THE EFFECT OF INTERMITTENT FASTING DURING RAMADAN ON ENERGY EXPENDITURE AND SUBSTRATE OXIDATION IN 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

“Exercise and work physiology”

School of Human Kinetics and Recreation

Memorial University of Newfoundland

June 2016

St. John’s Newfoundland
ABSTRACT
The study aimed to examine the effect of Ramadan fasting (RF) on substrate partitioning, energy production, blood lipids and glucose as well as body composition. Nine healthy Muslim men (FAST group) and eight healthy men (CNT group) were assessed pre- and post-RF. FAST were additionally assessed at 10th, 20th and 30th day of RF in the morning (a.m.) and evening (p.m.). Results showed a significant reduction in body mass and fat mass in FAST with no statistical differences pre- vs. post-RF for all other variables in both groups. A significant daytime fasting effect [a.m. vs. p.m.] on substrates oxidation (fat and carbohydrate) and blood parameters (glucose, insulin, total cholesterol, and triglycerides) was observed. In conclusion, although RF brings about an acute metabolic response that shifts substrate partitioning towards lipids, no chronic metabolic response was observed despite the extended daily fasting period (18.0±0.3 hrs) and changes in body composition.

Key words: Indirect calorimetry, substrate oxidation, intermittent fasting, Ramadan, lipids profile, insulin, blood glucose, body composition.
ACKNOWLEDGEMENT

I would like to express sincere gratitude to my graduate supervisors Dr. Fabien Basset and Dr. Sukhinder Cheema who have supported me through my graduate studies with their patience and knowledge while allowing me the room to work in my own way. Furthermore, I would like to thank Dr. Denis Joanisse (Laval University) for his assistance to accomplish my work, Dr. Thamir (Tim) Alkanani for his assistance in the exercise physiology laboratory and encouragement to continue on the path of academia, and to my colleagues: Alicia, Adebayo, and Jason for their support and help. A special thanks to my parents along with the rest of my family, whose support made this possible. Finally, I would like express deepest appreciation to my beloved husband Mohammad who is always by my side and makes sacrifices every day to enable me to continue with my studies.
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<tr>
<td>BM</td>
<td>Body mass</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>br•m⁻¹</td>
<td>Breath per minute</td>
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<td>b•m⁻¹</td>
<td>Beat per minute</td>
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<td>BS</td>
<td>Blood sample</td>
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<td>CNT</td>
<td>Control group</td>
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<td>DBP</td>
<td>Diastolic blood pressure</td>
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<td>EE</td>
<td>Energy expenditure</td>
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<td>EP</td>
<td>Energy production</td>
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<td>FAST</td>
<td>Fasting group</td>
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<td>FM</td>
<td>Fat mass</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
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<td>HR</td>
<td>Heart rate</td>
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<td>IF</td>
<td>Intermittent fasting</td>
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<td>IC</td>
<td>Indirect calorimetry</td>
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<td>LM</td>
<td>Lean mass</td>
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<td>LDL</td>
<td>Low density lipoprotein</td>
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<td>MR</td>
<td>Metabolic Rate</td>
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<td>R1</td>
<td>10&lt;sup&gt;th&lt;/sup&gt; day of RF</td>
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<td>R2</td>
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<td>R3</td>
<td>30&lt;sup&gt;th&lt;/sup&gt; day of RF</td>
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<td>RER</td>
<td>Respiratory exchange ratio</td>
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<td>RF</td>
<td>Ramadan fasting</td>
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<td>RR</td>
<td>Respiratory rate</td>
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<td>SBP</td>
<td>Systolic blood pressure</td>
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Chapter I
Introduction
1.1 Background of study

An overnight fast of about 8-12 hours is common for most individuals. Fasting can be defined as the absence of food and fluid intake (Maughan, Fallah, & Coyle, 2010). Intermittent fasting (IF) is an interventional strategy wherein individuals are subjected to varying periods of fasting (Azevedo, Ikeoka, & Caramelli, 2013). Intermittent fasting involves a complete or partial restriction in energy intake (Rothschild, Hoddy, Jambazian, & Varady, 2014), daily or on alternative days. However, many people undergo periodic fasts for cultural, health, and religious reasons. Many religions recommend periods of fasting and, among these, followers of Islam fast during daylight hours in the holy month of Ramadan (Azizi, 2010). Ramadan is the 9th month of the Islamic calendar. The Islamic calendar, being a lunar calendar, is 11 days shorter than the Gregorian calendar and therefore Ramadan moves forward by 11 days each year. For this reason, physiological changes during Ramadan fasting may be influenced by seasonal conditions, that is, whether it falls during summer or winter (Azizi, 2002). The duration of the fast thus depends on the geographical location and the season of the year, and can last 19-hrs a day in the summer of temperate region compared to the 12-hrs experiences by followers living near the equator (Azizi, 2002). Muslims believe that Ramadan fasting (RF) improves mental and spiritual discipline, and increases the awareness of the misery suffered by those who do not have enough food and frequently become hungry with no choice. Fasting during Ramadan requires to abstain from food, smoking, and fluid intake during daylight hours from dawn to sunset for a whole month (29-30 days) (El Ati, Beji, & Danguir, 1995). The annual RF is not mandatory for children, the sick and travelers, as well as for
menstruating women; pregnant and lactating women are also exempted and permitted to postpone their fasting to a later time (Azizi, 2010). Typically during Ramadan, two meals are eaten on daily basis, one after sunset and the other just before dawn that shift the pattern of caloric intake from daytime to the hours of darkness. There is, however, no restriction on the quantity or type of food to be consumed during the night (Chaouachi, Leiper, Souissi, Coutts, & Chamari, 2009). These changes in the timing of food intake as well as in the types of diet can influence substrate availability and utilization (Bouhlel et al., 2006). In addition, acute diurnal dehydration observed might influence metabolic responses (Leiper, Molla, & Molla, 2003). Fasting is characterized by a coordinated set of metabolic changes designed to spare carbohydrate and increase reliance on fat as a substrate for energy supply. It increases the rate of gluconeogenesis, that is, the formation of new glucose by the liver from amino acids, glycerol and lactate to help in maintaining the supply of glucose (Maughan et al., 2010). This religious fasting, practiced by a large number of Muslims all over the world, provides a unique opportunity to investigate the effect of RF over an extended period of time on energy expenditure, substrate oxidation, and serum lipids [total cholesterol (TC), and triglycerides (TG)] and glucose levels in the human blood.

1.2 Purpose of study

Metabolic regulation during fasting dictates substrate contribution to energy production (EP) through selected endocrine responses. However, while many studies have assessed potential variations in metabolic responses and anthropometric changes, few studies have assessed energy expenditure and fuel oxidation induced by RF. To the best
knowledge of the authors, no study extensively examined the effect of fasting on energy expenditure and fuel oxidation throughout Ramadan in healthy men. The study aims to examine the effect of RF on fuel oxidation. Acute (through the day) and long-term (through the month) alterations of fuel oxidation will be investigated.

It is hypothesized that IF induced by the strict Ramadan regimen will acutely alter the contribution of substrates to energy production and be magnified over time by the cumulative metabolic stress. It is expected that the contribution of lipid as a substrate to EP will increase during the day as well as during the month. Along with substrate oxidation alteration, the blood serum level of TC, TG, glucose, and insulin should reflect the change in mobilization, transport and oxidation of substrates.

1.3 Significance of study

Disturbances in lipid metabolic regulation can lead to obesity, diabetes, and cardiovascular disease (CVD). Cardiovascular diseases are the leading cause of death in western countries (Brooks, Fahey, & Baldwin, 2005). Hyperlipidemia and hypertension are considered the main risk factors for developing such a condition that could result in sudden heart attack (Fuster, Gotto, Libby, Loscalzo, & McGill, 1996). The progression of chronic disease can be delayed or prevented through lifestyle modifications such as smoking cessation and diet therapy. Furthermore, regular exercise programs are important components of a healthy lifestyle. Moderate intensity and long duration rhythmic exercise programs showed increasing fat oxidation and some beneficial effects on plasma lipid profile (Jeukendrup & Wallis, 2005). However, IF is currently generating a lot of interest as a non-pharmacological approach to address these health issues. Intermittent fasting was
reported to promote optimal health and reduce the risk of many chronic diseases such as CVD, cancer, and diabetes mellitus, particularly for those who are overweight and sedentary (Varady & Hellerstein, 2007). In addition, IF lowers blood pressure, total cholesterol and triacylglycerol concentrations and also reduces body fat (Longo & Mattson, 2014). Ramadan fasting is one type of IF. It provides a unique model to study the effect of IF on metabolism, especially energy expenditure and substrate oxidation. Information obtained from the time of fasting will help in understanding the regulation of metabolic changes, substrate partitioning and its impact on health.
Chapter II
Review of Literature
2.1 Metabolism and heat production in human

Many years of research have led to a great improvement in our understanding of human physiology. The eighteenth-century respiratory physiologist Antoine L. Lavoisier contributed to better understanding of the relationship between respiration and combustion that led to further investigation in chemistry and biochemistry (Brooks et al., 2005). Early in the eighteenth century, Chevreul (1813) published the first paper on the composition of animal fats and the description of fatty acids. Later during the same century, Berthelot (1854) described for the first time the synthesis of neutral lipids in combining glycerol with fatty acids, synthesis of mono-, di-, and triacylglycerol (Carpenter, 1998). In 1856, the German pathologist Rudolf Virchow described lipid accumulation in arterial walls (Brown & Fee, 2006). Some of these seminal scientific works provided the foundation for Francis G. Benedict and his associates in the early twentieth century to develop a calorimetric system coupled with a spirometry apparatus for measuring oxygen uptake and for determining metabolic rate in humans. They performed a series of experiments to measure human metabolic rate at rest and during steady-rate exercise (Brooks et al., 2005). The estimation of energy production (or metabolic rate, MR) allowed quantifying substrate utilization by the human tissue. In other words, the ultimate goal of substrates metabolism (i.e., carbohydrate, fats, and proteins) is to produce energy through biological oxidation, so measuring the rate of O$_2$ uptake yields a good estimate of the rate of heat production (Lighton, 2008). The heat generated by biologic combustion is utilized to maintain body temperature. In addition, the mechanical work (muscular contraction) of human body is thus made possible through the free energy produced by the oxidizable substrates.
(Ferrannini, 1988). However, there is an organized system of enzymes and coenzymes specialized in catalyzing foodstuff into substrates. Fatty acids are oxidized in the cell mitochondria through β-oxidation while the substrate-level phosphorylation of glucose to pyruvate and lactic acid (glycolysis) is completed in the cytoplasm. It is evident that the available energy from adenosine triphosphate (ATP) and creatine phosphate (CP) is very limited (few seconds). Therefore, the energy production from the oxidation of carbohydrate (CHO) and fatty acids is extremely important (Astrand & Rodahl, 1970).

**2.2 Indirect calorimetry and substrate oxidation**

Indirect calorimetry (IC) is the technique for the measurement of whole-body substrate oxidation. Indirect calorimetry can provide quantitative information about the type of substrate oxidized (Simonson & DeFronzo, 1990). This technique could measure the energy cost of a great variety of human activities. Early in the nineteenth century, Zuntz and Schumburg (1901) developed tables relating metabolic rate to O₂ uptake and CO₂ production, and the contribution of carbohydrate and fat to energy production as cited in Brooks et al. (2005). Later in the same century, Lusk (1927) developed the non-protein caloric equivalents based on the respiratory quotient (RQ) to analyze the oxidation of carbohydrate and fat mixtures and remove the energy released from basal protein oxidation to account for heat released from CHO and fat (Lusk, 1927). The estimation of total body carbohydrate and fat oxidation during rest and exercise were obtained by different studies through IC and other valid methodologies. As shown by several studies, it is evident that, after ingestion of a mixed meal, blood glucose, fat, and amino acid concentrations rise, and that the insulin secretion stimulates storage of substrates and
suppresses their mobilization (Maughan et al., 2010). In addition, increasing the availability of carbohydrate increases its oxidation and decreases lipid oxidation at rest and during exercise. In fact, Coyle et al. (1997) showed that the oxidation of intramuscular lipid is suppressed when carbohydrate is consumed before exercise (Coyle, Jeukendrup, Wagenmakers, & Saris, 1997). However, the intracellular mechanisms regulating substrate oxidation in human skeletal muscle is still unclear (Maughan et al., 2010). Back in the 1960s, Phillip Randle and associate examined the effect of elevated free fatty acids (FFAs) on the suppression of glucose oxidation in isolated heart muscle fibers, and showed that when β-oxidation produces high flux of citrate, it inhibits the phosphofructokinase (PFK) enzyme, thereby slowing down carbohydrate catabolism. These outcomes led to the glucose-fatty acid cycle theory (Randle cycle). This paradigm helped to understand how mitochondrial metabolic response to endurance exercise promotes lipid oxidation and spares carbohydrate (Brooks et al., 2005). The Randle cycle describes the dynamic interaction between substrates without hormonal mediation. However, this cycle must not be confused with the metabolic cycles in which sequential chemical reactions add and remove chemical compounds to form intermediate or end products (Hue & Taegtmeyer, 2009).

