo (1990)). No physiologist will question the relationship between substrates' contribution to energy production and the intensity of physical activity. Our metabolic outcomes are no exception in regard to these metabolic mechanisms. Examining the pooled participants’ substrate partitioning data, it is evident that they switched from fat to CHO as a preferred fuel directly as a function of intensity; however, this trend did not hold for FAT_{ox}. In fact, the highest rate of fat oxidized was at 5 METs. The latter outcomes do not align with Venables et al. (2005) data that showed an increase in FAT_{ox} from ~35%$\dot{V}O_{2max}$ to a maximal score of 48 ± 1% $\dot{V}O_{2max}$. Although participants in our study showed a higher metabolic rate of FATox at 5 METs compared to self-pace, the substrate contribution to energy production did not differ. The results based on Figure 3 showed a significant absolute FAT_{ox} rate difference between self-pace and 7 METs Despite a huge variance at 7 METs (CV = %125); a result that could be attributed to a high disparity in participants’ fitness levels (Klein S. et al., 1994). Further to the above outcomes, in all intensities, the population displayed a greater absolute FAT_{ox} variability compared to CHO. Once again, this acute metabolic response might mirror the fitness disparity among the participants. In fact, the variation of metabolic response was higher in 5, and 7 METs compared to self-pace and 3 METs, confirming the impact of fitness. In light of those results, one can argue that intensities revolving around natural walking speed should display a lower variance in substrate partitioning if we consider evolutionary factors. It seems logical to observe a higher contribution of fat to energy production at these conditions (self-pace and 3 METs) as they may reflect a greater metabolic efficiency and CHO sparing (perhaps a survival mechanism). Therefore, it can be concluded that oxidizing fat as an energy resource in self-pace and 3 METs is higher than 5 and 7 METs, as already shown by Christensen & Hansen (1939) and Maunder et al. (2018).

3.5.4 Metabolic Efficiency

As discussed above, from a FAT_{ox} point of view in absolute terms, the optimal workload seems to be between self-pace and 5 METs. Therefore, the argument must be made in terms of metabolic efficiency or CUC, that is, the ability to cover large distances by favouring FAT_{ox} and minimizing the depletion of body CHO reserve. The main result of the study supports this postulate; in fact, at self-pace, 50% of energy was produced by FAT_{ox} when body weight and distance covered are terms of the equation ($kcal \bullet km^{-1} \bullet kg^{-1}$). By moving away from self-pace, as the intensity went up, CUC_{CHO} rose as CUC_{FAT} fell. This is in agreement with the result of an earlier manuscript by Holloszy (1996), who stated that the absolute exercise intensity rate determines the total amount of fuel required, while relative work rate is involved in ascertaining the proportions of CHO and fat oxidized by the working muscles, such that as relative exercise intensity increases, the substrates' contribution to energy production switches from fat to CHO_{ox} (Holloszy, J., 1996). The results of the current study aligned with Holloszy's statement. The changes in relative FAT_{ox} occurred concurrently with changes in relative CHO_{ox}, an observation that is also consistent with what would have been predicted from previous work (Romijn et al., 1993). Overall, the results confirm that metabolic efficiency or CUC occurs at a self-selected pace even though CHO remained a major source of energy in both absolute and relative terms in all four conditions and both genders.

Human metabolism has evolved to preserve energy as an economic, biological system and adapt to harsh environments. The human metabolic machinery functions so that no stress beyond its safety capacity and “critical” disturbances to homeostasis occur (Noakes et al., 2001; St. Clair Gibson et al., 2006). As a matter of fact, CNS selects preferred walking speed to reduce EE (enhanced metabolic efficiency) to move the body mass over a distance (the energy cost of transport). The self-selection of stride frequency causes the least metabolic energy cost (Cotes & Meade 1960). Hence, in a study by Zarrugh and Radcliffe (1978), when participants were asked to perform at frequencies above and below the preferred frequency, the curve of metabolic cost (O_2 uptake) displayed a U-shaped as a function of frequency. A given subject chooses a "natural" or “comfortable” speed of walking relative to a minimum value of the EE expressed relative to body weight and distance covered (Ralston HJ, 1958). Self-paced physical activities also correspond to a lower perceived exertion (Ekkekakis & Lind, 2006) and a better emotive experience (i.e., self-reported pleasure) (Lind E. et al., 2005), which in turn, affects adherence. Hence, these could explain the reason why peak FAT_{ox} occurs at the self-pace condition in the present study.

