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THE ACUTE EFFECTS OF WEIGHTED VEST RUNNING ON SUBSTRATE UTILIZATION AND ENERGY EXPENDITURE

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**THE ACUTE EFFECTS OF WEIGHTED VEST RUNNING ON SUBSTRATE
UTILIZATION AND ENERGY EXPENDITURE**

BY

TROY MATHEW PURDOM

Submitted in Partial Fulfillment of the
Requirements for the Degree of

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BY

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ABSTRACT

This study evaluated the effect of weighted vest running (WVR) on fatty acid oxidation (FAox) and caloric expenditure at pre-designated exercise intensities (60,65,70,75,80% VO_{2max}).

Seventeen recreationally trained runners (9 men and 8 women) performed four separate graded exercise tests (GXT) separated by 24 hours each. The first graded exercise test (GXT) was performed to establish the workloads at specific exercise intensities. The following three GXTs tested WVR with a control (no vest), 5% body mass (BM) vest, and 10%BM vest, using 3-minute incrementally increasing steady rate stages. Indirect calorimetry was used to measure both FAox (g/min) and caloric expenditure (kcal/min). The ANOVA/ANCOVA analysis revealed that WVR significantly increased caloric expenditure ($p < 0.05$) and reduced FAox ($p < 0.05$). Pairwise comparisons revealed that caloric expenditure was significantly increased in the 10%BM condition at all exercise intensities compared with the control and 5%BM (except at 60% VO_{2max}). Post-hoc comparisons showed that FAox was significantly decreased in the 10%BM WV at the 70 and 75% VO_{2max} conditions only while all exercise intensities in the control and 5%BM trials were not significantly different from each other. As well, this study

demonstrates that maximal FAox occurs at 60% $\text{VO}_{2\text{max}}$ in all conditions (control, 5% WV and 10% WV). When sex + fat free mass (FFM) + fat mass (FM) were included as covariates, FFM (kg) was found to have a significant influence ($p < 0.001$) on caloric expenditure. Lastly, fat mass (kg) was found to have the strongest influence on FAox ($p = 0.07$) as compared to FFM and sex.

Key Words: Fatty Acid Oxidation, Caloric Expenditure, Substrate Oxidation, Sex Differences, Body Composition

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Figure 2: Pairwise comparisons show that as exercise intensity increases, FAox decreases. Data are presented as mean \pm SEM, n = 17.

Abbreviations Used in this Study:

\geq : greater than or equal to

$>$: greater than

\leq : less than or equal to

$<$: less than

\pm : plus or minus

\sim : approximately

ACSM: American College of Sports Medicine

ANOVA: analysis of variance

ATGL: adipose triglyceride lipase

ATP: adenosine triphosphate

Beta-ox: beta oxidation

BF%: body fat percentage

bpm: beats per minute

BW: body weight

CHO: carbohydrate

CHOOx: carbohydrate oxidation

CONT: control

CPT-1: carnitine palmitoyltransferase-1

DAG: diacylglycerol

df: degrees of freedom

dy: day

EE: energy expenditure

FA: fatty acid

FAox: fatty acid oxidation

FFM: fat free mass

FM: fat mass

g/min: grams per minute

GXT: graded exercise test

H₂O: water

HAD: β -hydroxy acyl-CoA dehydrogenase

hr: hour

HR: heart rate

HRmax: maximal heart rate

HSL: hormone sensitive lipase

IMTG: intramuscular triacylglyceride

Kcal: kilocalorie

kg: kilogram

LPL: lipoprotein lipase

LCFA: long chain fatty acids

MFO: maximal fat oxidation

mo: months

n: number of subjects

PDH: pyruvate dehydrogenase

Ra: rate of appearance

ANOVA: analysis of variance

RER: respiratory exchange ratio

RPE: rating of perceived exertion

SE: standard error

TAG: triacylglycerol

TCA cycle: tricarboxylic acid cycle

VO₂: volume of oxygen consumed

VO_{2max}: maximal oxygen consumption

WV: weighted vest

WVR: weighted vest running

WV5: weighted vest w/ an additional 5% body weight

WV10: weighted vest condition w/ an additional 10% body weight

yrs: years

CHAPTER I

INTRODUCTION

Lipids are the substrate largely responsible for energy supply during submaximal exercise (Achten, J. Gleeson, M. Jeukendrup, A., 2002; Venables, MC. Achten J. Jeukendrup, A., 2004). However, the definitive role of lipids and their contribution to cellular respiration during endurance exercise has yet to be fully elucidated. Subcutaneous adipose, intramuscular triacylglycerides (IMTG), and dietary fat all contribute to fatty acid oxidation (FAox) (Achten and Jeukendrup, 2004). Further, the energy contribution from lipid oxidation during submaximal exercise is in addition to carbohydrate oxidation (CHOox) (Achten et al. 2002; Stephens, 2007). As exercise intensities increase to >65% of VO_{2max} , the contribution of energy from fat begins to decline, favoring CHOox (Achten et al., 2002; Brooks & Mercier, 1994). Maximal fat oxidation (MFO) is a term used to describe the point when lipid oxidation reaches maximum (Achten and Jeukendrup, 2004; Venables et al., 2004). Maximal fat oxidation typically occurs between 45-65% of VO_{2max} , and is often expressed as (g/min) (Achten et al., 2002; Achten and Jeukendrup, 2004).

The point that FAox reaches maximum and begins to decline is further described as the crossover concept (Brooks and Mercier, 1994). Brooks and Mercier (1994) defined the crossover point as, “. . . the power output at which energy derived from oxidation of CHO-based fuels predominates over that derived from lipids, with further increases in power eliciting a relative increment in energy from CHO utilization and a relative decrement in energy from lipid oxidation” p.2253. Separately, weighted vest running (WVR) has been shown to acutely alter exercise intensity (Abe et al., 2011; Epstein Y. Stroschein, L. Pandolf, K., 1987). Therefore, WVR could alter the exercise intensity relative to a specific running velocity which can

potentially alter when the crossover point occurs. The influence WVR has on exercise intensity may impact substrate oxidative properties.

Fat oxidation is impacted by several factors including: exercise duration (Turcotte, Richeter, Kiens, 1992), training status, exercise intensity, nutrition, and sex (Achten and Jeukendrup, 2004). These factors have been shown to alter circulating hormone concentrations, intramuscular substrate concentrations, and cellular expression, all of which impact FAox. Each of these factors facilitate or inhibit physiological changes that may impact FA/CHO metabolism. Currently it is unknown if WVR has influence on the factors that dictate lipid oxidation.

Studies have consistently reported that pre-menopausal women have a significantly greater ability to oxidize fat at relative exercise intensities compared to men (Astorino, 2000; Venables et al., 2005). In a comprehensive investigation with over 300 men and women, the energy contribution of fat was found to be significantly higher in women vs. men at all exercise intensities measured ranging from 41-61% $\text{VO}_{2\text{max}}$ (Venables et al., 2005). Women's greater ability to oxidize fat during exercise is attributed to the increased circulation of estrogens (Isacco et al., 2012; (B) Maher, 2010). Evidence suggests that beta oxidative proteins are regulated in part by estrogens resulting in an increase in FAox ((A) Maher et al., 2010; Oosthuysen and Bosch, 2010). The sex-specific fluctuation (or lack thereof) of substrate oxidation during WVR has yet to be investigated.

Weighted vest running has been shown to acutely modify running mechanics, which can impact exercise intensity (Abe et al., 2011; Barnes et al., 2015). Furthermore, biomechanical data suggest that changes in running mechanics can positively influence running economy (Heise et al., 2008). Weighted vest running has been shown to have an effect on running mechanics which

resulted in a reduction of exercise intensity (Abe et al., 2011.). The acute effects of WVR on substrate oxidation kinetics as a function of exercise intensity are not known.

During WVR, variations in the amount of external mass as well as the distribution of the mass can impact exercise intensity at a given workload (Knapik et al., 2004; Watson et al., 2008). The placement and distribution of additional mass on the body can destabilize posture, and impact metabolic cost (Knapik et al., 2004). The influence of the location and distribution of the mass on exercise intensity may impact substrate oxidative properties. Asymmetrical placement of the mass, as well as positioning the mass directly superior/posterior to the pelvis have been shown to increase metabolic cost (Knapik et al., 2004). The ideal WVR load and exercise intensity for optimizing FAox and caloric expenditure is unknown. Therefore, testing the FA and CHO oxidative changes that occur while running with an added 5% & 10% of body mass is the purpose of the study.

PROBLEM STATEMENT

The effect of a 5% and 10% BM WV condition on substrate oxidation and energy expenditure at various running intensities has yet to be elucidated in the research. If WVR does prove to have an effect on substrate oxidation and energy expenditure, the practical application of this knowledge will have widespread appeal.

PURPOSE OF THE STUDY

The purpose of this study is to investigate caloric expenditure and substrate oxidation that occur while running with an additional 5% and 10% BM at various exercise intensities (60, 65, 70, 75, 80% VO_{2max}) in men and women.

HYPOTHESIS

In this study we will test the following hypotheses:

1. Hypothesis 1: Substrate oxidation and caloric expenditure will be altered significantly ($p < 0.05$) due to WVR.

Rationale: Changes in exercise intensity as a result of placement of external mass on the body have been observed. A higher exercise intensity will be sufficient enough to alter fat oxidation and caloric expenditure. Higher exercise intensities have been shown to suppress total fat oxidation as compared to moderate intensity exercise (Jeppesen and Kiens, 2012).

2. Hypothesis 2: The WV dose effect (5% of BM vs 10% of BM) will alter substrate oxidation significantly ($p < 0.05$) for a given exercise running intensity.

Rationale: Varying the amount and placement of external mass worn on the body has been shown to affect exercise intensity (Abe et al., 2011; Barnes et al., 2015; Knapik et al., 2004). Changes in exercise intensity are linked to changes in substrate oxidation (Achten et al., 2002; Jeukendrup and Wallis, 2005).

3. Hypothesis 3: Women will significantly ($p < 0.05$) oxidize FAs differently compared to men at a given workload (running velocity + WV condition).

Rationale: Although it has been shown that pre-menopausal women oxidize fat at higher rates compared to men at a variety of exercise intensities (Venables et al., 2005), the influence of WVR on sex-specific exercise intensity changes is

unknown. However, it is hypothesized that there will be a difference due to the body composition and hormonal differences between men and women.

SCOPE OF THE STUDY

Based on an *apriori* power analysis using existing research, we required 16 recreationally trained runners (8 men and 8 women) to determine if the various WV conditions impact FAox and caloric expenditure. Nine men and eight women volunteered to participate. Height and weight were measured, body fat was estimated, and participants completed a health history questionnaire, HIPPA authorization form, informed consent, a 24hr food recall, and a series of four VO_{2max} graded exercise tests (GXT) over four visits. Each participant was instructed to avoid moderate to intense physical activity for 24hrs prior to testing. Participants refrained from taking any caffeine and supplements for 12hrs prior to testing. No food was consumed during the 4hrs prior to each trial. In addition, subjects completed a 24hr food recall to be replicated prior to the subsequent visits for standardization purposes. All female subjects were administered a urine pregnancy test to ensure they were not pregnant. The first visit consisted of a GXT with no weighted vest (no-vest) to assess workloads, exercise intensities and VO_{2max} . After the 24hr food recall replication was verified, the subsequent visits consisted of a GXT with a randomized vest condition (no-vest, 5%BM, and 10%BM) for 3min stages at specific running velocities correlating with 60, 65, 70, 75, 80% VO_{2max} and VO_{2max} . During the last minute of each stage, VO_2 , respiratory exchange ratio (RER), heart rate (HR), rating of perceived exertion (RPE), FAox (g/min), CHOox (g/min), energy expenditure (EE), and FA/CHO percentage of EE (%) were measured. Mixed ANCOVA models were used to test these variables, the vest conditions, and exercise intensities.

LIMITATIONS OF THE STUDY

The limitations of the study were as follows:

1. The methodology cannot distinguish FAox sources from peripheral adipose tissue, IMTG, cholesterol, glycerol, amongst others.
2. Results of the study may not be applicable for all populations.
3. Macronutrient consumption relevant to total energy consumption has been shown to affect substrate oxidation was not measured. Alternatively, subjects were asked to follow the same precise diet for 24hrs prior to each trial to control for test-retest reliability, which study administrators verified prior to each trail. Nonetheless, macronutrient specificity was not tabulated.
4. Heavy exercise <24hrs prior to testing has been shown to alter substrate oxidation. Subjects were asked to refrain from heavy exercise during the testing period, but study administrators had no way to verify this.