### 2.3 Risk factors in the development of cardiovascular disease

In terms of energy metabolism, lipids are essential for energy maintenance and other biological processes in the human body (Brooks et al., 2005). However, lipids can also be problematic. Disturbances in lipid metabolic regulation can lead to obesity, diabetes, and CVD (Despres et al., 1990). Atherosclerosis is an example of CVD in which
fibrotic and lipid-filled plaques (mainly low density lipoprotein- LDL) are developed in the wall of large arteries such as coronary arteries. Hyperlipidemia and hypertension are considered the main risk factors for developing such a condition that could result in sudden heart attack (Fuster et al., 1996). Clinical studies report that a 10% reduction of total cholesterol reduces the risk of coronary artery disease (CAD) mortality by 13% and a 1% reduction of LDL reduces the risk of major coronary events by approximately 2% (Martins, Verissimo, Coelho e Silva, Cumming, & Teixeira, 2010). The progression of CAD can be delayed or prevented with cholesterol reduction therapy involving lifestyle modifications and/or drug therapy. Aggressive dietary modification, smoking cessation, and drug treatment contribute to the regression of atherosclerosis and may prevent myocardial infarction. Drugs used to treat dyslipidemia lower TC, TG, and LDL and increase high density lipoprotein-HDL (Hausenloy & Yellon, 2008). Furthermore, other interventions such as exercise programs aimed at increasing fat metabolism showed some beneficial effects on plasma lipid profile (Jeukendrup & Wallis, 2005). These exercise programs could help prevent the development and/or alleviate the symptoms of metabolic disruption such as obesity and diabetes (Jeukendrup & Wallis, 2005). The IF is currently generating a lot of interest as a non-pharmacological approach to address these health issues. The benefits of IF on metabolic disorders have been reported in many studies (Longo & Mattson, 2014).

2.4 Intermittent fasting approach

Fasting can be defined as the absence of food and fluid intake, but there is no clear defined time window, after the last food intake, at which fasting might be said to begin. It
depends on the amount and type of food ingested (Maughan et al., 2010). An overnight fast of about 8-12 hrs on daily basis is common for most people; however, IF can be undertaken in several ways. The basic pattern alternates days of normal calorie intake with days of calorie deficit [severely restricted]; a dietary strategy as effective as continuous modest calorie restriction (Rothschild et al., 2014). Therapeutic fasting in humans using either short periods or prolonged periods of underfeeding first appeared for the treatment of diabetes and was prescribed from 1913 until the first use of insulin in 1922 as cited in (Mazur, 2011). As early as 1915, prolonged fasting was also described as a possible treatment for obesity (Folin & Denis, 1915). Many studies have been conducted to investigate the effect of IF on substrate metabolism (Benedict, 1915; Cahill, 1970; Kerndt et al., 1982; Owen et al., 1967). These studies shed some light on some of the metabolic mechanisms underlying the responses to the absence of food. The transition from fed to a fasted state starts at the end of the postprandial period (post-absorptive state); however, the digestive tract may still absorb nutrients during that period (Lignot & LeMaho, 2012). The time window depends on the composition and size of meal, but varies between 3-4 hrs and 7-8 hrs during which 75 % of blood glucose levels are maintained via glycogenolysis (hydrolysis of glycogen stores in the liver) (Maughan et al., 2010). The reminder amount of blood glucose comes through gluconeogenesis process, that is the formation of new glucose from amino acids, lactate, pyruvate and glycerol (Kerndt, Naughton, Driscoll, & Loxterkamp, 1982). Lactate is metabolized from muscle glycogen and resynthesized into glucose by the liver and kidney while glycerol results from the hydrolysis of TG that also releases of FFA. However, amino acids provides 15% of the substrate in the early fasting
period, reaching a peak at approximately on the fourth fasting day (Benedict, 1915). Beyond ten days of fasting, the protein catabolism decreases and the energy demands are met mainly through fat oxidation (Kerndt, Naughton, Driscoll, & Loxterkamp, 1982). Although it has been known for over a century that fat is the principal storage form of energy, only in the recent past have the physiological mechanisms of esterification and mobilization been partially clarified (Cahill, 1970). The metabolic responses during fasting have been the interest of different investigators and the regulation of glucose and lipids oxidation is quite well understood (Maislos et al., 1993). Benedict (1915) in his classic study on a normal man, who fasted 30-days, noted that carbohydrate stores provide small but significant component of body fuel at the beginning of the fast. Thereafter, fat provided more than 75 percent of the energy production after the first few days of food deprivation. In addition, he reported that prolonged period of fasting could completely deplete body lipid reserves (Benedict, 1915). Cahill et al. (1966) repeated the classical study done by Benedict (1915) on fasting humans and showed that the rate of carbohydrate oxidation decreases in the fasted state and that the energy demand is met by an increased rate of lipid and protein oxidation (Cahill et al., 1966). However, during fasting, plasma FFA levels increased within 14-hrs after the last meal; a response that contributes to spare the limited carbohydrate reserve for the central nervous system and erythrocytes (Maughan et al., 2010). Moreover, Owen et al. (1967) performed catheterization of cerebral vessels in three obese patients undergoing 5-6 weeks of fasting. The authors showed that the production of β-hydroxybutyrate and acetoacetate in the liver through fatty acid β-oxidation replaced glucose as the predominant fuel for brain
metabolism (Owen et al., 1967). Finally, Kerndt et al. (1982) reported increased lipolysis and ketogenesis, while glycogenolysis was reduced to an undetectable level during the period of 36-days of complete fasting (Kerndt, Naughton, Driscoll, & Loxterkamp, 1982). Until the end of 1960, prolonged fasting was believed to be safe, however, several negative side effects and complications were observed, including breakdown of electrolyte homoeostasis after continuous fasting of 60-days (Runcie & Thomson, 1970), cardiac arrhythmias (Duncan, Duncan, Schless, & Cristofori, 1965), and severe orthostatic hypotension, as well as severe normocytic, normochromic anemia, and gouty arthritis (Drenick, Swendseid, Blahd, & Tuttle, 1964). Prolonged continuous fasting was finally stigmatized as an unsafe procedure exposing the patient to an undue risk of physiological stress. As an alternative, short period of fasting, fasting on alternative days or intervals between food intake and fasting was highly recommended to health minor metabolic disorders such as induced by chronic diseases (Lignot & LeMaho, 2012). Short periods of fasting were assessed by Nilsson and Hultman (1973) who reported that blood glucose is well maintained during the early stage of fasting as the liver glycogen store is progressively hydrolyzed and released as glucose into the circulation. These results were obtained by repeated percutaneous biopsies of the liver in 19 fasting participants. Another study on healthy subjects who volunteered to fast 60-hrs reported a decrease in plasma glucose and insulin, a significant increase in lipolysis and fat oxidation, and moderate increase in proteolysis and protein oxidation (Carlson, Snead, & Campbell, 1994). Finally, a study conducted with the isotope technique found that short periods of fasting resulted in some loss of lean tissue (Krempf et al., 1993). Fasting has been practiced for millennia,
but, only recently, studies have shed light on its health benefits. Intermittent fasting was reported to promote optimal health and reduce the risk of many chronic diseases, particularly for those who are overweight and sedentary (Longo & Mattson, 2014). In addition, IF reduces level of blood pressure and body fat. Fasting triggers adaptive cellular stress responses, which result in an enhanced ability to cope with more severe stress and counteract disease processes (Longo & Mattson, 2014). Moreover, Varady et al. (2007) reviewed animal and human evidence of alternative-day fasting (ADF) and its effects on chronic disease, such as cardiovascular disease, cancer, and type II diabetes mellitus. In this review, animal studies found lower incidences of diabetes and lower fasting glucose and insulin concentrations following ADF. In addition, ADF data showed lower total cholesterol and triacylglycerol concentrations, lower heart rate, improved cardiac response to myocardial infarction, and decrease in lymphoma incidence. While in humans, the review reported greater insulin-mediated glucose uptake, higher HDL and lower triacylglycerol concentrations (Varady & Hellerstein, 2007).

### 2.5 Ramadan intermittent fasting model

As mentioned earlier, fasting activates lipolysis, and FFA becomes the preferred fuel for cellular respiration. In the liver, β-oxidation of FFA fulfills the energy needs. This metabolic adjustment will spare glucose to be used by central nervous system (Hue & Taegtmeyer, 2009). In addition, intermittent or periodic fasting protects against diabetes, cancers, improves blood lipid profile and as a consequence, decreases the risk factors associated with high incidence of CVD (Longo & Mattson, 2014). Ramadan fasting differs from other fasting models used by the previously mentioned studies in that it involves
repeated days without food and fluid intake during the hours of daylight but with no restriction on food intake from sunset until dawn (Chaouachi et al., 2009). In addition, the total hours of fasting vary depending on the season and climate condition; factors that affect the physiological response of the human body (Azizi, 2002). However, since this type of fasting is being practiced by a great number of Muslims yearly, it provides a unique opportunity to examine the effect of fasting on human health and metabolism especially blood lipid profile and substrates oxidation. Many studies have been conducted during the month of Ramadan (El Ati et al., 1995; Maislos et al., 1993; Rahman et al., 2004; Ziaee et al., 2006) that examined blood lipid level and/or substrates oxidation.

2.5.1 The effect of Ramadan fasting on blood lipid profile

There exists extensive literature on the effects of RF on various aspects of health and on the risk factors for various diseases involving blood lipids serum levels and changes in body weight. However, on-going debate remains regarding the effects of RF on health. Early in the eighties, a study by Fedail et al. (1982) reported a rise in concentration of TC with a significant reduction in body weight, and no changes in TG level. Blood samples were taken on the 1st day and on the last day of RF after fasting 16-hrs (Fedail, Murphy, Salih, Bolton, & Harvey, 1982). Ten years later, Maislos et al. (1993) found an increase in HDL level with no change in TG, LDL, and TC when blood samples were taken at the end of RF and one month after RF (Maislos et al., 1993). Ten years ago, Rahman et al. (2004) examined the effect of fasting during Ramadan on blood lipid serum level in 20 healthy male participants. Blood samples were drawn before and after RF (after an overnight fast) and during RF (just after breaking the fast with a glass of water). Diet
information was obtained from all participants. From blood lipid analysis, only a significant increase in HDL was observed. This result was accompanied by significant weight reduction (Rahman, Rashid, Basher, Sultana, & Nomani, 2004). Ziaee et al. (2006) two years later conducted a study on the effect of RF on plasma lipids and lipoproteins in healthy participants. The authors found a decrease in blood glucose, LDL, body weight, and a decrease in HDL. Blood samples were taken after a night fast before Ramadan and 12-hrs after last meal on the 26th day of Ramadan. In the light of the outcomes the authors concluded that the effect of fasting on serum lipid levels was perhaps related to the nutritional content of food or metabolic response to diet restriction. However, authors acknowledged that no measure of participants’ physical activity, that might have potentially affected blood lipid levels, was recorded (Ziaee et al., 2006). A recent study by McNeil et al. (2014) compared normal and obese men during RF. They investigated variations in body weight, metabolic profile (LDL, TC, TG, HDL, insulin, and glucose), and energy expenditure. Measurements were taken prior to, during as well as one and four months after RF. Significant increases in blood glucose, total cholesterol, and LDL concentrations were observed in normal and obese men during RF. However, these findings could be questionable owing to the small sample size (n=10 in each group). In addition, participants did not complete food diaries, which might have affected the blood measurements as acknowledged by the authors (McNeil et al., 2014).

2.5.2 The effect of Ramadan fasting on sport performance and metabolic response

The combined effect of fasting and sport performance during Ramadan was
examined by Ramadan et al. (1999) who investigated the effect of fasting during the month of Ramadan on steady state submaximal exercise, body fluid, and energy balance in sedentary and active men. Fasting blood samples were obtained before the exercise test on first week, after 2 weeks, and at the fourth week of Ramadan. The authors reported lower body weight in the active group, and no changes in the TC and TG levels in both groups. In addition, the authors found a lower exercise respiratory exchange ratio due to increased lipid usage in the active group (Ramadan, Telahoun, Al-Zaid, & Barac-Nieto, 1999). Another study by Chennaoui et al. (2009) investigated the effect of RF on physical performance and metabolic, hormonal, and inflammatory parameters in eight middle-distance runners. A maximal aerobic power test was performed 5-days before RF, and on day 7 and 21 of RF. Blood samples were collected before RF, at the end of RF, and one week post-RF. Researchers reported no significant changes of body mass, TG, LDL, HDL, and LDL. Higher FFA level were noticed at the end of RF in endurance athletes (Chennaoui et al., 2009).

2.5.3 The effect of Ramadan fasting on body composition

Some studies have examined the effect of RF on body fat mass and fat-free mass. For instance, Sweileh et al. (1992) reported a significant decrease in fat mass with no change in fat-free mass during RF using hydrostatic weighing (Sweileh, Schnitzler, Hunter, & Davis, 1992), while El Ati et al. (1995) did not find significant differences in fat mass and fat-free measured using skinfold thickness (measured with a Harpenden caliper) and body density calculated with the equation of Durnin and Rahaman (El Ati et al., 1995). Another study examined body composition using bioelectrical impedance analysis
(BIA) reported a reduction in fat mass in all fasting subjects except in women aged 36-70 years. Fat-free mass was also significantly reduced in all participants (Norouzy et al., 2013). Finally, using the Dual-energy x-ray absorptiometry (DXA), McNeil et al. (2014) did not find changes in body total fat mass and total fat-free mass during RF. In conclusion, the contradictory results of the above-mentioned studies may depend on the validity and the reliability of the measurements, total hours of fasting, demographics, total caloric intake during dark hours, hours and quality of sleep, amount of physical activity and the degree of weight changes (Azizi, 2010).

