The Relationship Between Habitual Incidental Physical Activities
And Cardiovascular Disease Risk

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ABSTRACT

**Purpose:** The purpose of this study was to examine the association between the intensity and duration of incidental physical activity (IPA) with cardiovascular health markers. **Methods:** 41 participants (ages 18-70) were assessed for cardiovascular disease risk and wore an activPAL activity monitor for 7 consecutive days to track habitual IPA (light intensity activity [LPA] + sporadic < 10 min duration moderate to vigorous physical activity [MVPA]). Total and compartmental body composition were derived from dual energy x-ray absorptiometry, vascular hemodynamics of the carotid and popliteal arteries were assessed with Doppler ultrasonography, cardiorespiratory fitness was obtained from maximal aerobic capacity via indirect calorimetry, and inflammatory (tumor-necrosis-factor-alpha, C-reactive protein) and cardiometabolic (lipid panel, HbA1c, glucose, insulin) biomarkers were obtained from serum concentration of fasting blood samples. Unadjusted linear regressions and multivariate linear regressions adjusting for confounders (age, gender, accelerometer wear time, bouted ≥ 10 min duration MVPA) were used to determine associations between cardiovascular health and IPA outcomes. A p < 0.05 was set for significance and a p < 0.10 was set for interaction. **Results:** IPA (96.4% LPA) and LPA were significantly (p < 0.05) inversely correlated with resting systolic blood pressure (IPA: $r^2 0.122$, p 0.031; LPA: $r^2 0.118$, p 0.035) and triglycerides (IPA: $r^2 0.157$, p 0.018; LPA: $r^2 0.165$, 0.015), and positively correlated with high-density lipoprotein cholesterol (IPA: $r^2 0.252$, p 0.002; LPA: $r^2 0.267$, p 0.001) in univariate linear regressions. Only resting systolic blood pressure remained statistically significant (IPA: $r^2 0.224$, p 0.042; LPA: $r^2 0.227$, p 0.039) after controlling for the abovementioned confounders. Increasing sporadic moderate intensity physical activity (MPA) was significantly associated with decreasing popliteal artery peak systolic diameter ($r^2 0.133$, p 0.025) and peak diastolic diameter ($r^2 0.123$, p 0.031) in univariate linear regressions, but
associations were no longer evident after controlling for the abovementioned confounding variables. Changes observed in high density lipoprotein from tertile 1 to 3 of IPA and LPA accumulation represented a significant (p 0.002) shift (21.4 mg/dL, 95% CI 8.4 – 35.4) from levels of increased cardiovascular disease risk to levels protective of cardiovascular disease. Changes observed in systolic blood pressure from tertile 2 to 3 of IPA and LPA represented a significant (p 0.05) shift (-11.2 mmHg, 95% CI -0.1 – -22.6) from prehypertension to normal blood pressure. **Conclusions:** Results indicate that the duration of daily time spent in IPA and LPA has a greater influence than the intensity of IPA in improving resting systolic blood pressure and lipid profile. Highly sedentary populations may be more sensitive to associations between sporadic MVPA and cardiovascular health markers.
CHAPTER I: INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the U.S., accounting for 1 in 3 deaths (Mozaffarian et al., 2014; S. L. Murphy, Xu, & Kochanek, 2013). Modifiable risk factors attributable to lifestyle including physical inactivity, hyperglycemia, hypertension, overweight and obesity, hypercholesterolemia and hyperlipidemia increase risk for CVD by contributing to atherosclerosis. Atherosclerosis is a slow process of endothelial injury and vascular inflammation, which can begin in childhood and early adulthood before clinical signs of CVD are evident (Ross, 1999; Rubanyi, 1993). Early adulthood presents a clinically significant time point for assessment of CVD risk and introduction of behavior modification interventions to protect against cardiovascular pathogenesis.

Aerobic PA protects against the development of CVD. Earliest evidence of this comes from population studies in the 1950s by Morris et al. among double decker bus driver and conductors where increased PA among conductors was associated with a reduced incidence of coronary heart disease (CHD) as compared to sedentary bus drivers (Morris, Heady, Raffle, Roberts, & Parks, 1953). Since then multiple large-scale epidemiological observational studies and randomized control trials (RCT) have shown that a dose-response relationship exists between aerobic PA and morbidity and mortality due to CVD (D. C. Lee et al., 2011b; Manson et al., 2002; Paffenbarger et al., 1993; Rockhill et al., 2001; Tanasescu et al., 2002; Yu, Yarnell, Sweetnam, & Murray, 2003). The current U.S. federal guidelines for aerobic PA recommends at least 150 weekly minutes of moderate intensity PA (MPA), or 75 weekly minutes of vigorous intensity PA (VPA) accumulated in periods of at least 10 consecutive minutes (United States. Dept. of Health and Human Services. Physical Activity Guidelines Advisory, 2008). These guidelines are based on evidence of an inverse relationship between increasing aerobic PA and
premature mortality from CVD and coronary artery disease (CAD) and risk factors and co-morbidities for these, such as hypertension, hyperglycemia, dyslipidemia, hypercholesterolemia, obesity, and type 2 diabetes mellitus (DM2) (United States. Dept. of Health and Human Services. Physical Activity Guidelines Advisory, 2008).

**Statement of Problem**

It is possible that the federal guidelines’ strong emphasis to accumulate moderate to vigorous intensity PA in durations of 10 min or more is due to the availability of evidence on the health benefits of purposeful (planned and structured) PA in the form of exercise. Data to date are scarce and varied to on the cardiovascular benefits of light intensity PA (LPA) or sporadic (accumulated in bouts < 10 minutes) moderate to vigorous intensity PA (MVPA) for protection from CVD. These types of activities fall under the umbrella of incidental PA (IPA) (Tremblay, Esliger, Tremblay, & Colley, 2007) and are accumulated through non-purposeful (unplanned and unstructured) activities of daily living. Thus IPA can be classified as all non-sedentary activity composed of LPA and sporadic MVPA that does not meet the PA guideline criteria of being accumulated in bouts >10min. While current data may be inconclusive to define prescriptions of LPA or sporadic MVPA for health benefits protective of CVD, such activity should not be overlooked, as non-exercise PA may play an important role in sustaining cardiovascular health. Several CVD risk factors, including plasma triglycerides, HDL cholesterol, plasma glucose, blood pressure, central adiposity, waist girth, and atherosclerosis are inversely related to non-exercise activity (Ball, Brown, & Crawford, 2002; Bertrais et al., 2005; W. J. Brown, Miller, & Miller, 2003; Wendy J. Brown, Williams, Ford, Ball, & Dobson, 2005; Cameron et al., 2003; D. Dunstan et al., 2005; D. W. Dunstan et al., 2007; D. W. Dunstan et al., 2004; E. S. Ford, Kohl, Mokdad, & Ajani, 2005; Fung et al., 2000; Gao, Nelson, & Tucker, 2007; Gustat, Srinivasan,
Elkasabany, & Berenson, 2002; G. Healy et al., 2007; Jakes et al., 2003; Kronenberg et al., 2000; J. A. Levine et al., 2005; Mummery, Schofield, Steele, Eakin, & Brown, 2005; Salmon, Bauman, Crawford, Timperio, & Owen, 2000).

Current PA trends suggest that adherence to the 2008 federal aerobic PA guidelines is low among adults ranging from 5% using objective estimates (R. P. Troiano et al., 2008) to 21% in self reports (Statistics, 2013), and only slightly more than half of the U.S. college age (18-25) adult population participates in the regular MVPA dose advocated for protection against CVD (Pescatello & American College of Sports, 2014b). It is unclear whether habitual PA accumulated in either LPA or sporadic bouts of MVPA <10 min through the activities of daily living have an inverse dose-response relationship with risk factors and clinical markers of CVD. Ross and McGuire (2011) noted a significant positive association between both duration and intensity of IPA with cardiorespiratory fitness (CRF) in healthy inactive middle-aged men and women (McGuire & Ross, 2011). Since increasing CRF decreases the risk of CVD morbidity and mortality (S. Blair et al., 1995; D. C. Lee et al., 2011b; X. Sui, M. Lamonte, & S. Blair, 2007; Xm Sui, Mj Lamonte, Jn Laditka, et al., 2007), Ross and McGuire’s findings suggest that health benefits attainable from regular PA may not be exclusive to MVPA accumulated in bouts greater than 10 minutes. Additionally, there is no evidence on associations between IPA and other CVD risk factors.

In 2003, the American Heart Association (AHA) and the Centers for Disease Control (CDC) reported that strong associations exist between several inflammatory and cardio-metabolic blood biomarkers and CVD risk, and advocated for the use of these biomarkers in clinical CVD assessment (T. A. Pearson et al., 2003). C-reactive protein (CRP) and Tumor Necrosis Factor Alpha (TNF- alpha) are two important inflammatory biomarkers evident in CVD
pathogenesis (John Danesh, Collins, Appleby, & Peto, 1998; J. Danesh et al., 2000; P. M. Ridker, M. J. Stampfer, & N. Rifai, 2001; Tuomisto, Jousilahti, Sundvall, Pajunen, & Salomaa, 2006). There is strong consensus establishing the role of inflammation in the atherosclerotic process of CVD (Ross, 1999; Tracy, 1998), and aerobic PA has been shown to inhibit the biological inflammatory cascade (Mark Hamer et al., 2012; Kasapis & Thompson, 2005).

Additionally, several studies have explored the relationship between sedentary behavior (SED) and blood biomarkers of CVD (G. Healy et al., 2007; Healy, Matthews, Dunstan, Winkler, & Owen, 2011; G. Healy et al., 2008; E. Levitan, Y. Song, E. Ford, & S. M. Liu, 2004; McGuire, Ross, & Earnest, 2011; Owen, Healy, Matthews, & Dunstan, 2010). While results are mixed, these studies suggest SED and low caloric energy expenditure accompanying it may be key contributors in the development of CVD risk biomarkers. Breaking up SED with intermittent PA may be beneficial for cardiovascular health and yield comparable results to participation in planned aerobic PA. Yet, no research to date has examined the relationship between habitual IPA and CVD blood biomarkers in healthy inactive adults. Inactive individuals can be defined as those who fail to meet the federal PA guidelines advocated for health benefits.

In addition to the commonly assessed blood biomarkers discussed above, several non-invasive measures of vascular and cellular metabolism may be used to improve our understanding of PA and CVD risk. Structural and functional measures of vascular health that correlate with CVD risk include but are not limited to arterial hemodynamics (arterial peak systolic velocity and lumen diameter) (Grant et al., 2003), intima media thickness (Bots, Hoes, Koudstaal, Hofman, & Grobbee, 1997; Bucciarelli, Sramek, Reiber, & Rosendaal, 2002), and endothelial function (Celermajer et al., 1992; Verma, Buchanan, & Anderson, 2003). The relationship between habitual IPA and these factors is unknown.
Statement of Purpose

The purpose of this study is to examine associations between the volume and intensity of objectively measured habitual IPA (LPA + sporadic MVPA) with various CVD risk factors including lipid, metabolic and, inflammatory blood biomarkers and variables describing arterial structure and function among healthy inactive adults (age: 18-70).

Delimitations

Given the cross-sectional nature of this study, the scope of the results are limited to associative relationships between IPA and CVD risk measures. Thus, no causal relations between IPA and cardiovascular health can be inferred from the results of the study.

Limitations

This study is limited to the assessment of to the relationship between PA and cardiovascular health, and does not factor in the role of diet in CVD risk factors.

Hypotheses

1. A greater volume of IPA accumulation will have a stronger inverse relationship with CVD risk factors compared lower volume.

2. Higher intensity bouts of IPA will have a stronger inverse relationship with CVD risk factors compared to lower intensity bouts.
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Active Energy Expenditure</td>
<td>AEE</td>
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<td>American Heart Association</td>
<td>AHA</td>
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<td>Centers for Disease Control and Prevention</td>
<td>CDC</td>
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<td>C-Reactive Protein</td>
<td>CRP</td>
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<td>Cardiorespiratory Fitness</td>
<td>CRF</td>
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<tr>
<td>Cardiovascular Disease</td>
<td>CVD</td>
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<tr>
<td>Coronary Artery Disease</td>
<td>CAD</td>
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<tr>
<td>Coronary Heart Disease</td>
<td>CHD</td>
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<tr>
<td>Diastolic Blood Pressure</td>
<td>DBP</td>
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<tr>
<td>Dual Energy X-ray Absorptiometry</td>
<td>DEXA</td>
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<td>Flow Mediated Dilation</td>
<td>FMD</td>
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<td>High Density Lipoprotein</td>
<td>HDL</td>
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<td>Glycosylated Hemoglobin</td>
<td>HbA1c</td>
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<td>Incidental Physical Activity</td>
<td>IPA</td>
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<td>Interleukin-6</td>
<td>IL-6</td>
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<td>Intima Media Thickness</td>
<td>IMT</td>
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<td>Low Density Lipoprotein</td>
<td>LDL</td>
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<td>Light Intensity Physical Activity</td>
<td>LPA</td>
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<td>Maximal Oxygen Uptake</td>
<td>VO$_2$max</td>
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<td>Metabolic Equivalent</td>
<td>MET</td>
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<tr>
<td>Moderate Intensity Physical Activity</td>
<td>MPA</td>
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<tr>
<td>Moderate to Vigorous Intensity Physical Activity</td>
<td>MVPA</td>
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<tr>
<td>National Health &amp; Nutrition Examination Survey</td>
<td>NHANES</td>
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<tr>
<td>Non-Exercise Activity Thermogenesis</td>
<td>NEAT</td>
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<tr>
<td>Physical Activity</td>
<td>PA</td>
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<tr>
<td>Resting Metabolic Rate</td>
<td>RMR</td>
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<tr>
<td>Sedentary Behavior</td>
<td>SED</td>
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<tr>
<td>Systolic Blood Pressure</td>
<td>SBP</td>
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<tr>
<td>Total Energy Expenditure</td>
<td>TEE</td>
</tr>
<tr>
<td>Tumor Necrosis Factor-alpha</td>
<td>TNF-alpha</td>
</tr>
<tr>
<td>Type II Diabetes Mellitus</td>
<td>DM2</td>
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<tr>
<td>Vigorous Intensity Physical Activity</td>
<td>VPA</td>
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CHAPTER II: REVIEW OF LITERATURE

The following chapter reviews associations of PA and SED with health and CVD risk factors. An overview of traditional biomarkers and noninvasive techniques for screening CVD risk are also presented.