The study was designed as a repeated measures design that compares men and women under varying exercise intensity and weighted vest running conditions. The WV conditions were randomized to avoid a possible learning effect. We also attempted to control for the nutritional influence by asking subjects to replicate their diet prior to each trial, caffeine intake by mandating that they refrain from stimulants for >12hrs prior to the trial, pregnancy by requiring women to do a urine pregnancy test, and the pre-exercise effect on substrate oxidation by asking subjects to limit physical activity 24hrs prior to each trial. Additionally, due to the vigorous testing procedures, we tested endurance-trained individuals who exercise frequently.

SIGNIFICANCE OF THE STUDY

Exercise training with WV for professional and recreational athletes is increasing. No prior investigation has assessed the effects of WVR on substrate oxidation or caloric expenditure. Furthermore, sex differences in substrate oxidation using additional mass have yet to be investigated. Outcomes from this study will contribute to the literature and expand the understanding of the effects of WVR for recreationally trained runners. The results from this study can be used for exercise prescriptions and dietary recommendations that will elicit specific adaptations germane to a specific training plan.

DEFINITION OF TERMS

Lipids: Organic compounds that include FAs and their derivatives. They are insoluble in water.

Triglyceride: The largest energy/fat depot in the body. Triglycerides can be stored in subcutaneous adipose tissue or in various tissues such as muscle.

Intramuscular triglyceride: Triglycerides stored primarily within type I muscle fibers in close proximity to the mitochondria.

Fatty acid oxidation: The systematic removal of H atoms from carbon chain molecules. The oxidation of FAs, called beta-oxidation, produce acetyl-CoA used in cellular respiration.

Maximal fat oxidation: The maximal amount of FA that can be oxidized in a given time frame. Typical values are expressed as (g/min) or as a percentage of energy expenditure.

Glycolysis: An anaerobic process that occurs in the cytosol to facilitate the breakdown of glucose by cellular proteins. The product of glycolysis is pyruvate, which is used in cellular respiration.

Caloric expenditure: A small calorie is the amount of energy (heat) needed to raise one gram of water 1°C while large calories (kcal) is the amount of heat needed to raise 1000g of water 1°C.

Caloric expenditure is the number of kilocalories used during an activity or during a specific length of time.

VO_{2max}: The maximal amount of oxygen the body can use during a specified amount of time during intense exercise.

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CHAPTER II

This chapter presents a research manuscript entitled, “Understanding the Factors That Effect Fat Oxidation During Exercise.” The manuscript follows the formatting and style guidelines of The Journal of the International Society of Sports Nutrition. It is authored by Troy Purdom, Len Kravitz, Karol Dokladny, Christine Mermier.

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Understanding the Factors That Effect Fat Oxidation During Exercise

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Scholarly Review

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CHAPTER II

Abstract

Lipids as a fuel source for energy supply during submaximal exercise originates from subcutaneous adipose tissue derived long-chain fatty acids (LCFA), intramuscular triacylglycerides (IMTG), cholesterol and dietary fat. These sources of fat contribute to fatty acid oxidation (FAox) in various ways. The regulation and utilization of fatty acids (FA) occurs primarily at exercise intensities between 55-65% $\text{VO}_{2\text{max}}$, and is known as maximal fat oxidation (MFO), measured in g/min. Fatty acid oxidation occurs during submaximal exercise intensities, but is also complimentary to carbohydrate oxidation (CHOox). Due to limitations within FA intracellular transport across the cell and mitochondrial membranes, FAox is limited at higher exercise intensities. The point at which FAox reaches maximum and begins to decline is referred to as the crossover point. Exercise intensities that exceed the crossover point ($\sim 65\% \text{VO}_{2\text{max}}$) utilize CHO as the predominant fuel source for energy supply. Training status, exercise intensity, exercise duration, sex differences, and nutrition have all been shown to affect cellular expression responsible for FAox rate. Each stimulus affects the process of FAox differently, resulting in specific adaptations that influence endurance exercise performance. Endurance training, specifically long duration ($>2\text{hrs}$) facilitate adaptations that alter the origin of FAs and FAox rate. Additionally, the influence of sex and nutrition on FAox is discussed. Finally, the role of FAox in the improvement of performance during endurance training is discussed.

Key Words: Fat Oxidation, Substrate Utilization, Dietary Fat Oxidation, Crossover Concept

Introduction

Lipids are the substrate largely responsible for energy supply during submaximal exercise [1-3]. However, the definitive role of lipid contribution during cellular respiration has yet to be fully elucidated. Subcutaneous adipose tissue, intramuscular triacylglycerides (IMTG), cholesterol, and dietary fat all contribute to fatty acid oxidation (FAox) [1]. Moreover, the energy contribution from lipid oxidation during submaximal exercise is in addition to carbohydrate oxidation (CHOox) [4]. However, as exercise intensity increases, the contribution of carbohydrate oxidation increases in proportion to lipid oxidation [4]. Nonetheless, the oxidation of lipids is the predominant fuel source (%) during submaximal exercise intensities <65% VO_{2max} [1,2,5]. Increases in exercise intensity that exceed 65% of VO_{2max} produces a shift in energy contribution favoring CHOox. A term used to describe the point when lipid oxidation reaches maximum is maximal fat oxidation (MFO). Exercise intensities that exceed MFO oxidize CHO in greater proportion to fat [2,4,5].

Maximal fat oxidation has been shown to occur between 47-75% of VO_{2max} , and is different between trained and untrained men and women [1,5,6]. Factors that alter lipid oxidation rates are exercise intensity, duration, training status, sex, exercise mode, and nutritional intake [1]. Each of these factors facilitate or inhibit physiological changes that influence FAox [1] and are discussed in subsequent sections.

Lipid Oxidation

Lipolysis

Triacylglycerol (TAG) is the stored form of fat found in adipocytes and striated muscle, which consists of a glycerol molecule (a three-carbon molecule) that is bound to three fatty acid

(FA) chains. Fatty acid chains are carbon molecules linked together with accompanying hydrogen atoms. The intercellular process of liberating the FAs from the glycerol backbone is called lipolysis [7,8]. Once this occurs, FAs are released into the blood and transported to working muscle for oxidation.

Adipose tissue reserves can store a significant amount of TAG and deliver a seemingly endless supply of energy for prolonged exercise performance. A person with 7-14% body fat has >30,000 kcal of energy reserves stored in adipose tissue [3]. Therefore, if exercise intensity is maintained below 65% VO_{2max} , exercise can theoretically be maintained for longer durations because of the oxidation of endogenous TAG stores. However, when exercise intensities exceed ~65% VO_{2max} , FAox is reduced increasing the reliance on CHO for energy [2,4,9].

The process of lipolysis is largely controlled via the endocrine system [10,11]. The release of epinephrine stimulates lipolysis and therefore increases serum FA concentrations. At rest, catecholamine (epinephrine) concentration in the blood is low. As exercise intensity increases, there is a simultaneous and progressive increase in epinephrine [12] release from the adrenal glands. Depending on exercise intensity and/or duration, catecholamine concentrations can increase >20 times above basal levels [13]. The exercise-induced catecholamine release stimulates lipolysis, liberating FAs from the glycerol molecule [7,14]. During exercise intensities equating to ~60% VO_{2max} , serum FA concentrations increase 2-3 times resting values [15].

The binding of epinephrine to the β -adrenergic receptor on adipose cell membranes triggers a cascade of events that begin with the phosphorylation of adipose triglyceride lipase (ATGL) [7,8]. Recent findings indicate that lipolysis is under a hierarchal regulation by ATGL and hormone sensitive lipase (HSL) [7,8,16]. Recent studies show greater sensitivity of ATGL (a 10-fold increase) to epinephrine compared with HSL [7]. Therefore, ATGL disassociates the first

FA from the glycerol molecule forming diacylglycerol+FA or (DAG), whereas HSL is responsible for the second FA chain disassociation [7].

Endogenous skeletal muscle FAs, termed IMTGs, may contribute to overall FAox independent of serum FA contribution [17,18]. Intramuscular triacylglycerides are arranged within striated muscle, primarily in type I fibers in close proximity to the mitochondria [18,19]. The process of liberating intramuscular FAs from the TAG molecule for oxidation is slightly different from peripheral adipose tissue. Transport across the cell membrane is not a limitation to IMTG oxidation due to the fact that they are stored within the cell. However, the lipolytic enzymes lipoprotein lipase (LPL) and HSL are necessary to mobilize FAs (lipolysis) from the intracellular glycerol molecule [8]. Lipoprotein lipases are lipoproteins bound to the intramuscular capillary endothelium, and responsible for liberating the first FA from the TAG molecule within the cell, forming DAG [20].

The process of oxidizing IMTGs is facilitated by HSL and is similar to subcutaneous adipose tissue derived HSL. Hormone sensitive lipase has three important characteristics that impact DAG oxidation. First, HSL demonstrates a 10-fold higher affinity to DAG compared to TAG [19]. Secondly, HSL operates optimally at a pH of 7.0, which suggests that HSL activity is reduced at higher exercise intensities due to greater H⁺ production [19]. Lastly, HSL is directly stimulated by epinephrine and independent of the energy sensitive cAMP cascade known to stimulate lipolysis [17,19].

Despite the presence of IMTG within muscle, the overall IMTG concentration and energy contribution is still under debate due to tissue variabilities [8,17,21]. Some of the speculation is that ~10% of serum derived FAs are used to replenish IMTGs during exercise [12]. This makes it difficult to quantify the actual contribution of IMTGs to exercise substrate demands.

Additionally, variation in methodologies, e.g. muscle biopsy, isotope tracers, magnetic resonance spectroscopy make comparative efforts challenging [22]. Lastly, disparity in training status and dietary macronutrient specificity further complicate the ability to obtain definitive conclusions. More research in the area of IMTG energy flux is necessary to determine IMTG influence on energy contribution during exercise.

Fatty Acid Transport

Limitations to FAox are due in part to a multi-faceted delivery system that has a series of regulatory events [17]. Once FAs leave the adipocyte they first bind to albumin, which can bind as many as 12 FA molecules [14]. Interestingly, due to poor circulation in peripheral adipose tissue and an increased ratio of FA:albumin after exercise, the albumin binding capacity may be surpassed and high levels of free fatty acids can create a harmful condition [14]. Due to the poor circulation in type II diabetics, a high percentage of liberated FAs as a result of exercise-induced, catecholamine-stimulated lipolysis are not released into the circulation during high intensity exercise [12]. However, endurance training has been shown to increase blood flow to subcutaneous adipose tissue by 2-3 fold [12], which can increase overall FA transport to working muscle. Despite the positive circulatory effects of endurance training, limitations to the rate of FAox appear to be more dependent on cellular transport rather than systematic transport of serum FAs from adipose tissue [23].

Fatty acid transport across the muscle cell membrane occurs via transport proteins, mainly CD36 [23,24]. CD36 appears within the plasma membrane in as little as 1 min after the initiation of muscle contraction [24]. Research shows that sedentary obese women training at $>70\%HR_{max}$ increased CD36 expression by 25%. The result of increasing CD36 within muscle

cell membranes is highly correlated ($R^2= 0.857$, $P <0.003$) with a 23% increase in FAox [25]. Moreover, CD36 upregulation occurs rapidly and remains elevated for three days post exercise. Schenk and Horowitz (2006) [25] showed that the plasticity of the cellular changes positively influences resting FAox by 23% for days after exercise concludes.

In humans, sex differences have been shown to effect CD36 expression [26,27] due to circulating estrogen concentrations [28]. After 90 min of cycling at 60% VO_{2max} , CD36 mRNA was 85% higher in women vs men. Interestingly, there is a 49% greater FA uptake ability due to greater CD36 protein concentrations in trained women compared to trained men [29]. Additionally, CD36 protein concentrations are 49% higher in women compared to men, irrespective of training status.

In summary, transport of FAs across the cell membrane positively affects FAox [12,25,29]. Endurance training increases CD36, thereby increasing intracellular transport for oxidation. Increasing transport of FAs into the cell for oxidation spares CHO stores for both high intensity exercise and prolonged exercise [9].

Within-Cell FA Transport into Mitochondrion

Within the cell, serum derived long chain FAs (LCFAs) and IMTGs (>12 carbons) require a mitochondrial transport protein for FA transport [30]. The transport protein known as carnitine palmitoyltransferase-1 (CPT-1) is located on the outer mitochondrial membrane and is responsible for the transportation of LCFA into the mitochondria [31-33]. Fatty acids with 12 or fewer carbons are classified as medium chain FAs and can pass through the mitochondrial membrane independently of protein transporters [32]. CPT-1 is a product of free carnitine, which can be found in both the cytosol and mitochondrial matrix [32].