2.5.4 The effect of Ramadan fasting on body’s hydration status

The strict RF followers do not hydrate during the day. This restriction could lead to dehydration especially under tropical and equatorial climates or during the very long summer day of the septentrional countries (Maughan & Shirreffs, 2012). Loss of water can occur through sweating, urine, feces, and breathing. Excessive dehydration could result in a decreased rate of sweating, plasma volume, and, consequently, reduced cardiac output, maximal oxygen uptake, and muscle strength (Naghii, 2000). More importantly, it lowers liver glycogen content, a mechanism that potentially influences substrate metabolism (Leiper et al., 2003). Even if water is continuously formed by oxidation of substrate, the amount of water added (≈ 500 ml per day) to the body’s water pool is not sufficient to match the rate of water loss, so an oral intake is necessary otherwise dehydration occurs (Maughan & Shirreffs, 2012). In the absence of fluid intake during RF, a progressive loss of body water occurs over the course of the day. A small loss in body mass can come from a decrease of glycogen-bound water stores, change in extracellular fluid volume due to a
lower sodium intake, and to some moderate degree, hypo-hydration with little loss of body tissue (Leiper et al., 2003). In addition, evidence provided by Lang (2011) suggests that cell volume is influenced by hydration status and tissue osmolality, which has major effects on the metabolism of carbohydrate and of protein (Lang, 2011). The effect of dehydration during RF might, therefore, be of concern and was examined in several studies. In the study of Husain et al. (1987), fluid intake and urine output were measured in twelve men participants for one day during each week of fasting and one day during the pre-fasting control period. Among the participants examined, the RF regimen did not result in marked changes in fluid intake and output volumes (Husain, Duncan, Cheah, & Ch'ng, 1987). On the other hand, Trabelsi et al. (2012) examined the hydration status of 19 male participants during RF in fasting compared to postprandial condition during aerobic exercise state. The authors concluded that individuals engaging in aerobic exercise during RF should drink plentiful amounts of fluid during the night time to compensate for the dehydration that occurs during daylight hours (Trabelsi et al., 2012). Finally, a study has reported that a complete replenishment of water deficit will occur with sufficient water intake during the night (Maughan & Shirreffs, 2012).

2.5.5 The effect of Ramadan fasting substrate oxidation

As noted earlier, lipids are an important energy source. The previously mentioned studies to a large extent examined lipid/lipoprotein levels in the blood and although the outcomes greatly helped to increase our knowledge on the relationship between blood lipid content and chronic disease, these studies mainly focused on blood circulating lipids but did not really address blood lipids disappearance or oxidation. It has been known for
quite some time that substrate oxidation depends on tissue metabolic rate or energy demand and that substrate contribution to energy production depends on metabolic flux and substrate pool, conditions that could vary according to muscle contraction (intensity of exercise) or induced-energy deficit (restrictive diet) (Astrand & Rodahl, 1970). For instance, fasting during Ramadan induces additional metabolic stress on human body that alters substrate pools and consequently substrate oxidation (Azizi, 2002). Human body adjusts to the absence of nutrients and maintains the levels of blood glucose through progressively hydrolysing some liver storage of glycogen (Maughan et al., 2010). Furthermore, with longer fasting hours, evidences suggested that human body shifts from carbohydrate to fat oxidation to meet energy demands (Cahill, 1970; El Ati et al., 1995). Unfortunately, only few studies have addressed the potential variations in resting energy expenditure (REE) and substrate oxidation during RF. El Ati et al. (1995) examined the effect of RF on REE and substrate oxidation in healthy women. They measured EE through indirect calorimetry two days before, the second day, the 28th day, and one month after RF. Blood samples were obtained to evaluate serum lipids and energy intake was recorded for all participants. Concomitant decreases of plasma insulin concentrations and energy expenditure during RF as well as alterations in nutrient oxidation were noticed. In fact, fat oxidation increased and carbohydrate oxidation decreased within the fasting day of Ramadan (El Ati et al., 1995). However, these results must be interpreted with caution because women have higher lipolytic rate compared to men due to the differences in body fat distribution and sex hormones (Tarnopolsky, Atkinson, Phillips, & MacDougall, 1995). Recently, Stannard & Thompson (2008) conducted a study to examine the effect of RF on
substrate selection during submaximal cycling exercise. Eight men participated in the study and underwent three 10-min cycling exercise bouts at 45, 60 and 75% VO$_{2\text{peak}}$ one week before, at the end of first week, and during the final week of RF. Expired gas was collected via IC and substrate partitioning was calculated through stoichiometric equations. The results showed that RER during exercise at the end of the first week was significantly lower than pre-Ramadan. In addition, the rate of lipid oxidation increased by the first week of Ramadan, however, the effect was normalized by the final week (Stannard & Thompson, 2008). Finally, the effect of RF on fuel oxidation during steady-state exercise was evaluated by IC in nine trained male rugby players and showed increased lipid oxidation during submaximal exercise. The authors concluded that decreased body mass was the reason for increased fat utilization (Bouhlel et al., 2006). In general, RF seems to have the same effect on substrates oxidation as exercise or diet restriction, shifting substrate utilization from carbohydrate to lipid as a fuel of choice as reported in most studies. Unfortunately, none of the above-mentioned studies have examined the mechanisms underlying the change in substrate contribution to energy production. What leads to shift from utilizing glucose as a main fuel into oxidizing fat to meet the energy demand during fasting hours?
2.5.6 Concluding remarks

Some of the above-mentioned studies have been included in a meta-analysis examining the effects RF from which it has been concluded that RF can affect body weight, and reduce TC and LDL levels in men (Kul, Savas, Ozturk, & Karadag, 2014). However, given the different ways RF is practiced in different populations, the seasonal and climatic differences, differences in gender, health, fitness and physical activity levels of the studied population in addition to differences in study experimental designs, it is difficult to reach firm conclusions about the health benefits of fasting from the current body of literature (Alkandari, Maughan, Roky, Aziz, & Karli, 2012). In addition, while many studies have assessed potential variations in metabolic responses and anthropometric changes, only one study had assessed resting energy expenditure and fuel oxidation induced by RF in healthy women. Therefore, conducting a study to examine the change in the contribution of substrates to energy production in healthy men seems relevant to better understand the mechanisms underlying the metabolic adjustments to IF. The current study aims to examine the effect of RF on fuel oxidation. Acute (through the day) and chronic (through the month) alterations of fuel oxidation will be investigated. It is hypothesized that RF will acutely shift fuel oxidation from carbohydrates to fats during the daytime fasting and be magnified over time by the cumulative metabolic stress. It is expected that the shift in fuel oxidation will be mirrored by changes in blood serum levels of TC, TG, glucose, and insulin.
Chapter III
Manuscript

To be submitted in Nutrition and Metabolism
3.1 Introduction

Ramadan is the ninth month of the lunar year and is the fasting month of Muslims. During this month, Muslims all over the world abstain from eating, drinking and smoking from sunrise till sunset. Based on the lunar calendar, Ramadan occurs 11 days earlier every year and thus over time may occur in any of the four seasons. Therefore, depending on the season and the geographical location of the country, daytime fasting varies from 11- to 18-h, being longer in the summer and in the temperate regions (Azizi, 2010). During Ramadan, food (and water) is usually consumed in two meals, in the morning before sunrise and in the evening after sunset, shifting the pattern of caloric intake from daytime to the hours of darkness (Chaouachi et al., 2009). These changes in the timing of food intake as well as in the composition of diet can influence substrate availability and utilization, and a shift from carbohydrate to fat oxidation has been reported (Bouhlel et al., 2006; El Ati et al., 1995). In addition, acute diurnal dehydration has been observed which might influence the metabolic response (Leiper et al., 2003). The metabolic effect of Ramadan fasting (RF) on body weight is variable, though most studies have reported a decreased in body mass (Bouhlel et al., 2006; Sweileh et al., 1992; Ziaee et al., 2006), but not all (El Ati et al., 1995). Changes in blood lipid profile are also variable depending on the quantity and quality of the diet and weight changes (Azizi, 2010). In 2015, Muslims of Newfoundland fast approximately 18-hrs per day, the fasting season (summer) and location (temperate region) providing a unique opportunity to investigate the effect of a relatively long period of IF during Ramadan on body metabolism. Therefore, the aim of this study was to examine the effects of RF, if any, on substrate utilization, energy
expenditure, body composition and blood serum lipids and glucose. It was hypothesized that IF induced by the long fasting period during Ramadan will acutely alter the contribution of substrates to energy production and be magnified over time by the cumulative metabolic stress. It was expected that the contribution of lipid as a substrate to energy production will increase during the day as well as during the month. Along with substrate oxidation alteration, the blood serum level of total cholesterol (TC), triglycerides (TG), glucose, and insulin should reflect the change in mobilization, transport and oxidation of substrates.

3.2 Materials and methods

3.2.1 Participants

Nine healthy adult Muslim males – who are strict of Muslim faith – formed the Ramadan fasting group (FAST), and eight healthy adult men who do not fast during Ramadan formed the control group (CNT). Both groups were recruited from the local Muslim community and Memorial University (Newfoundland, Canada). Participants provided written informed consent in compliance with the declaration of Helsinki and Memorial University ethics committee regulations. Participants completed the Physical Activity Readiness Questionnaire (PAR-Q) to screen for any medical conditions including hypertension, cardiorespiratory disease, diabetes, musculoskeletal injuries, or a family history of these conditions. Individuals who did not pass the PAR-Q were excluded from the study. Screened participants attended an orientation session in which they were given information about equipment used in the study and the experimental design, in addition to
undertaking anthropometrics measurements [height and weight]. Participants were instructed to manually record their food intake and to wear a physical activity tracker in order to monitor their physical activity level. Anthropometric characteristics of the participants are reported in Table 1.

### 3.2.2 Study timeline

An orientation session was conducted 20 days before the onset of RF in which all participants were informed of the study procedures and received instructions regarding the completion of physical activity and diet log. Anthropometrics, vital signs (VS), blood samples, and metabolic rate (MR) were collected between 7:00-9:00 a.m. 10 days pre- and 10 days post-RF for all participants, and on the 10th (R1), 20th (R2), and 30th (R3) days of RF in the morning (between 7:00-9:00 a.m.) and the evening (between 6:00-8:00 p.m.) for the FAST. The study timeline can be seen in Figure 1.
Table 1 Anthropometric characteristics and vital signs variables of FAST (n=8) and CNT (n=8) subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>FAST</th>
<th></th>
<th></th>
<th>CNT</th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre-RF</td>
<td>Post-RF</td>
<td>Mean Δ</td>
<td>Pre-RF</td>
<td>Post-RF</td>
<td>Mean Δ</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>----</td>
<td>35.0±9.4</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Height (cm)</td>
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<td>----</td>
<td>----</td>
<td>178±8</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>BM (kg)</td>
<td>82.9±15.8</td>
<td>80.8±15.3</td>
<td>-2.1±1.1</td>
<td>86.8±16.5</td>
<td>87.2±16.6</td>
<td>0.4±2.2</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>25.7±10.8</td>
<td>24.3±10.6</td>
<td>-1.4±1.1</td>
<td>21.3±9.0</td>
<td>22.2±8.9</td>
<td>0.93±1.3</td>
</tr>
<tr>
<td>LM (kg)</td>
<td>57.3±7.4</td>
<td>56.5±7.2</td>
<td>-0.7±0.7*</td>
<td>65.5±9.2</td>
<td>65.0±9.6</td>
<td>-0.5±2.0</td>
</tr>
<tr>
<td>% FAT</td>
<td>29.6±8.4</td>
<td>28.8±8.4</td>
<td>-0.83±1.0</td>
<td>23.9±5.9</td>
<td>24.9±5.8</td>
<td>1.02±1.3</td>
</tr>
<tr>
<td>BMI (kg•m²)</td>
<td>26.5±5.0</td>
<td>25.9±4.9</td>
<td>-0.6±0.4</td>
<td>27.4±4.6</td>
<td>27.5±4.6</td>
<td>-0.1±0.7</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
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<td>-10±13</td>
<td>111±12</td>
<td>106±9</td>
<td>-6±14</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
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<td>-6±9</td>
<td>74±9</td>
<td>69±11</td>
<td>-5±14</td>
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<tr>
<td>HR (bpm)</td>
<td>68±5</td>
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<td>54±4</td>
<td>57±6</td>
<td>3±3*</td>
</tr>
<tr>
<td>RR (br•m⁻¹)</td>
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<td>17±2</td>
<td>-1±3</td>
<td>15±3</td>
<td>15±3</td>
<td>0±2</td>
</tr>
</tbody>
</table>

Values are Mean & standard deviation, * significantly different from pre-RF (p < 0.05), † significantly different from pre-RF (p < 0.01).

RF: Ramadan fasting, BM: body mass, FM: fat mass, LM: lean mass, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, RR: respiratory rate, bpm: beat per minute, br•m⁻¹: breath per minute
Figure 1 Experimental design. Throughout the study, food and physical activity logs were recorded for FAST and CNT groups.

3.2.3 Anthropometric variables

Body height (using stadiometer, ±0.1 cm – Perspective Enterprises, Portage, Michigan, USA) was measured throughout the study (all sessions) for all participants. Body mass was recorded with a weighing chair connected to a load cell (S-type, model LC1010-500; Omegadyne Inc, Sunbury, Ohio) interfaced with a computer. Body mass and height were used to calculate body mass index (BMI, kg m⁻²). Waist circumference was measured with an anthropometric fiberglass tape.