PA and Health

Classification of PA, Inactivity, and SED

While data supporting cardiovascular health benefits from PA really heavily on RCTs of structured exercise interventions, there is limited evidence on the cardiovascular health effects of non-purposeful PA and LPA (Garber et al., 2011). PA is any bodily movement due to muscle contraction that results in energy expenditure above resting metabolic rate (RMR) of 1 metabolic equivalent (MET)(Caspersen, Powell, & Christenson, 1985). One met is equivalent to 3.5 ml of O$_2$ consumed/kg body weight/ min or 1 kcal/kg/hour (Ainsworth et al., 2011; Balke, 1960; E. Howley, 2000; United States. Dept. of Health and Human Services. Physical Activity Guidelines Advisory, 2008). Exercise is a specific subset of PA that is planned, structured, and usually repetitive to improve or maintain physical fitness (Caspersen et al., 1985).

METs are also the unit of measure used to quantify activity by absolute intensity. PA intensity can be measured as the ratio of a person’s work metabolic rate to their RMR (Ainsworth et al., 2011). PA categorization by intensity level into LPA, MPA, and VPA are based on MET thresholds. Absolute MET thresholds for activity intensity are as follows: SED < 1.5 METs, LPA > 1.5 and < 2.99 METs, MPA ≥ 3.0 and < 6.0 METs, and VPA ≥ 6 METs (Norton, Norton, & Sadgrove, 2010; Pate, Neill, & Lobelo, 2008).

SED encompasses almost all resting activities, such as sleeping, sitting quietly, sitting reading, sitting watching television, riding a car, or standing quietly (Ainsworth et al., 2011).
Compared to SED, physical inactivity has been distinctly defined as failing to meet the federal aerobic PA guidelines (McGuire & Ross, 2011, 2012), and is defined as such in the context of this study.

Example of LPAs in domestic, occupational, and leisure activities of daily living include dishwashing, laundry tasks, ironing, cooking, eating, performing office duties at a desk or computer station, gardening, light walking or other household and occupational tasks (Ainsworth et al., 2011; Gunn et al., 2002). LPAs encompass the majority of habitual behaviors required for daily living, and have been shown to be a major contributing factor of energy expenditure variability across individuals (Donahoo, Levine, & Melanson, 2004). Objectively assessed (accelerometers) estimates indicate that LPA comprises of approximately 39% (6-7 hours) of overall PA in an average adult’s pattern of daily activity during waking hours (G. N. Healy et al., 2007). Therefore, it represents a substantial component of energy expenditure that should not be overlooked.

Most MPA is typically accumulated in durations ≥ 10 minutes because they are usually purposeful or planned activities. Examples of occupational and leisure MPAs include regular walking, brisk walking, light swimming, recreational sports, carrying light loads or bicycling at a regular pace ((AIHW), 2003; "The International Physical Activity Questionnaire (IPAQ)," 2005). However, MPA can also be accumulated in shorter durations through non-leisure intermittent PA, such as using the stairs over the elevator. Exercise prescriptions usually comprise of MPA because studies have shown it to provide sufficient physiological stress to protect the body from chronic disease and premature mortality.
VPA encompasses exercise type activities such as jogging, fast cycling, aerobics, competitive sports, or heavy lifting that physiologically stress the cardiovascular system to the point where one has to breathe hard and puff or pant; ((AIHW), 2003).

**PA Guidelines for Health Benefit**

In 2008 the U.S. federal government updated their recommendations, which included those on aerobic PA necessary to maintain adequate CRF for good health. These latest PA guidelines placed a strong emphasis on frequency, volume, intensity and dose of PA necessary to maintain general health. The guideline’s are as follows: “most adults should engage in moderate-intensity cardiorespiratory exercise training for \( \geq 30 \) minutes per day on \( \geq 5 \) days per week for a total of \( \geq 150 \) minutes per week, vigorous-intensity cardiorespiratory exercise training \( \geq 20 \) minutes per day on \( \geq 3 \) days per week, or a combination of moderate and vigorous intensity exercise to achieve a total energy expenditure of \( \geq 500-1000 \) MET minutes per week” accumulated in exercise bouts \( \geq 10 \) min (Garber et al., 2011). These recommendations stem from evidence linking regular PA and exercise with decreased risk for development of major chronic diseases, such as CHD, stroke, DM2 and CVD (United States. Dept. of Health and Human Services. Physical Activity Guidelines Advisory, 2008). Given that CHD and CVD are the leading causes of death in the U.S. (S. L. Murphy et al., 2013), it is a particularly important target in the promotion of PA among the American public.

**Dose Response for Attainment of Health Benefits from PA**

**Dose Response: Volume of PA**

Volume recommendations of PA stem largely from epidemiological studies that assessed associations between levels of planned and structured PA quantified in terms of energy expenditure, such as kilocalories per week, or MET-hours (or minutes) per week (Garber et al.,
Weekly energy expenditures of approximately 1000 Kcal of MPA have been shown to be associated with a lower incidence of CVD morbidity and mortality in large prospective cohort studies (I. M. Lee, Rexrode, Cook, Manson, & Buring, 2001; Manson et al., 2002; Sesso, Paffenbarger, & Lee, 2000; Tanasescu et al., 2002). 1000 kcal of energy expenditure can be achieved through accumulation of 10 weekly MET-hours, which is equivalent to either 150 weekly minutes of MPA or 75 weekly minutes of VPA (Garber et al., 2011). In addition, significant risk reductions in CVD morbidity and mortality have been observed in individuals meeting as little as half of this weekly energy expenditure volume recommendation at 500 kcal per week (I. min Lee & Skerrett, 2001; Manson et al., 2002; Sesso et al., 2000; Tanasescu et al., 2002).

**Dose Response: Intensity of PA**

Epidemiological studies to date have only quantified the intensity dose response relationship to health benefits from exercise, which by definition falls under the umbrella of MVPA. In addition, most studies examining the effects of exercise training on chronic disease outcomes have not controlled for total volume of energy expenditure (Shephard, 2001). As a result, studies concluding that VPA is more favorable than MPA for chronic disease protection are confounded by greater total volumes of energy expenditure in the VPA groups (Garber et al., 2011).

Exercise Intensity has been found to be an important determinant of the positive physiological outcomes of aerobic training on health (Fletcher et al., 2001; Medicine, 1998; Pescatello & American College of Sports, 2014b). However, a large portion of the evidence supports higher intensity exercise for beneficial CRF and risk factor adaptations (Asikainen et al., 2003; Helgerud et al., 2007; Rognmo, Hetland, Helgerud, Hoff, & Slordahl, 2004; Swain,
2005; Warburton et al., 2005) with fewer data on lower exercise intensity thresholds (Butcher et al., 2008; Dipietro, Dziura, Yeckel, & Neufer, 2006). Additionally, these studies are confounded by inconsistencies in the initial state of fitness, health, and conditioning of the subjects (Garber et al., 2011). As a result, it is unclear if an intensity threshold for physiological adaptions exists in highly sedentary individuals. One of the few interventional studies on threshold intensity for cardiometabolic modification found significant improvements in resting glucose utilization of SED elderly men and women engaged in 300 kcal VPA sessions on 4 days per week for 9 months, but not in those engaged in MPA with the same exercise energy expenditure (Dipietro et al., 2006). Significantly, this finding was contradicted by a later study (Butcher et al., 2008), which found that sedentary subjects who walked a fixed volume of 10,000 steps on 3 days per week at a self selected pace for 8 weeks improved lipoprotein profiles and lipid clearance from the vasculature. While Butcher and colleagues did not control for intensity or energy expenditure, the walking modality of the intervention suggests the activity was below the vigorous threshold Di Pietro and colleagues indicate is necessary for cardiometabolic physiological adaptations. These inconsistencies indicate further research is necessary to establish the role of PA intensity for sustaining cardiovascular health.

Therefore, the largest barrier to prescribing PA of intensities below 3.0 METs for cardiovascular health benefits seems to be that epidemiological studies of PA and chronic disease incidence have not quantified whether benefits of PA occur along an energy expenditure continuum because most have subjectively measured PA. The question of whether higher accumulation of total habitual energy expenditure results in greater protection from CVD regardless of the intensity utilized to achieve such energy expenditure remains to be answered.
Another important component of the PA guidelines recommended for cardiovascular health is that PA must be accumulated in a specific pattern to induce significant health benefits. The current guidelines suggest that MPA is beneficial for cardiovascular health only when accumulated in bouts $\geq 10$ minutes to sum up to at least 30 minutes per day of PA (Haskell W.L., 2007; Nelson et al., 2007; Pescatello & American College of Sports, 2014a; Shephard, 2001; United States. Dept. of Health and Human Services. Physical Activity Guidelines Advisory, 2008). This guideline stems from a comparatively larger body evidence that long bouts of PA are more effective than short bouts to improve CRF (Gordon-Larsen et al., 2009; Mark Hamer & Chida, 2008). Given that the necessary intensity of PA to provide health benefits, including improvements in CRF, may differ depending on individual basal levels of activity, there is some evidence (I. M. Lee, Sesso, & Paffenbarger, 2000) to suggest exercise durations shorter than 10 minutes result in fitness and health benefits for sedentary individuals. A study of exercise duration and prediction of CAD by Lee, Sesso, and Parfenbarger (2000) noted that overall energy expenditure is more important than accumulating PA in 10-min bouts to reduce CVD risk. The PA guidelines can be misunderstood to imply that PA obtained below moderate intensity or in episodes of less than 10 minutes may not confer health benefits and reduce CVD risk. Additionally, there is recent cross-sectional and prospective data supporting a beneficial role of LPA in reducing CVD risk, which suggests that just breaking up and limiting SED with PA (LPA or IPA) is associated with improvements in various CVD risk factors, such as triglycerides, HDL, CRP, glucose metabolism, insulin resistance, and waist circumference (Healy et al., 2011; G. N. Healy et al., 2007; G. N. Healy et al., 2008; E. B. Levitan, Y. Song, E. S. Ford, & S. Liu, 2004; McGuire et al., 2011; Owen et al., 2010).
IPA, distinct from planned and structured PA, is expressed as the combination of LPA and any sporadic MVPA (accrued in bouts ≤ 10 min) because it may or may not be purposeful and habitual (McGuire & Ross, 2011, 2012). These non-exercise accumulations of PA can result from short bouts of being active, such as taking the stairs over the elevator, walking to and from ones’ car, grocery shopping, playing with ones’ dog, washing dishes, cleaning and other household chores. Therefore, a large portion of daily activities may qualify as IPA. Evidence to show the impact of LPA on CVD risk reduction is limited to cardio-metabolic markers, and evidence to show the association of sporadic MVPA with cardiovascular health is sparse. Yet few cross-sectional studies of IPA shed light on the need for further examination of IPA’s relationship to CVD risk factors (McGuire & Ross, 2011, 2012).

Effects of PA on Specific CVD Risk Factors

Inflammation

Atherosclerosis: The Inflammatory Etiology

To understand the role of risk factors in the progression of CVD it is important to examine the etiology of CVD development due to atherosclerosis. Atherosclerosis is a slow and steady process of inflammation that can begin as early as childhood (Ross, 1999; Tracy, 1998). The first identifiable steps in atherosclerosis are arterial endothelial cell injury resulting from varying primary and secondary factors (e.g. blood flow turbulence elevated LDLs, hyperglycemia and hypertension), proceeded with inflammation, which promotes the accumulation of harmful oxidized lipids at the injury sites (Ross, 1999). Endothelial cell injury is characterized by atherogenesis, which is the formation of abnormal lipid masses on the arterial wall known as “fatty streaks” (Ross, 1999). These are commonly evident in children as a purely inflammatory lesion consisting of reparative immune cells (macrophages and T-lymphocytes)
In adolescence and adulthood, and with the onset of hypercholesterolemia, these “fatty streaks” are accompanied with the deposition of oxidized lipids (Napoli et al., 1997; Stary et al., 1994). Oxidized lipids progress to form a fibrous fatty lesion, or cap, and ultimately a hardened fibrous plaque (Ballinger, 2005). This process of inflammatory injury and repair ultimately leads to endothelial dysfunction, which alters the hemostatic properties of the endothelium, impairing its vasodilatory and vasoconstrictive properties. In a state of dysfunction, the endothelium has increased adhesiveness and permeability to leukocytes and platelets, which promote coagulation at the injury site (Ross, 1999). Additionally, this pro-inflammatory state stimulates the endothelium to release harmful vasoactive molecules, cytokines, and growth factors (Ross, 1999) that produce a chronic low-grade inflammatory state. This process continues indefinitely if the inflammatory cascade is not halted through neutralization of the molecules injuring the vessel. This ultimately leads to vascular “remodeling” characterized by recruitment and proliferation of smooth muscle cells that form a fibrous cap (plaque), thickening the arterial wall and narrowing the lumen and occluding blood flow (Ross, 1999). Arterial wall thickening stiffens the arterial wall and decreases compliance and distensibility (Cecelja & Chowienczyk, 2012). Occlusion results in a higher pulse pressure and pulse wave velocity of blood in order to maintain the steady blood flow rate needed to meet the body’s oxygen demands (Cecelja & Chowienczyk, 2012). These two factors create a dangerous high pressure and low elasticity arterial state that can lead to clinical manifestations of CVD including plaque rupture and clot formation, peripheral and myocardial ischemia, and heart attack or stroke.
**PA Mitigates the Inflammatory Cascade**

PA can attenuate CVD risk by halting the endothelial inflammatory cascade. The most studied markers of chronic low-grade inflammation that have been examined in relation to PA are IL-6, TNF-alpha and CRP. PA reduces the expression of these inflammatory markers in several ways. PA results in increased energy expenditure that reduces risk of accumulating excess visceral adipose tissue, which promotes inflammation (Gleeson et al., 2011). PA also reduces the risk of atherogenic inflammation by improving lipid profiles through the reduction of free floating triglycerides and LDLs (Kraus et al., 2002), in effect decreasing the likelihood of oxidized lipid accumulation on the vascular walls.

Some proposed mechanisms of the mitigatory effects of PA on selected inflammatory markers are as follows. Anti-inflammatory cardiovascular health benefits from PA have been proposed to arise from the suppression of TNF-alpha and subsequent protection from TNF-alpha induced insulin resistance (Petersen & Pedersen, 2005). Increased levels of TNF-alpha also contribute to CVD by promoting hyperglycemia and hypertension. There is minimal evidence on the relationship between PA that falls below the threshold of exercise (MVPA) and the inflammatory cytokine response of TNF-alpha.

Additionally, exercise, markedly increases the release of IL-6 from contracting muscle fibers (Gleeson et al., 2011), which may promote the release of anti-inflammatory cytokines (Steensberg, Fischer, Keller, Moller, & Pedersen, 2003). However, LPA and sporadic MVPA are unlikely to induce these effects. Reports suggest duration of PA is the most important factor determining prolonged systemic IL-6 increases (Fischer, 2006). Short PA bouts or PA bouts of low intensity are unlikely to increase IL-6 concentrations promoting systemic anti-inflammatory effects (Fischer, 2006). Therefore, reduced risk of heart disease attributable minimal increases of
PA in sedentary individuals (Miyashita, Burns, & Stensel, 2008; M. Murphy, Nevill, Neville, Biddle, & Hardman, 2002) is most likely the result of an alternate anti-inflammatory pathway.