CPT-1 concentration during exercise appears to be regulated in part by exercise intensity [23,32]. No significant changes in CPT-1 concentrations were observed in subjects exercising at lower exercise intensities compared to rest [23]. However, exercising at 60% $\text{VO}_{2\text{max}}$ has been shown to increase concentrations. At exercise intensities $>75\%$ $\text{VO}_{2\text{max}}$ [23], free carnitine and therefore CPT-1 can be a FA transport limitation, ultimately reducing FAox [32].

CPT-1 catalyzes the transfer of an acetyl group from acyl-CoA to free carnitine to form acyl-carnitine. During moderate intensity exercise, CPT-1 catalyzes the reaction primarily with FA derived acyl-CoA, and is transported across the outer mitochondrial membrane. CPT-1 is then translocated to the mitochondrial matrix [23,31,32]. During high intensity exercise, large quantities of acetyl-CoA are also produced via fast glycolysis [23,31]. The abundance of glycolytic and FA derived acetyl-CoA at high exercise intensities supersedes tricarboxylic acid cycle (TCA cycle) utilization, and thus free carnitine is used to buffer excess acetyl-CoA by forming acetyl-carnitine [23].

At exercise intensities $>75\%$ $\text{VO}_{2\text{max}}$, free carnitine concentrations in working muscle decrease to $\sim 20\%$ of resting concentrations [32]. Thus, free carnitine serves as an acceptor of acetyl-CoA from both beta-oxidation and glycolysis [23]. Therefore, carnitine can be a limitation of FA substrate utilization due to the formation of glycolytic acetyl-carnitine during high intensity exercise [23,31,32]. The result of the abundant fast glycolysis derived acetyl-carnitine concentrations at high exercise intensities directly limits FA-acetyl transport into the mitochondria, limiting FAox potential [23,31,32].

Fatty Acid Oxidation

Fatty acid oxidation or beta-oxidation (beta-ox) is a catabolic process of the removal of H^+ ions from FAs while producing acetyl-CoA, which is further metabolized in the TCA cycle. One of the key enzymes of beta-ox, known as β -Hydroxy acyl-CoA dehydrogenase (HAD) positively influences FAox in working muscle [17]. Additionally, aerobic training and fat-rich diets stimulate HAD activity in mitochondria. FAox within the mitochondria is directly influenced by HAD [1,17] as well as the transport of FAs across the mitochondrial membrane [23,31,32].

While FAox fluctuates continuously, the endocrine system is principally responsible for the regulation of lipid oxidation at rest and during exercise [14]. The feedback mechanisms of lipid metabolism are based primarily on catecholamines [11], cortisol, growth hormone, and insulin [15]. Because FAox has a maximal rate, it is important to identify at what exercise intensity MFO occurs for current maximal fat burning potential, exercise prescription, and dietary recommendations. Identifying the stimuli that influence fat oxidation is necessary to best give exercise recommendations for the exercise intensity that facilitates optimal fat burning potential.

Factors That Influence Maximal Fat Oxidation

Training Status

The result of maintaining an elevated training status impacts FAox potential due to the increase in cellular/mitochondrial protein changes and hormonal regulation. The adaptations that occur due to endurance training over time favors the ability to oxidize fat at higher workloads [34,35]. The physiological changes that occur due to endurance training increase MFO potential in addition to the ability to maintain FAox at higher workloads [34,35]. Increased fat oxidation

has been shown to improve with endurance training and therefore changes in training status. Bircher and Knechtle, (2004) [36] demonstrated this concept by comparing sedentary obese subjects with athletes and found that MFO was highly correlated with respiratory capacity, and thus training status

The correlation between respiratory capacity and MFO is demonstrated by similar serum glycerol concentrations in sedentary vs. trained subjects, with a significantly greater ability to oxidize fat seen in trained subjects [26,36,37]. These results, however, conflict with results from Lanzi et al. (2014) [38] who showed that obese subjects had higher serum FA rate of appearance, likely due to increased total adipose tissue mass (kg). Furthermore, sedentary/obese subjects have a reduced ability to oxidize fat, therefore maintaining higher serum FA concentrations [38]. Trained women were observed to significantly oxidize more fat compared with obese women (1.09 ± 0.36 ; 0.63 ± 0.27 g/kg/min respectively) [36].

The training effect, and therefore an increase in respiratory capacity is partially due to an increase in MFO. Scharhag-Rosenberger et al. (2010) [35] conducted a prospective study to demonstrate this concept using sedentary subjects who met or exceeded ACSM's minimum cardiorespiratory exercise recommendations for a period of 1yr. Maximal fat oxidation (rate) increased over 12 months of training (pre-training 0.26 ± 0.10 ; post-training 0.33 ± 0.12 g/min) and it occurred at a higher exercise intensity (pre-training $35 \pm 6\%$ $\text{VO}_{2\text{max}}$; post-training $50 \pm 14\%$ $\text{VO}_{2\text{max}}$). The training status effect on MFO further applies to athletic populations. In moderate vs highly trained subjects, the exercise intensity ($\% \text{VO}_{2\text{max}}$) that MFO occurred was not significantly different, but MFO was elevated (while not significantly) for the highly trained subjects (0.29 ± 0.10 vs 0.47 ± 0.17 g/min, respectively) [34]. Furthermore, mitochondrial enzymes citrate synthase and HAD were found to be significantly increased in highly trained (49%) vs.

moderately trained (35%) participants [37]. Increasing HAD directly effects beta-ox rate while citrate synthase increases the TCA cycle rate [39]. This evidence suggests that lipolysis and systemic FA delivery are not limitations to FAox at higher exercise intensities. Therefore, FA cellular transport proteins (CD36 and CPT-1) [23,24] and mitochondrial density (HAD) are likely the limitation of FAox during high intensity exercise [37].

Acknowledging the occurrence of large inter-individual differences in MFO, differences in MFO relative to training status are still observed. Lima-Silva et al. (2010) [34] showed that differences in the lipid oxidative potential may exist in high vs. moderately trained runners referenced above. However, while no differences were observed between groups at the exercise intensity that MFO occurred, there was an increased capacity to oxidize fat in the highly trained subjects. The increased performance capacity in highly trained runners is most likely attributed to an increased CHO oxidative potential at higher exercise intensities in order to maintain higher steady state running workloads [34]. Subsequently, cellular protein expression, oxidative capacity and therefore training status do have the ability to influence fat oxidation.

Training status further influences maximal fat oxidative potential by increasing endogenous substrate concentrations [18,19]. Endurance training enhances type I fiber IMTG concentrations as much as three-fold compared with type II fibers. Increased MFO potential due to training is further influenced by FA-liberating HSL [21] and LPL proteins [19] which are responsible for the liberation of intramuscular FAs from the IMTG molecule. However, during exercise, the IMTG pool is constantly being replenished with plasma-derived FAs during exercise. Nonetheless, the reliance of IMTG during submaximal exercise durations lasting <2hrs is essential to maintaining workload [40]. The exercise duration effect could be due to β -adrenergic receptor saturation, which has been shown to occur during prolonged bouts of

exercise [10,15]. Furthermore, HSL has been shown to increase initially within 10-60min, but returns to resting levels after 120min of exercise, increasing reliance on serum derived FAs [19]. More research in the area of hormone related FA kinetic limitations is warranted.

Intensity

The exercise intensity that MFO occurs has been reported to range from 45-75% VO_{2max} [1,4,6,36,38] depending on factors such as exercise intensity, training status, sex, and nutrition [1]. Exercise intensity has the most profound effect on MFO based on a combination of events which include FA transport changes [23,24] and hormone fluctuation, which can increase lipolytic rate [7]. The cellular and hormonal changes that occur during exercise are directly related to exercise intensity which can influence FAox [42].

The increased expression of FAox transport and oxidative cell proteins (CD36, CPT-1, HAD, etc.) that results in an increase FAox are a result of exercise intensity [23,43]. Bergomaster et al. (2008) [43] suggests a minimum training volume of two weeks is necessary, independent of training status, for sufficient cellular adaptation to occur. The Lima-Silva et al. (2010) [34] data, however, show that subjects who trained for a minimum of 3yrs at various exercise intensities have variable fat oxidation rates. Thus, FAox adaptations are related to exercise training intensity rather than chronic adaptation. It has also been shown that carnitine concentrations are a direct limitation of FAox at higher exercise intensities ($>65\% VO_{2max}$) to both IMTG [23] and serum FAox [32], regardless of mitochondrial enzymatic activity in untrained and moderately trained subjects.

Exercise intensity may affect MFO by influencing catecholamine concentrations which have a regulatory effect on lipolysis [15], glycogenolysis, as well as gluconeogenesis [11]. The

corresponding increase in epinephrine concentrations that parallel increases in exercise intensity stimulate both glycogenolysis and gluconeogenesis [11]. Glycogenolysis and gluconeogenesis elevate blood glucose levels at higher exercise intensities to meet energy demand. As exercise intensity increases, so does catecholamine concentrations, facilitating a concurrent increase of serum CHO and FAs into the blood [11]. However, the body still favors FAox at exercise intensities $<65\% \text{ VO}_{2\text{max}}$ [5,16]. When exercise intensity exceeds MFO, FAox begins to decline; this process is described as the crossover point [4].

The concept of the crossover point represents a theoretical means to understand the effect of exercise intensity on the balance of CHO and FA oxidation [4]. More specifically, the crossover concept describes the point that exercise intensity influences when the CHO contribution to energy demand exceeds FAox. The limitations of FAox at higher intensities is due to the vast amount of acetyl-CoA produced by fast glycolysis [23,32]. The abrupt increase in acetyl-CoA production at high intensity due to fast glycolysis floods the cell with potential energy suppressing FAox potential. Notably, the large inter-individual fluctuation of when the crossover point occurs at a given exercise intensity can be attributed to training status [34,35]. Training status has been shown to effect catecholamine release and receptor sensitivity [11], endogenous substrate concentrations, and cellular transport protein expression all of which contribute to the variability of when MFO occurs relevant to exercise intensity [1].

The reduction in FAox at higher exercise intensities can be explained in part by examining lipid oxidation at specific exercise intensities. At $25\% \text{ VO}_{2\text{max}}$, plasma FAs make the largest energy contribution, whereas muscle glycogen and IMTG contribute very little [14]. At $<65\% \text{ VO}_{2\text{max}}$, muscle glycogen and IMTG constitute as much as 50% of energy expenditure, depending on exercise duration [14,44]. Bergomaster et al. (2008) [43] compared 6wks of sprint

interval training (Wingate Tests) to endurance training ($\sim 65\%$ $\text{VO}_{2\text{max}}$) and found no differences in MFO. These findings suggest that training above $\geq 65\%$ $\text{VO}_{2\text{max}}$ will not increase MFO potential, which is in disagreement with current literature. Previous research suggests that exercise intensity greatly influences substrate utilization [5,37,41]. However, Bergomaster et al. [43] used untrained subjects, where the research cited above used trained subjects. Maximal fat oxidation occurs in all populations regardless of training status, nutritional influence, etc., and is decidedly dictated in large part by exercise intensity [5,6,37].

Duration

Another factor that significantly influences FAox is the duration of exercise [12,40,44]. Throughout a prolonged exercise bout, changes in hormonal and substrate concentrations trigger systematic changes in substrate oxidation [19,45]. Studies show that endurance training promotes reliance on endogenous fuel sources for up to 120min of submaximal exercise [40,45,46].

Exercise duration has a large effect on the origin of FAs for oxidative purposes. Reductions in IMTG concentrations has been shown to occur when exercise duration exceeds two hours [40]. Beyond 120min of submaximal exercise ($\geq 65\%$ $\text{VO}_{2\text{max}}$), IMTG oxidation is reduced to resting levels due to the inhibition HSL by the increase of serum derived LCFAs [19,40]. From 120-240min of exercise, IMTG oxidation returned to resting values and was offset by a 46% increase [45] in serum FA delivery and oxidation [40]. Additional evidence shows that after 12hrs of prolonged exercise, IMTG stores are 50-80% of pre-exercise concentrations despite the extreme duration of exercise [12].

The shift from intramuscular fuel sources to serum derived FAs after 2hrs of submaximal exercise parallel changes in blood glucose concentrations. Untrained subjects who completed

3hrs of knee extensions at 60% of 1RM had a 66% increase in serum glucose concentrations during the second to the third hour of exercise [45]. Trained subjects however, reduced muscular CHO uptake during the same time frame, suggesting that trained subjects were able to maintain FAox (despite substrate origin) during prolonged exercise to stave off CHO usage for high intensity exercise [45]. While the exercise intervention used in this study is not typically classified as endurance exercise, the exercise protocol does clarify the variation in the origin of substrate oxidation over time, and expands on the diverse effect of exercise duration on substrate oxidation.