Body composition was assessed by hydrodensitometry technique for both groups 10 days pre-RF and 10 days post-RF, following the method of Behnke and Wilmore (1974) by measuring body mass and height prior to hydrostatic weighing (±0.1 cm, ±0.1 kg) (Behnke & Wilmore, 1974). Participant body mass while seated was collected using an S-type load cell (Megadyne Inc. Sunbury, OH) interfaced with a data acquisition system (Biopac Inc., Quebec, Canada) in two static positions: dry and body immersed with head out and once while completely submerged in water with head in. Prior to weighing, forced vital capacity (FVC) was recorded out-of-water in a seated position while participants were connected to a spirometer (Micro Medical Inc., Basingstoke, U.K.). Measurements were taken until three trials within 0.5 ml were recorded. The average of these trials was calculated and served as a reference during submerged mass trials. An average of three trials for each static mass measurement was taken from a plateau in the transducer signal. During submerged trials, participants were asked to perform a FVC, submerged themselves and wait for five seconds. Submerged mass was taken from a plateau in the transducer signal during submersion. Out-of-water FVC values were then compared with those prior to
submersion to confirm valid submerged mass measurement: if FVC during submerged trials was within 0.15 ml of the out-of-water reference the measure of submerged mass was considered valid. The procedures were run until three successful trials of submerged mass were obtained.

An average of the three successful measurements of submerged mass was taken. Body density was calculated from the formula of Brozak & associates (1963) and fat percentage according to the following predictive equations (Brozek, Grande, Anderson, & Keys, 1963; Siri, 1961).

\[ D_b = \frac{\text{Dry mass} - \text{sub mass}}{\text{water density} - (\text{residual volume} - 0.1)} \]  
\[ \text{Percentage fat} = \left[ \frac{4.95}{D_b} - 4.5 \right] \times 100 \]  
\[ \text{Percentage fat} = \left[ \frac{4.57}{D_b} - 4.142 \right] \times 100 \]

where \( D_b \) = body density

The scores were then averaged and compared with Brozek and Siri calculations.

3.2.4 Vital signs (VS)

At the beginning of each session, after obtaining body mass (BM), VS were collected. Body temperature (BT) was measured with an ear thermometer (Braun trademark, Kaz Europe Sàrl, Kronberg, Germany) to control for any deviation from normal values, blood pressure (systolic – SBP; diastolic – DBP) was recorded with a sphygmomanometer, and heart rate (HR) and respiratory rate (RR) were determined by count over 1-min timed on a stop watch.
3.2.5 Blood analyses

A blood sample of approximately 5 ml was drawn from the antecubital vein into vacutainers tubes to obtain serum and plasma for analysis. These samples were collected under standardized conditions in a supine position after an overnight fast 10 days pre-RF and 10 days post-RF, and on the 10th, 20th, 30th day of RF in the morning (post-prandial, between 7:00 and 9:00 a.m.), and in the evening (day fasted, between 6:00 and 8:00 p.m.) for FAST group.

After collecting blood, samples were allowed to coagulate and were then centrifuged at 2500g at 4°C for 15 min. Plasma and serum were stored in cryo-tubes at -20°C until further analysis. Blood parameters were determined through colorimetric method using commercially available assay kits; glucose (Cayman Chemical Company, Ann Arbor, MI, U.S.A), TG (Cayman Chemical Company, Ann Arbor, MI, U.S.A), and TC (Cell Biolabs, Inc., CA, U.S.A). Insulin was determined by an enzyme-linked immunosorbent assay (ELIZA) method (Life Technologies Corporation, Carlsbad, CA, USA). All assays were performed according to manufacturer instructions.

3.2.6 Energy balance

Throughout the entire experiment, participants were required to manually record their food intake over three days before each session. Food logs were then entered into a web-based program [TotalCoaching.com] in order to quantify energy intake. Daily physical activity was monitored by having participants wearing a physical activity tracker (Garmin International Inc. Kansas, USA), which approximates daily energy expenditure (EE), the number of steps and distance walked (Alsubheen et al. 2016). Scores extracted from the
physical activity tracker website (www.garminconnect.com) for the purpose of estimating EE of daily physical activity, step count, and distance covered were averaged and compared to the values determined by IC (see appendix A). Daily energy intake (kcal•day⁻¹), carbohydrate (g•day⁻¹), fat (g•day⁻¹), and protein (g•day⁻¹) values were determined by entering the food logs information into the Total Coaching website that follows the Canadian nutrient guidelines. Foods consumed were selected from a pre-existing list of foods with complete nutritional information. All food logs were reviewed by a trained nutritionist.

3.2.7 Estimation of dehydration

Due to the fact that water intake was not allowed during RF days, water loss was expected. This loss affects substrate metabolism (Leiper et al., 2003), so it is imperative to estimate this loss in order to accurately analyze the effect of RF on substrate oxidation. Dehydration was estimated through measuring the change in BM during RF day for FAST group. Body mass was recorded on the 10th, 20th, and 30th days of RF, between 7:00 and 9:00 a.m., and between 6:00 and 8:00 p.m.

3.2.8 Metabolic rate

Metabolic rate was measured through indirect calorimetry. Participants were required for all sessions to rest supine while MR was recorded under a canopy in a thermo-neutral environment (22-24ºC) with dimmed lights. Metabolic rate was measured 10 days pre-RF and 10 days post-RF for both FAST and CNT groups. Measurements were taken in the morning between 7:00 and 9:00 a.m., for a duration of 45-min and in an overnight (12-hrs) fasted state except for ad libitum water. Participants were also instructed to avoid physical
activity for 12-hrs before coming to the laboratory. To assess the potential effect of energy deficit and the altered substrate partitioning induced by RF, MR of the FAST group was recorded during two different time points on the 10th, 20th, 30th day of RF, in the morning (post-prandial, between 7:00 and 9:00 a.m.), and in the evening (day fasted, between 6:00 and 8:00 p.m.). It is important to mention here that only the measurements of pre- and post-RF sessions followed the basal metabolic rate guidelines.

3.2.8.1 Indirect calorimetry

An indirect calorimetry (IC) system (Sable Systems International, Las Vegas NV, USA) recorded MR. This system measures oxygen uptake (\(\dot{V}O_2\)) and carbon dioxide production (\(\dot{V}CO_2\)) simultaneously through a hood canopy. 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\(^{-1}\) during MR. 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 gases). In addition, propane gas calibration was performed to ensure accuracy of the reading at a low metabolic rate. 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).

### 3.2.8.1 Calculations

The fraction of gases from the IC system was corrected for temperature and barometric and water vapour pressures. The respiratory data were truncated by 10-min (5-min at each end) in order to nullify any metabolic rate fluctuation due to familiarization with the ventilated hood and the expected termination of data collection. Respirometry data (\(\dot{V}O_2\) and \(\dot{V}CO_2\)) were then integrated, normalized over time, and corrected for protein oxidation at a constant oxidation rate of 0.06 g•min\(^{-1}\), and were finally included in the calculation of substrate oxidation [carbohydrate (\(G_{DIS}\)) and fat (\(L_{DIS}\))]. The non-protein adjusted volumes were obtained by subtracting volume of oxygen and carbon dioxide pertaining to protein oxidation from the total volume of gases., and the substrate oxidation values were used to calculate energy production expressed in kcal•min\(^{-1}\), according to the formulas of (Simonson & DeFronzo, 1990):

\[
G_{DIS} = 4.57 \dot{V}CO_2 - 3.23 \dot{V}O_2 \quad \text{(Eq. 4)}
\]

\[
L_{DIS} = 1.69 \dot{V}O_2 - 1.69 \dot{V}CO_2 \quad \text{(Eq. 5)}
\]

\[
EP = 3.74 G_{DIS} + 9.46 L_{DIS} + 4.32 P_{DIS} \quad \text{(Eq. 6)}
\]

### 3.3 Statistical analyses

Statistical analyses were performed using SPSS, version 23 (SPSS Inc., Chicago, IL, USA). All values are reported as mean ± standard deviation, unless otherwise
specified, and an alpha level ($p$) of 0.05 was used to indicate statistical significance.

Descriptive statistics were planned to explore the data set (homogeneity, sphericity, and heteroscedasticity) to test normality assumptions. For pre- to post-RF comparisons, paired $t$-tests were run on anthropometrics, vital signs, and metabolic data (MR and substrate oxidation) for FAST and CNT groups. Paired $t$-tests were also performed to detect the difference in blood parameters between pre- and post-RF and between a.m. and p.m. sessions within the fasting day for FAST participants. To detect the changes induced by the daytime fasting, a 3 (R1, R2, R3) X 2 (a.m./p.m.) repeated measures ANOVA was performed on MR, substrate oxidation, blood parameters (glucose, insulin, TC, and TG), vital signs (HR, DBP, SBP, and RR), and anthropometrics for FAST only. Finally, to assess physical activity and energy intake, a 2 (FAST & CNT) X 5 (pre, R1, R2, R3, post) ANOVA with repeated measures was run on physical activity scores (kcal, step count, and distance), and food log outcomes (kcal, fat, protein, and carbohydrate contents). Significant interactions were followed by pairwise comparisons using a Bonferroni correction.

### 3.4 Results

#### 3.4.1 Pre- and post-RF outcomes

Anthropometric data in the FAST and CNT groups is given in Table 1. The results show that (a) BM was decreased in FAST while CNT stayed stable, (b) FM was decreasing in FAST while no change was detected in CNT; (c) %FAT, although non-significant, displayed an opposite pattern showing a decrease and an increase for FAST and CNT, respectively; (d) LM was significantly decreased in FAST with no change in CNT; (e)
BMI decreased in FAST while no change was observed in CNT.

Systolic and diastolic blood pressure, heart rate, body temperature, and respiratory rate were assessed through paired t-tests that revealed a significant increase in HR for CNT while FAST HR decreased by 2 bpm (non-significant). There was a trend toward a decrease in SBP in FAST ($p = 0.06$) with no changes in all other parameters. Substrate oxidation (glucose and lipids), RER, and energy production (EP) remained unchanged pre- and post-RF in both groups. FAST pre- and post-RF blood parameters were not significantly different for glucose, insulin, TC, and TG.

3.4.2 Within RF outcomes (FAST only)

A significant main effect of daytime fasting [a.m. vs. p.m.] as well as a significant main effect of month-time [R1 to R3] on BM were observed. Body mass loss from a.m. to p.m. was 1.4±1.4 kg of magnitude as a result of dehydration. Further, the magnitude of decrease between R1 and R2, R2 and R3 were 1.0±2.3 and 0.6±3.4 kg, respectively. For SBP, DBP, RR, and HR results showed no significant main effect of time [R1 to R3]. However, there was a significant main effect of daytime fasting [a.m. vs. p.m.] on RR showing lower values during p.m. sessions (15.9±2.6 br•m⁻¹) compared with am sessions (17.1±4.8 br•m⁻¹) as summarized in table 2.
<table>
<thead>
<tr>
<th>Variables</th>
<th>R1</th>
<th></th>
<th></th>
<th>R2</th>
<th></th>
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<tr>
<td></td>
<td>a.m.</td>
<td>p.m.</td>
<td>a.m.</td>
<td>p.m.</td>
<td>a.m.</td>
<td>p.m.</td>
<td>a.m.</td>
<td>p.m.</td>
<td>p.m.</td>
</tr>
<tr>
<td>BM (kg)</td>
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<td>82.3±15.4</td>
<td>81.0±15.3*</td>
<td>81.8±15.1</td>
<td>80.1±14.8*</td>
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<td>SBP (mmHg)</td>
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<td>63±3</td>
<td>62±4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR (br•m⁻¹)</td>
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<td>17±2</td>
<td>16±2</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean & standard deviation, * significantly different a.m. vs. p.m. (p < 0.05)
BM: body mass, R1: 10th day of RF, R2: 20th day of RF, R3: 30th day of RF, RF: Ramadan fasting, am: morning, pm: evening, SBP: systolic blood pressure, DBP: diastolic blood pressure, RR: respiratory rate, HR: heart rate, bpm: beat per minute, br•m⁻¹: breath per minute.
A significant effect of daytime fasting was observed on carbohydrates and fat oxidation during RF at R1, R2, & R3 (Figure 2). These changes in substrate oxidation occurred between a.m. and p.m. sessions and were mirrored by a decrease in RER values ($p = 0.001$; a.m. = 0.88±0.04, p.m. = 0.82±0.05). In contrary to the above outcomes, EP was not significantly different from a.m. to p.m. (a.m. = 1.3±0.4, p.m. = 1.2±0.5 kcal•min$^{-1}$). Mirroring the substrate oxidation outcomes, there was a significant main effect of daytime fasting [a.m. vs. p.m.] on glucose (a.m. = 5.5±2.5, p.m. = 4.5±1.2 mmol•L$^{-1}$); insulin (a.m. = 169.5±380.7, p.m. = 74.9±279.0 pmol•L$^{-1}$); TC (a.m. = 4.2±2.9, p.m. = 3.7±2.3 mmol•L$^{-1}$), and TG (a.m. = 1.6±3.4, p.m. = 0.68±1.8 mmol•L$^{-1}$) (Table 3).

3.4. 3 Energy balance from pre- to post-RF for both groups

Recall that for physical activity and food intake FAST and CNT were on same timeline, that is, data were collected for both groups on pre-, R1, R2, R3, and post-RF. Therefore, a two-way ANOVA (2 groups X 5 periods) with repeated measures was run on physical activity tracker scores and caloric intake. The statistical analysis revealed no significant difference between FAST and CNT or between periods (pre-, R1, R2, R3, post-RF) as shown in table 4.
Figure 2: Morning (a.m.) and evening (p.m.) carbohydrate (CHO) and fat oxidation (mg•min⁻¹) during RF.