The cardio-protective anti-inflammatory (Gleeson et al., 2011; Mark Hamer et al., 2012; M. Hamer & Stamatakis, 2009; Mora, Cook, Buring, Ridker, & Lee, 2007; Rana et al., 2011) and anti-atherosclerotic effects (Mark Hamer, 2007) of PA have been documented both longitudinally and cross-sectionally.

One such longitudinal study (Mark Hamer et al., 2012) looked at 10-year changes in inflammatory markers (cytokines IL-6 and CRP) relative to self-reported frequency and duration of LPA, MPA, and VPA in a cohort of 4289 middle-aged men and women. However, this study did not objectively measure PA, which limits the inference that can be drawn about the dose and pattern response relationship of PA with the reduction in inflammatory markers. Yet, after adjustment for confounders, physically active participants maintaining or increasing their levels of PA over 10-years had lower levels of CRP and IL-6. Since high and low PA groups were stratified by estimated time adhering to the PA guidelines (≥ 2.5 hours per week), no conclusions can be drawn regarding relationships between patterns of accumulating PA and levels of inflammatory markers. Hamer, Sabai, Batty, Shipley et al. established that long term sustained regular PA attenuates increases in vascular inflammation. This is an important finding regarding the role of PA in the prevention of CVD.

Another study (M. Hamer & Stamatakis, 2009) reported that a cluster of biological risk factors arising from physical inactivity, which included CRP, fibrinogen, adiposity, cholesterol, and hypertension, explained between 22.6% and 39.2% of the cardio-protective effects of PA. Differences in inflammatory markers and hypertension accounted for the variance in CVD
incidence. Significantly, engaging in MPA for less than 30min/day reduced cardiovascular risk by approximately 40%.

Additionally, an inverse relationship between PA and CRP levels has also been shown in multiple cross-sectional studies (Abramson & Vaccarino, 2002; Geffken et al., 2001; Isasi et al., 2003; Koenig et al., 1999; Pitsavos et al., 2003; Rohde, Hennekens, & Ridker, 1999; Taaffe, Harris, Ferrucci, Rowe, & Seeman, 2000; Tomaszewski et al., 2003; Wannamethee et al., 2002), but the majority of these have focused on exercise. These studies mostly examined the effects of self-reported exercise on inflammation with little attention to habitual PA. A 20 year prospective study (Wannamethee et al., 2002) of PA changes in elderly men found that individuals who were initially inactive and then became lightly active reduced their CRP levels comparable to lightly active individuals, and those who became inactive to have similar CRP levels to those who remained inactive. Therefore, it was established that sustaining CRP related CVD risk attenuation required regular adherence to PA. Limitations in the measurement of PA, which was done via self-reporting of the participant’s usual PA pattern, limit the inference that can be drawn, but these results suggest high volumes of habitual IPA could be protective of CRP induced inflammation. The association between these two factors needs to be examined along with other inflammatory markers of CVD.

*Importance of mitigating the inflammatory cascade: Links between Inflammation, Insulin Resistance, and Atherosclerosis*

It has not been conclusively determined if IL-6 has an effect on insulin resistance, and only exercise has been shown to increase IL-6 sufficiently to induce systemic anti-inflammatory effects. Unlike IL-6, TNF-alpha and CRP are known to have an effect on insulin resistance, but the role of non-exercise PA on attenuating these inflammatory risk factors remains to be examined. TNF-alpha is particularly important because data suggests it is a key player in
metabolic syndrome, which is a clustering of 3 of 5 metabolic risk factors (Petersen & Pedersen, 2005). Metabolic risk factors are comprised of: 1) an elevated waist circumference, 2) high triglyceride level, 3) low HDL cholesterol, 4) resting hypertension, and 5) fasting hyperglycemia (National Heart, 2011). The 3rd report of the National Cholesterol Education Program’s Adult Treat Panel (2002) has identified 6 factors that are typically associated with metabolic syndrome that contribute to increasing the abovementioned metabolic syndrome risk factors: 1) abdominal obesity, 2) atherogenic dyslipidemia (high triglyceride, low HDL), 3) hypertension, 4) Insulin resistance, 5) glucose intolerance, and 6) pro-inflammatory state (TNF-alpha & CRP) (Grundy et al., 2002).

Evidence of TNF-alpha’s role in metabolic syndrome stems from studies of diabetic patients showing high intramuscular (Saghizadeh, Ong, Garvey, Henry, & Kern, 1996) and free floating plasma (Feingold & Grunfeld, 1992; Mishima et al., 2001; Winkler et al., 1998) levels of TNF-alpha correlated with adipose tissue as a main source of circulating TNF-alpha production (Coppack, 2001; Hotamisligil, Shargill, & Spiegelman, 1993). The physiological pathway by which TNF-alpha contributes to metabolic syndrome is linked to insulin signaling. While this pathway has been difficult to examine in vivo, one study (Halse, Pearson, McCormack, Yeaman, & Taylor, 2001) demonstrated that TNF-alpha impairs glucose uptake regulated by insulin in cultured human cells, and another (Youd, Rattigan, & Clark, 2000) demonstrated the same effect in rats. In addition to having a direct inhibitory effect on insulin mediated glucose uptake, TNF-alpha indirectly causes insulin resistance by stimulating lipolysis (Ryden et al., 2002; Zhang, Halbleib, Ahmad, Manganiello, & Greenberg, 2002) in humans, in vivo. Insulin-resistance induced lipolysis results in a greater than normal release of free-fatty acids from adipose tissue into the blood stream (Botion, Brasier, Tian, Udupi, & Green, 2001; Gasic, Tian, & Green, 1999;
High levels of free fatty acid feed into the negative cycle of atherosclerosis by inhibiting the release of anti-inflammatory adipokines, such as adiponectin. Insulin resistance also creates a state of hyperglycemia whereby the elevated blood glucose competes with oxygen for hemoglobin binding sites forming glycosylated hemoglobin. In addition to limiting the oxygen carrying capacity of the blood, elevated levels of free-floating glucose causes hypertension that further stress the narrowing vasculature.

CRP is a known marker of vascular inflammation, and has received a lot of attention with regards to CVD because of advancements in detection methods. The National Health and Nutrition Examination Survey (NHANES) (1999-2002) collected CRP data of representative sample of the U.S. adult population consisting of 22,403 participants (E. Ford, Giles, Myers, & Mannino, 2003). This has allowed the CDC and AHA to specify CVD risk assessment cut points for CRP: <1.0 mg/dL (low risk), 1.0-3.0 mg/dL (average risk), >3.0 mg/dL (high risk), and anything ≥10 mg/dL associated with injury or bacterial induced inflammation (G. Myers et al., 2004). While the direct physiological pathway by which CRP feeds into the chronic low-grade inflammatory state of atherosclerosis is unclear, hepatic secretion of CRP is known to be mediated by IL-6 and TNF-alpha (Kasapis & Thompson, 2005). CRP levels have also been documented to be higher in individuals with insulin insensitivity or adipocyte-mediated inflammation (McLaughlin et al., 2002; Yudkin, Stehouwer, Emeis, & Coppack, 1999). Nonetheless, there is a great deal of evidence to show a dose response relationship between levels of CRP and risk of CAD (Thomas A. Pearson et al., 2003) and peripheral arterial disease (P. Ridker, M. Stampfer, & N. Rifai, 2001). Meta analyses of prospective population based studies (John Danesh et al., 1998; J. Danesh et al., 2000), including prominent cohorts such as...
NHANES, have shown coronary events are twice as likely to occur in high CRP risk groups compared to low CRP risk groups.

**CRF and CVD**

CRF has an inverse relationship with physical inactivity and poor health, and is protective against CVD. Healthy individuals with higher CRF or those who increase their CRF over time have lower risks of CVD morbidity and mortality (S. N. Blair et al., 1995; D. C. Lee et al., 2011a; Xm Sui, Mj Lamonte, & Sn Blair, 2007; X. Sui, M. J. LaMonte, & S. N. Blair, 2007). Increasing CRF decreases the risk of future clinical cardiovascular events among individuals with pre-existing CVD (Church, Lamonte, Barlow, & Blair, 2005; D. C. Lee et al., 2011a; McAuley et al., 2009; J. Myers et al., 2002; Xuemei Sui et al., 2007). Therefore, substantial evidence indicates CRF is an important link between regular PA and decreased risk of CVD.

**The Relationship Between IPA and CRF**

Ross and McGuire (2011) examined the association of objectively measured IPA with CRF in a population of inactive abdominally obese men and women. They sought to determine whether duration and intensity of objectively measured IPA was associated with CRF (McGuire & Ross, 2011). Ross and McGuire recruited inactive individuals, defined as not meeting the federal aerobic PA guidelines, as this subject population increases the sensitivity to observe changes in CRF with participation in IPA.

PA of 135 abdominally obese middle-aged men and women was assessed with actigraphy using acceleration cut points to quantify intensity. It is important to note subjects in this study had relatively low CRF (mean: men, 31.0 ± 3.9 ml/kg/min; women, 24.9 ± 3.8 ml/kg/min) and accumulated no MVPA in bouts ≥10 min. Despite some accumulation of sporadic MPA, an average of 94% of IPA in this study was accumulated as LPA. Positive significant association of
both duration and intensity of IPA with CRF were found for both men and women after controlling for gender and BMI. However, LPA alone did not significantly correlate with CRF after controlling for gender, BMI, and MPA, whereas sporadic MPA did show to be a significant predictor of CRF after controlling for the same confounders. When clustering individuals based on tertiles of low, moderate, and high accumulation of sporadic MPA, significant increases in CRF were found with increases in rank.

The authors concluded that non-purposeful IPA accumulated sporadically throughout the day is associated with higher CRF. Ross and McGuire’s work suggests health benefits of PA do occur along an energy expenditure continuum, and suggests that promoting IPA throughout the day may be an alternative and practical approach to improve cardiovascular health in inactive individuals. In line with the inverse relationship between CRF and CVD events quantified in a meta-analysis by Kodama, Saito, Tanaka et al. (2009), Ross and McGuire noted that small 10-minute increases in total accumulation of sporadic MPA between consecutive tertiles resulted in a corresponding 7.5% lower risk of CVD.

**Body Fatness, PA and CVD**

Body fatness is also closely associated with the cardio-metabolic risk factors of CVD mediated by the inflammatory cascade. Therefore, higher overall and abdominal obesity, is associated with higher risk of CVD morbidity and mortality (de Koning, Merchant, Pogue, & Anand, 2007; Reis et al., 2009). Excess body weight in the form of abdominal obesity results in production of pro-inflammatory cytokines that cause or exacerbate cardio-metabolic CVD risk factors, such as hypertension, dyslipidemia, insulin resistance, and glucose intolerance (Cannon, 2007). Unlike inflammatory markers, the relation of body fatness with non-exercise PA has been studied extensively. Evidence indicates that LPA and incidental movement are associated with a
lower waist circumference (Strath, Holleman, Ronis, Swartz, & Richardson, 2008; Tremblay et al., 2007).

**Relationship Between Markers of Obesity and Sporadic MVPA**

Adherence to a single prolonged 30 min bout of PA may be burdensome for most individuals. Thus, it has been recommended that daily doses of PA for health benefit be accumulated in several bouts of 10 minutes (Hill, Wyatt, Reed, & Peters, 2003; J. A. Levine et al., 2005). However, Strath, Holleman, and Swartz (2008) suggest that regularly engaging in short activities lasting 1-3 min such as taking the stairs instead of the elevator or parking farther from one’s car, may help to transition from a sedentary to an active lifestyle.

Strath et al. used data from the 2003-2004 NHANES on body mass index (BMI), waist circumference, and objective measures PA on 3,250 participants to predict markers of obesity from PA accumulated in PA guideline bouts (≥ 10 min) and non-bouts (< 10 min) (Strath et al., 2008). Linear regressions showed that the strength of the association with decreased BMI was 4 times greater for MVPA accumulated in bouts than for MVPA duration in non-bouts. Authors concluded that sporadically accumulated MVPA is not significantly associated with BMI reduction. However, MVPA accumulated in non-bouts was significantly associated with waist circumference reduction. Similar to BMI, the association of decreased WC was 3 times greater for total MVPA in bouts than for MVPA in non-bouts. However, a significant association remained for MVPA accumulated in non-bouts. Overall, it was quantified that people who accumulated a total of 30 minutes of MVPA in non-bouts had a waist circumference that was 0.9 cm smaller than those who did not. People who accumulated MPVA in bouts ≥ 10 minutes had a waist circumference that was 2.7 cm smaller compared to those who failed to accumulate such activity. While MVPA accumulated consecutively in episodes ≥ 10 min may result in more
pronounced reduction of markers of obesity, Strath et al’s. findings that sporadic MVPA can also significantly reduce body girth are noteworthy. This study highlights the discrepancies of the PA guidelines for health benefit with regard to dose response for PA prescription. Findings from Strath et al. suggests that waist circumference, which is a direct indicator of abdominal fat, may be reduced through sporadic PA.

*The Role of IPA in Increasing Total Energy Expenditure*

IPA, which falls outside the scope of the PA guidelines for health benefit, contributes towards increasing total energy expenditure (TEE) as non-exercise activity thermogenesis (NEAT). NEAT includes active energy expenditure (AEE), which encompasses brief interment activities accumulated throughout the day (Tremblay et al., 2007) that are not performed as exercise and encompasses all activities that result in muscular contraction (e.g. maintenance of posture, chewing gum, fidgeting, and other physical activities of daily life) (J. Levine, Eberhardt, & Jensen, 1999).

In a position paper on lifestyle incidental movement, Tremblay, Tremblay, and Colley (2007) suggest that while the energy expenditure of a single brief movement classified under NEAT may be small, the cumulative effect of many of these throughout the day make a significant contribution to TEE. Since little is known about the health benefits of incidental movement (non-exercise or non-purposeful) Tremblay et al. sought to explain how factors other than leisure time PA contribute to TEE, including NEAT, physical inactivity, sleep, and behavioral compensation (Tremblay et al., 2007). They presented results from two studies (J. Levine et al., 1999; J. A. Levine et al., 2005), which indicated that low NEAT plays a potential role in obesity. These studies (J. Levine et al., 1999; J. A. Levine et al., 2005) showed low NEAT led to excess weight gain by resulting in an average differences of > 350 kilocalories in energy.
expenditure among lean individuals compared to obese individuals who sat on average 2 
hours/day longer.

Therefore, the influence of PA on adiposity extends beyond purposeful activities as 
suggested by the PA guidelines because simply replacing sitting time with incidental standing or 
walking behavior contributes substantially to energy expenditure. A positive energy balance of 
\leq 210 \text{kcal/day} has been reported among adults Americans ages 20-40 (Lanningham-foster, 
Nysse, & Levine, 2003). Even though this is a small excess of calories per day it may account for 
weight gain over the course of a year (Lanningham-foster et al., 2003). IPA thus presents an 
intriguing avenue to reduce energy imbalances that lead to excess accumulation of body fat.