Training duration has a large influence on FA and CHO oxidation during prolonged submaximal exercise. However, training status has little influence on the origin of FAs during the first 120min of submaximal exercise. Nonetheless, trained subjects are able to maintain workloads for longer periods compared to untrained individuals based on the ability to maintain FAox for longer durations. Despite the training status effect on FAox, exercise duration will dictate substrate origin [19,40,45].

Sex Differences

Variability in FAox owing to sex exist due to the inherent hormonal differences specific to men and women [47-50]. In a comprehensive study with over 300 men and premenopausal women, the energy contribution of fat was significantly higher in women vs. men at all exercise intensities measured ranging from 41-61% $\text{VO}_{2\text{max}}$ [2]. Studies have consistently shown that premenopausal women have a significantly greater ability to oxidize fat during exercise [2,51,52].

The sex differences in fat oxidation [52,53] during exercise is attributed to the increased circulation of estrogens [47,48,54]. Evidence suggests that estrogen directly stimulates AMPK [28] and PGC-1 α activity [54], which is thought to increase the downstream FAox transport protein CD36 and beta-oxidative protein glyceraldehyde-3-phosphate dehydrogenase (HAD) [28]. Beta-oxidative proteins that oxidize LCFA oxidation are regulated in part by estrogen [48,54]. The result of increased beta-oxidative proteins is directly related to increased FAox potential [28,48]. Interestingly, when men were supplemented with estrogen, increases in FAox were observed along with increased cellular expression of beta-ox proteins within eight days of supplementation [54].

Circulating estrogen is naturally higher for premenopausal women compared to men. Additionally, fluctuation in estrogen levels is inherent throughout the menstrual cycle [47,53]. Estrogens are generally higher during the follicular phase of the menstrual cycle compared to the luteal phase [27]. Paradoxically, elevated estrogens during the follicular phase do not affect FAox when compared to the luteal phase [28,47]. Nevertheless, elevations in endogenous circulating estrogens inherent to premenopausal women increase the expression of cellular proteins responsible for increased FA transport and oxidation compared to men.

Nutrition

Cellular protein expression and the corresponding endogenous vs. systematic substrate oxidation vary according to dietary macronutrient intake [18,33]. It has been recently shown that high fat diets promote FAox and have performance enhancement capabilities [3,55]. However, definitive conclusions regarding macronutrient specificity and exercise performance

improvements are contingent on specific exercise applications [56] that are directed by exercise duration and intensity [57-59].

Diets that have higher proportions of a specific macronutrient (e.g. fat/CHO) have shown an increased ability to oxidize the primary macronutrient consumed [60-62]. Furthermore, endogenous substrate concentrations increase after acclimating to high fat/high CHO diets [59,62,63]. High fat diets increase IMTG concentrations while decreasing glycogen levels within muscle [16,33]. Alternatively, high CHO diet conditions increase glycogen concentrations while IMTGs decrease [16]. During exercise, the body favors oxidation of specific substrates based on long-term cellular adaptation in accordance to macronutrient consumption [3,33,63]. However, post-exercise macronutrient consumption has been shown to influence cellular protein expression in as little as 2hrs [63]. The plasticity of cellular changes relevant to chronic adaptation are compromised when macronutrient content is altered [59,61].

Macronutrient proportion and timing has been shown to have effects on cellular adaptation [33] as well as the physiological response to exercise [64-66]. High fat diets increase beta-ox potential at rest and during exercise, however, the limitations of high fat diets (including short term adaptation (24hrs)) reside with high intensity exercise [61]. High intensity exercise (>75% VO_{2max}) eclipses the FAox oxidative potential relying on CHO-derived acetyl-CoA [23] contingent on pyruvate dehydrogenase (PDH) for ATP re-synthesis [32,61].

High fat diets (>68% total daily calorie intake) have had positive effects on lowering RER values [58,65,66] during high intensity exercise, indicating an increase in FAox. Adapting the body to high fat diets allows the body to increase IMTG storage as well as oxidize fat more effectively [18,33], however, PDH activity is compromised along with high intensity exercise

performance [18,33]. Alternatively, after adapting the body to a high fat diet, short term high CHO loading can increase glycogen stores [66], increase glycolytic enzymes, as well as partially restore CHOox [61]. The current literature shows that high fat diet adaptation with short term (36-72hrs) CHO loading maintains IMTG stores while glycogen stores increase with short-term CHO loading [59,66].

Alternating macronutrient availability has the potential to be effective in accommodating the stress of high intensity exercise. Pyruvate dehydrogenase is the enzyme responsible for oxidizing pyruvate as the final substrate of the glycolytic pathway. Fat adaptation has been shown to reduce PDH activity [33] by 59% at rest and 29% at 70% VO_{2max} [61]. The reduction in PDH activity due to high fat diets is a limiting factor in CHO oxidation at high intensity exercise. However, fat adaptation with short-term CHO loading (compared with high fat diet) maintains IMTG concentrations and restores PDH activity, while maintaining 80% HSL activity [61]. Maintaining the ability to store and oxidize fat after acclimating to a high fat diet while restoring the ability to oxidize CHO with short-term CHO loading is an ideal physiological state for endurance exercise performance. Furthermore, glycogenolysis is elevated during exercise after CHO loading [61] indicating an increase in both glycogen storage as well as an increased ability to produce/maintain CHO availability during intense exercise [65].

Current research asserts that high fat diets favorably enhance FAox at both rest and during exercise [3,55]. However, exercise intensity dictates substrate utilization regardless of dietary influence, training status, and exercise duration. During moderate intensity exercise, serum FAs are primarily oxidized as opposed to IMTGs [44]. Because of this, high fat diets are sometimes encouraged during preparatory training when training volumes are high and exercise intensities are low to moderate [55]. However, during sustained high intensity exercise (>70%

VO_{2max}) which is common during competition, CHO is the primary substrate relied upon despite short and long term fat acclimation [65,67]. More research into the short-term macronutrient manipulation effect on endogenous substrate concentrations, plasticity of cellular expression, and preferential substrate oxidation are necessary to ascertain if there is benefit on exercise performance outcomes.

Conclusion

In summary, FAox is contingent on many factors which can modify cellular expression in a short amount of time. Macronutrient availability, training status, sex, exercise intensity, and duration all influence cellular adaptation, systematic FA transport, and FAox. Exercise intensity dominates substrate oxidation acutely, regardless of training status and/or nutritional influence. Additionally, more investigation into the ideal nutritional timing and content that will favorably influence the physiological adaptations of FAox during endurance exercise is warranted. Nonetheless, exercise prescriptions and dietary recommendations need to take into account specific exercise goals (duration, intensity, sport specific) to facilitate a training plan that will elicit the ideal substrate oxidation adaptations relevant to improve sport performance.

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CHAPTER III

This chapter presents a research manuscript entitled, “The Acute Effects of Weighted Vest Running on Substrate Oxidation and Energy Expenditure.” The manuscript follows the formatting and style guidelines of The Journal of Strength and Conditioning Research.

Running Title: The acute effects of submaximal weighted vest running on substrate utilization and energy expenditure.

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CHAPTER III

ABSTRACT

This study evaluated the effect of weighted vest running (WVR) on fatty acid oxidation (FAox) and caloric expenditure at pre-designated exercise intensities (60,65,70,75,80% VO_{2max}). Seventeen recreationally trained runners (9 men and 8 women) performed four separate graded exercise tests (GXT) separated by 24 hours each. The first GXT was performed to establish the workloads at the pre-specified exercise intensities. The following three GXTs tested WVR with a control (no vest), 5% body mass (BM) vest, and 10% BM vest, using 3-minute incrementally increasing steady-rate stages. Indirect calorimetry was used to measure both FAox (g/min) and caloric expenditure (kcal/min). The ANOVA/ANCOVA analysis revealed that WVR significantly increased caloric expenditure ($p < 0.05$) and reduced FAox ($p < 0.05$). Pairwise comparisons revealed that caloric expenditure was significantly increased in the 10% BM condition at all exercise intensities compared with the control and 5% BM (except 60% VO_{2max}), while FAox was significantly decreased in the 10% BM WV at the 70% and 75% VO_{2max} conditions only. Our study demonstrates that maximal FAox occurs at 60% VO_{2max} in all conditions (control, 5% WB and 10% WV). When sex + fat free mass (FFM) + fat mass (FM) were included as covariates, FFM (kg) was found to have a significant influence ($p < 0.001$) on caloric expenditure. Lastly, it appears that fat mass (kg) was found to have the strongest influence on FAox ($p = 0.07$) as compared to FFM and sex.

Key Words: Fatty Acid Oxidation, Caloric Expenditure, Substrate Oxidation, Sex Differences

INTRODUCTION

Lipids are the substrate largely responsible for energy supply during submaximal exercise (2,3,23). Subcutaneous adipose, intramuscular triacylglycerides (IMTG), and dietary fat all contribute to fatty acid oxidation (FAox) (3). Furthermore, the energy contribution from lipid oxidation during submaximal exercise is in addition to the carbohydrate oxidation (CHOox) (11,42). Maximal fat oxidation (MFO) is the term used to describe the point when lipid oxidation reaches maximum (39), and typically occurs between 60-70% of VO_{2max} in trained individuals (2,3,8). When exercise intensity exceeds MFO, FAox begins to decline while CHO becomes the primary substrate oxidized to maintain workload. The point that FAox reaches maximum and begins to decline is referred to as the crossover effect (11,39).

The contribution of substrates (FA and CHO) oxidized for energy permutate as exercise intensity increases, eventually leading to the crossover point where CHO is the dominant substrate oxidized (11). Weighted vest running (WVR) has been shown to acutely modify running kinematics, which can impact exercise intensity (1,5). Weighted vest running may affect when the crossover point occurs by influencing exercise intensity. However, the acute effects of WVR on substrate oxidation as a function of exercise intensity are not well understood.

During WVR, variations in the amount of external mass, as well as the distribution of the mass can impact exercise intensity at a given workload (14,19,38) and therefore influence substrate utilization. Asymmetrical placement of the mass (38), as well as positioning the mass lower on the spine relevant to the pelvis have been shown to increase metabolic cost (19). Moreover, the addition of too much mass can increase exercise intensity beyond MFO, increasing the reliance on glycolytic metabolism (26). Knapik et al. (18) reported military applications that use extreme weighted conditions (up to 46kg) marching over long distances (up

to 20km) which are quite demanding. Contrariwise, for recreational usage, WVR applications with up to 10% body mass (BM) may be ideal for optimizing changes in exercise intensity (9), and thus favorably influence FAox and caloric expenditure. Therefore, the purpose of this study was to assess the influence of running with an additional 5% BM and 10% BM on caloric expenditure and FAox.

EXPERIMENTAL APPROACH TO THE PROBLEM

Using a repeated measures design, this study investigated the impact of WVR on substrate oxidation and caloric expenditure. Seventeen (9 men and 8 women) recreationally trained runners completed a series of graded exercise tests (GXTs) to assess substrate oxidation and caloric expenditure at pre-designated intensities (60, 65, 70, 75, 80% $\text{VO}_{2\text{max}}$). There were four trials, a no-vest demographic GXT and three randomized vest conditions: control (no-vest), and two weighted vest trials at 5% BM and 10% BM. Participants completed 3-minute stages that progressively increased to the desired intensities. Metabolic gases were averaged over the last minute and used to analyze fat oxidation (g/min), caloric expenditure (kcal/min), and FA contribution to energy expenditure (%) during each stage. Participants completed a 24-hr food recall log prior to the first exercise trial, which was replicated prior to subsequent visits. This study was approved by the University Institutional Review Board.

PARTICIPANTS

Seventeen (9 male and 8 female) recreationally trained runners participated in the study. Demographic information for the study participants are presented in Table 1. Inclusion criteria for both men and women were defined as running (>10 - <50mi/wk) and were above the 80th aerobic power percentile defined by American College of Sports Medicine (ACSM) Guidelines for Exercise Testing (specific to sex and age) (4). All participants provided informed written

consent and completed a health history questionnaire prior to participation. Participants were excluded if they self-reported any symptoms/diagnosis with any cardiovascular, pulmonary, or metabolic disease, complained of any orthopedic pain, were taking any nutritional supplements, or were amenorrheic. Participants who were currently participating in any diet that encouraged specific macronutrient restrictions were excluded from the study. Additionally, all female participants were required to take a pregnancy test as pregnancy was an exclusion criterion. Volunteers were asked to complete a 24hr food recall prior to participation and preceding each exercise trial to minimize inter-individual fluctuation in substrate oxidation (32). Additionally, participants arrived to the lab for each trial having fasted for 4hrs, abstained from caffeine and any supplements for 12hrs prior to testing, and refrained from exercise for a minimum of 24hrs prior to each trial. Each participant completed four GXTs separated by a minimum of 24hrs which was shown to have a high substrate oxidation day-to-day reliability by Siveira et al. (29).