Substrate oxidation was measured by indirect calorimetry (IC) and calculated using the equations of Simonson and DeFronzo (1990). *, significant time effect (p < 0.05).
Figure 3  Morning (a.m.) and evening (p.m.) changes in blood parameters (glucose, TC, and TG mmol•L⁻¹) within RF (R1 to R3).

*, significant time effect (p < 0.05).
Table 3 Changes in blood parameters of the FAST group during RF (n=9)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-RF</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Post-RF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a.m.</td>
<td>p.m.</td>
<td>a.m.</td>
<td>p.m.</td>
<td>a.m.</td>
</tr>
<tr>
<td>Glucose (mmol•L⁻¹)</td>
<td>4.4±0.6</td>
<td>5.3±1.1</td>
<td>4.7±0.6</td>
<td>5.3±1.3</td>
<td>4.3±0.3</td>
</tr>
<tr>
<td>Insulin (pmol•L⁻¹)</td>
<td>86±81</td>
<td>125±93</td>
<td>40±23*</td>
<td>239±296</td>
<td>135±249</td>
</tr>
<tr>
<td>TC (mmol•L⁻¹)</td>
<td>3.8±0.8</td>
<td>4.1±1.0</td>
<td>3.7±0.8*</td>
<td>4.2±1.1</td>
<td>3.9±0.8</td>
</tr>
<tr>
<td>TG (mmol•L⁻¹)</td>
<td>0.8±0.6</td>
<td>1.5±0.8</td>
<td>0.6±0.5*</td>
<td>2.1±1.9</td>
<td>0.9±0.9*</td>
</tr>
</tbody>
</table>

Values are Mean & standard deviation, * significantly different from a.m. (p < 0.05)
RF: Ramadan fasting, R1: 10th day of RF, R2: 20th day of RF, R3: 30th day of RF, am: morning, pm: evening, TC: total cholesterol, TG: triglycerides.
The contribution of carbohydrate, fat, and protein to the total energy intake did not change throughout the study in either group. However, it is worth noting that, in FAST, carbohydrates intake increased from pre- to R1 by 15% and then decreased from R1 to post-RF by 29%. The fat intake slightly decreased throughout the study compared to pre-RF values in both groups (FAST 8%; CNT 16%). For protein content of food, there was no variation in CNT while a slight increase by 9% occurred in FAST during RF.

Physical activity tracker (Vivofit) does not distinguish between physical activity and non-physical activity thermogenesis (NEAT). Vivofit merges the above-mentioned parameters in one category called “active calories”. Therefore, for the following statistical analysis active calories will include all physical activity intensities. Note that a study from our laboratory (Alsubheen et al. 2016) has reported a 29% EE under-estimation with Vivofit (Refer to the appendix A). In the current study, active calories \((p = 0.03)\), daily step count \((p = 0.03)\), and total distance \((p = 0.04)\) were all significantly different between groups.

The CNT actively spent \(286\pm265 \text{ kcal}\cdot\text{day}^{-1}\) compared to \(181\pm121 \text{ kcal}\cdot\text{day}^{-1}\) for FAST. Accordingly, daily step count and total distance covered were also higher in CNT compared to FAST. Indeed, CNT daily cumulated \(9685\pm7126\) step and covered a total distance of \(7.4\pm5.8 \text{ km}\cdot\text{day}^{-1}\) while FAST amounted a daily step count of \(6850\pm2079\) step and summed a total distance of \(5.1\pm1.8 \text{ km}\cdot\text{day}^{-1}\).

### 3.5 Discussion

The aim of this study was to examine the effect of the extended daily fasting period \((18.0\pm0.3 \text{ hrs})\) during the holy month of Ramadan on substrate oxidation, EP, serum lipids
and glucose, and anthropometrics (body mass and body composition) in healthy men. The major outcome of the study revealed that the daytime fasting significantly altered fuel oxidation regardless of time (i.e., the fasting month of Ramadan). This acute response (transient) was of a magnitude large enough to have an impact on blood markers, and body mass and composition.

3.5.1 The effect of daytime fasting on FAST group

There was a main effect of the daytime fasting [a.m. vs. p.m.] on substrate oxidation; fat oxidation increased by 62% or by 2.2 kcal•min⁻¹ and carbohydrate oxidation decreased by 55% for an equivalent of 3.1 kcal•min⁻¹. However, EP was not dramatically affected in the evening sessions during RF in contrast to El Ati et al. (1995) who reported significant decreases in EP from 11:00 to 17:00 during the daytime fasting. However, as reviewed by Maughan et al. (2010), EP is not considerably affected by fasting and the energy demands are met by the increase in the rate of fat oxidation to spare the limited availability of carbohydrate during the postprandial phase (Maughan et al., 2010). In fact, the metabolic stress induced by RF alters substrate storage and usage (Azizi, 2002). Among the many studies investigating RF, only one study examined the change in substrate oxidation in resting state from morning to evening (El Ati et al., 1995). The participants of that study were all healthy women who had shifted from carbohydrate to fat oxidation as did our men participants.

In the current study, the shift in fuels oxidation from a.m. to p.m. was mirrored by the changes in blood parameters (glucose, insulin, TC, and TG). Although blood glucose level significantly declined by 18%, the values remained within the normal range (Table 3). Our
data support previous reports in which blood glucose concentration is reduced during evening sessions and the new glucose level is maintained by an increased rate of fat oxidation due to the decrease in carbohydrate oxidation in the fasted state (El Ati et al., 1995). The decreased levels of both TC by 12% and TG by 58% in the evening sessions provide evidence of increased fat oxidation. The formation of new glucose from fat oxidation can be illustrated through gluconeogenesis mechanism. During fasting, the level of carbohydrate intake decreases, as a result, the rate of TG mobilization to FFA and glycerol increases. Thus, FFA are metabolized to provide direct source of energy in the skeletal muscles and the glycerol is transferred through circulation to the liver to serve a role as a gluconeogenic precursor (Brooks et al., 2005). Moreover, Fakhrzadeh et al. (2003) measured the level of TC during RF and concluded that the reduction of TC levels in blood indicates increased fat oxidation (Fakhrzadeh, Larijani, Sanjari, Baradar-Jalili, & Amini, 2003). These integrated metabolic responses to fasting are regulated by changes in the hormonal environment including a change in the plasma insulin concentration as our results showed. The reduced insulin levels by 56% observed during evening sessions can promote lipolysis as reported by El Ati et al. (1995). In addition, the significant lower RER values provided indirect evidence of predominant lipolysis process in the evening sessions. In contrast to the current study, Sweileh et al. (1992) assessed RER in men by collecting resting oxygen uptake for 5-min and reported non-significant RER values between morning and evening sessions during the first and the fourth weeks of RF (Sweileh et al., 1992). This non-significant result likely stems from a too short (5-min) data collection period to reach metabolic steady-state. When MR was recorded on a longer
period of time, El Ati et al. (1995) reported a significant decline in RER similar to our results and concluded that fat oxidation was dominant in the evening sessions during RF (El Ati et al., 1995). Vital signs including SBP, DBP, RR, and HR were monitored during the daytime fasting. Our results showed no significant changes in these parameters. However, a significant decrease in HR of 2.7 bpm in the morning and 14 bpm in the evening ($p = 0.048$) was observed by Sweileh et al. (1992). Finally, the significant decrease in body mass during the daytime fasting might be secondary to dehydration since Muslim fasting requires abstinence from water during the day (Leiper et al., 2003).

### 3.5.2 The overall effect of RF on FAST compared with CNT

It is important to mention that all participants remained healthy throughout RF and did not complain of any disorders. The major significant result is the reduction in the BM, FM, LM, as well as BMI values post-RF as determined by hydrostatic data in FAST compared with CNT. This reduction could be explained by the fact that fat stores are mobilized from adipose tissue and oxidized to meet body energy demands during RF as supported by lower RER values. This could partially be due to the small reduction in energy intake of about 347 kcal•day$^{-1}$ pre- to post-RF. These results agree with previous findings of hydrostatic weighing measurement that reported significant decreases in BM (1.92 kg, $p = 0.000$) and %FAT (2.3%, $p = 0.02$); an outcomes that was attributed to a deficit in caloric
## Table 4 Energy balance of FAST (n=8) and CNT (n=8) groups over the course of the study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-RF</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Post-RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (kcal/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAST</td>
<td>2162±802</td>
<td>2291±765</td>
<td>2146±682</td>
<td>2030±893</td>
<td>1815±549</td>
</tr>
<tr>
<td>CNT</td>
<td>2184±516</td>
<td>2011±683</td>
<td>2129±746</td>
<td>1945±727</td>
<td>2013±783</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAST</td>
<td>60±27</td>
<td>52±25</td>
<td>53±28</td>
<td>57±24</td>
<td>55±29</td>
</tr>
<tr>
<td>CNT</td>
<td>80±24</td>
<td>63±28</td>
<td>73±35</td>
<td>65±25</td>
<td>67±24</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAST</td>
<td>89±23</td>
<td>98±28</td>
<td>98±48</td>
<td>98±57</td>
<td>72±16</td>
</tr>
<tr>
<td>CNT</td>
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<td>106±37</td>
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<td>Carbohydrates (g/d)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAST</td>
<td>319±160</td>
<td>376±168</td>
<td>328±104</td>
<td>286±130</td>
<td>266±89</td>
</tr>
<tr>
<td>CNT</td>
<td>261±99</td>
<td>259±114</td>
<td>250±125</td>
<td>232±131</td>
<td>243±139</td>
</tr>
<tr>
<td>Active EE (kcal/d)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAST</td>
<td>171±63</td>
<td>166±49</td>
<td>172±57</td>
<td>181±60</td>
<td>175±49</td>
</tr>
<tr>
<td>CNT (n=7)</td>
<td>254±96</td>
<td>290±131</td>
<td>286±99</td>
<td>289±95</td>
<td>330±118</td>
</tr>
<tr>
<td>Steps (#/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAST</td>
<td>6368±1556</td>
<td>6252±1954</td>
<td>6498±1832</td>
<td>6714±1627</td>
<td>6483±1383</td>
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<tr>
<td>CNT (n=7)</td>
<td>9377±2529</td>
<td>10116±3539</td>
<td>9679±2980</td>
<td>9485±2204</td>
<td>10655±3888</td>
</tr>
<tr>
<td>Distance (km/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAST</td>
<td>4.8±1.1</td>
<td>4.8±1.3</td>
<td>4.9±1.5</td>
<td>5.1±1.3</td>
<td>4.9±1.2</td>
</tr>
<tr>
<td>CNT (n=7)</td>
<td>7.1±2.0</td>
<td>7.7±2.9</td>
<td>7.4±2.4</td>
<td>7.3±1.8</td>
<td>8.2±3.0</td>
</tr>
</tbody>
</table>

Values are Mean & standard deviation, RF: Ramadan fasting, R1: 10th day of RF, R2: 20th day of RF, R3: 30th day of RF, d: day.
intake and as such the utilization of body storage of fat increases (Sweileh et al., 1992). The significant reduction in body lean mass was supported by other studies (McNeil et al., 2014; Norouzy et al., 2013). Short periods of fasting will result in some loss in body lean mass because the rate of protein breakdown exceed the synthetic rate during the post-absorptive phase (Maughan et al., 2010). These findings are important because RF is an integral part of Muslim religion and changes in body composition can be additive and may have various implications on energy requirements over the long term (Norouzy et al., 2013). However, other studies showed contradictory results as reported in table 5. The discrepancy between studies might be due to the validity and reliability of the techniques, seasonal differences across different years leading to a daily fasting time that varies between 10- and 18-h, demographic location, total caloric intake, type of foods consumed, and amount of physical activity (Azizi, 2010; Maughan, Bartagi, Dvorak, & Zerguini, 2008).

Our study demonstrated no changes in EP, substrate oxidation, and RER values post-RF in both groups, similar to other studies (El Ati et al., 1995; Husain et al., 1987; McNeil et al., 2014). In addition, we observed no changes in vital signs in agreement with Fakhrzadeh et al. (2003) but in disagreement with Unalacak et al. (2011) and Rahman et al. (2004) who reported significant decrease in BP, and with the study of Husain et al. (1987) in which a reduction in HR was observed and explained by an increase in religious activity that altered mental state and reduced sympathetic activity (Fakhrzadeh et al., 2003; Husain et al., 1987; Rahman et al., 2004; Unalacak, Kara, Baltaci, Erdem, & Bucaktepe, 2011). Interestingly, blood parameters (glucose, insulin, TC, and TG) were not changed post-RF.
This was expected, as RF is not a total starvation but a one month of change in eating schedule (Khan & Khattak, 2002). These findings were similar to those of many studies (El Ati et al., 1995; Fedail et al., 1982; Khan & Khattak, 2002; Maislos et al., 1993; McNeil et al., 2014; Unalacak et al., 2011). However, a significant decrease in glucose levels \( (p < 0.0001, p < 0.05) \) was reported by Fakhrzadah et al. (2003) and Rahman et al. (2004) and a significant decrease in TG levels was reported in two other studies (Fakhrzadeh et al., 2003; Unalacak et al., 2011). This diversity in the results might depend on the quality and quantity of food consumed during RF and to the degree of body mass changes (Azizi, 2010). Additionally, RF can occur during all seasons, the time of fasting thus varying between 10- to 18-hrs, in addition to regional and cultural difference between countries in terms of physical activity performed during RF; these could also reasonably explain inter-study differences in results (Maughan et al., 2008).