**IPA and Abdominal Adipose Tissue**

The indication that non-purposeful PA plays a role in mediating energy balance to 
attenuate weight gain led to a study by McGuire and Ross examining the association between 
objectively measuring IPA and SED with abdominal obesity (subcutaneous + visceral adiposity) 
(McGuire & Ross, 2012). All participants failed to accumulate MVPA in periods \geq 10 \text{ min.} 
Neither SED nor total IPA was found to have an association with abdominal obesity, but 
sporadic MVPA was inversely associated with visceral adipose tissue. This study confirms Ross 
and McGuire’s (2011) previous finding that IPA has an intensity dose response relationship with 
health benefits. Although further research is needed to quantify the exact role of sporadic MVPA 
in stimulating this type of lipolysis, this study suggests sporadic MVPA typical of IPA may play 
a role in preventing central visceral adiposity that increases risk for CVD.

**SED and IPA**

Prolonged SED may be detrimental to health and Owen, Healy, Mathews and Dunstan 
have demonstrated a distinction between the independent effects of SED and the lack of MVPA
on disease (Owen et al., 2010). However links between disease prediction and breaking up SED with IPA have yet to be fully established.

SED and Cardio-Metabolic Risk: Implications for CVD

Mcguire and Ross examined the relationship between SED with 2-hour plasma glucose and insulin resistance, as well as LPA and sporadic MVPA with glucose metabolism and other cardio-metabolic risk factors (triglycerides, total cholesterol, LDL, HDL, and blood pressure) in 135 active abdominally obese adults (McGuire et al., 2011). All participants minimally accumulated MVPA in periods $\geq$ 10 minutes. Neither SED nor LPA were found to be associated with 2-hour plasma glucose or insulin insensitivity, but sporadic MVPA was significantly associated with total cholesterol and triglycerides. These results provide further evidence indicating health benefits attainable from IPA may be determined by its intensity of accumulation. Further research is needed to see if these associations of IPA with cardio-metabolic makers of CVD hold true in younger adult populations.

Other observational studies of cardio-metabolic risk, SED and PA confirmed significant associations exist between SED, LPA, and MVPA with glucose metabolism (G. N. Healy et al., 2007; G. N. Healy et al., 2008). However, clear inferences cannot be drawn about the effect of PA accumulation patterns relevant to IPA since it is unclear whether MVPA in these studies was accumulated sporadically or in bouts $\geq$ 10 min.

One such study (G. N. Healy et al., 2008) examined the associations of objectively measured sedentary time and PA with waist circumference, triglycerides, HDL cholesterol, resting blood pressure fasting plasma glucose, and clustered metabolic risk scores among 169 non-diabetic adults of the Australian Diabetes, Obesity, and Lifestyle Study. The authors reported significant independent associations of SED and LPA with waist circumference and
clustered metabolic risk score as well as SED and MVPA with triglycerides. On average, each 10% increase of SED was associated with a 3.1 cm increase in waist circumference, and SED and LPA were strongly inversely correlated. This same correlation was not evident between SED and MVPA, nor LPA and MVPA. Therefore, this study suggests replacing SED with LPA provides cardio-metabolic health benefits. Thus, IPA mainly comprising of LPA, may provide cardio-metabolic protective effects of CVD when replacing SED.

Another paper from the Australian Diabetes, Obesity, and Lifestyle Study compared the association of objectively measured SED, LPA, and MVPA with 2-hour plasma glucose of 173 non-diabetic participants (G. N. Healy et al., 2007). SED was positively associated with 2-hour plasma glucose and negatively associated with LPA and MVPA. LPA also remained significantly correlated after adjusting for the effect of MVPA. This study provides important evidence of the role between objectively measured LPA and breaking up SED on glucose metabolism. Healy and colleagues (2007) noted that breaking up SED has the potential to be a “practical and achievable” behavior change method to reduce DM2 and CVD risk. Since no indication was given about the pattern of MVPA, further studies like McGuire and Ross (2011) work are needed to quantify the association of sporadic MVPA with glucose metabolism to fully quantify the association of IPA with glucose metabolism. The potential role of IPA in attenuating glucose intolerance is important because hyperglycemia has been shown to prominently increase CVD risk. A meta-analysis of 38 prospective studies by Levitan, Song, Ford, and Liu found a linear association between 2-hour plasma glucose and CVD mortality in non-diabetic hyperglycemia (E. B. Levitan et al., 2004).

Healy, Matthews, Dunstan, Winkler, and Owen recently conducted a similar analysis of cardio-metabolic biomarkers in US adults from a sample of the NHANES cohort, a multi-ethnic
and diverse US population sample (Healy et al., 2011). This was a cross-sectional analysis of 4737 participants on SED and breaks in SED in relation to cardio-metabolic and inflammatory risk factors. Significant detrimental linear associations were found between SED with waist circumference, CRP, triglycerides, and insulin sensitivity. The differences observed between the lowest and highest quartiles of SED were clinically significant for triglycerides, and insulin sensitivity. Additionally, after adjusting for total SED and exercise, breaks in SED were significantly inversely associated with waist circumference and CRP. This study further stresses the importance of reducing and breaking up SED for CVD risk prevention.

Morbidity and mortality of CVD attributable to physical inactivity may not entirely result from failure to adhere to the PA guidelines. Insufficient breaking up of SED may play a role as well. Consciously increasing IPA may be a more practical means to reduce burden of SED to improve health outcomes. Lack of time is a commonly indicated barrier to participating in regular PA (Kodama et al., 2009). IPA is less burdensome to implement in daily life than planned and structured PA currently advocated by the federal guideline because IPAs are non-purposeful and short lived.

Cellular Metabolic Health and CVD

Muscular contractions function as the central driver of whole body protein metabolism, which is a necessary response to combat (Wolfe, 2006), oxidative stress, a sub-cellular feature of poor metabolic and cardiovascular health. Yet, the role of muscle metabolism is rarely considered as a target of interest for combating chronic diseases. Cardiac failure, which is a common endpoint of CVD, is associated with extensive loss of skeletal muscular metabolic function (Wolfe, 2006). Endurance training has been widely documented to improve muscle metabolic function through structural and functional changes, including increased mitochondrial
volume, density and enzyme activity; and enhanced regulation of mitochondrial respiration (Holloszy & Coyle, 1984; Hoppeler & Fluck, 2003; Tonkonogi, Harris, & Sahlin, 1998; Zoll et al., 2002). The role of LPA and sporadic MVPA for health benefits has never been studied in the context of muscle metabolic function. Although it is unknown whether altered cellular metabolic function or vascular oxidative stress occurs first in the generation of CVD, simply increasing muscle activation in the form of LPA and MPA has been shown to result in improved muscular mitochondrial function (Brierley, Johnson, James, & Turnbull, 1996) is associated with improvements in cardio-metabolic health (Palmeira, 2007; Rolo, 2006). Furthermore, Haseler and colleagues found that muscle oxidative rate in sedentary individuals, unlike active individuals, is limited by mitochondrial capacity and not oxygen availability (Haseler, Lin, & Richardson, 2004). Based on these results, the authors suggested that mitochondrial dysfunction may contribute to CVD risk even in the absence or reduced blood flow resulting from atherosclerosis.

Summary

The high rate of CVD morbidity and mortality can be partially attributed to modifiable lifestyle physical inactivity, which contributes to hyperglycemia, hypertension, weight gain, hypercholesterolemia, hyperlipidemia, and atherosclerosis. Cardiovascular health benefits of structured MVPA in bouts of at least 10 minutes are well established and strongly emphasized in the 2008 federal PA guidelines, but little is known about the role of IPA (LPA + short duration <10 min MVPA bouts) in CVD risk attenuation. Duration and intensity of IPA accumulation has been found to be positively associated with CRF, which is strongly correlated to cardiovascular health, but its relationship to additional cardiovascular health markers has yet to be investigated. These CVD risk markers include body girth and body composition variables, central and
peripheral vascular health markers, cardiometabolic health markers and oxidative stress (vascular and cellular). The latest research has distinguished that sedentary lifestyles are an independent risk factor for adverse cardiometabolic health and body girth irrespective of participation in structured MVPA. Non-exercise PA may be important to sustain cardiovascular health because several CVD risk factors, which include high plasma triglycerides, low HDL cholesterol, high plasma glucose, high blood pressure, high central adiposity, and atherosclerosis, are strongly correlated with low NEAT. IPA variability in inactive populations may result in marked differences in NEAT that explain differences in cardiovascular health. Yet, it is unclear whether IPA has a volume and intensity dose-response with risk factors and clinical markers of CVD.
CHAPTER III: METHODS

All procedures and protocols in this study were approved by Northeastern University’s Institutional Review Board. The study was conducted at Northeastern University’s Human Performance and Exercise Science Lab.

Participants

41 volunteers from Northeastern University and surrounding communities were recruited for this study. Recruitment was conducted through fliers, email, word of mouth, and craigslist. Volunteers were (1) between the ages of 18 and 70, (2) inactive and not engaged in regular (> 1 days/week past year) planned exercise (3) able to safely exercise to maximal exertion (4) not on medications that altered heart rate, blood pressure, glucose intolerance or metabolic function (5) did not have a pacemaker implanted (6) weighed > 110 pounds, and (7) had no previous history of fainting during medical procedures. Women who were pregnant or planning to become pregnant were not eligible to participate. For the purposes of screening for enrollment, habitual inactivity was defined as failing to meet the federal PA guidelines recommended for health benefits, which is consistent with previous examinations of IPA (McGuire & Ross, 2011, 2012; McGuire et al., 2011; Tremblay et al., 2007). All volunteers were screened for eligibility through email and phone calls. Eligible volunteers were enrolled on a first come basis, and were provide written informed consent (Appendix).

Study Protocol

This study had a cross-sectional design where all outcome measurements were conducted over the course of 2 laboratory-testing sessions at the Human Performance and Exercise Science Lab, each lasting for approximately 2.5 hours. Anthropometric variables, body composition, bone mineral content, resting heart rate and blood pressure, vascular hemodynamics, CRF and
muscle metabolism were measured during visit 1. RMR, inflammatory biomarkers and cardio-
metabolic biomarkers were measured during visit 2. Prior to the second lab visit, participants
came to the lab in a fasted 12-hour overnight condition. Daily habitual free living PA was
objectively monitored for a period of 7 days in between lab visits one and two using the Activpal
activity monitor to measure IPA.

*Anthropometrics*

Anthropometric variables included height, weight, waist and hip circumference. Height
was measured using a stadiometer and weight was measured using a digital electronic scale. Hip
and waist circumference were measured using a Gulick tape. Circumference measurements were
taken according to the American College of Sport Medicine’s guidelines (Pescatello & American
College of Sports, 2014a). With the subject standing erect and feet together, hip circumference
was measured as the widest horizontal circumference of the buttocks. With the subject standing
upright and relaxed, waist circumference was measured as the horizontal circumference of the
greatest anterior extension of the abdomen. An average measure was calculated from three
measurements at each site, and was retested if the three measures did not fall within 1 cm of each
other. During each measure, the Gulick tape was placed such that it was snug without pinching
the skin. A spring gauge measurement improves measurement consistency by reducing skin
compression (Pescatello & American College of Sports, 2014a).

*Body Composition*

Body composition was measured using Dual Energy X-ray Absorptiometry (DEXA)
(Lunar DXA, GE HealthCare, Fairfield, CT). During the test, participants were asked to remove
all metallic objects and lie down on the testing bed. DEXA measures whole body or regional
bone, fat, and muscle tissue. The densitometer utilizes two thin beams of very low dose X-rays
with distinct energy peaks that are absorbed by bone and soft tissue for a gram-by-gram assessment of bone mineral mass, fat mass, and lean mass. Body composition variables including total fat mass, total lean mass and compartmental fat and lean mass were derived using proprietary software (encore 2002, version 6.70.021, GE Lunar Corp., Madison, WI).

**Resting Heart Rate and Blood Pressure**

Resting heart rate and blood pressure were measured after 10 minutes of seated rest with the subject sitting in the upright position on a chair with a backrest and both feet firmly planted on the floor. Both measurements were made using an Omron upper arm digital blood pressure monitor. Digital monitors measure blood pressure via the oscillometric technique rather than by auscultation technique, which is done with a stethoscope and sphygmomanometer. Unlike auscultation, which relies on technician expertise to listen for specific heart beat sounds to determine systole and diastole, the oscillometric method of blood pressure measurement does not require such skill. Oscillometric technology measures vibrations of blood traveling through the arteries and converts them into digital readings of systolic and diastolic blood pressure. Omron blood pressure monitors are clinically validated to be accurate within +/- 3 mmHg (roughly 2%) for blood pressure measurement and accurate within +/- 5% for pulse readings (Coleman, Freeman, Steel, & Shennan, 2006; El Assaad, Topouchian, & Asmar, 2003; Stergiou, Yiannes, & Rarra, 2006).

**Vascular Hemodynamics**

Measurements of arterial intima media thickness (IMT), peak systolic and diastolic velocities, and peak arterial lumen diameter during systole and diastole were obtained using an Ultrasound Doppler System (Sonosite Micromaxx Ultrasound, 13.6 MHz transducer). Before beginning ultrasonography, the subjects were connected to a three lead (limb leads) EKG to sync
the ultrasound measurements with the subject’s cardiac cycles. Measurements were taken at the common-carotid artery, which is located in the mid-lateral anterior portion of the neck, and popliteal artery, which is located on the posterior mid-leg beneath the popliteal fossa. All measurements were obtained from the right side of the body with the subject lying down. Measurements from four cardiac cycles were obtained for each variable of interest.

**IMT**

Carotid IMT is an accepted surrogate measure of atherosclerosis because it is a direct measurement of structural changes in the vascular wall, which lead to vessel narrowing (Espeland et al., 2005). Minor alterations in the anatomy of the inner arterial wall preceding the development of atherosclerosis can be used to assess CVD risk (Paul S. Sidhu, 2000). Ultrasonography was used to longitudinally image the boundaries of the inner arterial wall. Proprietary software of the Micromaxx Ultrasound was utilized to measure the mean arterial wall thickness. Briefly, the ultrasound IMT tool displays two line segments that can be aligned with the luminal-intimal interface and the media-adventitia interface, i.e. IMT (Pignoli, Tremoli, Poli, Oreste, & Paoletti, 1986). The mean distance between these two line segments was measured at peak systole and diastole from a sample of 4-6 cardiac cycles. The reason for obtaining IMT from both segments of the cardiac cycle is because carotid IMT sizes vary considerably between systole and diastole. During diastole, when the lumen diameter is at its maximum, carotid IMT is at its maximum (Gonzalez, Wood, Dorey, Wren, & Gilsanz, 2008). While there is no consensus for an ideal measurement site of IMT, the most common protocol was used. IMT is most commonly measured along the far wall of the common carotid artery, within 1 cm proximal to the carotid bulb (P. S. Sidhu & Desai, 1997). Median adult IMT ranges have been reported to be between 0.5-1.0 mm, and values greater than 1 mm are considered abnormal (Ebrahim, 1994;
Howard, Manolio, Burke, Wolfson, & Leary, 1997). Since the common carotid artery branches directly out of the aorta to supply blood to the brain, its close proximity to the heart and brain make it an ideal vessel to non-invasively assess central CVD risk. Therefore carotid IMT measurement serves as risk factor for clinical manifestations of CVD (i.e., myocardial infarction and stroke).