PROCEDURES

Body composition and Anthropometry

Anthropometric and demographic data were collected prior to the first GXT. Height, weight, and skinfold thickness were collected using a stadiometer (Tanita HR-200, Arlington Heights, IL), electronic scale (SECA Model #741-0142, Chino, CA), and skinfold calipers (Lange, Cambridge Scientific Industries, Cambridge, MD), respectively. After height and weight were measured, the sum of three skinfold sites (men-chest, abdomen, and thigh; women-triceps, suprailiac, thigh) were used to estimate body density (12) before being converted to percent body fat according to ACSM's Guidelines for Exercise Testing (4). Skinfold thickness was based on the average of two trials. If the skinfold measurements varied for a particular site by more than

0.5mm, the technician obtained a third measurement and the mean value of the two closest measures were used. All measurements were obtained by a single trained technician.

Exercise Protocols

For the initial maximal aerobic capacity test, resting VO_2 was measured for 1min using a metabolic cart (ParvoMedics TrueOne 2400, Sandy, UT). Heart rate (HR) was collected for 1-min in the standing position prior to exercise using a wireless signal integrated into the metabolic cart from a (Polar T31, Warminster, PA) chest strap. Participants then ran a self-selected warm-up and familiarization on a treadmill (Precor 956i Treadmill, Woodinville, WA) for 5-min. Participants then performed a GXT without a weighted vest until volitional fatigue while VO_2 and HR were collected at 1min intervals. Each participant began the initial GXT at a walking pace with increasing velocity every minute until volitional fatigue. Each exercise test protocol was developed to ensure that volitional fatigue would occur within 8-12min as described by Yoon et al. 2007 (41). Standardized criteria for the determination of $\text{VO}_{2\text{max}}$ was used as described by Robergs et al. (27). The pre-designated exercise intensities (e.g., 60%, 65%, 70%, 75%, 80% $\text{VO}_{2\text{max}}$) for the subsequent trials were identified using the $\text{VO}_{2\text{max}}$ obtained in the trial described above. Each specified exercise intensity was calculated as a percentage of $\text{VO}_{2\text{max}}$, and the corresponding workloads were identified for later trials.

Participants were then randomly assigned to one of three weighted vest (WV) (Ironwear Long/Short Speed Vest, Pittsburg, PA) conditions: control (CONT), WV with 5% BM (WV5), or WV with 10% BM (WV10). If the participant was scheduled to run with a WV, the added mass was first secured onto the participant with the mass uniformly distributed and secured prior to completing the warm-up. Participants then completed a GXT that consisted of running at pre-

designated relative intensities for 3-min stages. For each specified exercise intensity, the data were averaged in the last minute of each stage to assess steady rate FAox (g/min), caloric expenditure (kcal/min), and percent substrate contribution (%). Subjects then completed the subsequent two GXTs following the protocol above with or without the WV in a randomized fashion.

STATISTICAL ANALYSIS

Substrate oxidation and caloric expenditure were analyzed using mixed ANCOVA/ANOVA models. Variables considered within the model were sex, age, body weight (BW), body fat percentage (BF%), fat free mass (FFM), and fat mass (FM). Statistical tests were conducted using R (version 3.2.2; R Foundation for Statistical Computing; Vienna, Austria). Mixed effects ANCOVA models were analyzed with the lme4 package (version 1.1-10), using restricted maximum-likelihood (REML) estimation. Values are presented as mean \pm standard error (SEM) and F values. The level of statistical significance was set to $p < 0.05$. When significant differences were found, the Bonferroni post hoc comparison was used to investigate any significant interaction. An *a priori* power analysis (G*Power) indicated that 8 men and 8 women participants with a power of .97 was needed to find a statistically significant difference in the dependent variables (fat oxidation (g/min) and caloric expenditure (kcal/min)).

RESULTS

Participant characteristics are presented in Table 1. Caloric expenditure was significantly affected by both the vest condition and exercise intensity ($F(2,205) p < 0.001$; $F(4,205) p < 0.001$) in all statistical models, though no significant interaction between intensity and condition was detected ($F(8,205) p = 0.96$). ANOVA results as seen in Table 2 show that the increase in

exercise intensity increased kcal/min ($p < 0.05$) for all exercise intensities and vest conditions compared with the control trial, except the 5%BM, 60% stage. Caloric expenditure for the 10%BM trial was significantly different ($p < 0.001$) compared with the control trial for all exercise intensities except the 60% stage, which remained significantly different ($p = 0.04$) (Figure 1). Caloric expenditure for the 10%BM trial was significantly greater than the 5%BM trial ($p < 0.05$) for all exercise intensities except the 60% stage ($p = 0.51$).

Table 1. Characteristics (mean \pm SEM) of men (n=9) and women (n=8).

	Men	Women	P Value
Age (yrs)	24.7 \pm 1.9	30.0 \pm 2.9	$p = 0.13$
Ht (cm)	177.9 \pm 3.2	164.7 \pm 1.7	$p = 0.003^*$
Wt (kg)	78.6 \pm 3.9	58.2 \pm 2.5	$p < 0.001^*$
FFM (kg)	68.3 \pm 3.3	46.9 \pm 2.0	$p = 7.40$
FM (kg)	10.3 \pm 1.5	11.3 \pm 1.0	$p = 0.58$
Body Fat (%)	13.1 \pm 1.7	19.5 \pm 1.4	$p = 0.01^*$
VO _{2max} (ml/kg/min)	54.8 \pm 1.9	50.9 \pm 1.8	$p = 0.14$
Heart Rate Max (b/min)	184.8 \pm 2.8	187.3 \pm 3.4	$p = 0.57$

*Represents a significant difference using independent t-tests ($p \leq 0.05$).

Table 2 displays the adjusted means for fat oxidation rate and caloric expenditure across the pre-specified exercise intensities for each condition. The FAox rate declined progressively through the steady rate exercise intensities with MFO occurring at 60% VO_{2max} in all conditions. The 5%BM and 10%BM weighted vest conditions increased VO₂ by an average of .08% and 4.6%, respectively, across all pre-designated steady rate exercise intensities as compared to the control condition. Despite the increase in exercise intensity, WVR elicited an average decrease in FAox for the 5%BM and 10%BM by 4% and 10%, respectively. The modest increase in exercise intensity due to the weighted vest did not significantly alter fat oxidation ($p > 0.05$) in the 5%BM condition. However, the Bonferroni post hoc comparisons indicate that the 10%BM trial significantly reduced fat oxidation in the 70% and 75% VO_{2max} running intensities ($p = 0.036$, and $p = 0.015$, respectively), compared to the control condition.

Table 2: Output of the Bonferroni post hoc comparisons (mean \pm SEM) of vest condition with pre-designated exercise intensities of both FAox (g/min) and caloric expenditure (Kcal/min).

% VO2max	Control				5% BM				10% BM			
	g/min	SEM	Kcal/min	SEM	g/min	SEM	Kcal/min	SEM	g/min	SEM	Kcal/min	SEM
60	0.60	0.04	11.5	0.34	0.59	0.04	11.7	0.34	0.54	0.04	12.0 *	0.34
65	0.53	0.04	12.7	0.34	0.50	0.04	13.0	0.34	0.47	0.04	13.5 * σ	0.34
70	0.44	0.04	13.9	0.34	0.41	0.04	14.2	0.34	0.34 *	0.04	14.7 * σ	0.34
75	0.31	0.04	15.2	0.34	0.28	0.04	15.4	0.34	0.20 *	0.04	16.0 * σ	0.34
80	0.18	0.04	16.3	0.34	0.19	0.05	16.7	0.35	0.10	0.05	17.1 * σ	0.36

*- Statistically Different from Control

σ - Statistically Different from 5% BM

An ANCOVA analysis indicated that BW, independently, was found to significantly influence caloric expenditure ($F(1,15) p < 0.001$), but did not reach the threshold for significance regarding fat oxidation ($F(1,15) p = 0.08$). In a separate ANCOVA model, FFM was observed to be primarily responsible for the increase in caloric expenditure ($p < 0.001$). Additionally, FM is a possible contributing factor of fat oxidation ($F(1,13) p = 0.07$). Exercise intensity and vest condition each independently influenced fat oxidation ($F(4,209) p < 0.001$; $F(2,209) p < 0.001$) in all ANCOVA models examined, but no significant interaction between intensity and condition was detected ($F(8,209) p = 0.96$) in any model.

While participant sex, BW, FM, FFM, BF%, and age were each evaluated as covariates; only sex and a 2-compartment body composition model in kilograms (FM + FFM) were included in the final analyses. Age (18-38yrs) was found to offer little predictive value. Any significant effects attributable to sex were no longer apparent after accounting for the difference in body composition between sexes. Log-likelihood comparisons indicated no significant difference between ANCOVA models including both (sex + FFM (kg) + FM (kg)) and those containing

(FM + FFM) alone as covariates within ANCOVA models. Therefore, sex was not considered as a cofactor to influence FAox ($F(1,13) p = 0.57$) or energy expenditure ($F(1,13) p = 0.67$).

Conversely, body composition (FM + FFM), was found to have an effect. Fat free mass was independently found to influence caloric expenditure ($p < 0.001$), while FM did not reach the threshold for significance ($p = 0.07$) regarding FAox. Both intensity and condition were included within all models. Both FFM and FM displayed a significant main effect in all caloric expenditure and FAox models, but no significant interaction between these factors were identified.

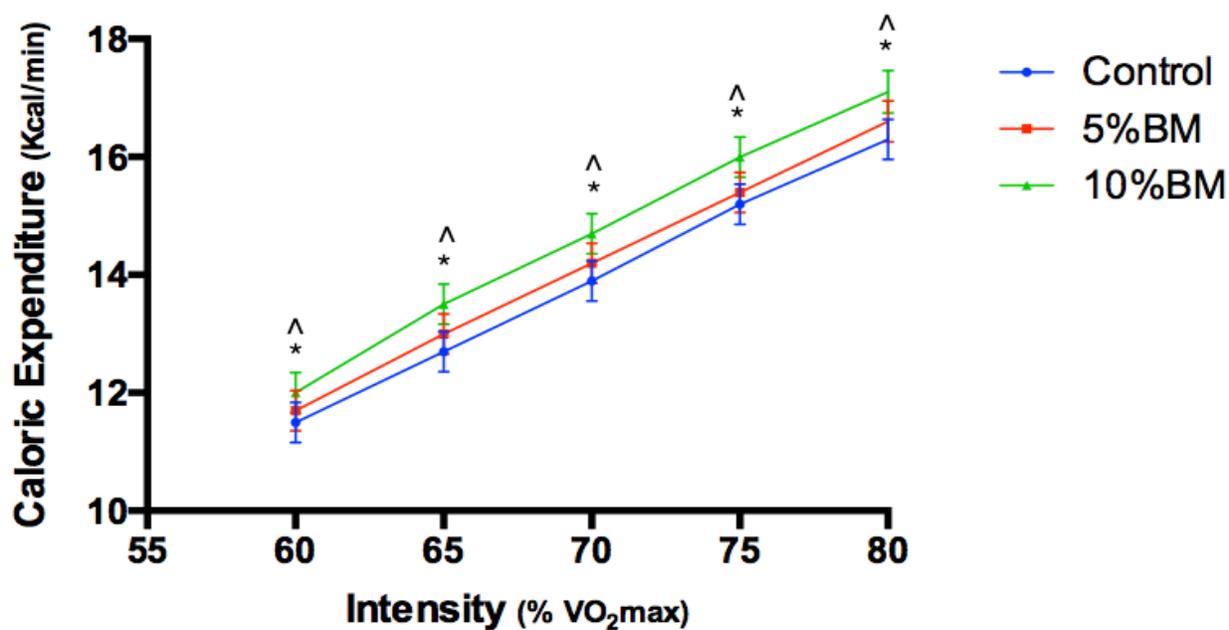


Figure 1: The Bonferroni post hoc comparison show that as exercise intensity increases, caloric expenditure increases. Data are presented as mean \pm SEM, $n = 17$.

* Designates the 10%BM significant difference ($p < 0.05$) compared control trial.

^ Designates 10%BM significant difference ($p < 0.05$) compared to 5%BM.