### 3.5. 3 Energy balance

The daily energy intake within RF although statistically insignificant has a biological relevance. In fact, there was a decrease of 261 kcal\( \text{day}^{-1} \) from R1 to R3 compared with that of pre- and post- RF, which decreased by 347 kcal\( \text{day}^{-1} \) in the FAST as well as by 66 kcal\( \text{day}^{-1} \) and 171 kcal\( \text{day}^{-1} \) for R1 to R3 and pre- to post-RF in CNT. Similar findings were reported by El Ati et al. (1995) and Norouzy et al. (2013).
Table 5 Comparison of anthropometric findings of the current study with other published studies

<table>
<thead>
<tr>
<th>Source</th>
<th>Parameter</th>
<th>Results</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>BM</td>
<td>-2.1±1.1</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>FM</td>
<td>-1.4±1.1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>-0.7±0.7</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>% Fat</td>
<td>-0.83±1.0</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.6±0.4</td>
<td>0.003</td>
</tr>
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<td>BM</td>
<td>-1.9 kg</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>0.4 kg</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>% Fat</td>
<td>-3.3 kg</td>
<td>0.02</td>
</tr>
<tr>
<td>El Ati et al. (1995)</td>
<td>BM</td>
<td>-0.7 kg</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>FM</td>
<td>-0.6 kg</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>LM</td>
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<td>NS</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>No change</td>
<td>NS</td>
</tr>
<tr>
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<td>FM</td>
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</tr>
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<td>NS</td>
</tr>
<tr>
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<td>0.002</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.4 kg/m2</td>
<td>0.004</td>
</tr>
<tr>
<td>Akanji et al. (2000)</td>
<td>BM</td>
<td>-0.2 kg</td>
<td>NS</td>
</tr>
<tr>
<td>Norouzy et al. (2013)</td>
<td>BM</td>
<td>-1.7 kg</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>FM</td>
<td>-0.9 kg</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>-1.2 kg</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>% Fat</td>
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<td>0.029</td>
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<td></td>
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<td>Husain et al. (1987)</td>
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<td>-0.3</td>
<td>NS</td>
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<td>Skinfold thickness</td>
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<td>Muscle girth</td>
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<td>NS</td>
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<td>NS</td>
</tr>
<tr>
<td>Fedail et al. (1982)</td>
<td>BM</td>
<td>-1.8 kg</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Khan &amp; Khattak (2002)</td>
<td>BM</td>
<td>-3.2 kg</td>
<td>-</td>
</tr>
<tr>
<td>Hajek et al. (2012)</td>
<td>BM</td>
<td>-0.84 kg</td>
<td>&lt;0.0001</td>
</tr>
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<td>Rahman et al. (2004)</td>
<td>BM</td>
<td>-1.98 kg</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.76 kg/cm$^2$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ziaee et al. (2006)</td>
<td>BM</td>
<td>-1.2 kg</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.6</td>
<td>0.136</td>
</tr>
</tbody>
</table>

BM: body mass, FM: fat mass, LM: lean mass, BMI: body mass index.
These authors concluded that such findings negate the common belief that Muslims tend to overcompensate in terms of food intake during RF (El Ati et al., 1995; Norouzy et al., 2013). However, our observation contradicts the results of some previous studies that showed decreased total caloric intake (Fakhrzadeh et al., 2003; Husain et al., 1987; Khan & Khattak, 2002; Sweileh et al., 1992). Although there were insignificant changes in food composition, there was a reduction in fat and an increase in protein consumption within RF in FAST. El Ati et al. (1995) reported a relative increase in the fat and protein contents, with a corresponding decrease in carbohydrate as the total energy intake was unchanged. However, some other studies reported significant decreases in fat and protein consumption ($p = 0.04$) (Sweileh et al., 1992), a decrease in protein intake ($p = 0.032$) (Norouzy et al., 2013), and finally, a decrease in fat intake ($p = 0.01$) (Rahman et al., 2004). Certainly, these variations in total caloric intake and food composition might be explained by the food habits in different Islamic countries (El Ati et al., 1995). Regional and cultural differences in the observation of RF should also be taken into consideration (Maughan et al., 2008).

Energy expenditure (EE) recorded through the physical activity tracker revealed significant differences between groups within RF. CNT was more active in terms of total energy expenditure, steps, and distance covered per day as shown in table 4. However, no differences in the level of physical activity were recorded in FAST. Similarly, McNeil et al. 2014 reported insignificant differences in physical activity EE measured through accelerometer during RF in healthy men. The authors suggested that the absence of difference in EE measurements within RF is in line with the lack of change in body mass.
and body composition (McNeil et al., 2014). Furthermore, Leibel et al. (1995) observed that a 10% variation in body mass is associated with little change in EE (Leibel, Rosenbaum, & Hirsch, 1995). In the present study, there were no changes in EP and EE, thus the reduction in BM and FM post-RF might be attributed to the metabolic stress resulting from fasting. As a consequence, fuel oxidation shifted from carbohydrate to fat throughout the month.

### 3.6 Methodological consideration

The current study has some methodological considerations that need to be addressed. First, the present findings are limited to a small sample size of normal-weight men living in Newfoundland, Canada, which limits generalizability to other populations (e.g. residents of other countries). Furthermore, this study represents a healthy population and it is not representative of individuals with chronic disease or metabolic disorders. Second, the body composition was evaluated only pre- and immediately post-RF; thus, it is not known whether changes persisted after Ramadan and for how long. Lastly, similar to other published and ongoing studies, food logs completed by the participants are probably underestimated and this can explain the insignificant results of energy intake.

### 3.7 Conclusion

Collectively, our results demonstrated that the intermittent fasting induced by the strict Ramadan regimen acutely altered the contribution of substrates to energy production within RF that partially confirming our initial hypothesis. Fat oxidation assumed a greater role in the evening after day-time fasting as indicated by lower RER values. These
alterations are associated with changes in metabolic profile markers that reflect the mobilization, transport and oxidation of fat compared with carbohydrates oxidation. Moreover, increased fat oxidation is also associated with the decrease in body mass and fat mass post-RF since there were small energy deficit pre- and post-RF. In general, the insignificant changes in energy production, substrate oxidation, and blood biomarkers post-RF do not support our hypothesis, that is, RF induces no chronic metabolic response and that our bodies are very conservative in adjusting metabolic response to a certain extent (metabolic flexibility). Further, our results indicate that human body can quickly adjust to maintain biological responses within physiological range after removal of metabolic stress. Future studies would be needed to support the changes in the daytime fasting of Ramadan.
3.9 Acknowledgments

The work was supported by an internal grant from the School of Human Kinetics and Recreation, Memorial University, St. John’s, NL, Canada. The authors would like to thank Mr. David Gill, the owner of TotalCoaching.com, for providing access to his platform and technical support. Finally, we would like to thank the participants for their time, commitment and patience.

3.10 Declaration of interest

The authors report no declarations of interest.
Chapter IV
Overall Summary of Study
It is well established that the progression of many chronic diseases can be delayed or prevented through lifestyle modifications (Brooks et al., 2005) such as IF that is considered to have a positive effect on human health (Unalacak et al., 2011). Ramadan is an example of IF and is the holiest fasting month in the Islamic calendar. Muslims from all over the world abstain from eating, drinking and smoking from dawn until sunset (Chaouachi et al., 2009). The duration of fasting varies from 11- to 18-hrs per day depending on the season and the geographical location (Azizi, 2010). These differences in RF timing can affect the metabolic state and anthropometric measurements of Ramadan observers (Unalacak et al., 2011). The potential effects of RF on metabolic and biochemical parameters, as well as anthropometric measurements, have been investigated by many studies (El Ati et al., 1995; Khan & Khattak, 2002; Maislos et al., 1993; McNeil et al., 2014; Unalacak et al., 2011) with varied results depending on the quantity and quality of food, total fasting hours, and level of physical activity (Azizi, 2010; Maughan et al., 2008).

Few studies investigated the effect of RF on resting energy production and reported no changes in EP pre- to post-RF (El Ati et al., 1995; McNeil et al., 2014). Only one study investigated the change in substrate oxidation in resting state within the fasting day of Ramadan in healthy women and reported a shift from carbohydrate to fat oxidation (El Ati et al., 1995). The current study examined the effect of RF on body composition, blood serum lipids and glucose, as well as EP and fuel oxidation in healthy men. Acute and chronic alterations of fuel oxidation were investigated. It was hypothesized that RF will acutely shift fuel oxidation from carbohydrate to fat and this will be magnified over time.
by the cumulative metabolic stress of the fasting month. This shift will be mirrored by changes in the blood serum level of TC, TG, glucose, and insulin.

The present study consisted of nine healthy Muslim men who formed the FAST group and eight healthy men who formed the CNT group. Participants were assessed 10 days pre- and 10 days post-RF. FAST were additionally assessed at the 10th, 20th, and 30th days in the morning and in the evening during RF. After applying proper statistical plan, results showed no statistical differences pre- vs. post-RF for any of the dependent variables in both groups, except for body composition in FAST; there was a significant reduction of BM, LM, BMI and FM. During RF, statistical analyses revealed significant main effects of daytime fasting [a.m. vs. p.m.] of RF on carbohydrate oxidation, fat oxidation, and RER. In addition, there was a significant main effect of daytime fasting on glucose, insulin, TC, and TG. Although insignificant, energy intake reduced in FAST group. However, energy expenditure was not significantly different throughout RF in both groups.

The results of the present study partially supported our hypothesis that RF acutely shifted substrate oxidation from carbohydrates to fat to meet the energy demand. This shift was supported by the reduction in blood TC and TG concentrations in the evening sessions. Blood glucose level although significantly decreased, it was maintained within normal range due to gluconeogenesis mechanisms, the formation of new glucose from the oxidation of fat (TG) stores that release glycerol and fatty acids. Fatty acids are delivered by the circulation to be oxidized within the skeletal muscle while glycerol is used by the liver for the purpose of making new glucose. The integrated hormonal response represented by the decrease in insulin level promoted fat oxidation. Moreover, the
decrease in body mass and fat mass post-RF can be attributed to the increase in fat oxidation and to the small deficit in the energy intake.

In general, the insignificant changes in EP, substrate oxidation, and blood biomarkers post-RF do not support our hypothesis, that is, RF induces no chronic metabolic response and that our bodies are very conservative in adjusting metabolic response. Further, our results indicate that the human body can quickly adjust to maintain biological responses within physiological range after removal of metabolic stress. Future studies would be needed to support our findings and to further explain the changes in the metabolic response within daytime fasting of Ramadan. Furthermore, future studies should find more accurate ways for better food intake estimation. Finally, the specific research question may address the effect of diet composition on energy expenditure.
References


Appendix A: Monitoring physical activity using Vivofit activity tracker

Physical activity has great impact on human metabolism and health. Therefore, accurate measuring of physical activity level is important to estimate the body’s total energy expenditure. One method of tracking physical activity involves the use of accurate and objective activity trackers. Throughout the entire current experiment, daily physical activity was monitored using Vivofit activity tracker (Garmin international Inc. Kansas, USA). Participants were required to wear the activity tracker on their wrist over the course of the study and the collected data were synced every 10 days. The use of the Vivofit activity trackers allowed more detailed activity information to be collected such as energy expenditure and step count. However, it is important to ensure that the information provided is both reliable and accurate. Therefore, a study was conducted in our laboratory to assess the accuracy of these activity trackers as illustrated in the following document (Alsubheen et al., 2016).
Accuracy of the vivofit activity tracker

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Keywords: physical activity, energy expenditure, step count, treadmill walking test.
Abstract

The purpose of this study was to examine the accuracy of the Vivofit activity tracker in assessing energy expenditure and step count. Thirteen participants wore the Vivofit activity tracker for five days. Participants were required to independently perform one hour of self-selected activity each day of the study. On day four, participants came to the lab to undergo BMR and a treadmill-walking task. On day five, participants completed one hour of office-type activities. BMR values estimated by the Vivofit were not significantly different from the values measured through indirect calorimetry. The Vivofit significantly underestimated EE for treadmill walking, but responded to the differences in the inclination. Vivofit underestimated step count for level walking but provided an accurate estimate for incline walking. There was a strong correlation between EE and the exercise intensity. The Vivofit activity tracker is on par with similar devices and can be used to track physical activity.

Introduction

Physical activity is an important component to maintaining a healthy lifestyle. Approximately 31% of adults above the age of 15 are insufficiently active, leading to increases in obesity, chronic illness, and global mortality (Organization, 2009; Organization, 2016). The Canadian Society for Exercise Physiology (CSEP) recommends adults obtain at least 150 minutes of moderate to vigorous intensity physical activity per week (American Heart Association, 2015; Tremblay et al., 2011). Accurately measuring one’s level of physical activity can be important for many individuals who are trying to
maintain a healthy lifestyle to aid in decreasing the risk of chronic disease and obesity (Fruin & Rankin, 2004). One method of tracking physical activity involves the use of accurate and objective activity trackers.

In recent years, various activity trackers, using accelerometer-based technology, were released into the market. These activity trackers are preferred over basic pedometers (Mekky, 2014) – which were commonly used before accelerometer-based technology was widely available to the general public – as pedometers are often inaccurate (Schneider, Crouter, Lukajic, & Bassett, 2003). Pedometers generally measure movement in one direction (up and down) whereas accelerometers measure acceleration across multiple axes. The use of accelerometer-based activity trackers allows more detailed activity information to be collected such as energy expenditure and step count. However, it is important to ensure that the information provided is both reliable and accurate.