In addition to carotid IMT, popliteal artery IMT was measured as well. The popliteal artery IMT has been shown to have a strong correlation with plaque formation of atherosclerosis (Bucciarelli, Srámek, Reiber, & Rosendaal, 2002), and is thus indicative of peripheral CVD risk. Similar to the carotid artery, popliteal artery images were taken from 4-6 cardiac cycles and analyzed to obtain measurements of arterial IMT.

**Arterial Endothelial Function**

In addition to the aforementioned variables, flow mediated dilation (FMD) was used to measure peripheral endothelial function during longitudinal imaging of the popliteal artery. Measurements of peak systolic velocity and peak systolic diameter were obtained before and after an arterial occlusion protocol (blood pressure cuff inflation). The cuff inflation condition consisted of a blood pressure cuff placed 3-4 centimeters above the knee and was inflated to 50 mmHg above the subject’s resting systolic blood pressure for a period of 5 minutes. Endothelial function measured via FMD has been proposed to represent the functionality of nitric oxide (NO) bioavailability in humans (D. Green, 2005), and thus can be used to assess the compliance of the vasculature for maintaining blood flow. Circulatory occlusion utilized in FMD results in an acute increase in blood flow known as hyperemia, which increases shear stress of the vessel walls (Niebauer & Cooke, 1996). This mechanical signal is detected by receptors on the vascular endothelial cells resulting in a chemical cascade that terminates with the release of NO along the
vascular wall (Sessa, 2004), inducing relaxation of smooth muscle and subsequent vasodilation (R. A. Harris, Nishiyama, Wray, & Richardson, 2010). Change in vessel diameter during and after hyperemia was expressed as the percentage of baseline diameter (%FMD). Consistent with a consensus protocol recommendation compiled by Harris et al. (2010) from analysis of previous studies, the following guidelines were utilized:

1. **Transducer Angle**

   For optimal readings, velocity and diameter measurements were obtained at a transducer angle of 60 degrees, as this has been noted to produce the least errors in velocity readings while ensuring adequate image clarity for diameter readings (Jogestrand et al., 2003; Logason et al., 2001; Rizzo et al., 1990).

2. **Baseline Measurements**

   Baseline diameter was measured as the distance between the far and near wall of the vascular lumen. Since shear stress resulting from hyperemia is the primary stimulus for FMD response, accurate measurement of resting blood velocity is critical. Resting blood velocity can vary considerably in a short period of time. So it is recommended that measures be averaged over a 10-20 second period (Gill, 1985). Peak systolic velocity was obtained from the 4-6 cardiac cycles present on the digital screen with one scan. Velocity readings were obtained from a central blood flow sample by placing the Doppler in the center of the lumen with the smallest Doppler gate size. This controlled for consistency between pre and post measurements.

3. **Arterial Occlusion**

   Vascular occlusion creates a region of ischemia distal to the occlusion site that results in accumulation of cellular metabolic byproducts in the absence of oxygen,
thereby allowing robust vasodilation typical of reactive hyperemia (Celermajer et al., 1992). A rapid (0.3 seconds) cuff inflator (Hokanson E20 Rapid Cuff Inflator) powered by a 20 gallon air compressor (Campbell Hausfeld) was used to inflate a blood pressure cuff (Hokanson SC5, Bellevue, WA) placed on the thigh, proximal to the measurement site. Several studies have utilized 10-minute occlusion protocols. However, a 5-minute occlusion period was utilized over longer periods because tests of 10-minute versus 5-minute occlusions have been shown to yield over 50% greater vasodilation (R. Harris et al., 2009), which is indicative of non-endothelium dependent vasodilators being active following longer periods of ischemia. Cuff inflations of 25-50 mmHg above resting systolic blood pressure are recommended to elicit reactive hyperemia. Fifty mmHg above resting systolic blood pressure has been shown to consistently induce arterial occlusion of blood flow (Corretti et al., 2002), but some individuals may have a lower threshold for occlusion. If individuals experienced discomfort at 50 mmHg above systolic blood pressure, the pressure of the cuff was lowered to a threshold level where occlusion was visually spotted on the ultrasound Doppler reading.

4. Reactive Hyperemia Measurements

Peak systolic velocity was obtained from a series of 4-6 cardiac cycles during the first 15 seconds following cuff release, and peak diameter was obtained from a series of 4-6 cardiac cycles in the 45-80 second window following cuff release. Peak velocity during FMD has been documented to occur within the first 15 seconds following cuff release, and peak vasodilation has been documented to occur within 45-80 seconds following cuff release (Black, Cable, Thijssen, & Green, 2008).
5. *Calculation of Shear Rate*

Similar to blood velocity, shear rate is affected by the width of the blood flow sample volume and the gate size of the Doppler measurement within the vessel lumen. Shear rate can be estimated from Poiseuille’s law as the ratio of mean blood velocity to the internal diameter of the lumen. The ratio of mean blood velocity to lumen diameter was multiplied by 4 to correct for the small volume of blood flow collected in the center of the vessel. FMD protocols call for a correction factor of 8 if using a large, centered sample volume, and a correction factor of 4 if using a small, centered sample volume. The difference in these two correction factors results in varying necessity to account for slower moving red blood cells flowing along the edge of the vessel (R. A. Harris et al., 2010). Therefore, the small, centered sample volume formula was used to calculate shear rate, and is as follows:

\[
\text{Shear Rate} = 4 \times \left( \frac{\text{mean blood velocity}}{\text{Internal Diameter}} \right)
\]

6. *FMD Calculation*

FMD was expressed as the percent change in vessel tone, and was calculated as follows:

\[
\text{FMD (\%)} = \left( \frac{\text{Peak Diameter} - \text{Baseline Diameter}}{\text{Baseline Diameter}} \right) \times 100\%
\]

7. *FMD Normalization*

%FMD was normalized by dividing it by shear rate. Since FMD is believed to result from shear stress on the vascular walls, it is proposed that FMD is proportional to reactive hyperemia (Corretti et al., 2002). (Pyke & Tschakovsky, 2007).
Peak VO$_2$ Measurement for Assessment of CRF

Whole body maximal oxygen uptake (VO$_2$max) was obtained from an indirect calorimeter (COSMED, Quark CPET Metabolic Cart) using an open-circuit spirometer for a breath-by-breath analysis during a standard Bruce protocol treadmill maximal exercise protocol. During the procedure subjects wore a sterilized mask on their nose and mouth with the attached open-circuit spirometer. Indirect calorimetry determines the rates of oxygen consumption, carbon dioxide production, and respiratory rate for determination for exercise metabolic rate. The Bruce protocol is comprised of seven three-minute stages of incremental increase in speed and inclination. The details of the protocol procedure are presented in the Appendix- Figures and Tables section.

Participants were asked to exercise to maximal volitional exhaustion. Heart rate and rate of perceived exertion (RPE) were monitored and recorded the last 15 seconds of every 3 minute stage of the Bruce graded exercise protocol. Test termination criteria and determination of achievement of VO$_2$max were based on a respiratory exchange ratio (RER) $\geq$1.10, achievement of age-predicted maximal heart rate ($208 – 0.7 \times \text{age}$) (Tanaka, Monahan, & Seals, 2001), a plateau in oxygen uptake (VO$_2$ diff $\leq 2.1$ ml/kg/min) during the last two minutes of the test, or upon voluntary exhaustion (E. T. Howley, Bassett, & Welch, 1995).

Participants were categorized based on gender specific VO$_2$max norms. The norms are presented in the Appendix- Figures and Tables section.

Inflammatory and Cardio-metabolic Biomarkers

Fasting (12 hours) blood samples were obtained from the antecubital vein using a standard venipuncture procedure with a butterfly needle while the subject was seated. A total of 47.5 mL of blood were collected in four vacutainer tubes and centrifuged (eppendorf centrifuge
5702) for 15 minutes at a relative centrifugal rate of 1500 G-forces. Samples were sent to Laboratory Corporation of American (LabCorp, Burlington, NC) for analysis of serum concentrations of total cholesterol (mg/dL), triglycerides (mg/dL), HDL cholesterol (mg/dL), LDL cholesterol (mg/dL), HbA1c (%), Insulin (uIU/mL), CRP (mg/dL), TNF-alpha (pg/mL) and plasma glucose (mg/dL).

PA Monitoring and IPA Measurement

Activity Monitors

PA was measured in a free-living environment over a period of 7 consecutive days to determine habitual SED, IPA, and MVPA accumulation. Participants were asked to wear 2 small (20 g, 35 mm x 53 mm x 7 mm) activPal™ motion monitors (PAL Technologies Ltd., Glasgow, UK). One device was worn on the on the midline of the thigh a distance midway between the knee and the hip. A second device was worn on the anterior-lateral torso midway between the inguinal crease and the inferior border of the rib cage. Both monitors were adhered to the skin using a hypoallergenic medical tape (3M Tegaderm). The activPal™ captures PA and SED information using an 8-bit digital capacitive accelerometer that samples at a frequency of 20 Hz. These data were post processed to quantify daily total and hourly time spent sitting/lying, standing and stepping, and step count and provide MET.hours of activity. Since the activPal™ is unable to distinguish between sitting and lying sedentary postures, use of the two monitors allowed for differentiation of these activities (Bassett et al., 2014). When both monitors detected sedentary postures the subject was said to be lying. When the torso monitor returned to the standing posture and the thigh returned to the sitting/lying posture, the subject was said to be sitting. Previous studies objectively measuring IPA have utilized the Actigraph GTX3 accelerometers, which quantify time spent in different activity intensities based on accelerometer
frequency cut points, with counts per minute (CPM) below 100 representing SED (Freedson, Melanson, & Sirard, 1998). The activPal™ was chosen over the Actigraph because it has been shown to be more reliable at distinguishing between SED and PA since it uses postural changes to quantify activities (Godfrey, Culhane, & Lyons, 2007; Hart, Ainsworth, & Tudor-Locke, 2011).

**Quantification of IPA and SED**

IPA was quantified using the classification set forth by Tremblay and Colleagues (2007), which categorizes IPA as all activities that fail to meet the PA guidelines: LPA and sporadic MVPA (bouts < 10 consecutive min) accrued typically through activities of daily living. In addition to providing outputs of time spent lying/sitting, standing, and stepping, the activPal provides an output in the form of events files, which provide a data output for every bout of activity. The output provides information on the type of activity (1=sit/lie, 2= stand, 3= step), the time spent in the bout of activity, and the volumetric representation of the activity (MET-hours), which is a product of the time spent in the activity and the associated energy expenditure in METs. The activPal had a default setting that classified sit/lie postures as 1.25 METs and idle standing postures as 1.4 METs. These values were multiplied by the duration of the activity to provide a MET-hour output in the events file.

Activities from the event files were first sorted to separate the three types of postures by bouts. Sit/lie activities were classified as SED. To separate stepping bouts, the files were sorted to provide a time stamp along with the sum of MET-hours, steps, and duration of the activity for stepping periods. MET-hour outputs were then converted to average intensity (METs) of the bout by dividing the sum of MET-hours by the duration of the activity. Utilizing the PA intensity ranges defined previously (Norton et al., 2010; Pate et al., 2008), bout intensities were used to
further classify stepping activity bouts into intensity categories (stepping LPA, MPA, VPA). The durations of the stepping bouts for MPA and VPA bouts were then used to sort MPA and VPA into sporadic (< 10 min duration) and bouted (≥ 10 min duration) categories. To account for periods of standing rest during activity, participants were required to spend at least 80% of bouts within the MET intensity category range for the bout to be counted. To do so, stepping periods were given an allowance of 120 total seconds of idle non-stepping time within the bout. The rationale for this was to follow the NHANES model of “modified 10-min bouts” for distinguishing between sporadic and bouted MVPA, whereby 10 minute activity bouts are defined as 10 or more consecutive minutes above the respective MET threshold with an allowance of 2 minutes of interruptions below the threshold within the bout (R. Troiano et al., 2008). Therefore, when classifying the events, a bout was ended and a new activity began every time the total idle non-stepping time within a stepping bout exceeded 120 seconds. Standing bouts that fell outside of stepping periods were classified as standing LPA.

A visual representation detailing the process for sorting the activPal data is presented in the Appendix.

Activity bouts were quantified on the basis of total weekly and daily average duration of time spent in in SED, total LPA (standing LPA + stepping LPA), sporadic MVPA and MVPA in bout ≥10min. IPA was reported as average daily duration, average daily intensity (expressed in categorical terms), and average daily EE volume (MET-hours).

Statistical Analysis

Data analysis was conducted using Microsoft Excel and STATA 12 (STATACorp. LP). PA data were analyzed to determine the distribution characteristics of average daily and weekly time spent in SED, LPA, MVPA in bouts ≥ 10 min, sporadic MVPA, total IPA. Distributions
were defined as normally distributed if their skewness was between -0.5 and 0.5. Gender differences in descriptive characteristics, cardiovascular health and PA variables were determined using independent student’s t-tests. Univariate and multivariate linear regressions were used to determine the relationships and associations of average daily time spent in total IPA, LPA, and sporadic MVPA with CVD risk measures (total cholesterol; triglycerides; HDL cholesterol; LDL cholesterol; HgbA1C; resting insulin and glucose; CRP; TNF-alpha; CRF, total and compartmental adiposity; peripheral FMD; peripheral and central resting peak systolic and diastolic velocity, lumen diameter and IMT. Age, gender, accelerometer wear time, and average daily time spent in MVPA in bouts ≥10 min were entered as covariates in multivariate regressions. Percentiles derived from PA pattern distributions were used draw comparisons among subjects for significant relationships between IPA, LPA, and sporadic MPA with CVD risk measures. Results are reported as coefficients of determination (R²) with beta 95% confidence interval for changes between tertiles of IPA accumulation. Significance was set at a p<0.05 for main effects, and p<0.1 for interaction.

Participants were then divided into tertiles based on accumulation of IPA, LPA and sporadic MPA. In order to determine the effect of IPA on CVD risk, changes between tertiles were compared for cardiovascular health outcomes that were significantly correlated to IPA or its subcomponents.
CHAPTER IV: RESULTS & DISCUSSION

This chapter presents and discussion of the statistical analysis performed to investigate whether there was an association between intensity and duration of IPA with CVD risk measures in inactive individuals.

Results

Forty-one participants (n=41) completed tests of CVD risk assessment (anthropometrics, body composition, vascular hemodynamics, CRF, inflammatory biomarkers, and cardiometabolic biomarkers) and wore activPal PA monitors for 1-week. Average daily intensity and duration of SED, LPA, sporadic ≤ 10 minute duration MVPA, total IPA, and MVPA in bouts ≥ 10 minutes were calculated from MET-hour outputs of the activPal PA monitors.

3 participants were excluded from the analysis due to malfunctioning of activity monitors. Tests for normality revealed non-normal distributions of cardiovascular and PA measures. All cardiovascular measures and PA variables were logarithmically transformed to correct for skewed distributions. Gender differences were only detected for android to gynoid % fat ratios and carotid artery PD diameter; therefore analyses were collapsed across gender for all other variables.

Demographics and Cardiovascular Health Characteristics

The mean age of participants was 33.6 ± 14.4 years for males (n=23) and 33.8 ± 15.7 years for females (n=17). Minorities (Black, Hispanic, Asian) represented a large portion of the subject population with 42% of male subjects and 67% of female subjects being minorities. BMI indicated 53% of subjects were overweight with an average BMI of 26.7 ± 5.0 kg/m² in males and 25.7 ± 5.4 kg/m² in females. Total % body fat indicated subjects were obese (ACE, 2009) on
average with an average of 26.2 ± 10.1 % body fat in males and 37.1 ± 8.6 % body fat in females.

Table 4 (Appendix, pg. 73) presents the demographic and cardiovascular health characteristics of participants by gender.

**PA Outcomes**

On average, subjects were 76% ± 10.4% sedentary by spending 18.3 ± 2.5 hours of every 24-hour day in either seated or lying postures. There was large variability in accumulation of all PA outcomes. Daily LPA accumulation for every 24 hours of monitor wear time ranged from 1.9 to 15.4 hours with an average of 4.9 ± 2.63 hours spent in LPA. Subjects did not spend any time in VPA. Daily sporadic MPA accumulation ranged from 42 seconds to 27.6 minutes with an average of 10.65 ± 6.7 minutes spend in sporadic MPA. Daily IPA, which consisted mostly of LPA (i.e., 96.4%), accumulation ranged from 2 to 15.5 hours with an average of 5.1 ± 2.6 hours spent in IPA. 58% of subjects met or exceeded the daily PA outcomes of at least 30 daily minutes of MVPA accumulated in bouts of at least 10 consecutive minutes (United States. Dept. of Health and Human, 2008). The remaining 62% of subjects accumulated either a negligible amount or zero daily minutes in bouted MPA. Daily bouted MPA accumulation ranged from 0 to 2.3 hours with an average of 36 ± 34.8 minutes spent in MPA. Subjects accumulated on average 8.7 ± 4.1 daily MET-hours in LPA, 0.60 ± 0.38 daily MET-hours in sporadic MPA, 2.1 ± 2.1 daily MET-hours in bouted ≥ 10-minute duration MPA, and 9.3 ± 4.2 MET-hours in IPA, which consisted primarily of LPA (i.e., 93.5%). Sporadic MPA accumulated on average was equivalent to 50.4% ± 31.9% of the recommended weekly EE volume of 500 MET-min per week from MVPA accumulated in bouts of at least 10 consecutive minutes.

Table 5 (Appendix, pg. 74) presents PA outcomes by gender.
Linear and Multivariate Regressions

Daily total IPA accumulation was not a significant predictor of anthropometrics, body composition, vascular hemodynamics, CRF, or inflammatory biomarkers. Average daily IPA accumulation was significantly inversely associated with resting SBP ($r^2 0.122$, $p 0.031$; Table 6) and triglycerides ($r^2 0.157$, $p 0.018$; Table 6), but only resting SBP remained significantly correlated after controlling for age, gender, bouted MPA duration, and accelerometer wear time ($r^2 0.118$, $p 0.042$; Table 6). Additionally, average daily IPA accumulation was significantly positively correlated with HDL cholesterol ($r^2 0.252$, $p 0.002$; Table 6), but only showed trends towards significance after controlling for age, gender, bouted MPA duration, and accelerometer wear time ($r^2 0.369$, $p 0.087$; Table 7).

Similarly, LPA was not a significant predictor of anthropometrics, body composition, vascular hemodynamics, CRF, or inflammatory biomarkers. Average daily LPA accumulation was significantly inversely associated with resting SBP ($r^2 0.118$, $p 0.035$, Table 8) and triglycerides ($r^2 0.165$, $p 0.015$, Table 8), but only resting SBP remained statistically significant after controlling for age, gender, bouted MPA duration, and accelerometer wear time ($r^2 0.227$, $0.039$, Table 9). Additionally, average daily LPA accumulation was significantly positively associated with HDL cholesterol ($r^2 0.267$, $p 0.001$, Table 8), but only showed trends towards significance after controlling for age, gender, bouted MPA duration, and accelerometer wear time ($r^2 0.378$, $p 0.070$, Table 9).

Sporadic MPA was a significant predictor of a few peripheral vascular hemodynamic variables, but was not a significant predictor of anthropometrics, body composition, CRF, inflammatory biomarkers, or cardiometabolic biomarkers or central vascular hemodynamics (carotid). However, these relationships disappeared after controlling for covariates. Average
daily sporadic MPA accumulation was significantly inversely associated with popliteal artery PSD ($r^2 0.133$, $p 0.025$, Table 10) and PDD ($r^2 0.123$, $p 0.031$, Table 10), but neither remained statistically significant after statistical control for age, gender, bouted MPA duration, and accelerometer wear time. Sporadic MPA showed positive interactions with popliteal artery PDV ($r^2 0.102$, $p 0.054$, Table 10) and HDL cholesterol ($r^2 0.093$, $p 0.076$, Table 10). These interactions were no longer evident after controlling for age, gender, bouted MPA duration, and accelerometer wear time. Also, sporadic MPA showed inverse interactions with android % body fat ($r^2 0.25$, $p 0.068$, Table 11) and total % body fat ($r^2 0.344$, $0.099$, Table 11) after controlling for these same confounders.

Tables 6-11 (Appendix, pgs. 74-75) present the results of linear and multivariate regressions between cardiovascular health outcomes and IPA, LPA, and sporadic MVPA. Only variables that displayed an alpha level below 0.05 ($p < 0.05$) or 0.10 ($p < 0.10$) are presented. Linear regressions with an alpha level $< 0.05$ are represented with two stars (**), and linear regressions with an alpha level $< 0.10$ are represented with one star (*).

**Tertile Distributions of PA and Cardiovascular Health Outcomes**

Participants in tertile 1 of IPA accumulation accumulated an average of $3.21 \pm 0.59$ daily hours of IPA ($3.06 \pm 0.59$ hours in LPA and $4.25 \pm 1.92$ minutes in sporadic MPA); participants in tertile 2 accumulated an average of $4.52 \pm 0.48$ hours daily hour of IPA ($4.34 \pm 0.51$ hours in LPA and $9.32 \pm 1.96$ minutes in sporadic MPA); and participants in tertile 3 accumulated an average of $7.75 \pm 3.2$ daily hours of IPA ($7.55 \pm 3.25$ hour in LPA and $18.28 \pm 4.88$ minutes in sporadic MPA).

Triglyceride levels across tertiles of average daily LPA accumulation ranged from $83.7 \pm 26.02$ mg/dL of blood in tertile 1 to $65 \pm 29.98$ mg/dL of blood in tertile 3. There were no
statistically or clinically significant differences in triglyceride levels among the tertiles. The normal range of fasting triglyceride concentration, is defined as less than 150 mg/dL of blood (Miller et al., 2011). However, statistically significant and clinically relevant differences were observed in HDL cholesterol from tertiles 1 to 2 (14.47 mg/dL, p 0.016, 95% CI 3.0 – 26.2) and tertiles 1 to 3 (21.4 mg/dL, p 0.002, 95% CI 8.4 – 35.4). On average, participants in tertile 1 were in the CVD risk threshold level for HDL concentrations, which is defined as < 45 mg/dL of blood for men and < 55 mg/dL of blood for women (Adult-Treatment-Panel-III, 2001). Participants in tertile 2 were in the good category, which is associated with reduced CVD risk, and participants in tertile 3 were in the excellent category, which is considered protective against CVD, for HDL concentrations (Adult-Treatment-Panel-III, 2001).

Figure 1 (Appendix, pg. 76) displays tertiles of daily LPA and IPA accumulation with changes observed in HDL cholesterol across tertiles.

Changes in resting SBP from participants in tertile 2 to tertile 3 of average daily LPA accumulation were statistically significant (-11.2 mmHg, p 0.05, 95% CI -0.1 – -22.6). Changes across tertiles were clinically significant as average changes in SBP from tertile 1 to 3 and tertiles 2 to 3 represented a shift from prehypertension, which is defined as a resting SBP between 120 and 139 mmHg or a DBP between 80 and 89 mmHg, to normal blood pressure (James et al., 2014).

Figure 2 (Appendix, pg. 77) displays tertiles of daily LPA and IPA accumulation with changes observed in SBP across tertiles.

Popliteal artery PSD and PDD changes across tertiles of sporadic MPA were not statistically or clinically significant. Participants in all tertiles had popliteal arterial diameters within a narrow range between 5.1 and 5.9 mm, which falls under the normal popliteal artery
size defined as ranging from 5 to 9 mm (J.H. Van Bockel, 2005; Johnston et al., 1991; R.E. Zierler, 1983).

Discussion

Associations between IPA and CVD Markers

The results of this study indicate that IPA has a volume dose-response relationship with triglyceride concentrations, HDL cholesterol concentrations, and resting SBP, but not an intensity dose-response relationship with such factors as only LPA was associated with the abovementioned cardiovascular variables. Resting SBP was the only variable that remained significantly correlated to LPA after adjusting for confounders of age, gender, accelerometer wear time, and time spent in bouted ≥ 10 minute duration MVPA. This study contrasted the results of previous studies of IPA and health markers (McGuire & Ross, 2011, 2012; Strath et al., 2008), as IPA accumulation was not found to be significant predictor of CRF, body girth, or glucose and insulin metabolism. These discrepancies most likely stem from differences in subject populations. Unlike previous studies of IPA and health markers, abdominal obesity was not an inclusion criterion for this study and efforts to recruit inactive individuals were ineffective as 58% of subjects met or exceeded the MVPA guidelines.

Additionally, significant associations found between peripheral vascular hemodynamics and sporadic MPA were of little clinical relevance. The changes observed in popliteal arterial diameter across tertiles of sporadic MPA accumulation represented marginal decreases within a healthy range of arterial lumen diameter. The observed lack of association after controlling for confounders can be explained by the fact that arterial diameter is strongly related to age, gender, and body size (Sandgren, Sonesson, Ahlgren, & Länne, 1998). Therefore, the large age and body size variability of our subject population is more likely to be the cause of the observed small
popliteal diameter differences than IPA accumulation volume. The observed popliteal arterial
diameter differences may have also been attributable to the high levels of structured PA
accumulated by the majority of the study population. Therefore, intensity and duration of IPA is
not associated with central or peripheral vascular hemodynamics in this population.

The inverse relationship observed between volume of IPA accumulation and triglycerides
may be attributable to more frequent muscle activation from postural changes between SED and
LPA. The Framingham heart study has reported that triglyceride concentrations have strong
associations with both subcutaneous and visceral adipose tissue, but that elevated visceral
adipose tissue is a greater contributor to hypertriglyceridemia (Fox et al., 2007). Catecholamine
release resulting from muscle activation during PA stimulates lipid oxidation, which facilitates
the breakdown and utilization of triglycerides by skeletal muscle (Martin, 1996). Visceral
adipose tissue is more sensitive to catecholamine-induced lipolysis in response to PA compared
to abdominal subcutaneous adipose tissue (Richelsen, Pedersen, Mollerpedersen, & Bak, 1991).
Therefore, the high volume of intermittent muscle activation that accompanies high volumes of
LPA results in a large amount of transient catecholamine activation that may play a role in
stimulating triglyceride utilization throughout the day. The results observed in shifts of
triglycerides concentrations across tertiles in this study population, which were associated with
increases of average LPA accumulation from 3 to 7.5 hours per day, were not of clinical
relevance to this subject population because all subject were within the normal range. However,
the observed 18 mg/dL average concentration difference, while not statistically significant,
between tertile 1 and 3 may be of clinical relevance to individuals with elevated triglycerides.

The positive relationship observed between accumulation of LPA and HDL cholesterol
concentrations suggests LPA is of clinical relevance for the prevention of low levels of HDL
cholesterol. Shifts in HDL concentrations from tertile 1 to 3 of LPA accumulation in this population represented a difference between being at risk of CVD and having levels of HDL protective of CVD. According to The Quebec Cardiovascular Study, which was a 5 year prospective study of middle aged men, CAD risk increases by 13% with every 10% decrease in HDL below the normal range (Després, Lemieux, Dagenais, Cantin, & Lamarche, 2000). Therefore, under this premise, males in tertile 1 of this study had an 8.7% increased risk of CAD compared to males in tertiles 2 and 3. Exercise is well known to increase activity of lipoprotein lipase, which is the enzyme catalyst for the synthesis of HDLs (Kantor, Cullinane, Sady, Herbert, & Thompson, 1987), but it is unknown whether LPA stimulates the same physiological mechanism. Given that LPA did not remain a significant correlate for HDL after adjusting for time spent in ≥ 10-minute duration MVPA bouts, it is unlikely LPA is involved in the same mechanism of HDL synthesis regulation as exercise, but it may play stimulate HDL synthesis via another pathway. Results of this study pertaining to HDL may also be affected by a non-uniform distribution of inactivity in the subject population.

Limitations

A major limitation of this study was that subjects were not homogenous with respect to inactivity. Despite efforts to recruit inactive individuals, the study population was fairly active, as greater than half met or exceeded the PA guidelines for cardiovascular health. Even though time spent in MVPA in bouts ≥ 10-minutes was controlled for in the statistical analysis, active individuals included in the study may have reduced the sensitivity of the subject population to observe associations between IPA and cardiovascular health variables if they were present among inactive individuals.
Summary

Definite conclusions cannot be made about the association between intensity and duration of IPA with overall cardiovascular health. The small effects of LPA on HDL cholesterol, triglycerides, and SBP may be more pronounced in populations that are abdominally obese or sedentary. Different results may be evident with respect to body composition, inflammatory markers, and vascular hemodynamics if structured MVPA did not contribute to the subject populations’ PA. Future studies seeking to find associations between intensity and duration of IPA with CVD risk markers should screen for sedentary lifestyles.
CHAPTER V: CONCLUSION

Summary

The purpose of this study was to examine the association between the volume and intensity of IPA accumulation and cardiovascular health markers. 41 subjects between the ages of 18 and 70 completed assessment of body composition, vascular hemodynamics, CRF, cellular metabolism, and cardiometabolic health, and their habitual PA was objectively monitored for 7 days to quantify average daily durations of time spent in SED, LPA, sporadic < 10 min duration MVPA, and bouted ≥ 10 min duration MVPA. A significant inverse correlation between LPA and triglycerides and a positive correlation between LPA and HDL cholesterol was only evident before adjusting for confounders, which included average daily accumulation of bouted ≥ 10 min duration MVPA. LPA and sporadic MPA were not associated with any other cardiovascular health markers after controlling for confounders.