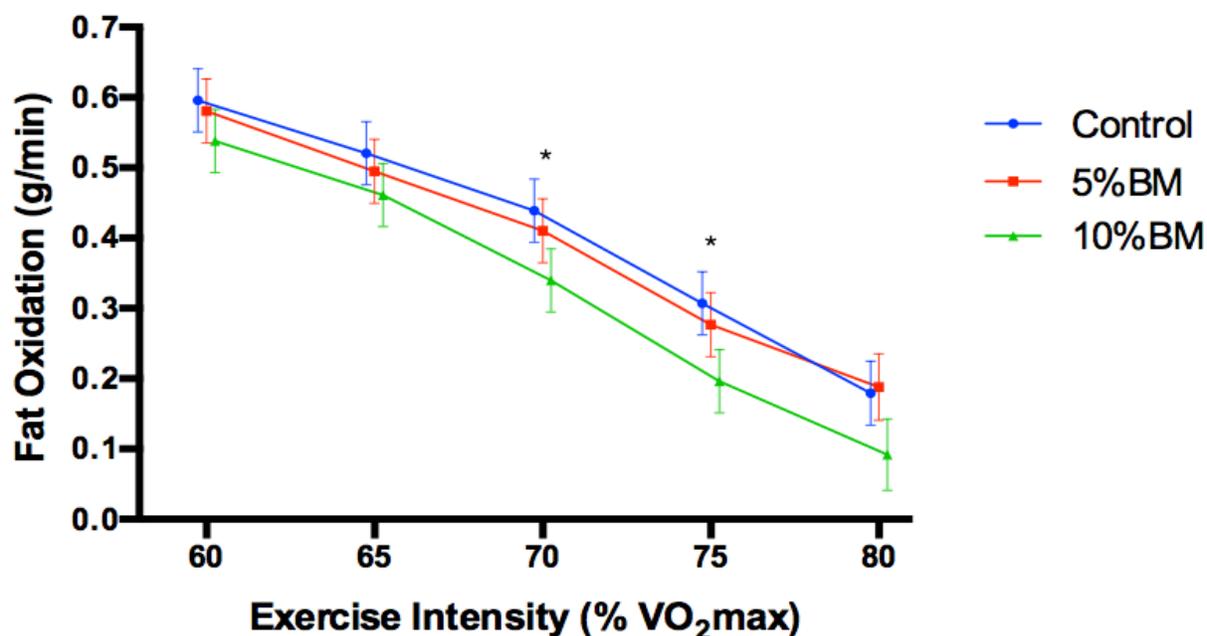


Figure 2: The Bonferroni post hoc comparison show that as exercise intensity increases, FAox decreases. Data are presented as mean \pm SEM, n = 17.

* Designates 10%BM significantly different ($p < 0.05$) compared to the control trial.

DISCUSSION

Caloric Expenditure

The original hypotheses for this study were to compare men and women's caloric expenditure and substrate oxidation changes that occur while running with an additional 5% and 10% BM at predesignated exercise intensities (60,65,70,70,75,80% VO_{2max}). Our results show that, as expected, exercise intensity and the 10%BM vest condition increased caloric expenditure ($p < 0.05$) shown in Table 2. In the control trial, each exercise intensity increased caloric expenditure an average of 1.23 Kcal/min, all of which were significantly different from each other ($p < 0.05$). Post hoc comparisons revealed that at all exercise intensities the 5%BM trial was not different from the control. However, caloric expenditure during the 10%BM vest condition was significantly greater than the control trial at all exercise intensities, and significantly greater than the 5%BM condition at all exercise intensities except the 5%BM, 60%

VO_{2max} trial. These results indicate that the 5%BM condition is not enough of a stimulus to increase caloric expenditure compared to the control trial, but the 10%BM condition does significantly increase caloric expenditure.

ANCOVA models included the covariates sex, age, BW, BF%, FM, and FFM to predict caloric expenditure. When only sex was included in the model, the effect of sex on caloric expenditure was significant ($p < 0.001$). When controlling for body mass, BW in kg was a strong predictor of Kcal/min ($p = 0.001$) in this sample, while the effect of sex was meaningful, it did not attain significance ($p = 0.056$). When two-compartment estimations of body composition were included within the ANCOVA model (FFM + FM + Sex), sex was no longer found to be a significant predictor ($p = 0.42$) of caloric expenditure in this sample. In contrast, FFM (kg) clearly explained the increase in caloric expenditure ($p < 0.001$). Venables et al. (36) agreed with these findings that FFM is the likely tissue responsible for substrate oxidation (FAox) and caloric expenditure.

Our results concur with Tarnoplosky (33) who suggests that sex differences exist when whole body substrate oxidation is considered. There was a mean difference of 20.4kg in BW between men and women (Table 1) in our sample. Interestingly, FFM was responsible for the majority of the weight discrepancy, where men had an average of 21.5kg higher mean FFM compared to women, while the average mean difference in FM was just 1.0kg. With higher FFM than women, men had the capability to oxidize more substrate, therefore increasing caloric expenditure. Furthermore, running with an average of 21.5kg increase in BW increases the workload when running at the same relative exercise intensity. However, when body composition was included within the ANCOVA model, sex was no longer a significant determinant of caloric expenditure. Conversely, Pauley et al. (23) examined the impact of body

composition on energy expenditure during running and found significant differences between the sexes ($p = 0.05$). Furthermore, our results agree with previous research that increasing FFM rather than body mass will increase caloric expenditure potential (23).

Fat Oxidation

An additional study hypothesis was that 5%BM and 10%BM WVR would have an effect on FAox as compared to running with no additional mass. This supposition was based on the notion that WVR alters running economy/intensity (1), and therefore would influence FAox which agrees with previous research that exercise intensity effects FAox (2,11,36). ANCOVA models indicated that both exercise intensity and WVR had a significant main effect on FAox ($p < 0.05$). However, the Bonferroni post hoc comparison revealed that FAox was significantly reduced only in the 10%BM condition at 70% and 75% VO_2 (-0.10 g/min; -0.11g/min respectively). The lack of difference in FAox between the control and vest conditions (despite the increase in workload) at other time points could possibly be attributed to the alterations in running mechanics described by Heise et al. (15). Heise et al. found that increasing the biarticular co-contraction of the leg muscles improved running economy. By increasing the contraction force of the muscles surrounding the knee during the stance phase of running, the body is able to acquire more potential energy using the elastic properties of muscle to reduce energy expenditure. Furthermore, by increasing the bi-articular co-contraction, the body can access neuromuscular properties such as the stretch shortening cycle (SSC). Accessing both the elastic potential energy and SSC has potential to reduce the metabolic cost of running (10). For example, despite the acute increase in workload (additional mass), Abe et al. (1) reported a reduction in exercise intensity due to the mechanical changes incurred with WVR.

It is well-established in the literature that exercise intensity has a profound effect on substrate utilization at submaximal exercise intensities (2,3,11,36). In the present study, our results agree with previous research that exercise intensity influences FAox ($p < 0.001$). Previous research has employed various techniques as an intervention to alter exercise intensity. Knapik et al. (19) reviewed the history of military-based load applications identifying asymmetrical load placement, unbalanced anterior/posterior distribution of mass, and velocity (specifically walking) to increase exercise intensity. They determined that equal distribution of mass close to or near to the body's center of gravity mimics non-weighted locomotion, regardless of the amount of mass, therefore minimizing the effect on metabolic cost. The uniformly distributed mass WVR application in our study concurs with the Knapik et al. (19) conclusions that mass evenly distributed reduces energy cost. Watson et al. (38) further agreed that the asymmetrical distribution of mass significantly increases exercise intensity. Watson et al. (38) used kettlebells, backpacks, and mannequins all with equal mass (20kg) to test the effects of symmetry and distribution. These findings confirm that external mass equally distributed minimizes the effect on exercise intensity. For the present study we also distributed the mass (5%BM, 10%BM) uniformly across the torso. The intervention showed a mild effect on exercise intensity for both vest conditions compared with the control, with an average increase in VO_2 across all exercise intensities (60-80% VO_{2max}) of +0.08% VO_2 (5%BM) and +4.6% VO_2 (10%BM), respectively. A recent study by Barnes et al. (5) used a vest with 20%BM during pre-run sprints (to test the vest pre-run effect on running without the vest), immediately prior to a no-vest middle distance run (5min run). This protocol reduced running intensity throughout the middle distance run without the vest by increasing the biarticular co-contraction of the leg muscles (5). Conversely, our pilot testing using 15%BM was deemed inappropriate for the middle distance running

velocities within our WVR protocol due to increasing exercise intensity beyond sustainable/practical applications.

Our research shows that an inverse relationship with exercise intensity and FAox exists (Figure 1), which agrees with previous research (2,11,36). The relationship between exercise intensity and FAox can be partially explained by the crossover concept (11). As exercise intensity increases beyond ~60% of VO_{2max} , the contribution of energy from fat begins to decline favoring carbohydrate oxidation (2,11). Achten et al. (2) further examined this concept with trained subjects by progressively increasing exercise intensity resulting in a progressive decline in fat oxidation, with which our results agree. However, in the current study, the pairwise comparisons (Table 2) revealed that the increase in exercise intensity resulted in the decrease in FAox was due to the increase in running velocity, not WVR in all conditions and intensities (except 10% BM at 70 and 75% VO_{2max}).

The addition of mass while running has been shown to alter running mechanics by changing how the lower limb muscles contract during running (1,5). Additionally, a notable change that has been shown to alter running mechanics is to improve joint stiffness (5,31), or the co-activation of the bi-articular muscles of the knee (10,15). It is surmised that these changes also occur with WVR, but have yet to be investigated. Increasing joint stiffness favorably increases the co-concentric/eccentric muscular activation surrounding the knee during the stance phase in normal running gait (15). The preparatory bi-articular co-contraction activates the SSC, reducing metabolic cost for a given running intensity. Increasing joint stiffness allows the body to access elastic potential energy in working muscle (10,15), increasing muscle power during the stance phase (7). The increase in joint stiffness increases the eccentric contraction (compared with normal running) while loading the limb (10), therefore increasing the concentric contraction

during the propulsion phase (1). Thus, the mechanical changes shown to impact running economy could result in a decrease in energy expenditure.

Methodology using EMG analysis further confirms that mechanical changes that occur with WVR modify running mechanics and effect exercise intensity (1). The results from Abe et al. (1) agree with previous research that accessing stored elastic potential energy (eccentric/concentric muscle contraction) reduces metabolic cost (5,7,10,15,31). The impact WVR could have on running mechanics could positively impact FAox. Conversely, in our sample the ANCOVA model revealed that WVR was significantly different between the control and 10%BM, and 5%BM and 10%BM. While previous research has shown a decrease in energy cost (5) with WVR (1), our results demonstrate that WVR (symmetrically distributed) can limit the effect added mass on exercise intensity. The 5%BM and 10%BM conditions only marginally affected exercise intensity (5%BM increased VO_2 0.08% or .11 ml/kg/min; 10%BM increased VO_2 4.6% or 2.01ml/kg/min) when averaged across all intensities tested (60-80% $\text{VO}_{2\text{max}}$).

In the present study, we used multiple ANCOVA models with the covariates sex, age, BW, BF%, FM, and FFM to evaluate the effect on FAox. When included as a lone covariate, sex was not found to significantly affect FAox ($p = 0.24$). However, when BF% + sex were included within the ANCOVA model, sex appeared have a meaningful impact ($p = 0.07$) while BF% was not a significant predictor ($p = 0.14$). Alternatively, an ANCOVA model using a two compartment body composition model (FM+FFM) and sex as covariates indicated that sex and FFM were not predictors of FAox ($p = 0.46$), but FM was consequential ($p = 0.07$). These findings show that the body composition of the study population should be considered when making conclusions regarding fat oxidation. In our sample, FM was very similar between the sexes (men 10.3 ± 1.5 kg; women 11.3 ± 1.0 kg), while BF% was significantly different (men

13.1% \pm 1.7; women 19.5% \pm 1.4) as shown in Table 1. The results of our study indicate that FM (kg) has a stronger influence on FAox than sex alone.

Previous research has reported differences in FAox between men and women (3,28,33,35,36), which our statistical analysis does not support. Venables et al. (36), in a comprehensive examination of sex based differences with over 300 subjects, suggests using regression analysis that FFM is likely the reason for the sex based FAox discrepancy ($p = 0.001$). However, the authors state that FFM explains only 12% of the variance. In our sample a 20.4 kg mean difference in BW exists between men and women. Despite the BW discrepancy largely attributed to FFM (21.4kg), FFM was not an influential predictor ($p = 0.86$) of FAox in this sample. When BW was considered alone with the ANCOVA model, BW did display a direction towards significantly influencing FAox ($p = 0.08$), but when sex + FFM + FM replaced total BW within the model, FM was more strongly associated with differences ($p = 0.07$) in FAox as compared to FFM ($p = 0.85$) or sex ($p = 0.45$). As sex was not an influential predictor of FAox in any model when body composition was considered ($p > 0.05$), BW and FFM (inherent to each sex) seem likely to account for some of the sex-based differences in FAox that have been previously reported (33,36).