Previous work has explored the output from accelerometer-based trackers using a walking protocol. During flat walking the accelerometers tended to overestimate energy expenditure, but underestimate energy expenditure during inclined walking (Fruin & Rankin, 2004). Energy expenditure calculations are unique to each activity tracker and while many companies choose to keep their formulas secret (Mackinlay, 2013), the validity of published equations indicates that energy expenditure is overestimated during resting and walking, and underestimated during sports (such as racquetball and basketball) and running, when compared to indirect calorimetry (Crouter, Churilla, &
When encouraging people to be active it is important for monitoring devices to quantify their activity level accurately. Due to differences in formulas between companies, the accuracy of different trackers requires further investigation to determine which devices provide the most reliable results.

In addition to energy expenditure, many activity trackers provide other information such as daily step counts. As many activity trackers are worn on the wrist, an overestimation of actual steps taken may occur. Significant amounts of vigorous hand movement (such as brushing teeth or doing dishes) can be mistaken for steps. The level of accuracy between activity trackers has a great deal of variability (Stackpool, 2013). Despite this, activity trackers have been shown to provide useful data about energy expenditure at rest (Fruin & Rankin, 2004) and to monitor energy expenditure during activity (Noah, Spierer, Gu, & Bronner, 2013).

The Garmin Vivofit is one of the newer activity trackers on the market and is able to track steps, distance, calories, and sleep activity. Additionally, it can be connected to a heart rate sensor to provide more accurate workout session data. The device is water resistant, able to sync wirelessly, and is wrist worn (like a watch). It also displays an inactivity bar that becomes red when the user is inactive for more than an hour, serving as a visual reminder to stay active. Activity files are automatically created for more than 10 minutes of continuous walking/running when the heart rate sensor is worn (Rainmaker,
The data recorded by the Garmin Vivofit is stored in the device for up to three weeks and can be synced with the Garmin Connect website and viewed at any time to provide progress reports based on individual activity. To the best of the authors’ knowledge, there are currently no published studies examining the accuracy of the Garmin Vivofit. While many activity trackers have been validated (Dannecker et al., 2011; Noah et al., 2013; Schneider et al., 2003; Stackpool, 2013), the Vivofit has not yet undergone the same scrutiny.

Therefore, the objective of the present study was to assess the validity and reliability of the Vivofit activity tracker by comparing its values of energy expenditure and step count to the gold standard methods of indirect calorimetry and kinematics technique (video camera recording). Due to the variability of accuracy levels of previously tested activity trackers, there were no prior hypotheses regarding the outcome.
Methodology

Participants

Thirteen active healthy adult participants (5 women and 8 men) were recruited from Memorial University and the local community (Newfoundland, Canada). Participants read and signed the written informed consent in compliance with the declaration of Helsinki and Memorial University ethics committee regulations. Participants completed the Physical Activity Readiness Questionnaire (PAR-Q) to screen for any medical conditions including hypertension, cardiorespiratory disease, diabetes, musculoskeletal injuries or a family history of any of the above conditions. Individuals who did not pass the PAR-Q were excluded from the study. Anthropometric characteristics of the participants are reported in table 1.

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Insert table 1 about here

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Study timeline

Data collection consisted of three sessions over five days. On day one, participants were informed of the study procedures and received instructions regarding the completion of physical activity and diet logs. On day four, participants completed a basal metabolic rate (BMR) determination and a treadmill-walking task (TWT). On day five, participants completed an office task performance (OTP). The study timeline can be seen in figure 1.

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Insert figure 1 about here

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Procedures:

Session 1: After signing the consent form, the participant’s gender, age, height, and weight were recorded. In addition, participant walked over a 50-m distance to determine personalized step count as recommended by the manufacturer. All information was entered into the Garmin Connect website (https://connect.garmin.com) and wirelessly synced with the Vivofit activity tracker. Next, participants were instructed how to wear the Vivofit on the wrist, how to put it in and out of sleep mode, and how to wear and connect the heart rate sensor. Participants were instructed to wear the Vivofit for the duration of the study, and the heart rate sensor during both the testing sessions and self-selected exercise sessions. For the duration of the study, participants recorded physical activity and food intake on the web-based survey (www.totalcoaching.com). Participants were instructed to continue with their usual daily activities but with the addition of one hour of self-selected exercise each day while wearing the heart rate sensor. Participants were also requested to upload their physical activity (self-selected exercise) and food intake on the Total Coaching website each day.

Session 2: For at least 12-hours prior to the morning laboratory session, participants were instructed not to consume any food or energy-containing beverages, except for water ad libitum, and to avoid physical activity. Participants underwent BMR and TWT to assess
energy expenditure through indirect calorimetry. BMR measurements lasted 45-minutes, starting at 07:00 and were recorded in a supine position with the participant’s head supported by a single pillow in a thermo-neutral environment (22-24°C), with dimmed lighting. Participants were instructed to lie motionless but awake and not to talk. Upon completion of the BMR, participants then completed the TWT that consisted of three 10-minutes walking conditions at a self-selected pace (ranging from 4.0 to 7.2 km•hr\(^{-1}\)), with inclines of 0%, 5%, and 10% (Quinton fitness equipment, Bothell, USA). The walking conditions were interspersed with 10-minutes of rest to afford a clear demarcation between walking conditions so that the three EE periods from the Vivofit could be accurately identified. During the TWT a video camera (Sony-HDR-FX1 12X HD, Mini DV Camcorder) was placed 1.5-m away from the treadmill in 90° coordinates for yaw, pitch, and row to record stride frequency (SF) at 30 Hz.

Session 3: The following day participants returned to the laboratory at midday to complete 1-hour of office task performance (OTP) including computer work, reading articles, and writing, during which metabolic rate was recorded. For this measurement, participants were instructed to fast for 4-hours and to avoid vigorous exercise 24-hours prior to testing.

**Physical activity and food intake**

Throughout the study, participants uploaded their physical activity and food intake to the Total Coaching website for the purposes of monitoring physical activity and energy intake. Total Coaching is personal training software for health and fitness management.
using training and nutrition logs. Participants navigated the website and recorded activity by selecting exercise type, time, and intensity. Foods consumed were selected from a pre-existing list of foods with complete nutritional information. Nutritional information for items not on the food list could be manually entered.

**Apparatus**

**Activity tracker**

The Vivofit is a lightweight activity tracker that contains a micro-electromechanical triaxial accelerometer and uses an algorithmic equation to estimate energy expenditure. The Vivofit is worn around the wrist and has one screen with multiple displays; step count (total daily steps), goal (daily step goal), distance (kilometers or miles), calories (daily calories burned), time (12-hour or 24-hour format) and date (month and day). Heart rate can be displayed when the strap is worn around the chest. The strap contains a built-in sensor and transmitter that wirelessly transfers heart rate data (refer to Garmin user manual for further technical specifications). Additionally, the Vivofit can be placed in sleep mode to track sleep time, amount of restful sleep, and movement during sleep. The Vivofit data can be uploaded to a personal computer via a wireless USB ANT StickTM and viewed on the software created by the manufacturer (www.GarminConnect.com/vivofit).

**Indirect calorimetry**

An indirect calorimetric (IC) system (Sable Systems International, Las Vegas NV, USA) collected oxygen uptake (\(\dot{V}O_2\)) and carbon dioxide production (\(\dot{V}CO_2\)) simultaneously through a Hans Rudolph two-way non-rebreathing valve during the TWT and OTP, and
through a canopy during the BMR. 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 during BMR and OTP, and of 200 L•min⁻¹ during TWT. 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 gases). In addition, propane gas calibration was performed to ensure accuracy of the reading at a low metabolic rate. Water vapor pressure was zeroed after drying by passing through a column of magnesium percolate and the sub-sampler pump was calibrated using a flow meter (Gilmont Rotameter).

Data analysis and reduction

The fraction of gases from IC was corrected for temperature, and barometric and water vapor pressures. For BMR and OTP, respirometry data were truncated by 10-minutes (5-minutes at each end) in order to nullify any metabolic rate fluctuation due to familiarization with the ventilated hood or the mask and the expected termination of data collection. For TWT, respirometry data was reduced after reaching steady-state for each walking condition. Respirometry data was then integrated, normalized over time, and used for the calculation of EE. Energy expenditure was calculated from the VO₂ and VO₂C.
values using the Weir equation (Weir, 1949) and expressed in kcal•min⁻¹. The calculated values from IC were multiplied by 1440 (minutes per day) to be compared with the daily Vivofit BMR scores. Energy expenditure during TWT was recorded over a 10-minute period for each condition to match EE (active calories) from the Vivofit. For kinematics analysis (video), SF was calculated as the number of right foot contacts per minute, and multiplied by 2 for each condition. The outcomes were compared to the step count extracted from the Vivofit. Physical activity logs (exercise day and type) from the Total Coaching website were compared to the Vivofit physical activity logs. To determine the exercise intensity, maximal HR was calculated using the following equation (Tanaka, Monahan, & Seals, 2001):

\[
(1) \quad 208 - (0.7 \times \text{age})
\]

where 208 is the intercept that represents a constant HR, and 0.7 represents the slope. Next, the averaged exercise HR from the Vivofit was divided by the predicted maximal HR and multiplied by 100 to determine exercise intensity. Based on calculated exercise intensities and according to the American College of Sport Medicine instructions (Pollock et al., 1998), physical activities performed by the participants were classified into six categories (very light, light, moderate, hard, very hard, and maximal) as summarized in table 2.

Statistical analysis

Statistical analyses were performed using SPSS, version 23 (SPSS Inc., Chicago, IL, USA). All values are reported as mean ± standard deviation, unless otherwise specified,
and statistical significance was set at $\alpha = 0.05$. Descriptive statistics were used to explore the data set (homogeneity, sphericity, and heteroscedasticity) to test normality assumptions. First, paired samples t-tests were used to detect EE differences in BMR determined by IC and the Vivofit. A 2 (method) by 3 (condition) repeated measures ANOVA was used to compare the video camera SF with the Vivofit step count, and to compare the EE obtained via IC with the estimated score from Vivofit. Significant interactions were followed up with pairwise comparisons using a Bonferroni correction. Finally, simple linear regressions were performed to determine the coefficient of determination between EE and exercise intensity from IC and Vivofit, respectively.

Results

Characteristics of the participants are presented in table 1. Although men were 22% heavier, 3.5% taller and with a greater BMI (+4.4%) compared to women, the difference was not significant.

To test sensitivity, the Vivofit predicted basal EE was compared to the reliable and valid indirect calorimetric system. The analysis revealed no significant difference, $t_{(12)} = -1.902, p = .082$, in basal EE between IC ($1811\pm374$ kcal\(\cdot\)day\(^{-1}\)) and Vivofit ($1957\pm272$ kcal\(\cdot\)day\(^{-1}\)).

During TWT, a significant main effect of method, $f_{(1,2)} = 6.78, p = 0.024$, was observed on EE. The Vivofit significantly underestimated EE during TWT by 29.5% (IC, $m = 69.5\pm12.9$ kcal; Vivofit, $m = 49.07\pm16.6$ kcal). Despite no significant interaction, a trend towards decreasing differences between methods at higher inclinations was
observed (45%, 30%, and 18%, for 0%, 5%, and 10% inclination, respectively). When linear regressions analyses were performed the outcomes demonstrated a strong correlation between EE and increases in inclination for both methods (IC, $R^2 = 0.995$; Vivofit, $R^2 = 0.994$). However, the slope between the two differed by 1.2 kcal•min$^{-1}$ with a steeper slope for Vivofit (4.4 kcal•min$^{-1}$) compared to IC (3.2 kcal•min$^{-1}$), leading to a lesser difference as the intensity increases. Nevertheless, the statistical analysis showed a significant condition effect, $f_{(1,2)} = 138.08, p = 0.001$; higher inclinations were associated with higher EE for both methods (figure 2).

The indirect calorimetry outcomes were compared to Vivofit scores during an office task performance. Surprisingly, no change from baseline during OTP was detected by the Vivofit (data from the device website revealed EE equal to zero), which has resulted in no variance. Therefore, no comparison was made between the two methods. However, the EE recorded with IC during OTP showed an increase of 17.4±1.2 kcal•hr$^{-1}$ above the basal EE (i.e. from basal metabolic rate).

There was a significant interaction between method and inclination, $f_{(1,2)} = 5.03, p = 0.017$, for the step count data. Post-hoc analysis using the Bonferroni correction examined the interaction. Results showed that the Vivofit systematically underestimated step count only at 0% incline (930±148 steps) compared to the kinematics analysis (1052±69 steps) (figure 3).
During the daily one-hour of self-selected activity, participants performed different types of physical activity/exercises including, but not limited to, endurance activities, strength exercises, and team sports that lasted in average 46±23 minutes per day. Table 2 reports the physical activity intensity and frequency performed by the participants during the study. Low-to-moderate intensity activities comprised 67% of exercises (35 to 69 %HR_max); vigorous activities (>70%HR_max) comprised the remaining 33% of exercises. A linear regression analysis was performed to assess the degree of correlation between EE and exercise intensity [estimated through HR]. The analysis outcomes revealed a significant correlation between the two variables, $R^2 = 0.75, f(1,56)= 169.6, p = 0.001$.

Discussion

The aim of this study was to examine the accuracy of the Vivofit activity tracker in estimating EE and daily step count. For the user, a slight deviation in measuring total energy expenditure may have an impact on physical activity levels and therefore, on health issues. The first major outcome of the study revealed that the Vivofit significantly
underestimated EE during TWT by 29.5 %. It co-occurred with an underestimation of step count at 0% incline. Furthermore, the Vivofit was not sensitive enough to detect the small increase in EE induced by a free-living activity (i.e. office work).

**Basal and resting energy expenditure**

Our results indicate that the Vivofit provides valid and accurate estimates of basal EE, as there was no significant difference between IC and the Vivofit. As underlined by Chen and Sun (Chen & Sun, 1997), basal EE estimated by tri-axial accelerometers is as accurate as the algorithm implemented in the activity tracker. Most of these devices use a prediction equation based on age, gender, height, and weight (Chen & Sun, 1997). Since the Vivofit uses the same predictive model, it is not surprising that there was no significant difference. These results correspond, to some extent, with Fruin and Rankin (Fruin & Rankin, 2004) who found accurate basal EE predictions with a multiple sensors device (Sense Wear Armband, SWA) compared to IC measurements over a period of 3-hours using the same predictive equation (i.e., age, gender, weight, and height). The Sense Wear armband (SWA) estimates did not differ from the IC value confirming that the two methods were highly correlated ($p > 0.65; r = 0.76$). However, note that the Vivofit overestimated basal EE by 146 kcal•day$^{-1}$, which might have a significant impact over time. It is important to mention that the Vivofit did not detect the slight increase in EE over basal levels during light activities such as office work. Other studies have reported either an over- or underestimation of EE during resting activities including lying, static standing, doing computer work while sitting and filing articles. For instance, Nichols et al (Nichols, Morgan, Sarkin, Sallis, & Calfas, 1999) found that the Tritrac
accelerometer underestimated light activity, while Crouter et al (Crouter et al., 2006) found that the AMP-331 and Actigraph overestimated light activity. On the other hand, the Actical and Fitbit devices accurately estimated light activities (Crouter et al., 2006; Sasaki et al., 2014). These discrepancies are believed to be a result of differences in the regression equation implemented in the activity tracker (Crouter et al., 2006).

**Exercise energy expenditure**

As illustrated in figure 2, the Vivofit significantly underestimated EE during the TWT for all three inclinations (0%, 5%, and 10%) compared to IC values. Meanwhile, estimated EE from the Vivofit was linearly correlated with the inclinations. A linear regression revealed that the Vivofit had a steeper slope compared to IC; higher inclinations resulted in less difference between the methods. Therefore, Vivofit is more accurate during moderate to high intensity exercises. Several studies have assessed the accuracy of activity trackers for measuring caloric expenditure. Outcomes have varied (both over- or underestimation) depending on the activity, the intensity, and the device being tested. As summarized in table 3 for horizontal treadmill walking, most of the studies reported an overestimated EE at speeds ranging from 3.2 to 9.7 km•hr⁻¹ using several different activity trackers (Balogun, Martin, & Clendenin, 1989; Crouter et al., 2006; Fruin & Rankin, 2004; Nichols et al., 1999; Stackpool, 2013). In contrast, other studies reported an accurate estimation of EE using different activity trackers (Crouter et al., 2006; Stackpool, 2013). Finally, other studies reported an underestimated EE when different activity trackers were tested (CSA, Fitbit, Fitbit ultra, and Actical) (King, Torres, Potter, Brooks, & Coleman, 2004; Noah et al., 2013; Sasaki et al., 2014) – results
that agreed with ours. For inclined treadmill walking, few studies reported an underestimation of EE using different activity trackers. For instance, significant underestimations ranging from 22% to 40% and more were reported by Fruin and Rankin (Fruin & Rankin, 2004) for SWA, by Noah et al (Noah et al., 2013) for Actical, Fitbit and Fitbit Ultra, and by Sasaki et al (Sasaki et al., 2014) for Fitbit device at 2 speeds (4.8 and 6.4 km\(\text{hr}^{-1}\)). All concluded that the assessed activity trackers were inaccurate at detecting changes in EE as inclination increased. Considering the literature and the present study, we can conclude that activity trackers do not accurately estimate EE. Due to the variety of differences in the accuracy of activity trackers, it is important to consider the directionality of the impact. An awareness of whether a particular tracker is under- or overestimating activity levels can be useful when examining data to draw conclusions, both statistically and for real-world purposes. Further research is needed to investigate whether the cause of the differences can be determined; is it a result of the design of the tracker (hardware) or the programmed prediction equations (software)?

**Step Count**

For the step count data, the kinematics analysis revealed that Vivofit significantly underestimated step counts at 0 % incline while no significant differences were observed for the other two conditions (figure 2). Similarly, Stackpool (Stackpool, 2013) found that the Nike Fuelband underestimated step count during level treadmill walking by 6% at self-selected speed for 20-minutes. The author reported a moderate correlation \((R = 0.55)\) between the device and the hand counting steps technique. However, results should be
considered with caution due to potentially inaccurate methods of quantifying stride frequency in the above-mentioned study (Stackpool, 2013). In contrast, Nichols et al (Nichols et al., 1999) and Noah et al (Noah et al., 2013) both reported no significant difference between calculated step count and the activity tracker step count ($p >0.05$) for both level and incline treadmill walking. The discrepancy in results between the studies might be a result of the different type of accelerometers implemented in the activity trackers and the prediction equation used to quantify steps.

**Physical activity**

Participants were instructed to perform 1-hour of self-selected daily activity while wearing the Vivofit and the heart rate sensor. Compliance rates were high and participants achieved, on average, 46-minutes of exercise each day. The intent of performing physical activity was to validate the heart rate sensor for different types of physical activity. To do so, exercise intensity was estimated through the average heart rate collected from the Vivofit. Based on intensity, exercises were classified according to the ACSM guidelines (Pollock et al., 1998). The results showed a strong correlation ($R^2= 0.753$) between the EE and the exercise intensity as determined by HR, confirming that Vivofit HR sensor is an accurate device (see figure 4). However, none of the previously mentioned studies tested the validity of HR as a predictor of exercise intensity in their corresponding devices when the option was available which renders comparisons difficult.

Possible reasons for the systemic underestimation of EE and step count by the Vivofit might stem from the predictive algorithm implemented in the activity tracker by the manufacturer. This underestimation could be unfavorable for the consumer since an
appropriate level of physical activity is important to maintain good health. Although laboratory procedures can accurately measure physical activity, these are not widely accessible by the general population. The various activity trackers available provide information useful for health-oriented goals such as management of blood pressure, blood glucose levels, and weight loss. As previously shown (Bravata et al., 2007), pedometers have been effective in increasing physical activity even though inaccuracy in estimating step count was observed (Schneider et al., 2003). Most of the new physical activity trackers available on the market offer an interactive website where users can monitor physical activity and other options, depending on the services offered by the company. This mode of behavioural intervention seems to be an effective strategy to motivate users to increase physical activity time (Spring et al., 2012). Additionally, physical activity trackers might be useful when investigating exercise intervention in field studies. Therefore, validating the instrument against a gold standard measure is required to ensure the tracker provides reliable data.

**Methodological consideration**

There are some methodological considerations of this study. First, our results are only generalizable to healthy adults within the few physical activities assessed. The validity and reliability of this device needs to be determined with other populations such as obese individuals and with broader types of physical activity. Secondly, we only assessed a subset of the many options Vivofit offers. For instance, we did not test the reliability and validity of the instrument under stringent conditions such as vigorous...
exercise activities or under less demanding conditions like during sleep. In fact, unpublished observations from our laboratory revealed significant differences between EE estimated by the Vivofit and IC during cycling on a stationary cycle ergometer at different intensities for 30-minutes (261 and 360 kcal•min⁻¹ for Vivofit and IC, respectively), confirming similar findings (Crouter et al., 2006; Sasaki et al., 2014). Considering that this study only included a short-duration walking activity, future investigations should examine the impact of Vivofit EE underestimation over extended time periods in free-living settings with different physical activities and sports.
Conclusion

Despite some limitations, the Vivofit activity tracker is on par with similar devices and can be used to track physical activity. Although the Vivofit underestimates EE during low to moderate activity, modifications to the prediction algorithm may improve device performance. Manufacturers typically do not release their formulas to allow for periodic updates when required; as such, it is hopeful that with future updates more accurate results will be generated. This is important for ensuring that individuals obtain accurate feedback to self-monitor EE to become more active and for athletes to better monitor their training load. Activity trackers may also be beneficial for research purposes, allowing researchers to track individuals’ activity over extended periods of time without the use of expensive and complex laboratory equipment. The Vivofit is user-friendly in terms of easy attachment/detachment and minimal discomfort with little-to-no interference in activity. More research is needed to provide a feasible evaluation of free-living activities. It is important to further test the reliability and validity of the Vivofit in estimating the EE and step count of other activities before conclusions can be made about the overall reliability and validity.

References


Organization Press; 2009.


10. Dannecker KL, Petro SA, Melanson EL, Browning RC. Accuracy of fitbit activity
monitor to predict energy expenditure with and without classification of activities


18. Nichols JF, Morgan CG, Sarkin JA, Sallis JF, Calfas KJ. Validity, reliability, and


Acknowledgments

The work was supported by an internal grant from the School of Human Kinetics and Recreation, Memorial University, St. John’s, NL, Canada. The authors would like to thank Mr. David Gill, the owner of TotalCoaching.com, for providing access to his platform and technical support. Finally, we would like to thank the participants for their time, commitment and patience.
Declaration of interest

The authors report no declarations of interest.
Figures Caption

**Figure 1:** Experimental design consisting of three laboratory sessions – Day 1: orientation and anthropometric measurements – Day 4: basal and exercise metabolic rate determination plus kinematics analysis – Day 5: None-exercise metabolic rate determination (office work performance). Day 2 and 3 consisted of 1-hour of self-selected exercise. Throughout the study, food and physical activity log were recorded.

**Figure 2:** Energy expenditure (kcal•min\(^{-1}\)) plotted against three different treadmill inclinations (0%, 5%, and 10%) measured through indirect calorimetry (IC) and Vivofit activity tracker.

* represents the main significant time effect (\(p < 0.05\)) and # represents the main significant method effect (\(p < 0.05\)).

**Figure 3:** Step count (step•10-min\(^{-1}\)) plotted against three different treadmill inclinations (0%, 5%, and 10%) measured by kinematics technique (video camera) and Vivofit accelerometer.

* represents significant difference (\(p < 0.05\)) after decomposing the significant interaction using Bonferroni correction.

**Figure 4:** Correlation between energy expenditure (kcal•min\(^{-1}\)) and intensity expressed in percent of maximal heart rate (%HR\(_{max}\)) during the self-selected exercise.
Table 1: Physical characteristics of the participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male (n=8)</th>
<th>Female (n=5)</th>
<th>All participants (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>40.7± 4.4</td>
<td>39± 5.9</td>
<td>40±11.9</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>84.5± 4.9</td>
<td>75±6.3</td>
<td>81.0±13.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175± 2.9</td>
<td>169±4.4</td>
<td>172.8±8.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5±3.5</td>
<td>26.3±4.0</td>
<td>27.0±3.4</td>
</tr>
</tbody>
</table>

Mean±SD
Table 2: Physical Activity intensity and frequency performed by participants during the study

<table>
<thead>
<tr>
<th>Intensity</th>
<th>$\text{HR}_{\text{max}}$ (%)</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very light</td>
<td>&lt; 35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Light</td>
<td>35-54</td>
<td>16</td>
<td>27.6</td>
</tr>
<tr>
<td>Moderate</td>
<td>55-69</td>
<td>23</td>
<td>39.7</td>
</tr>
<tr>
<td>Hard</td>
<td>70-89</td>
<td>18</td>
<td>31.0</td>
</tr>
<tr>
<td>Very hard</td>
<td>&gt;90</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maximal</td>
<td>100</td>
<td>1</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Table 3: Summary of different studies results that compared different activity trackers with IC during horizontal treadmill walking (0%).

<table>
<thead>
<tr>
<th>Source</th>
<th>Activity Tracker</th>
<th>Speed (km•hr⁻¹)</th>
<th>Effect on EE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balogun et al. (1989)</td>
<td>Caltrac</td>
<td>3.2, 4.8, 6.2, and 7.8</td>
<td>over-estimate</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Nichols et al. (1999)</td>
<td>Tritrac-R3D</td>
<td>3.2, 6.4, and 9.7</td>
<td>Over-estimate</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>King et al. (2004)</td>
<td>Tritrac-R3D, RT3, SWA, and BioTrainer-Pro CSA</td>
<td>3.2, 4.8, and 6.4</td>
<td>Over-estimate</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fruin and Rankin, 2004</td>
<td>SWA</td>
<td>4.8 and 6.5</td>
<td>Over-estimate</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Crouter et al. (2006)</td>
<td>Actigraph and Actical AMP-331</td>
<td>-------</td>
<td>Over-estimate</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Stakpool (2013)</td>
<td>Adidas Mi coach, Nike Fuelband, Jawbone UP, BodyMedia FIT Core, Fitbit Ultra, and NL-2000i</td>
<td>-------</td>
<td>Over-estimate</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Noah et al. 2013</td>
<td>Fitbit, Fitbit Ultra, and Actical</td>
<td>5.6 and 8.8</td>
<td>Under-estimate</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sasaki et al. 2015</td>
<td>Fitbit</td>
<td>8.8</td>
<td>Under- estimate</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
$Y = 0.24x - 8.67$

$R^2 = 0.7518; p < 0.001$