Conclusions

1. Since only IPA and LPA were significantly associated with CVD risk factors, the hypothesis that greater volumes of IPA accumulation are associated with positive cardiovascular health outcomes is accepted. However, these associations are restricted to triglycerides and HDL concentrations and SBP.

2. Since there were no significant correlations between sporadic MPA and cardiovascular health outcomes after controlling for confounders, the hypothesis that higher intensity IPA has stronger associations with CVD risk factors is rejected.
Recommendations for Further Study

Future studies should aim to test these hypotheses in sedentary or at risk populations who may be more sensitive to the small effects LPA and sporadic MVPA may have on cardiovascular health.
APPENDIX

Figures and Tables

Study Protocol

Table 1 - Bruce Treadmill Maximal Exercise Protocol

<table>
<thead>
<tr>
<th>Stage</th>
<th>Minutes</th>
<th>% Grade (inclination)</th>
<th>MPH</th>
<th>Approximate Energy Expenditure (METs; 1 MET = resting level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-3</td>
<td>10</td>
<td>1.7</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>3-6</td>
<td>12</td>
<td>2.5</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>6-9</td>
<td>14</td>
<td>3.4</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>9-12</td>
<td>16</td>
<td>4.2</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>12-15</td>
<td>18</td>
<td>5.0</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>15-18</td>
<td>20</td>
<td>5.5</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>18-21</td>
<td>22</td>
<td>6.0</td>
<td>20</td>
</tr>
</tbody>
</table>

(Pescatello & American College of Sports, 2014a)

Table 2 - VO\textsubscript{2}\text{max} Norms - Men

<table>
<thead>
<tr>
<th>Age</th>
<th>Poor</th>
<th>Fair</th>
<th>Good</th>
<th>Excellent</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>\leq 36.1</td>
<td>36.2-42.2</td>
<td>42.3-45.7</td>
<td>45.8-51.1</td>
</tr>
<tr>
<td>30-39</td>
<td>\leq 36.7</td>
<td>36.8-41.0</td>
<td>41.1-44.4</td>
<td>44.5-47.5</td>
</tr>
<tr>
<td>40-49</td>
<td>\leq 34.6</td>
<td>36.5-38.4</td>
<td>38.4-42.4</td>
<td>42.5-46.8</td>
</tr>
<tr>
<td>50-59</td>
<td>\leq 31.1</td>
<td>31.2-35.2</td>
<td>35.3-38.3</td>
<td>38.4-43.3</td>
</tr>
</tbody>
</table>

(Pescatello & American College of Sports, 2014a)

Table 3 - VO\textsubscript{2}\text{max} Norms – Women

<table>
<thead>
<tr>
<th>Age</th>
<th>Poor</th>
<th>Fair</th>
<th>Good</th>
<th>Excellent</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>\leq 31.6</td>
<td>31.7-35.5</td>
<td>35.6-39.5</td>
<td>39.5-44.0</td>
</tr>
<tr>
<td>30-39</td>
<td>\leq 29.9</td>
<td>30.0-33.8</td>
<td>33.9-36.7</td>
<td>36.8-41.0</td>
</tr>
<tr>
<td>40-49</td>
<td>\leq 28.0</td>
<td>28.1-31.6</td>
<td>31.7-35.1</td>
<td>35.2-38.9</td>
</tr>
<tr>
<td>50-59</td>
<td>\leq 25.5</td>
<td>25.6-28.7</td>
<td>28.8-31.5</td>
<td>31.6-35.2</td>
</tr>
</tbody>
</table>

(Pescatello & American College of Sports, 2014a)
ActivPal Statistical Analysis Process

1. Step 1: sort stepping (2) bouts from sit/lie (0) and standing (1) events. EndBout = end of stepping time (allowance 120 second idle non-stepping time within bout)

<table>
<thead>
<tr>
<th>Time</th>
<th>DataCount</th>
<th>Interval (s)</th>
<th>ActivityCode (0=sedentary, 1=standing, 2=stepping)</th>
<th>Cumulative Activity Score (MET·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/7/15 3:17:26 PM</td>
<td>46592</td>
<td>3</td>
<td>1</td>
<td>1.17E-03</td>
</tr>
<tr>
<td>2/7/15 3:17:29 PM</td>
<td>46622</td>
<td>1.6</td>
<td>2</td>
<td>1.34E-03</td>
</tr>
<tr>
<td>2/7/15 3:17:31 PM</td>
<td>46638</td>
<td>1.2</td>
<td>2</td>
<td>1.19E-03</td>
</tr>
<tr>
<td>2/7/15 3:17:32 PM</td>
<td>46650</td>
<td>2.1</td>
<td>2</td>
<td>1.54E-03</td>
</tr>
<tr>
<td>2/7/15 3:17:34 PM</td>
<td>46671</td>
<td>0.8</td>
<td>2</td>
<td>1.03E-03</td>
</tr>
<tr>
<td>2/7/15 3:17:35 PM</td>
<td>46679</td>
<td>1.6</td>
<td>2</td>
<td>1.34E-03</td>
</tr>
<tr>
<td>2/7/15 3:17:37 PM</td>
<td>46695</td>
<td>21.6</td>
<td>1</td>
<td>0.0084</td>
</tr>
<tr>
<td>2/7/15 3:17:58 PM</td>
<td>46911</td>
<td>1.6</td>
<td>2</td>
<td>1.34E-03</td>
</tr>
<tr>
<td>2/7/15 3:18:00 PM</td>
<td>46927</td>
<td>1.3</td>
<td>2</td>
<td>1.23E-03</td>
</tr>
<tr>
<td>2/7/15 3:18:01 PM</td>
<td>46940</td>
<td>28.4</td>
<td>1</td>
<td>1.10E-02</td>
</tr>
<tr>
<td>2/7/15 3:18:29 PM</td>
<td>47224</td>
<td>1.2</td>
<td>2</td>
<td>1.19E-03</td>
</tr>
<tr>
<td>2/7/15 3:18:31 PM</td>
<td>47236</td>
<td>12.7</td>
<td>1</td>
<td>4.94E-03</td>
</tr>
<tr>
<td>2/7/15 3:18:43 PM</td>
<td>47363</td>
<td>1.5</td>
<td>2</td>
<td>1.31E-03</td>
</tr>
<tr>
<td>2/7/15 3:18:45 PM</td>
<td>47378</td>
<td>9.3</td>
<td>2</td>
<td>3.62E-03</td>
</tr>
<tr>
<td>2/7/15 3:18:54 PM</td>
<td>47471</td>
<td>1</td>
<td>3</td>
<td>1.11E-03</td>
</tr>
<tr>
<td>2/7/15 3:18:55 PM</td>
<td>47481</td>
<td>1.6</td>
<td>2</td>
<td>1.34E-03</td>
</tr>
<tr>
<td>2/7/15 3:18:57 PM</td>
<td>47497</td>
<td>175.1</td>
<td>1</td>
<td>6.81E-02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cumulative Step Count</th>
<th>total stepping bout duration</th>
<th>total time stepping</th>
<th>bout total Met-hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>2</td>
<td>1.6</td>
<td>1.6</td>
<td>0.001344444</td>
</tr>
<tr>
<td>CS</td>
<td>4</td>
<td>2.8</td>
<td>2.8</td>
<td>0.002533333</td>
</tr>
<tr>
<td>CS</td>
<td>6</td>
<td>4.9</td>
<td>4.9</td>
<td>0.004072222</td>
</tr>
<tr>
<td>CS</td>
<td>8</td>
<td>5.7</td>
<td>5.7</td>
<td>0.005105555</td>
</tr>
<tr>
<td>CS</td>
<td>10</td>
<td>7.3</td>
<td>7.3</td>
<td>0.006449999</td>
</tr>
<tr>
<td>CS</td>
<td>2</td>
<td>28.9</td>
<td>7.3</td>
<td>0.014849999</td>
</tr>
<tr>
<td>CS</td>
<td>2</td>
<td>30.5</td>
<td>8.9</td>
<td>0.016194443</td>
</tr>
<tr>
<td>CS</td>
<td>4</td>
<td>31.8</td>
<td>10.2</td>
<td>0.017422221</td>
</tr>
<tr>
<td>CS</td>
<td>2</td>
<td>60.2</td>
<td>10.2</td>
<td>0.028466661</td>
</tr>
<tr>
<td>CS</td>
<td>2</td>
<td>61.4</td>
<td>11.4</td>
<td>0.029655555</td>
</tr>
<tr>
<td>CS</td>
<td>2</td>
<td>74.1</td>
<td>11.4</td>
<td>0.034594439</td>
</tr>
<tr>
<td>CS</td>
<td>2</td>
<td>75.6</td>
<td>12.9</td>
<td>0.035899999</td>
</tr>
<tr>
<td>CS</td>
<td>2</td>
<td>84.9</td>
<td>12.9</td>
<td>0.039516662</td>
</tr>
<tr>
<td>CS</td>
<td>2</td>
<td>85.9</td>
<td>13.9</td>
<td>0.040627773</td>
</tr>
<tr>
<td>CS</td>
<td>4</td>
<td>87.5</td>
<td>15.5</td>
<td>0.041972217</td>
</tr>
<tr>
<td>EndBout</td>
<td>4</td>
<td>87.5</td>
<td>15.5</td>
<td>0.041972217</td>
</tr>
</tbody>
</table>
2. Step 2: sum stepping bout duration and MET-hours, and calculated average METs during bout.

<table>
<thead>
<tr>
<th>Step Time Stamp</th>
<th>Steps</th>
<th>Step Duration (min)</th>
<th>Step cadence (steps/min)</th>
<th>Step METs</th>
<th>Step EE Volume (MET hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/7/15 3:17:29 PM</td>
<td>4</td>
<td>1.46</td>
<td>2.74</td>
<td>1.73</td>
<td>0.041972</td>
</tr>
</tbody>
</table>

3. Step 3: sort standing bouts outside of stepping bouts, sum standing bout duration and MET-hours, and calculate average METs of standing bout.

<table>
<thead>
<tr>
<th>Stand Time Stamp</th>
<th>Stand Duration (min)</th>
<th>Stand METs</th>
<th>Stand EE Volume (MET-hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/7/15 3:17:26 PM</td>
<td>0.05</td>
<td>1.40</td>
<td>0.001167</td>
</tr>
<tr>
<td>2/7/15 3:18:57 PM</td>
<td>2.92</td>
<td>1.40</td>
<td>0.068094</td>
</tr>
</tbody>
</table>
4. Step 4: sort stepping bout activity into intensity category (stepping LPA, MPA, or VPA) based on average MET value of bout, and retain all information of bout (time stamp, step count, step duration, step cadence, step METs).

<table>
<thead>
<tr>
<th>LPA Stepping Bout Time Stamp</th>
<th>LPA Steps</th>
<th>LPA Step Duration (min)</th>
<th>LPA Step Cadence (steps/min)</th>
<th>LPA Step METs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/7/15 3:17:29 PM</td>
<td>4</td>
<td>1.46</td>
<td>2.74</td>
<td>1.73</td>
</tr>
</tbody>
</table>

5. Step 5: calculate summary statistics for each type of activity (LPA, sporadic MPA and VPA, bouted MPA and VPA)

<table>
<thead>
<tr>
<th>LPA Summary (Weekly/Daily [24 hours])</th>
<th>Absolute</th>
<th>Relative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T) Total Weekly LPA Time (hours)</td>
<td>17.91</td>
<td>13.81%</td>
</tr>
<tr>
<td>U) Ave. Daily LPA Time (hours)</td>
<td>3.31</td>
<td>13.81%</td>
</tr>
<tr>
<td>V) Ave. LPA Bout Time (min)</td>
<td>1.82</td>
<td>NA</td>
</tr>
<tr>
<td>W) LPA Bout Time Standard Deviation (min)</td>
<td>3.79</td>
<td>NA</td>
</tr>
<tr>
<td>X) Longest LPA Bout (min)</td>
<td>48.83</td>
<td>NA</td>
</tr>
<tr>
<td>Y) Shortest LPA Bout (min)</td>
<td>0.01</td>
<td>NA</td>
</tr>
<tr>
<td>Z) Median LPA Bout (min)</td>
<td>0.37</td>
<td>NA</td>
</tr>
<tr>
<td>AA) Total # LPA Bouts</td>
<td>590.00</td>
<td>NA</td>
</tr>
<tr>
<td>AB) Ave. LPA Bouts per day</td>
<td>109.16</td>
<td>NA</td>
</tr>
<tr>
<td>AC) Total LPA Standing Time (hours)</td>
<td>6.32</td>
<td>4.87%</td>
</tr>
<tr>
<td>AD) Total LPA Stepping Time (hours)</td>
<td>11.59</td>
<td>8.94%</td>
</tr>
<tr>
<td>AE) Average Total LPA Intensity (METs)</td>
<td>1.62</td>
<td>NA</td>
</tr>
<tr>
<td>AF) LPA Intensity Standard Deviation (METs)</td>
<td>0.43</td>
<td>NA</td>
</tr>
<tr>
<td>AG) LPA Max Intensity (METs)</td>
<td>2.99</td>
<td>NA</td>
</tr>
<tr>
<td>AH) LPA Min Intensity (METs)</td>
<td>1.40</td>
<td>NA</td>
</tr>
<tr>
<td>AJ) Ave. Daily LPA Standing Time (hours)</td>
<td>1.17</td>
<td>4.87%</td>
</tr>
<tr>
<td>AK) Ave. LPA Stepping Intensity (METs)</td>
<td>2.14</td>
<td>8.94%</td>
</tr>
<tr>
<td>DJ) Total Weekly LPA EE (MET-hours)</td>
<td>34.95</td>
<td>19.38%</td>
</tr>
<tr>
<td>DL) Average Daily LPA EE (MET-hours)</td>
<td>6.47</td>
<td>19.38%</td>
</tr>
</tbody>
</table>

Table 3

Results
Table 4. Demographic and Cardiovascular Health Characteristics of Participants by Gender