Current literature regarding the contribution of macronutrient consumption to substrate oxidation confirms that dietary fat consumption affects FAox (37,40,42). In the present study, we attempted to control for test-retest reliability regarding macronutrient consumption as described by Støa et al. (34), but we did not calculate total macronutrient intake. We did however exclude anyone who was on a diet that restricted any specific macronutrient, which has been shown to affect substrate oxidation (21,24,25). Nonetheless, the women in our sample had similar FM profiles compared with the men (10.3 \pm 1.5kg; 11.3 \pm 1.0kg for men and women, respectively),

which are common values between the sexes when expressed as a percentage of total body weight (33). Within the ANCOVA model, sex was not considered a predictor ($p > 0.05$) while FM tended to predict FAox more than any other covariate, which has not been previously reported. This suggests that in trained subjects maintaining a higher FM would increase FAox. However, increased dietary fat consumption has been shown to increase higher circulating FA concentrations, intramuscular triglyceride (IMTG) concentrations, and FAox (30,40). Fat consumption was not measured in our study which is a limitation. Additionally, our sample is comprised of trained runners who have been shown to maintain higher concentrations of IMTG, independent of dietary intervention (20,34). However, despite the BF% discrepancy, sex did not have a strong influence on FAox. In the current study, we did not control for specific macronutrient consumption, however trained subjects with increased FM could increase IMTG storage and oxidation in addition to increasing overall FAox compared with lean subjects.

PRACTICAL APPLICATIONS

In summary, our results agree with previous research that 60% VO_{2max} elicits maximal FAox. However, our results show that recreationally conditioned runners training with weighted vests at 5%BM and 10%BM will significantly increase caloric expenditure while maintaining fat oxidation. The 10% weighted vest had the most meaningful increase in caloric expenditure. Furthermore, our results confirm that symmetrical placement of mass has little effect on exercise intensity while running. These findings are relevant for running-based exercise prescriptions to increase caloric expenditure, but maintain fat specific substrate oxidation. Lastly, trained runners maintaining a higher FM (within reason) could positively impact FAox. Conversely, FFM precipitates total energy expenditure, therefore, increasing FFM can positively increase caloric

expenditure during running activities. Future investigations should consider body composition of importance when exploring substrate oxidation.

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CHAPTER IV

SUMMARY, SPECIAL FINDINGS, CONCLUSIONS, RECOMMENDATIONS AND FUTURE STUDIES

Summary

This study evaluated the effect of weighted vest running (WVR) on fatty acid oxidation (FAox) and caloric expenditure at pre-designated exercise intensities (60,65,70,75,80% VO_{2max}). Seventeen recreationally trained runners (9 men and 8 women) performed four separate graded exercise tests (GXT) separated by 24 hours each. The first graded exercise test (GXT) was performed to establish the workloads at specific exercise intensities. The following three GXTs tested WVR with a control (no vest), 5% body mass (BM) vest, and 10%BM vest, using 3-minute incrementally increasing steady rate stages. Indirect calorimetry was used to measure both FAox (g/min) and caloric expenditure (kcal/min). The ANOVA/ANCOVA analysis revealed that WVR significantly increased caloric expenditure ($p < 0.05$) and reduced FAox ($p < 0.05$). Pairwise comparisons revealed that caloric expenditure was significantly increased in the 10%BM condition at all exercise intensities compared with the control and 5%BM (except 60% VO_{2max}), while FAox was significantly decreased in the 10%BM WV at the 70 and 75% VO_{2max} conditions only. As well, our study demonstrates that maximal FAox occurs at 60% VO_{2max} in all conditions (control, 5% WB and 10% WV). When sex + fat free mass (FFM) + fat mass (FM) were included as covariates, FFM (kg) was found to have a significant influence ($p < 0.001$) on caloric expenditure. Lastly, it appears that fat mass (kg) was found to have the strongest influence on FAox ($p = 0.07$), as compared to FFM and sex.

Special Findings

In addition to the results addressed in this investigation, ratings of perceived exertion (RPE) were collected throughout the study for all participants, in all conditions. Our analysis revealed that WVR and increasing exercise intensity significantly increased RPE ($p < 0.001$). Moreover, we observed a significant interaction ($p < 0.001$) between WVR and exercise intensity with regard to RPE, implying that the effect of WVR on perceived exertion increased with increasing exercise workload. Post hoc comparisons indicated that both the 5%BM and 10%BM conditions elicited higher RPE than the control condition at all levels of exercise intensity, but the 10%BM conditions were only significantly different from the 5%BM condition at 75% VO_{2max} . These results parallel the increase in workload due to increased exercise intensity and increased mass (weighted vest), meaning that the perception that exercise is becoming more difficult aligns with the increase in workload.

Conclusions

This study shows that 5%BM and 10%BM WVR increases caloric expenditure. Caloric expenditure was meaningfully increased in the 10%BM condition at all exercise intensities compared with the control and 5%BM (except 60% VO_{2max}). Our study demonstrates that maximal FAox occurs at 60% VO_{2max} in all conditions (no vest, 5%WB and 10% WV). As exercise intensities increase, FAox is reduced. Sex differences in FAox were not apparent in any condition tested in this study after accounting for body composition. Finally, body composition (FM + FFM) appears to have an influence on FAox and caloric expenditure in males and females.

Recommendations

Our study was able to identify that WVR reduces FAox and increases caloric expenditure. However, this study exposed some limitations regarding the statistical methodology utilized within the previous literature. Further research evaluating the effect of FM and FFM on substrate oxidation is warranted. As well, current literature states that acute variation in nutrition (<24hrs) can influence substrate oxidation. Macronutrient consumption may vary substrate oxidation, and should be controlled in future research. Our study used trained subjects ($\geq 80^{\text{th}}$ percentile identified by ACSM). Subjects who are less trained or sedentary may respond differently to WVR compared to our sample. Therefore, exploring how different fitness level populations respond to WVR is another opportunity for future investigation. Lastly, more research is needed to better compare and explain the influence WVR between males and females.

Future Studies

- 1) Future studies should account for the effects of body composition (FM + FFM) when making conclusions regarding sex-based differences in substrate oxidation and caloric expenditure.
- 2) Investigating the effect of training status (sedentary vs trained) during WVR would help to address the value of differences in substrate oxidation for beginning training regimens and weight loss goals. How WVR influences FAox and caloric expenditure with beginning runners should be addressed in future studies.
- 3) The effect of diet on WVR has not been investigated. This research may provide potential ideas for optimizing substrate utilization with WVR.

APPENDICIES

- A. Informed Consent
- B. IRB Approval Form
- C. Health History Questionnaire
- D. Data Collection Sheet
- E. Recruitment Flyer
- F. Electronic Recruitment Prompts
- G. 24hr Food Recall

APPENDIX A

The University of New Mexico
Consent to Participate in Research

The Acute Effects of Weighted Vest Running on Substrate Oxidation
and Energy Expenditure

Purpose and General Information

You are being asked to participate in a research study that is being conducted by Dr. Len Kravitz, who is the Principal Investigator and his associates. This research is being done to evaluate the effect of weighted vest running on the variation of fat and carbohydrate utilization, and energy expenditure. Your trials will consist of running on a treadmill with and without a weighted vest. Evidence suggests that exercising with a weighted vest may influence the ideal carbohydrate and fat utilization in your body. You are being asked to participate because you are someone who regularly engages in running activities, and do not have any short or long-term medical conditions. Approximately 40 people will take part in this study at the University of New Mexico Exercise Physiology Lab.

This form will explain the study to you, including the possible risks as well as the possible benefits of participating. This is so you can make an informed choice about whether or not to participate in this study. Please read this Consent Form carefully and ask the study investigators or study staff to explain any words or information that you do not clearly understand.

What will happen if I participate?

If you agree to be in this study, you will be asked to read and sign this Consent Form. After you sign the Consent Form, the following things will happen:

First visit

1. You will be asked to visit the Exercise Physiology Lab in Johnson Center on the University of New Mexico main campus for 4 separate visits.
2. Each visit will be separated by a minimum of 24hrs.
3. Prior to each visit, you will be asked not to exercise for 24hrs, not to consume caffeine/supplements for 12hrs, and no food intake for 4 hours before testing. Furthermore, you will be asked to record your entire food and liquid consumption for 24hrs prior to your exercise tests. The 24hr food record is to be replicated prior to each exercise test. You may drink water at any time throughout the study.
4. All visits will be less than 1hr each.
5. During your first visit, you will fill out paperwork including a combined consent/HIPAA form and health history questionnaire. If you have any medical issues that the study team feels would prevent safe participation in the study, then you will not be able to continue. Your resting heart rate, height, and weight will be measured, and body fat will be estimated. Body fat estimates will be done using the skinfold method that involves a measurement of a double layer of skin and

underlying fat with the use of a skinfold caliper. A skinfold caliper is a hand-held device that measures the width of skin folds at 3 separate sites on the body such as: women- back of the arm, above your hip bone, thigh;

men- chest, by the belly button, and thigh. The skin is pinched together and the accumulated width of all sites will be used to estimate your body fat. Total time for the first visit will be approximately 1hr.

6. All women will be required to complete a urine pregnancy test to ensure they are not pregnant. If time between any of the visits exceeds 3wks, another pregnancy test will be administered.
7. You will be asked to stand while we collect resting heart rate and inhalation/exhalation gases for one minute prior to running a 5min warm-up at a speed of your choosing. Immediately following your warm-up, you will perform a maximal oxygen consumption exercise test (VO_{2max}). The VO_{2max} test will be performed without a weighted vest to measure your VO_{2max} . This test requires you to run on a treadmill for approximately 8-12min with increasing intensity until you voluntarily stop. Throughout the resting and exercise measurements you will wear a heart rate monitor (strap around chest), a mouthpiece, and noseclip setup that is connected to a gas analyzer to measure your exhaled oxygen and carbon dioxide throughout each trial. You will be asked to rate how hard you feel you are working using a rating of perceived exertion (RPE) scale by pointing to a number. The RPE scale is a 6-20 number scale rating with 6 being the least effort and 20 being your maximal effort. Once you have completed the VO_{2max} test, the mouthpiece and noseclip will be removed. You will be given a copy of your 24 hour food recall regarding what you ate and drank in order to replicate for the 24hrs prior to your next visit. Also your next visit will be scheduled before leaving.

Second Visit

8. You will arrive to the lab (the same location as visit 1) having not exercised for 24hrs, along with refraining from caffeine/supplements for 12hrs prior, and consuming any food for 4hrs. Study administrators will verify that you have replicated your food and liquid consumption for 24hrs prior to the trial. If you do not adhere to the 24hr food replication procedure, you will be re-scheduled for a later date.
9. You will again put on the mouthpiece and heart rate strap while standing so we can collect resting heart rate and expired gases. The following trials will be randomized and consist of three different conditions: a control (no-vest) trial, 5% of body weight, and 10% of body weight. If the trial consists of running with a weighted vest, the added mass will be first secured onto your shoulder/torso using a weighted vest prior to a 5min warm up. You will then run a 5min warm-up at a speed of your choosing with or without a weighted vest using either an additional 5% or 10% body weight. You will then run a second VO_{2max} test in the appropriate condition that will use 3min stages at pre-designated exercise intensities such as 60, 65, 70, 75, 80% VO_{2max} and VO_{2max} . The running velocities that correspond with the given exercise intensities will be determined from the first test. After the 80% VO_{2max} stage, 1min stages will be used to shorten the time required to reach your VO_{2max} . The remaining exercise bouts will follow this protocol. At the end of this test, the mouthpiece and noseclip will be removed and you will be given time to cool down.
10. Prior to leaving, you will be given a copy of your food recall to replicate prior to the next exercise tests and schedule the next visit.

Third Visit

1. You will arrive to the lab (the same location as visit 1) having not exercised for 24hrs, along with refraining from caffeine/supplements for 12hrs, and consuming any food for 4hrs. Study administrators will verify that you have replicated your food and liquid consumption for 24hrs prior to the trial. If you do not adhere to the 24hr food replication procedure, you will be re-scheduled for a later date.

2. You will be fitted with a heart rate monitor, noseclip, and mouthpiece and we will then collect resting heart rate and oxygen consumption as in previous trials.
3. You will then run a 5min warm-up with or without a weighted vest at a speed of your choosing. After, you will complete a VO_{2max} test with or without the weighted vest using the 3min staged protocol described above until you voluntarily stop.
4. You will then be allowed time to cool down.

Fourth Visit

1. The 4th visit will be identical to the 2nd and 3rd trials.

Participation in this study will take a total of approximately 3hrs over a period of one week.

What are the possible risks or discomforts of being in this study?

Risks associated with maximal/submaximal exercise testing including the following: brief feelings of nausea, lightheadedness, muscle cramps and soreness, or dizziness. Furthermore, due to the metabolic by-products produced during intense exercise, short-term general discomfort throughout your muscles is likely.