It is notable that even though in the absolute form 5 METs is the peak FAT_{ox} in those conditions (= 0.179 g•min^{-1}; self-pace = 0.178 g•min^{-1}), its optimum contribution was much higher during self-pace (= 0.501 kcal•km^{-1}•kg^{-1}). Hence, at self-pace, participants spared most of their glucose content. Therefore, it is concluded that people should normally be able to walk a long distance or do a particular physical activity for a longer period of time at self-pace comparing to any other imposed speed.

3.6 METHODOLOGICAL CONSIDERATIONS

The current study has some methodological considerations that need to be addressed. First, the present findings are limited to a small sample size of the population living in the St. John’s area, Newfoundland, Canada, which prevents the results from being generalizable to other populations (e.g., residents of other countries), in addition to the fact that 85% of our participants are 40 years old or younger. Second, the chosen sample was selected by convenience, and people voluntarily participated in the study; therefore, they were more likely to be health-conscious and not a good representative of individuals with chronic disease or metabolic disorders. Third, the applied protocol was restricted to four different types of activities, which show the intensity level of some, but not all, free-living activities. However, they should be illustrative of most daily locomotor activities. Third, the absolute and relative contributions of fat and CHO during exercise can be affected by age (Roberts S.B. & Rosenberg I., 2006), sex (Friedlander AL. et al., 1998), training status (Horton, T.J., 1998), diet (Coyle, E.F. et al., 2001), mode of exercise (Knechtle B. et al., 2004), duration (Henderson G.C. et al., 2007), and intensity (Romijn J.A., 2000). Therefore, future research should seek to control these factors and may wish to categorize individuals based on the metabolic profile. Next, most of the studies in the literature used incremental exercise testing, while in this study, we used steady state exercise. Looking at the differences between these exercise tests could be an interesting topic to investigate in future studies. Last, it would be important to assess EMG in later research to justify the fiber type being used during each exercise intensity to determine if the change in substrate partitioning due to a different muscle fiber recruitment.

3.7 CONCLUSION

In conclusion, as hypothesized, the peak FAT_{ox} occurs within the normal range of intensities during daily physical activities for the general Western population. In fact, self-pace, which also falls into this range, is revealed to be the most efficient metabolic state in oxidizing fat. In other words, this confirms that metabolic efficiency corresponds to the intensities that have been freely chosen. Contrary to the current literature regarding the effect of HIIT on obesity and chronic diseases, the study outcomes suggest that self-pace or free chosen intensities might lead to the same metabolic adjustments as for HIIT but with a lower risk of injury and a higher level of pleasure/enjoyment, despite its longer duration. Individuals participating in free chosen physical activities might be more inclined, due to its natural pattern of locomotion, into non-exercise physical activities throughout the day such as gardening, fishing, dancing, or simply going for a walk at preferred speed (e.g., walking a dog, trail hiking, &, etc.).

3.8 References

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CHAPTER FOUR: OVERALL SUMMARY OF STUDY

It is well known that lipids have several beneficial roles in the body (Fahy et al., 2011); however, body fat accumulation and impaired FAT_{ox} result in overweight and obesity (Kim J.Y. et al., 2000), known as the most significant public health challenge (WHO, n.d.). Over the past few years, modernization and automation (Thivel D. et al., 2018) resulted in increasing sedentary behavior, which is now known as a new health risk factor (Biskup M. et al., 2018). Physical activity can play an essential role in preventing morbidity and mortality (Willett W.C. et al., 2006; Miura, S. et al., 2014). Various research has been conducted to investigate the benefits of high-intensity exercise; however, there is not as much focus on lower intensity activities. One of the boldest setbacks of high-intensity protocols such as HIIT protocol is the lack of adherence. Bringing more activities such as 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 daily walks.

This study aimed to examine FAT_{ox} in an intensity range covering daily ambulatory activities (e.g., 3–7 METs) for the general population. It was hypothesized that peak FAT_{ox} occurs within this range. Ralston (1975) showed in his study that the CNS chooses the speed that feels most comfortable for the body, and in selecting such speed, it is led by the perception of effort (Gandevia & Rothwell, 1987). At this preferred walking speed, the body's dependence on CHO is minimal, and fat provides most of the required energy (Ganley K.J. et al., 2008; Willis W.T. et al., 2005). Therefore, the hypothesis was completed by stating that peak FAT_{ox} occurs specifically during self-selected intensity activities such as self-paced walking.

The present study consisted of forty-seven physically active males and females ranging from 20 to 64 years old who completed a 65-minute lab-based experimental protocol. The testing begins with sedentary conditions (i.e., lying on a cot and sitting on a chair) for 5 minutes each.

Immediately after, participants were asked to do four different activities on a treadmill (walking at self-pace, 3, 5, 7 METs). Participants had a 5-minute resting period (lying on a cot) between the activities. During the test, oxygen uptake and carbon dioxide output were collected from each participant by an indirect calorimetry system (Oxycon Pro, Jaeger, Hochberg, Germany). Later, daily EE, FAT_{ox}, and CHO_{ox} in absolute ($g \bullet min^{-1}$) and relative ($kcal \bullet kg^{-1} \bullet km^{-1}$) terms were calculated. After applying proper statistical analysis, results showed that although the highest value for absolute FAT_{ox} occurs at 5 METs, this would be at self-pace when expressed in terms of body weight and distance covered (CUC). However, evidence showed that absolute FAT_{ox} rates at self-pace and 5 METs are roughly the same. These findings confirmed our primary hypothesis of peak FAT_{ox} occurs within the normal range of daily activities and particularly at the self-selected intensity.

Tarnopolsky and associates (1990) show that male-female comparisons can happen when the study has controlled for factors such as subjects' training status, diet, and females' menstrual cycle. Since we used secondary data analysis, these factors were not assessed, and therefore, sex differences could be explored in future studies.

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APPENDIX A – FULL ETHICS CLEARANCE - ICEHR APPROVAL

MEMORIAL UNIVERSITY

Interdisciplinary Committee on Ethics in Human Research (ICEHR)

St. John's, NL Canada A1C 5S7
Tel: 709 864-2561 icehr@mun.ca
www.mun.ca/research/ethics/humans/icehr

ICEHR Number:	**20210663-HK**
Approval Period:	August 25, 2020 – August 31, 2021
Funding Source:	
Responsible Faculty:	Dr. Fabien Basset School of Human Kinetics and Recreation
Title of Project:	*Is Peak Fat Oxidation within Daily Locomotor Activity?*

August 25, 2020

Miss Setayesh Behnamfar
School of Human Kinetics and Recreation
Memorial University of Newfoundland

Dear Miss Behnamfar:

Thank you for your submission to the Interdisciplinary Committee on Ethics in Human Research (ICEHR), seeking ethical clearance for your research project. The Committee appreciates the care and diligence with which you prepared your application.

The project is consistent with the guidelines of the *Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans* (TCPS2). *Full ethics clearance* is granted for one year from the date of this letter. ICEHR approval applies to the ethical acceptability of the research, as per Article 6.3 of the *TCPS2* (2018). Researchers are responsible for adherence to any other relevant University policies and/or funded or non-funded agreements that may be associated with the project.

The *TCPS2* **requires** that you submit an Annual Update to ICEHR before August 31, 2021. If you plan to continue the project, you need to request renewal of your ethics clearance and include a brief summary on the progress of your research. When the project no longer involves contact with human participants, is completed and/or terminated, you are required to provide an annual update with a brief final summary and your file will be closed. If you need to make changes during the project which may raise ethical concerns, you must submit an Amendment Request with a description of these changes for the Committee's consideration. If funding is obtained subsequent to ethics approval, you must submit a Funding and/or Partner Change Request to ICEHR so that this ethics clearance can be linked to your award.

All post-approval event forms noted above must be submitted by selecting the ***Applications: Post-Review*** link on your Researcher Portal homepage. We wish you success with your research.

Yours sincerely,

Russell J. Adams, Ph.D.
Chair, Interdisciplinary Committee on Ethics in Human Research
Professor of Psychology and Pediatrics
Faculties of Science and Medicine

RA/bc

copy: Supervisor – Dr. Fabien Basset, School of Human Kinetics and Recreation