<table>
<thead>
<tr>
<th></th>
<th>Men (n=21)</th>
<th>Women (n=17)</th>
<th>p (diff.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>0.99</td>
</tr>
<tr>
<td>% Caucasian, % Minority</td>
<td>58%, 42%</td>
<td>33%, 67%</td>
<td>0.11</td>
</tr>
<tr>
<td>Resting HR (beats/min)</td>
<td>73.2 ± 9.7</td>
<td>74.5 ± 9.1</td>
<td>0.91</td>
</tr>
<tr>
<td>Resting SBP (mmHg)</td>
<td>128.0 ± 13.6</td>
<td>116.5 ± 13.0</td>
<td>0.83</td>
</tr>
<tr>
<td>Resting DBP (mmHg)</td>
<td>76.3 ± 10.6</td>
<td>73.2 ± 12.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>84.4 ± 17.3</td>
<td>70.5 ± 14.7</td>
<td>0.93</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.8 ± 6.5</td>
<td>165.6 ± 5.0</td>
<td>0.30</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 ± 5.0</td>
<td>25.7 ± 5.4</td>
<td>0.49</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>91.85 ± 17.0</td>
<td>82.1 ± 16.2</td>
<td>0.89</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>103.0 ± 9.4</td>
<td>104.0 ± 10.5</td>
<td>0.29</td>
</tr>
<tr>
<td>Waist to Hip Circumference Ratio (cm)</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Total % Body Fat</td>
<td>26.2 ± 10.1</td>
<td>37.1 ± 8.6</td>
<td>0.78</td>
</tr>
<tr>
<td>Android % Body Fat</td>
<td>34.7 ± 12.9</td>
<td>40.8 ± 9.6</td>
<td>0.27</td>
</tr>
<tr>
<td>Gynoid % Body Fat</td>
<td>30.0 ± 8.9</td>
<td>45.2 ± 6.3</td>
<td>0.28</td>
</tr>
<tr>
<td>Android to Gynoid % Body Fat Ratio</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Carotid Artery PS Diameter (cm)</td>
<td>0.66 ± 0.07</td>
<td>0.59 ± 0.04</td>
<td>0.10</td>
</tr>
<tr>
<td>Carotid Artery PD Diameter (cm)</td>
<td>0.65 ± 0.09</td>
<td>0.59 ± 0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Carotid Artery PSV (cm/sec)</td>
<td>79.0 ± 23.7</td>
<td>77.2 ± 24.4</td>
<td>0.99</td>
</tr>
<tr>
<td>Carotid Artery PDV (cm/sec)</td>
<td>24.9 ± 8.9</td>
<td>23.9 ± 4.9</td>
<td>0.32</td>
</tr>
<tr>
<td>Carotid Artery PS IMT (cm)</td>
<td>0.166 ± 0.087</td>
<td>0.201 ± 0.102</td>
<td>0.14</td>
</tr>
<tr>
<td>Carotid Artery PD IMT (cm)</td>
<td>0.153 ± 0.082</td>
<td>0.193 ± 0.088</td>
<td>0.80</td>
</tr>
<tr>
<td>Popliteal Artery PS Diameter (cm)</td>
<td>0.56 ± 0.13</td>
<td>0.50 ± 0.10</td>
<td>0.60</td>
</tr>
<tr>
<td>Popliteal Artery PD Diameter (cm)</td>
<td>0.55 ± 0.13</td>
<td>0.50 ± 0.10</td>
<td>0.44</td>
</tr>
<tr>
<td>Popliteal Artery PSV (cm/sec)</td>
<td>39.8 ± 16.4</td>
<td>32.9 ± 11.9</td>
<td>0.21</td>
</tr>
<tr>
<td>Popliteal Artery PDV (cm/sec)</td>
<td>18.8 ± 7.1</td>
<td>15.4 ± 2.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Popliteal Artery PS IMT (cm)</td>
<td>0.292 ± 0.173</td>
<td>0.297 ± 0.163</td>
<td>0.76</td>
</tr>
<tr>
<td>Popliteal Artery PD IMT (cm)</td>
<td>0.294 ± 0.167</td>
<td>0.287 ± 0.157</td>
<td>0.61</td>
</tr>
<tr>
<td>Popliteal Artery Absolute FMD (%)</td>
<td>1.8 ± 18.4</td>
<td>10.9 ± 20.2</td>
<td>0.45</td>
</tr>
<tr>
<td>Popliteal Artery Normalized FMD (%)</td>
<td>0.0022 ± 0.0356</td>
<td>0.0390 ± 0.0970</td>
<td>0.09</td>
</tr>
<tr>
<td>VO₂Max (ml O₂/kg/min)</td>
<td>43.9 ± 5.9</td>
<td>34.9 ± 4.8</td>
<td>0.40</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>160.8 ± 25.1</td>
<td>168.4 ± 35.4</td>
<td>0.50</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>86.9 ± 31.3</td>
<td>65.5 ± 27.0</td>
<td>0.58</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>47.0 ± 14.4</td>
<td>64.5 ± 17.2</td>
<td>0.28</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>96.4 ± 21.2</td>
<td>90.8 ± 30.3</td>
<td>0.68</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>4.3 ± 11.2</td>
<td>1.75 ± 2.5</td>
<td>0.17</td>
</tr>
<tr>
<td>TNF-alpha (mg/dL)</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>0.16</td>
</tr>
<tr>
<td>HgbA1c (%)</td>
<td>5.5 ± 0.2</td>
<td>5.6 ± 0.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Resting Plasma Glucose (mg/dL)</td>
<td>87.5 ± 9.5</td>
<td>83.8 ± 5.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Resting Plasma Insulin (uIU/mL)</td>
<td>9.2 ± 6.0</td>
<td>8.8 ± 7.5</td>
<td>0.87</td>
</tr>
</tbody>
</table>
### Table 5. PA Outcomes by Gender

<table>
<thead>
<tr>
<th></th>
<th>Men (n=21)</th>
<th>Women (n=17)</th>
<th>p (diff.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average ActivPal Monitor Wear Time (days-24 h)</td>
<td>6.3 ± 0.9</td>
<td>5.6 ± 2.2</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Duration & Intensity**

- Average Daily SED Time (hours) | 18.4 ± 2.5 | 18.2 ± 2.6 | 0.34 |
- Average Daily LPA Time (hours) | 4.7 ± 2.7  | 5.2 ± 2.6  | 0.49 |
- Average Daily Sporadic MPA Time (min) | 10.4 ± 6.1 | 11.0 ± 7.7 | 0.39 |
- Average Daily Bouted MPA Time (min) | 45.6 ± 36.8 | 24.7 ± 29.7 | 0.28 |
- Average Daily IPA Time (hours) | 4.9 ± 2.7  | 5.4 ± 2.6  | 0.48 |

**Energy Expenditure**

- Average Daily LPA EE (MET-h) | 8.6 ± 4.2  | 9.0 ± 4.1  | 0.45 |
- Average Daily Sporadic MPA EE (MET-h) | 0.56 ± 0.34 | 0.63 ± 0.44 | 0.31 |
- Average Daily Bouted MPA EE (MET-h) | 2.6 ± 2.2  | 1.4 ± 1.7  | 0.26 |
- Average Daily IPA EE (MET-h) | 9.1 ± 4.2  | 9.6 ± 4.2  | 0.41 |

### Table 6. Regression Analyses – Average Daily IPA Time (LPA + Sporadic MPA) Unadjusted

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>n</th>
<th>R-Squared</th>
<th>t-score</th>
<th>Beta 95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting SBP (mmHg)</strong></td>
<td>38</td>
<td>0.122</td>
<td>0.006</td>
<td>0.64 – 0.98</td>
<td>0.031</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dL)</strong></td>
<td>35</td>
<td>0.157</td>
<td>0.003</td>
<td>0.19 – 0.85</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>HDL Cholesterol (mg/dL)</strong></td>
<td>35</td>
<td>0.251</td>
<td>2500</td>
<td>1.45 – 4.67</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### Table 7. Regression Analyses – Average Daily IPA Time (LPA + Sporadic MPA) Adjusted for Confounders (Age, Gender, accelerometer wear time, and bouted MPA time)

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>n</th>
<th>R-Squared (adjusted)</th>
<th>t-score</th>
<th>Beta 95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting SBP (mmHg)</strong></td>
<td>34</td>
<td>0.224</td>
<td>0.007</td>
<td>0.64 – 0.99</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>HDL Cholesterol (mg/dL)</strong></td>
<td>31</td>
<td>0.369</td>
<td>60.3</td>
<td>0.92 – 3.30</td>
<td>0.087</td>
</tr>
</tbody>
</table>
Table 8. Regression Analyses – Average Daily LPA Time Unadjusted

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>n</th>
<th>R-Squared</th>
<th>t-score</th>
<th>Beta 95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting SBP (mmHg)</strong></td>
<td>38</td>
<td>0.118</td>
<td>0.006</td>
<td>0.65 – 0.98</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dL)</strong></td>
<td>35</td>
<td>0.165</td>
<td>0.003</td>
<td>0.20 – 0.83</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>HDL Cholesterol (mg/dL)</strong></td>
<td>35</td>
<td>0.267</td>
<td>5495</td>
<td>1.49 – 4.62</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 9. Regression Analyses – Average Daily LPA Time Adjusted for Confounders (Age, Gender, accelerometer wear time, and bouted MPA time)

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>n</th>
<th>R-Squared (adjusted)</th>
<th>t-score</th>
<th>Beta 95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting SBP (mmHg)</strong></td>
<td>34</td>
<td>0.227</td>
<td>0.007</td>
<td>0.64 – 0.99</td>
<td>0.039</td>
</tr>
<tr>
<td>*HDL Cholesterol (mg/dL)</td>
<td>31</td>
<td>0.378</td>
<td>79.4</td>
<td>0.95 – 3.30</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Table 10. Regression Analyses – Average Daily Sporadic MPA Time Unadjusted

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>n</th>
<th>R-Squared</th>
<th>t-score</th>
<th>Beta 95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Popliteal Artery PSD (cm)</strong></td>
<td>38</td>
<td>0.133</td>
<td>0.004</td>
<td>0.70 – 0.97</td>
<td>0.025</td>
</tr>
<tr>
<td><strong>Popliteal Artery PDD (cm)</strong></td>
<td>38</td>
<td>0.123</td>
<td>0.004</td>
<td>0.70 – 0.98</td>
<td>0.031</td>
</tr>
<tr>
<td>*Popliteal Artery PDV (cm/sec)</td>
<td>37</td>
<td>0.102</td>
<td>97.7</td>
<td>0.99 – 1.58</td>
<td>0.054</td>
</tr>
<tr>
<td>*HDL Cholesterol (mg/dL)</td>
<td>35</td>
<td>0.093</td>
<td>0.015</td>
<td>0.58 – 1.02</td>
<td>0.076</td>
</tr>
</tbody>
</table>

Table 11. Regression Analyses – Average Daily Sporadic MPA Time Adjusted for Confounders (Age, Gender, accelerometer wear time, and bouted MPA time)

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>n</th>
<th>R-Squared (adjusted)</th>
<th>t-score</th>
<th>Beta 95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Android % Fat</td>
<td>34</td>
<td>0.250</td>
<td>1.90</td>
<td>0.97 – 2.20</td>
<td>0.068</td>
</tr>
<tr>
<td>*Total % Fat</td>
<td>34</td>
<td>0.334</td>
<td>51.3</td>
<td>0.94 – 2.08</td>
<td>0.099</td>
</tr>
<tr>
<td>Variable</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPA Duration (hours/day)</td>
<td>3.06 ± 0.59</td>
<td>4.34 ± 0.41</td>
<td>7.55 ± 3.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IPA Duration (hours/day)</td>
<td>3.21 ± 0.59</td>
<td>4.52 ± 0.48</td>
<td>7.75 ± 3.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>42.2 ± 11.7</td>
<td>56.8 ± 16.6</td>
<td>63.6 ± 19.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) : significantly different than tertile 1, p 0.016
(b) : significantly different than tertile 1, p 0.002

**Figure 1** – Tertiles of Daily LPA Accumulation with HDL Cholesterol
### Table

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPA Duration (hours/day)</td>
<td>3.06 ± 0.59</td>
<td>4.34 ± 0.41</td>
<td>7.55 ± 3.25</td>
</tr>
<tr>
<td>Total IPA Duration (hours/day)</td>
<td>3.21 ± 0.59</td>
<td>4.52 ± 0.48</td>
<td>7.75 ± 3.20</td>
</tr>
<tr>
<td>Resting SBP (mmHg)</td>
<td>122 ± 14.0</td>
<td>127 ± 12</td>
<td>116 ± 15 (a)</td>
</tr>
</tbody>
</table>

(a) = significantly different than tertile 2, p 0.05

**Figure 2** – Tertiles of Daily LPA Accumulation with resting SBP
IRB Approval Form

Northeastern

Notification of IRB Action
Modification

Date: October 30, 2014

IRB #: 14-01-03

Principal Investigator(s): Dinesh John

Department: Health Sciences

Address: 316D Robinson Hall
Northeastern University

Title of Project: Examining Change in Metabolic Response to Differences in Posture and Muscle Activation under Controlled Energy Expenditure

Modification: a) use of DEXA for body composition; b) subject # increased to 50; c) reduced lab visits to 2; d) added Near Infrared Spectrometry; e) blood draws decreased from 5 to 3; f) VO2 test added; g) exclusion criteria added; h) activity monitors will be worn for one week; i) ultrasound of neck and knee arteries will be measured

Participating Sites: N/A

DHHS Review Category: Expedited #2(a), #4, #7 - Full board approval for DEXA scan

Original Protocol Approval: February 3, 2014

Most Recent Approval: March 20, 2014 (modification)

Informed Consents: One (1) signed consent form with Health Information Use and Disclosure Authorization with Detailed Procedures Addendum

Approval Expiration Date: FEBRUARY 2, 2015

Investigator’s Responsibilities:

1. The informed consent form bearing the IRB approval stamp must be used when recruiting participants into the study.
2. The investigator must notify IRB immediately of unexpected adverse reactions, or new information that may alter our perception of the benefit-risk ratio.
3. Study procedures and files are subject to audit any time.
4. Any modifications of the protocol or the informed consent as the study progresses must be reviewed and approved by this committee prior to being instituted.
5. Continuing Review Approval for the proposal should be requested at least one month prior to the expiration date above.
6. This approval applies to the protection of human subjects only. It does not apply to any other university approvals that may be necessary.

C. Randall Colvin, Ph.D., Chair
Northeastern University Institutional Review Board

Nan C. Regina
Director, Human Subject Research Protection

Northeastern University FWA #: 4630
REFERENCES


Abramson, J., & Vaccarino, V. (2002). Relationship between physical activity and inflammation among apparently healthy middle- aged and older US adults. *Archives Of Internal Medicine, 162*(11), 1286-1292.


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up in men and women from the Whitehall II cohort study. *Circulation, 126*(8), 928. doi: 10.1161/CIRCULATIONAHA.112.103879