Added weight while running has added risks of soreness or injury to bone and joints. We have tried minimize the additional stress by minimizing your time running with the vest and having you run on a treadmill, which provides a soft surface compared to running on the ground. There are risks of stress, emotional distress, injury, and inconvenience associated with participating in this study.

Every effort will be made to protect the information you give us. However, there is a small risk of loss of privacy and/or confidentiality that may result in hardship or inconvenience. If you have any follow up questions regarding the risks and discomforts, feel free to ask any of the researchers throughout the study.

How will my information be kept confidential?

Your name and other identifying information will be maintained in locked files, available only to authorized members of the research team for the duration of the study. For any information entered into a computer, the only identifier will be a unique study identification (ID) number. All data will be destroyed 5 years after data analysis is complete. Any personal identifying information and any record linking that information to study ID numbers will be destroyed when the study is completed. Information resulting from this study will be used for research purposes and may be published; however, you will not be identified by name in any publications.

Information from your participation in this study may be reviewed by federal and state regulatory agencies, and by the UNM Institutional Review Board (IRB), which provides regulatory and ethical oversight of human research. There may be times when we are required by law to share your information. However, your name will not be used in any reports about this study.

What are the benefits to being in this study?

There is no direct benefit to you from being in this study. However, your participation may help you find out more about your body in terms of body composition, aerobic capacity, and how your body responds to a new form of exercise.

What other choices do I have if I don't participate?

Taking part in this study is voluntary so you can choose not to participate.

Will I be paid for taking part in this study?

No compensation is available for participating in this study.

What will happen if I am injured or become sick because I took part in this study?

If you are injured or become sick as a result of this study, The University of New Mexico (UNM) will provide you with emergency treatment at your cost.

No commitment is made by UNM to provide free medical care or money for injuries to participants in this study.

In the event that a medical emergency occurs due to your participation in the study, 1st responders will be notified and appropriate care will be provided. If you have an injury or illness that is caused by your participation in this study, reimbursement for all related costs of care will be sought from your insurer, managed care plan, or other benefits program. If you do not have insurance, you may be responsible for these costs. You will also be responsible for any associated co-payments or deductibles required by your insurance.

It is important for you to tell the investigator immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Office of Institutional Review Board (OIRB) at (505) 277-2644 for more information.

How will I know if you learn something new that may change my mind about participating?

You will be informed of any significant new findings that become available during the course of the study. Further, you will be notified to any changes in risks or benefits resulting from participating in the research. Any new alternative to participation that might change your mind about participating you will also be made aware of.

Can I stop being in the study once I begin?

Yes. You can withdraw from this study at any time without affecting your access to future health care or other services to which you are entitled.

The investigators have the right to end your participation in this study if they determine that you no longer qualify to take part. Your participation could cease if you do not follow study procedures, if it is in your best interest, or the study's best interest to stop your participation.

HIPPA authorization for Use and Disclosure of Your Protected Health Information (HIPPA)

As part of this study, we will be collecting health information about you and sharing with others. This information is “protected” because it is not identifiable or “linked” to you.

Protected Health Information (PHI)

By signing this Consent Document, you are allowing the investigators and other authorized personnel to use your protected health information for the purposes of this study. This information may include: personal health information, and testing results. In addition to researchers and staff at UNM and other groups listed in this form, there is a chance that your health information may be shared (re-disclosed) outside of the research study, and no longer be protected by federal privacy laws. Examples of this include disclosures for law enforcement, judicial proceeding, health oversight activities and public health measures.

Right to Withdraw Your Authorization

Your authorization for the use and disclosure of your health information for this study shall not expire unless you cancel this authorization. Your health information will be used or disclosed as long as it is needed for this study. However, you may withdraw your authorization at any time provided you notify the UNM investigators in writing. To do this, please send a letter notifying them of your withdrawal to:

Len Kravitz

MSC 04 2610

1 University of New Mexico

Albuquerque, New Mexico 87131

Please be aware that the research team will not be required to destroy or retrieve any of your health information that has already been used or shared before your withdrawal is received.

Refusal to Sign

If you choose not to sign this consent form and authorization for the use and disclosure of your PHI, you will not be allowed to take part in the research study.

What if I have questions or complaints about this study?

If you have any questions, concerns or complaints at any time about the research study, Len Kravitz, PH.D. or his associates will be glad to answer them at (505) 277-4136 Monday-Friday 8am-5pm. If you need to contact someone after business hours or on weekends, please call (530) 237-7694 and ask for Troy Purdom. If you would like to speak with someone other than the research team, you may call the UNM IRB office at (505) 277-2644. The IRB is a group of people from UNM and the community who provide independent oversight of safety and ethical issues related to research involving human participants.

What are my rights as research participant?

If you have questions regarding your rights as research participant, you may call the Office of Institutional Review Board at Tel: (505) 277-2644, or via website at <http://irb.unm.edu>.

Consent and Authorization

You are making a decision whether to participate in this study. Your signature below indicates that you read the information provided (or the information was read to you). By signing this Consent Form, you are not waiving any of your legal rights as a participant.

I have had an opportunity to ask questions and all questions have been answered to my satisfaction. By signing this Consent Form, I agree to participate in this study and give permission for my health information to be used or disclosed as described in this Consent Form. A copy of this Consent Form will be provided to me.

_____ / _____

Name of Research Participant

Signature of Participant

Date

I have explained the research to the participant and answered all of his/her questions. I believe that he/she understands the information in this consent form and freely consent to participate.

_____ / _____

Name of Research Team Member

Signature of Research Team Member Date

APPENDIX B



DATE: May 3, 2016

REFERENCE #: 02716

PROJECT TITLE: [867698-5] The Acute Effects of Weighted Vest Running on Substrate Oxidation and Energy Expenditure

PI OF RECORD: Dr. Len Kravitz, Ph.D.

SUBMISSION TYPE: Response/Follow-Up

BOARD DECISION: APPROVED

EFFECTIVE DATE: May 2, 2016

EXPIRATION DATE: February 24, 2017

RISK LEVEL: More than Minimal Risk

REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Full Committee

SUBPART DECISION: Not Applicable

PROJECT STATUS: Active - Open to Enrollment

DOCUMENTS:

- HIPAA Consent/Authorization - Consent Form (UPDATED: 04/28/2016)
- Letter - Response Letter (UPDATED: 04/28/2016)
- Amendment/Modification - Amendment Application Form (UPDATED:04/13/2016)
- Application Form - Project Team Form (UPDATED: 04/13/2016)
- Conflict of Interest - Declaration - Nathan FCOI (UPDATED: 04/13/2016)
- Protocol - Protocol (UPDATED: 04/13/2016)
- Questionnaire/Survey - Health History Questionnaire (UPDATED: 04/13/2016)
- Training/Certification - CITI Galen (UPDATED: 04/13/2016)
- Training/Certification - CITI Nathan (UPDATED: 04/13/2016)

Full Committee review of this submission occurred on April 27, 2016 and requested modifications were reviewed using Expedited procedures on May 2, 2016.

Thank you for your submission of Response/Follow-Up materials for this project. The University of New Mexico (UNM) IRB Main Campus has APPROVED your submission. This approval is based on an acceptable risk/benefit ratio and a project design wherein the risks to human participants have been minimized.

This determination applies only to the activities described in the submission and does not apply should any changes be made to this research. If changes are being considered, it is the responsibility of the Principal Investigator to submit an amendment to this project for IRB review and receive IRB approval prior to implementing the changes. A change in the research may disqualify this research from the current review category.

The IRB has determined the following:

_____/_____/_____ average

Do you train in any activity (eg. jogging, cycling, swimming, weight-lifting)? Y N

How well trained are you? _____

Vigorous Exercise (>30 Minute sessions)

_____ Minutes/hours a week



WOMEN ONLY

Please check the response that most closely describes your menstrual status:

_____ Post-menopausal (surgical or absence of normal menstrual periods for 12 months)

_____ Eumenorrhic – Normal menstrual periods (~every 28 days)

_____ Amenorrhic – Absence of normal menstrual periods for at least 3 months

_____ Oligomenorrhic – Irregular menstrual periods with occasional missed cycles.



_____ Subject # _____

Name of Participant (print)

Phone #: home _____ cell _____ Phone

(W) _____

Date of Birth ___/___/___ Age _____

Address (home) _____ zip _____

email _____

Primary health care provider and health insurance _____

(Only for information/emergency contact)

Person to contact in case of emergency: **name** _____ **phone #** _____

APPENDIX D

VO2 Max Trial WVR and Substrate Utilization

Subject # _____

Study # 867698-4

Date/Time _____/_____/_____

TRIAL: Demographic Control 5% 10%

AGE: _____ yrs

HEIGHT: _____ cm _____ in

WEIGHT: _____ kg _____ lbs

Siri/ Brozak BODY FAT % _____

Women: _____ triceps _____ suprailiac _____ thigh

Men: _____ chest _____ abdomen _____ thigh

Ex Intensity (%) VO _{2max}	Stage (1min)	Speed (mph)	VO ₂ (ml/kg•min)	RER	HR (b/min)	RPE	Fatox g/min	CHOox g/min	Fatox (%)	CHOox (%)	Kcal/min
Resting	Standing	-----									
60% (3min Stage)	1										
65% (3min Stage)	2										
70% (3min Stage)	3										
75% (3min Stage)	4										
80% (3min Stage)	5										
(1min Stage)	6										
(1min Stage)	7										
(1min Stage)	8										
(1min Stage)	9										
	10										
	11										
	12										
	13										
	14										
	15										

VO2max: Breath by Breath _____ ml/kg/min 15sec _____ ml/kg/min 1min _____ ml/kg/min
 11br _____ ml/kg/min

NOTES: _____

APPENDIX E

Trained Male & Female Runners Needed

Institutional Review Board

Number: 02716
Version: 03/09/16
Approved: 03/15/16
Expires: 02/24/17**for a research project at the
UNM Exercise Physiology Lab
OIRB# 867698-1**

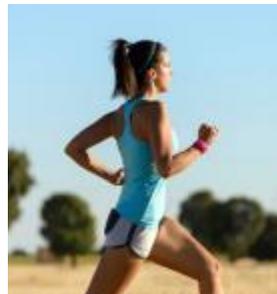
What is the study about? Testing fat oxidation energy expenditure changes with weighted vest running.

Who can volunteer? Men and women who regularly participate in running activities between the ages of 18-45 yrs. Need to be regularly training at a minimum of ~10miles/wk.

Is there any compensation for completing this study? There will be no monetary compensation for this study.

Who can I contact for more information?

Troy Purdom, 530-237-7694, tpurdom@unm.edu; Len Kravitz, 505-277-4136 lkraivitz@unm.edu



APPENDIX F

Electronic Media Prompts

Prompt intended for Facebook, Email, Forums, & Listserves:

“Hello Runners,

My name is Troy Purdom, a 4th year Ph.D. student here at UNM studying the effects of weighted vest running on fat utilization and total energy expenditure, which is relevant for training applications and weight management programs. I am looking for potential participants who are trained runners, both men and women aged 18-45yrs old whom have been running for at least 6mo.

If you or anyone you know are interested in this study, please contact me at 530-237-7694, or via email at tpurdom@unm.edu. My advisor and primary investigator of the study is Dr. Len Kravitz can be contacted at 505-277-4136, or via email at lkravitz@unm.edu.”

Twitter:

“Trained runners needed for a study conducted at the UNM Exercise Physiology Lab. The study is testing weighted vest running on fat utilization. Men and women aged 18-45 who have been training for ~6mo are encouraged to contact Troy Purdom @ 530-237-7694.”

APPENDIX G

24hr RECALL INSTRUCTIONS

- Please write down all foods and beverages consumed for a 24-hour time period. Each day record the time you began and continue until either the trial or 24hrs has concluded.
- List the approximate time the meal was consumed, place where it was consumed (home, work, name of restaurant, church, etc.), and the type of eating occasion or meal (breakfast, lunch, dinner, snack, or other).
- List each Food/Beverage item you consumed, including foods eaten between meals and all drinks, even if it is a non-caloric item like water, coffee, tea, or sugar free gum.
- Specify Details/Ingredients/Preparation of each food or beverage consumed.
- Record the Amount of each food or beverage consumed. Portion sizes can be recorded in a variety of ways, please use the method that works best for you. Portion sizes can be recorded using the following standard measurements:
 - Weight in grams or ounces (Not fluid ounces)
 - Solid foods – use volume in cups, tablespoons or teaspoons
 - Liquids – use volume in fluid ounces
 - Fraction of the whole (e.g. 1/8 of 9” pie)
 - Dimensions for the following shapes:

