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Does acute preprandial exercise modify postprandial inflammation after a high fat meal in young and older adults?

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Does acute preprandial exercise modify postprandial inflammation after a high fat meal in young and older adults?

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Abstract

A single high-fat meal (HFM) can increase systemic inflammation (postprandial inflammation; PPI) and may be attenuated by recent exercise. However, the effect of preprandial exercise on PPI in older adults (OA) is not clear. **Purpose**: To determine if preprandial exercise attenuates PPI in YA and OA. **Methods**: 12 YA (23.3±3.9 years n= 5 M/7 F) and 12 OA (67.7±6 years, n= 8 M/4 F) completed two HFM challenges in a randomized order. During HFM alone, participants abstained from exercise 48 hours prior to the HFM session (except for the exercise session) and adhered to a 12 hour overnight fast. Baseline measurements were taken immediately before participants consumed the HFM (12 kcals/kg BW: 57% fat, 39% CHO, 4% PRO), and then again three and six hours postprandially. In exercise + HFM (EX+HFM) participants performed exercise at a heart rate that corresponded to 65% VO_{2Peak} until caloric expenditure matched 75% of the caloric content of the HFM, then adhered to a 12 hour overnight fast before consuming the HFM. **Results**: All markers of inflammation were lower at three hours postprandially regardless of condition or age ($p<0.05$). Furthermore, IL-10 (93% of baseline) and TNFα (88% of baseline) remained below baseline concentrations at six hours ($p<0.05$) while IL-6, IL8, and IL-1β increased back to baseline levels ($p>0.05$). TNFα was greater in OA at baseline compared to YA ($p<0.05$), but not at three or six hours postprandially ($p>0.05$). There was no difference in inflammation between HFM and EX+HFM at any time point ($p>0.05$). **Conclusion**: Contrary to our hypotheses, a HFM did not elicit PPI in YA or OA. Additionally, exercise did not impact inflammation at any timepoint. Future work should be performed on the PPI response and the effect of preprandial exercise on individuals already afflicted with inflammation related diseases.

Chapter I

Western Diet

The typical western diet consists of nutrient poor and calorically dense foods, including red meats, simple refined carbohydrates, and saturated or trans fats. Research into the potential health risks of the western diet has increased over the past decades in reaction to the increased prevalence of lifestyle disease in the American population[1]. Specifically, the prevalence of obesity, cardiovascular disease, hypertension, and type II diabetes has increased in tandem with the proliferation of chronic high-fat and high calorie, westernized diets[2]. While the negative health effects from chronic westernized diets have been well characterized and may extend beyond obesity[3], the adverse health effects of regular high-fat meals may arise prior to the onset of obesity. It is likely that there are adverse metabolic and immune responses result from a single high-fat, westernized meal[3–5]. Research has shown the influence of a single HFM on complex physiological functions[6] (i.e. inflammation, immune system, metabolism), which provides insights into the mechanistic role a chronic westernized diet has on the risk of metabolic and cardiovascular disease development. A key mechanism initiating the development of chronic diseases may be the prolonged activation of innate and acquired immune pathways.

Innate Immune System and Inflammation

The innate immune system is the first response to tissue damage or the presence of invading antigens[7]. Activation of the innate immune system is characterized by the mobilization of neutrophils, macrophages[8], and natural killer (NK) cells[9] . These cells engulf the pathogens to be destroyed within the cell or secrete other proinflammatory

cytokines to mediate additional inflammatory effects[8]. Interleukin-6 (IL-6) is a pro-inflammatory cytokines that recruits T cells and B cells from the acquired immune system to aid in managing invading pathogens[10].

Inflammation is a complex, multi-regulated, physiological response to tissue damage or invading pathogens[11]. Classical inflammation is associated with swelling and tenderness from a laceration or damaged muscles that is easily visible and causes discomfort. However, classical inflammation is not an urgent problem for most people and is resolved by the coordination of innate immune responses and tissue healing within days of injury. Inflammation can also be caused by invading pathogens, which provoke an acute inflammatory response at a subclinical level. Regular exposure to such pathogens results in the prolonged presence of pro-inflammatory cytokines involved in the subclinical response and promotion of a state of persistent low-grade, systemic inflammation. It appears that this type of chronic, low-grade systemic inflammation plays a detrimental role in human health. The presence of low-grade inflammation has been associated with increased risk of cardiovascular disease[12,13], vascular diseases[14], atherosclerosis[15], type II diabetes[16], Alzheimer's disease[17], and various cancers[18,19].

Damage to the vascular tissue by penetration of pathogens triggers an innate immune response and a pro-inflammatory cascade that begins with monocytes arriving at the site of endothelial cell activation. Monocytes enter vascular tissue around activated endothelial cells and differentiate into macrophages[20] which absorb circulating pathogens in an effort to prevent further damage. Working macrophages subsequently secrete two types of acute phase inflammatory proteins, interleukin-6 (IL-6) and tumor necrosis factor-

α[21] (TNF-α). IL-6 and TNF-α are two upstream mediators that further contribute to the pro-inflammatory cascade[22,23].

IL-6 is a multifunctional, polypeptide protein hormone[24]. Depending on the interaction of IL-6 with either IL-6 receptor (IL-6R) or soluble IL-6 receptor (sIL-6R) [25,26], IL-6 can mediate either a pro or ant-inflammatory response. During an innate immune response, IL-6 is synthesized and secreted from active pro-inflammatory M1 macrophages. As a pro-inflammatory cytokine, IL-6 is the key regulator of secondary acute phase inflammatory protein synthesis in the liver, such as c-reactive protein[27] (CRP), a marker of chronic inflammatory.

IL-6 also contributes to creating an environment that is more prone to chronic inflammation[26]. Prolonged secretion of IL-6 stimulates more IL-6 synthesis and secretion, as well as B cell differentiation, T cell activation, and immunoglobulin secretion[26]. This leads to an increase in monocyte recruitment which then stimulates secretion of IL-8 and monocyte chemoattractant protein 1 (MCP-1)[28]. MCP-1 stimulates the recruitment of additional monocytes, which differentiate into inflammatory macrophages within the vascular tissue[29]. The shift to more tissue resident macrophages creates an environment that can more easily promote inflammation via IL-6 and TNF-α synthesis and secretion[26,30].

TNF-α is regulated by nuclear factor-kappa-B (NF-kB) and mediates inflammation by modifying endothelial leukocyte function. These cells exhibit altered combinations of leukocyte adhesion molecules E-selectin, vascular cell adhesion molecule-1(VCAM-1), and intercellular adhesion molecule-1[26] (ICAM-1). ICAM-1 and VCAM-1 are essential in promoting macrophage infiltration into vascular and adipose tissue. ICAM-1 and VCAM-

1 also increase the rate at which inflammatory cytokines adhere to the endothelium, which contributes to the development and progression of atherosclerosis.

Inflammation is a complex process involving collaborating systems, cytokines, and molecules. Chronic inflammation is achieved through continued activation of acute inflammation responses. These consequences of regular acute inflammation creates an environment of chronic, low-grade inflammation and highlights the importance of minimizing exposure to inflammatory stimuli. A frequent HFMs may be a stimuli for such consequences, and lead to chronic inflammation process through repeated increases in postprandial lipemia and acute inflammation.

Postprandial Lipemia and Inflammation

High and/or moderate fat meals consisting of 0.4-1.5 g of fat/kg bw can elicit a significant increase in postprandial lipemia[31] (PPL), typically measured through blood triglyceride (TG) concentrations. Even a single HFM increases circulating triglycerides and very low density lipoprotein (VLDL) concentration. Elevated levels of TG cause a decrease in high density lipoproteins (HDL) and an increase in low density lipoproteins[32] (LDL). LDLs are a smaller, denser derivative of VLDLs, formed as a result of the breakdown of circulating triglycerides. LDLs can penetrate endothelial cells of the vascular wall and initiate the inflammatory cascade previously mentioned[33]. Vascular tissue penetration and endothelial cell activation has an immediate detrimental effect on endothelial function[34]. The buildup of plaque on vascular walls enables greater monocyte penetration, ultimately contributing to an environment that promotes inflammation. Frequent consumption of 2-3 HFMs a day by many individuals means that they spend much of the day in a state of postprandial lipemia and inflammation.

Chronic, low-grade systemic inflammation has been reported to increase due to a Westernized lifestyle. The scale of the postprandial inflammatory response after a single HFM is dependent on the percent of saturated and trans fat within the meal as well as its caloric density. Typical western meals contain approximately 20-40 grams of saturated fat. Absolute saturated and trans fat content of 30-50 grams in a single meal causes significant increases in circulating triglycerides[35] (known as postprandial lipemia (PPL)) and possibly postprandial inflammation (PPI) as a result of PPL. HFMs with relative saturated and trans fat content in a meal of at least 60% also cause PPL and PPI.

Variations in Postprandial Inflammatory Responses

A wide range of macronutrient ratios have been used in PPI research. Increases in IL-6 have been observed with fat percentages as low as 30% of the meal[36], while meals with up to 85% of the macronutrients from fat and have seen similar responses in postprandial IL-6 concentrations[37]. Absolute versus relative total calories consumed is also a factor in the PPI. There is substantial variation in absolute calorie content between meals in the literature, ranging from 600[38] kilocalories[39,40] (kcals) to over 1470 kcals[41]. Kračmerová and colleagues used a HFM containing over 1470 kcals with 46% fat, resulting in significant increases in IL-6 and increased mRNA expression of TNF-α, MCP-1, and other inflammatory markers such as IL-1β, and IL-8 in young healthy men. In contrast, Drew and colleagues implemented a HFM containing 600 kcals with 50% fat and reported increases in IL-6 and TNF-α that were 7% greater than those of Kračmerová and colleagues. Relative caloric content is also commonly used in HFM challenges, because it is more applicable to real-world eating habits. Results for HFMs relative caloric content are consistent with those of the absolute caloric content[42,43]. Even though HFMs consisting

of large absolute caloric content elicit large pro-inflammatory changes in IL-6 and possibly TNF-α, more realistic meals with relative caloric content still elicit significant pro-inflammatory responses, though findings have been mixed.

It is understood that PPI may be dependent on the type of fat, the percentage of fat in the meal, and the relative versus absolute calories consumed. However, recent studies have also suggested that chronic physical activity level and participation in acute exercise may be an important consideration in reducing chronic low-grade systemic inflammation and acute PPI[44].

The Effect of Exercise on Postprandial Inflammation

Exercise is a natural anti-oxidant[45] and anti-inflammatory[46], and therefore may induce a systemic attenuation of PPI. The potential attenuation of PPI from exercise could occur through several mechanisms. Exercise can act indirectly on PPI through mitigation of the lipemic pathway of inflammation, through reduction of PPL via circulating LDL through [47] and/or increased plasma VLDL clearance[48], or by up-regulation of lipoprotein lipase levels (LPL) at the skeletal muscle[48], and increased lipid hydrolysis. LDL will decrease as a result of these attenuations in VLDL, which will reduce scale of endothelial activation and inflammation response. Alternatively, exercise can elicit a direct anti-inflammatory state through increased anti-inflammatory cytokine secretion[7].

Post exercise reductions in plasma VLDL levels results in decreased LDL concentration and potential for endothelial activation. Moderate intensity exercise (60% $VO2_{MAX}$) performed the afternoon before a morning HFM has been shown to reduce postprandial VLDL clearance by 40%, but not hepatic secretion[48]. Additionally, there

appears to be a nonlinear dose response to exercise's effect on VLDL dynamics. The effect of an acute one hour moderate intensity cycling (60% $VO2_{MAX}$) bout had a lesser effect on VLDL levels compared to a 90 minute exercise session[49], and changes in VLDL levels nearly plateau as exercise exceeded two hours[50,51]. Exercise induced caloric deficit may provide be the mediator for eliciting decreases in VLDL levels. Additionally, a threshold caloric deficit of 500 kcals may exist, as there is minimal or absent reductions in VLDL concentrations when energy expenditure is less than 500 kcals[52]. However, inconsistencies in changes in VLDL at this low caloric expenditure might suggest the need for a relative caloric expenditure threshold, additional research needs to be conducted to confirm this idea. The introduction of regular exercise in previously sedentary, healthy individuals as well as type II diabetics decreases hepatic VLDL secretion[47]. This suggests a protective benefit of chronic exercise. Exercise of similar duration and intensity needed to alter hepatic VLDL secretion also increases LPL activity 8-22 hours after exercise[53]. Greater mass and activity of LPL at the muscle and endothelial membrane upregulates lipoprotein hydrolysis and reduces circulating lipoproteins[52].

Regular physical activity appears to reduce fasting pro-inflammatory IL-6 levels in healthy middle aged men for at least 24 hours after an individual's last exercise bout. However, postprandial IL-6 levels are no different between regularly active and inactive men[54] when exercise is abstained for 24 hours. This indicates that there might be a narrow time frame for the direct anti-inflammatory effects of exercise. Working skeletal muscles secrete anti-inflammatory IL-6 in concentrations up to 100 times greater than fasting levels [55,56]. Prolonged muscle contraction[57] and the depletion of muscle glycogen stores[58] appear to be the main stimulus for increases in anti-inflammatory IL-6. IL-6 freely enters the

bloodstream after secretion from skeletal muscle, eliciting a systemic effect predominantly mediated by inhibiting the release of pro-inflammatory cytokines TNF-α and IL-1 in addition to upregulating the production of anti-inflammatory interleukin-10 (IL-10).

Circulating IL-10 mediates many of the systemic anti-inflammatory benefits of exercise[59]. IL-10 directly downregulates pro-inflammatory cytokines and inhibits production of ICAM1 which both reduce pro-inflammatory capacity[60]. Additionally, IL-10 down regulates macrophage concentrations and also reduce macrophage penetration into adipose and muscular cells in mice trials[61]. Thus, exercise can have a large and systemic anti-inflammatory response that directly counteracts multiple pro-inflammatory pathways.

Exercise Prescriptions for Attenuating PPI

Due to the link between PPL and PPI, the ability of exercise to attenuate the increase in postprandial circulating TG indicates its effectiveness at also attenuating PPI as well as increased anti-inflammatory IL-6 and IL-10 production. Research into type, duration, and intensity of exercise show that certain modalities and prescriptions are more effective than others[31]. In a review by Freese and colleagues, low to moderate intensity exercise (d = -0.58; $P < 0.0001$) and high intensity interval exercise (>90% VO_{2Max}) (d = -1.49; $P < 0.0001$) both significantly reduced PPL[31] with high intensity interval exercise having the largest impact. In contrast, resistance training did not significantly reduce PPL (d = -0.13; $P < 0.01$)[31]. Yet, the effect that exercise duration and intensity have on PPL and PPI is based on the caloric deficit they produce[6].

Additionally, the magnitude of the exercise induced caloric deficit varies widely in the literature. Both absolute and relative energy expenditures are used to prescribe preprandial and postprandial exercise. However, Katsanos and Moffatt suggest attenuation of PPL is most commonly observed with preprandial exercise that reaches an energy expenditure threshold of at least 500 kcals[62]. Several studies have utilized acute exercise bouts where energy expenditures were over 1000 kcals. While these types of exercise bouts elicit the greatest reductions in PPL, the intensity and duration of exercise to reach 1000 kcals is not feasible for most people and may not be true to life. To improve PPL and PPI outcomes, but keep exercise realistic for most individuals, recent studies employ exercise where caloric expenditure is in the range of 600-900 kcals, which may still attenuate PPL and PPI.

Older Adults and Inflammation?

Systemic, low-grade inflammation, termed inflammaging, increases through the aging process[12,63]. In addition, the immune system gradually deteriorates with age, known as immunosenescence. Immunosenescence worsens the immune system's response time and effectiveness when pathogens are present, increasing susceptibility to disease and illness[12]. A life time of poor lifestyle choices, including sedentary behavior and poor dietary choices, results in more frequent exposure to various pathogens[64]. Prolonged pathogen exposure may elicit subclinical inflammatory responses which mediate inflammaging[43] and cause greater damage to tissues and cells. In a 10 year longitudinal study of 249 male and female older subjects age 67.5 $\pm$ 2.4 years old, Bartlett and colleagues reported significant increases in IL-6, TNF-α, and CRP[63]. Additionally, there was a significant decrease in IL-10, indicating not only increased inflammation via greater

concentrations of pro-inflammatory cytokines, but also through a reduction in anti-inflammatory mediators.

Similar to low-grade, systemic inflammation that is not associated with age, inflammaging is also strongly associated with diseases commonly seen in older populations[64]. However, despite the growing numbers of older Americans, little research has been done to determine the impact of a HFM on those 60 and older[65,66]. Inflammation may magnify the normal PPI response observed in young adults. Furthermore, substantial decreases in skeletal muscle mass and resting IL-10 levels suggest a decreased ability to upregulate anti-inflammatory cytokines. Thus, the effect that acute and chronic exercise have on PPI in young adults may vary when compared to older adults.

Purpose and Hypotheses

The purpose of this study is to determine whether an acute bout of preprandial exercise attenuates the PPI response after a HFM in young and older adults. We hypothesize that (1) an acute bout of physical activity will attenuate the postprandial inflammatory response in young and older adults, (2) older adults will experience a greater attenuation from acute exercise than young adults.

Chapter II

Methods

Subjects

Twenty-four subjects will be recruited for this study, of which there will be twelve young adults between 18-35 years (n= 5 M / 7 F) and 12 older adults between 60-80 years (n= 8 M/ 4 F). Subjects will be asked to complete a physical activity readiness questionnaire (PAR-Q) to determine whether they are eligible to enroll in the study. They also will complete the international physical activity questionnaire (I-PAQ) to determine chronic physical activity level to estimate physical activity in MET-minutes/week.

Experimental Design

Each subject will complete a total of four laboratory visits. On the first visit, initial baseline measures of height, weight, bodyfat percentage, and blood pressure will be taken and VO_{2Peak} will be determined. Subjects will then be randomly assigned an order to perform two HFM challenges, either the HFM alone (HFM) or a HFM challenge with a preprandial exercise session twelve hours prior to the meal (EX + HFM) that will be conducted in the same lab. The experimental design can be seen displayed in Figure 1, below.

Initial Measurements

The first laboratory visit will be used to collect baseline measurements. Height will be taken with a stadiometer (Charder Model HM 200P, Charder Electronic Co Ltd., Taichung, Taiwan) and weight with a standard physician's scale (Dymo Pelouze model 4040, Newell Brands, Hoboken, NJ) to calculate body mass index. Following height and weight, a dual-energy x-ray absorptiometry (DEXA) (GE Lunar iDXA Fairfield, CT) scan

will be performed to calculate lean mass, fat mass, and percent body fat. Resting blood pressure (BP) will be taken immediately after the DEXA is completed, so that the subject will have been resting for at least five minutes. BP will be measured using an automatic Sphygmomanometer (ProBP 3400 Welch Allyn, Skaneateles Falls, NY) as detailed in American College of Sports Medicine (ACSM) Guidelines. The average of the two BP measurements will be recorded. Waist circumference will be taken at the narrowest part of the waist with a Gulick tape measurer (Creative Health Products, Ann Arbor, MI).

Maximal Exercise Testing

An incremental cycling test to exhaustion will be performed on a VIAsprint 150P cycle ergometer (Vyaire Medical, Mettawa, IL) to determine VO_{2Peak}. Expired air will be analyzed by a Vmax Encore metabolic cart (Vyaire Medical, Lake Forest, IL), while heart rate (HR) will be measured by a Polar Link heart rate strap and watch (Polar Lake Success, NY). Once the headset, mouthpiece, and nose plug are fitted the subjects will complete a five minute warm up at a self-selected cadence $\geq$50 revolutions per minute (rpm). During the warm up, power output will be either increased or decreased every minute until subjects identify a workload, they perceived as maintainable for approximately thirty minutes.

Based on the power output determined during the warm up, one of four potential test protocols will be chosen (Table 1) in order to reach VO_{2Peak}. Immediately after the warm up, the incremental test will begin, and wattage will be set according to stage one of the selected protocol. Resistance will then be increased every minute based on the protocol being used (10 Watts or 25 Watts). Subjects will be verbally encouraged to continue pedaling by the investigators throughout the exercise test. The test will be terminated when subjects reach volitional exhaustion.

Food Log and ASA24 Food Recall

Subjects will be given a food log and instructed to record, in detail, all foods and beverages consumed the day before their HFM Challenge. For the second HFM Challenge, subjects will be asked to duplicate their diet from the previous HFM Challenge as closely as possible, and once again record in detail their diet for that day.

Preprandial Exercise Bout

During the EX + HFM trial, subjects will perform an acute exercise bout twelve hours prior to their HFM challenge on the VIAsprint 150P cycle ergometer (Vyaire Medical, Mettawa, IL), which was previously used for the VO_{2Max} test. Wattage will be set so that subjects' HR stays within a HR range that corresponds to 60-70% of their VO_{2Peak} HR, for a duration long enough to achieve a total energy expenditure equivalent to 75% of the calories of the HFM challenge (Equation 1). However, subjects will not be permitted to exercise longer than 2-hours. After the exercise bout, subjects will be asked to adhere to the same 12 hour overnight fast as they would for the non-exercise trial.

HFM Composition

Subjects will be asked to adhere to an overnight twelve hour fast before consuming the HFM which will consist of a slice of Marie Callender's Chocolate Satin Pie (Conagra Brands, Chicago, IL) portioned out to a caloric density of 12 kcals/kg bw. Macronutrient composition of the HFM will be 57% fat (57% of which is saturated fat), 4% PRO, and 39% CHO.

HFM Challenge Day

After reporting to the lab, subjects will be seated in a reclining chair for the duration of the HFM session. After resting for at least five minutes before two baseline BP

measurements will be taken with at least 30 seconds of rest between each recording. An indwelling safelet catheter will be inserted into a forearm vein via a 22-gauge needle (Fisher Scientific, Waltham, MA) and kept patent with a 0.9% NaCl solution with a drip rate of one/second. A fasting blood draw will be taken once the catheter is in place via a 5 mL syringe (BD) and emptied into a 6-mL EDTA-coated vacutainer tube. Subjects' satiety will then be recorded via the satiety labeled intensity magnitude (SLIM) Satiety Scale. Following the baseline blood draw and SLIM rating, subjects will have 20 minutes to consume the HFM.

Minimal movement will be allowed for the duration of the six hours after consumption. The subject will not be allowed to stand up except for inside the restroom. At every hour postprandially, subjects' satiety will be recorded by the SLIM scale. Blood draws will be taken to assess inflammatory markers at baseline, three hours, and six hours. The experimental protocol is pictured in Figure 1.

Blood samples will be drawn every 30 minutes postprandially until the two hour mark at which point draws will be performed every hour. Plasma (for inflammatory markers), and blood glucose and lipids (Cardiochek Plus Analyzer, PTS Diagnostics, Indianapolis, IN) will be tested prior to the HFM challenge. Plasma samples will be taken at baseline, 3, and 6 hours. Blood glucose will be tested every 30 minutes up to 2 hours postprandial, and then every hour. Blood lipids will be tested every hour from baseline until 6 hours post-prandially.

Cytokine Analysis

After blood draws, samples will be transferred to six ml BD vacutainers and immediately centrifuged for 12 minutes at 1800 x *g* and four degrees Celsius. After being

separated blood plasma will be aliquoted into 0.5 ml microtainers and stored for later investigation in a freezer at -80 C. All plasma samples will be assayed at Eve Technologies (Calgary, Alberta, Canada). For this study, a Human High Sensitivity T-Cell Discovery Array 14-plex (HDHSTC14) will be used to measure IL-6, IL-1β, IL-8, IL-10, and TNF-a. Biomarkers will be analyzed in duplicate and the average measurement will be used for analysis of coefficient of variance for each analyte.

Statistical Analysis

Statistical analysis will be done with IBM SPSS Statistics v26.0 (IBM Corporation, Armonk, NY). To determine statistical significance with 80% power at an alpha level of 0.05 for determining differences in postprandial triglycerides based on age and activity level, five participants will be needed. However, 12 participants will be recruited in each group to detect changes in inflammatory responses. All data will be checked for normality before analysis using the Shapiro-Wilk test. When data is not normally distributed, it will be log-10 transformed and checked again for normality. A mixed factorial analysis of variance (ANOVA) will be done with the within-subjects' factor of time (baseline, three, and six hours for cytokines) and condition (HFM or EX+HFM) and the between subjects' factor of age (YA and OA) and if significant main effects of group or time are detected, first data will assessed to determine significance as a linear or quadratic function. Next, post hoc multiple comparisons will be conducted to test for group- or time-specific differences. In this instance, Bonferroni adjustment for multiple comparisons will be implemented to avoid type I error. Pearson's r comparisons will be also be performed to assess associations between PPL and PPI. For all analyses, significance will be set to $p<0.05$.

Chapter III

Manuscript

Does acute preprandial exercise modify postprandial inflammation after a high fat meal in young and older adults?

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Abstract

A single high-fat meal (HFM) can increase systemic inflammation (postprandial inflammation; PPI) and may be attenuated by recent exercise. However, the effect of preprandial exercise on PPI in older adults (OA) is not clear. **Purpose**: To determine if preprandial exercise attenuates PPI in YA and OA. **Methods**: 12 YA (23.3±3.9 years n= 5 M/7 F) and 12 OA (67.7±6 years, n= 8 M/4 F) completed two HFM challenges in a randomized order. During HFM alone, participants abstained from exercise 48 hours prior to the HFM session (except for the exercise session) and adhered to a 12 hour overnight fast. Baseline measurements were taken immediately before participants consumed the HFM (12 kcals/kg BW: 57% fat, 39% CHO, 4% PRO), and then again three and six hours postprandially. In exercise + HFM (EX+HFM) participants performed exercise at a heart rate that corresponded to 65% VO_{2Peak} until caloric expenditure matched 75% of the caloric content of the HFM, then adhered to a 12 hour overnight fast before consuming the HFM. **Results**: All markers of inflammation were lower at three hours postprandially regardless of condition or age($p<0.05$). Furthermore, IL-10 (93% of baseline) and TNFα (88% of baseline) remained below baseline concentrations at six hours ($p<0.05$) while IL-6, IL8, and IL-1β increased back to baseline levels ($p>0.05$). TNFα was greater in OA at baseline compared to YA ($p<0.05$), but not at three or six hours postprandially ($p>0.05$). There was no difference in inflammation between HFM and EX+HFM at any time point ($p>0.05$). **Conclusion**: Contrary to our hypotheses, a HFM did not elicit PPI in YA or OA. Additionally, exercise did not impact inflammation at any timepoint. Future work should be performed on the PPI response and the effect of preprandial exercise on individuals already afflicted with inflammation related diseases.

Introduction

The typical western diet consists of nutrient-poor and calorically-dense foods, much of which include meals high in saturated and trans fats (high-fat meals; HFMs). In addition, individuals may consume 2-3 HFMs a day, which means that they spend the majority of the day in the postprandial state of a HFM. The negative health effects of chronic high-fat diets may include obesity, type II diabetes, cardiovascular disease (CVD) and other chronic diseases. A key mechanism initiating the development of chronic diseases may be the prolonged activation of the innate immune system brought on by postprandial lipemia (PPL), mainly the elevated levels of low density lipoproteins (LDLs) in circulation after a HFM. Circulating LDLs cause damage to vascular tissue when they penetrate the endothelial cells[1]. An inflammation response is launched to remove the LDLs. Repeated exposure to PPL and the subsequent postprandial inflammation (PPI) creates a chronic low-grade inflammatory environment[2]. Specifically, persistent low-grade inflammation has been associated with increased risk of cardiovascular disease (CVD)[3,4], atherosclerosis[5], type II diabetes[6], Alzheimer's disease[7], and certain cancers[8,9]. Interestingly, even a single HFM can specific markers of inflammation which include c-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1B (IL-1ß), and tumor necrosis factor-a (TNF-α). Considering these inflammatory markers have been associated with increased risk of the previously mentioned chronic diseases in a fasted state, modifying the acute increase in these inflammatory markers may lead to a lower overall risk of chronic disease development.

While a HFM transiently increases inflammation, aging also independently increases the risk of chronic diseases, in part due to the decreases in immune function

(termed immunosenescence)[3,52], which occurs naturally with age (i.e. inflammaging). There is also an age-related increase in systemic markers of inflammation, including CRP, IL-8 and IL-6[10,11]. In addition, there may be a decrease in anti-inflammatory IL-10 with aging[11] which may be due to decreased muscle mass and ability to secrete anti-inflammatory cytokines[10]. Inflammaging, a westernized diet, and reduced anti-inflammatory capacity may work synergistically to amplify PPI, leading to greater risk of inflammation related disease for older adults (OA).

Fortunately, acute exercise has been utilized as a means to modify the acute inflammatory response, likely by altering blood lipid dynamics[12, 13] and/or by enabling the secretion of anti-inflammatory cytokines through prolonged muscle contraction[14]. A single bout of exercise within 24 of a HFM appears to have the greatest attenuation of PPL in young adults (YA)[15], however the impact of pre-prandial exercise on PPL remains to be elucidated in OA. Therefore, the purpose of this study is to determine whether an acute bout of preprandial exercise attenuates the PPI response after a HFM based on age. We hypothesize that (1) an acute bout of physical activity will attenuate the postprandial inflammatory response in YA and OA and (2) OA will experience a greater attenuation from acute exercise than YA.

Methods

Subjects

12 young adults (YA) between 20-33 yrs (23.3±3.9 yrs, n= 5 M/7 F) and 12 older adults (OA) between 62-77 yrs (67.7±6 yrs, n= 8 M/4 F) participated in this investigation. Subjects signed an informed consent, completed a physical activity readiness questionnaire (PAR-Q), and the International Physical Activity Questionnaire (I-PAQ) to determine chronic physical activity level in MET-minutes/week. Subject characteristics are displayed in table 2. All procedures were approved by the James Madison University Institutional Review Board in accordance with the Declaration of Helsinki.

Experimental Design

Each subject completed a total of four laboratory visits. On the first visit, initial baseline measures of height, weight, body fat percentage, and blood pressure were taken. The subjects then performed an incremental test to volitional exhaustion to determine peak oxygen consumption (VO_{2Peak}) and exercise intensity for the acute exercise condition. Subjects were then randomly assigned an order to perform two HFM challenges, either the HFM alone or exercise + HFM challenge with the preprandial exercise session occurring twelve hours prior to the meal (EX + HFM). Subjects began each experimental trial in the morning (starting time; 7:00-11:00 am) after a 12 hour overnight fast with identical starting times within subjects. The experimental design is displayed in Figure 1.

Initial Measurements

The first laboratory visit was used to collect baseline measurements. Height was taken with a stadiometer (Charder Model HM 200P, Charder Electronic Co Ltd., Taichung, Taiwan) and weight with a standard physician's scale (Dymo Pelouze model 4040, Newell

Brands, Hoboken, NJ) to calculate body mass index. Following height and weight, a dual-energy x-ray absorptiometry (DEXA) (GE Lunar iDXA Fairfield, CT) scan was performed to calculate lean mass, fat mass, and percent body fat. Resting blood pressure (BP) was taken immediately after the DEXA was completed, so that the subject had been resting for at least five minutes. BP was measured using an automatic Sphygmomanometer (ProBP 3400 Welch Allyn, Skaneateles Falls, NY) as detailed in the American College of Sports Medicine (ACSM) Guidelines. The average of the two BP measurements were recorded. Waist circumference was taken at the narrowest part of the waist with a Gulick tape measurer (Creative Health Products, Ann Arbor, MI).

Incremental Exercise Test

An incremental cycling test to exhaustion was performed on a VIAsprint 150P cycle ergometer (Vyaire Medical, Mettawa, IL) to determine VO_{2Peak}. Breath samples were analyzed by a Vmax Encore metabolic cart (Vyaire Medical, Lake Forest, IL), while heart rate (HR) was measured by a Polar Link heart rate strap and watch (Polar Lake Success, NY). Once the headset, mouthpiece, and nose plug were fitted, the subjects completed a five-minute warm up at a self-selected cadence $\geq$50 revolutions per minute (rpm). During the warm up, power output was either increased or decreased every minute until subjects identified a workload that they perceived as sustainable for approximately thirty minutes.

Based on the power output determined during the warm up, one of four potential test protocols was chosen (Table 1) in order to reach VO_{2Peak}. Immediately after the warm up, the incremental test began, and wattage was set according to stage one of the selected protocol. Resistance was increased every minute based on the protocol being used (10 Watts or 25 Watts). Subjects were verbally encouraged to continue pedaling by the

investigators throughout the exercise test. The test was terminated when subjects reached volitional exhaustion.

Food Log and ASA24 Food Recall

Subjects were given a food log and instructed to record, in detail, all foods and beverages consumed the day before their HFM challenge. For the second HFM challenge, subjects were asked to duplicate their diet from the previous HFM challenge as closely as possible, and once again record in detail their diet for that day.

Preprandial Exercise Bout

During the EX + HFM trial, subjects performed an acute exercise bout twelve hours prior to their HFM challenge on the VIAsprint 150P cycle ergometer (Vyaire Medical, Mettawa, IL), which was previously used for the VO_{2peak} test. Wattage was set so that the subjects' HR stayed within a HR range that corresponds to 60-70% of their VO_{2Peak} HR, for a duration long enough to achieve a total energy expenditure equivalent to 75% of the calories of the HFM challenge (Equation 1). However, subjects were not permitted to exercise longer than 2-hours which included four OA subjects. There was no caloric replacement after the exercise bout due to the potential impact of partial caloric replacement on PPL[16].

HFM Composition

The HFM which consisted of a slice of Marie Callender's Chocolate Satin Pie (Conagra Brands, Chicago, IL) portioned out to a caloric density of 12 kcals/kg bw. Macronutrient composition of the HFM was 57% fat (57% of which is saturated fat), 4% PRO, and 39% CHO.

HFM challenge protocol

After reporting to the lab, subjects were seated in a reclining phlebotomy chair and asked to remain sedentary for the duration of the HFM session, except when inside the restroom. In the HFM alone condition, subjects were asked to refrain from exercise for at least 48 hours prior to their visit to the laboratory. After five minutes, two baseline BP measurements was taken with at least 30 seconds of rest between each recording. An indwelling safelet catheter was inserted into a forearm vein via a 22-gauge needle (Fisher Scientific, Waltham, MA) and kept patent with a 0.9% NaCl solution with a drip rate of one/second. A fasting blood draw was taken once the catheter was in place via a 5 mL syringe (BD) and emptied into a 6-mL EDTA-coated vacutainer tube.

Blood samples were drawn every 30 minutes postprandially until the two hour mark at which point draws were performed every hour. Plasma (for inflammatory markers), blood glucose and lipids (Cardiochek Plus Analyzer, PTS Diagnostics, Indianapolis, IN) were tested prior to the HFM challenge. Plasma samples were taken at baseline, 3, and 6 hours. Blood glucose was tested every 30 minutes up to 2 hours postprandial, and then every hour. Blood lipids were tested every hour from baseline until 6 hours post-prandially. The experimental protocol is pictured in Figure 1.

Cytokine Analysis

After blood draws were transferred to vacutainer tubes, they were immediately centrifuged for 12 minutes at 1800 x *g* in 4 degrees Celsius. After being separated blood plasma was aliquoted into 0.5 ml microtainers and stored for later investigation in a freezer at -80 C. All plasma samples were assayed at Eve Technologies (Calgary, Alberta, Canada). A Human High Sensitivity T-Cell Discovery Array 14-plex (HDHSTC14) was

used to measure IL-6, IL-1β, IL-8, IL-10, and TNF-α. The major pro-inflammatory and anti-inflammatory cytokine are the primary focus of this paper, while the remaining cytokines were included as an exploratory aim and part of a larger study. Biomarkers were analyzed in duplicate and the average measurement was used for analysis.

Statistical Analysis

Statistical analyses were done with IBM SPSS Statistics v26.0 (IBM Corporation, Armonk, NY). All data were analyzed for normality using the Shapiro-Wilk test. Considering baseline TG and baseline inflammation markers passed the Shapiro-Wilk test of normality and appeared to be normally distributed upon inspection of central tendency, a three-factor analysis of variance was performed with the within-subjects' factor of time (baseline, three, and six hours for cytokines; baseline, two, and four hours for glucose; baseline and every hour postprandially up to 6 hours for lipids) and condition (HFM or EX+HFM) and the between subjects' factor of age (YA and OA), and. If significant main effects of group or time were detected, data were assessed to determine significance as a linear or quadratic function by contrast analysis. Next, post hoc multiple comparisons were conducted to test for group- or time-specific differences. In this instance, Bonferroni adjustment for multiple comparisons were implemented to avoid type I error. Pearson's r comparisons were also performed to assess associations between PPL and PPI. For all analyses, significance was set to $p<0.05$.

Results

Participant characteristics

There were no differences in body mass index (BMI) (p=0.454), body fat percentage (p=0.158), or absolute VO_{2Peak} (p=0.993) between YA and OA. However, there were significant differences in age (p<0.001), weight (p=0.047) and relative VO_{2Peak} (p =0.005) between YA and OA. Physical activity level in MET-minutes/week was not significantly different between age groups (YA=3,776 MET-min/week ± 3,532, OA=6,157 MET-min/week ± 5,456) (p=0.262).

Inflammatory Markers

Figure 3 displays the cytokine response to the HFM for both groups and both conditions. There was no main effect of time x condition across any of the cytokines reported at between any timepoints (p >0.05). Figure 3A displays the IL-6 response post-HFM. There was a time effect for IL-6 (p <0.001), which decreased from baseline to 3 hours. In examining the change from baseline to 6 hours, the change in IL-6 was significant as a quadratic function (p<0.001), where IL-6 decreased by 21 ± 23% at 3 hours but by 53 ± 95% from 3 to 6 hours back to baseline levels. There was no main effect by age (p=0.774) and no interaction effects for IL-6 by time x age, time x condition, or time x age x condition (all p-values>0.05). Figure 3B displays the IL-8 response post-HFM. There was a significant time effect for IL-8 (p<0.001), which was also significant as a quadratic function (p<0.001). IL-8 decreased from baseline to 3 hours by 8 ± 20% and increased from baseline to 6 hours by 2 ± 25% and increased from 3 to 6 hours by 13 ± 23%. There were no significant effects of time x age (p =0.133), time x condition (p =0.259), or of age

x condition (p =0.215). There was no main effect of age (p =0.423). Figure 3C displays the IL-10 response post-HFM. There was a significant effect of time for IL-10 (p<0.001), which was significant as a quadratic function (p <0.001). IL-10 decreased from baseline to 3 hours by 9 ± 28% and also decreased from baseline to 6 hours 4 ±26%, however increased from 3 to 6 hours by 7 ±28%. There were no interaction effects of time x condition (p =0.388), time x age (p =0.357), or in time x condition x age (p =0.641). There was also no main effect of IL-10 by age (p =0.746). Figure 3D displays the TNF- α response post-HFM. There was a significant main effect of time (p <0.001), which was also significant as a quadratic function. TNF- α decreased from baseline to 3 hours by 16.3 ± 19% and baseline to 6 hours by 10 ± 24%, however increased from 3 to 6 hours by 9 ± 28%. Where was also no interaction in time x condition x age (p =0.538). However, there was a significant interaction of time x age (p =0.036), where YA had a greater decrease in TNF- α post-HFM, with an attenuated decline from baseline to 6 hours when compared to OA (p =0.04). OA also had significantly higher TNF- α compared to YA (p =0.047).

Figure 3E displays the IL1-β response post-HFM. There was a significant main effect of time that decreased linearly from baseline to 6 hours (p <0.001). There were no interaction effects of time x condition (p =0.485), time x age (p =0.197), or of time x condition x age (p =0.355). There was also no main effect of age(p =0.553).

Metabolic Data

TG, GLU and LDL are displayed in Figure 4. TG results are displayed from baseline to 6 hours post-prandially in Figure 4A. There was a significant time effect for TG (p <0.001), which increased from baseline to 6 hours. There was a trend toward a time x condition interaction (p =0.06), in the direction of an attenuated increase in TG in the

EX+HFM condition. There was also a time x age interaction (p =0.035), where the OA had a greater TG response compared to YA, however there was no interaction time x condition x age interaction (p =0.714). There was no difference in TG by condition alone between HFM and EX+HFM (p =0.278) or in age alone between YA and OA (p =0.187).

Figure 4B shows LDL hourly post-HFM. There was a significant time effect for LDL, where it decreased from baseline to 6 hours post HFM (p =0.001). There was also a significant time x age interaction (p <0.001), where the OA had an attenuated decline compared to the YA. There was also a trend for a time x condition x age interaction (p =0.078), where the OA had greater LDL decline in the EX+HFM condition compared to the YA EX+HFM condition. LDL was not different between the HFM alone or EX+HFM condition (p =0.870), however LDL was higher in OA compared to YA (p =0.005).

Post-prandial GLU responses are shown in Figure 4C. There was a significant time effect for GLU from baseline to 2 hours post-prandially (p<0.001). There was also a significant time x condition interaction (p =0.002), where EX+HFM lowered post-prandial glucose compared to the HFM condition alone. There was no time x age or time x condition x age interaction between time and age (p =0.690 and p =0.728, respectively). There were no main effects for condition or age (p =0.174 and p =0.457, respectively).

Assosiation Between Metobolic and Inflammatory Markers

Change in TG from baseline to two hours was weakly, negatively associated with change in IL-3 from baseline to three hours (r=-0.31, p=0.034). There was no association between TG, GLU, or LDL and any other inflammatory cytokine at any time point either in absolute value or change score (p>0.05). HDL was weakly, positively associated with IL-1β at baseline (r=0.345, p=0.018) and at 3 hours postprandially (r=0.378, p=0.008).

Discussion

The purpose of this study was to determine whether an acute bout of preprandial exercise would attenuate the PPI response to a HFM in YA and OA. We hypothesized that (1) an acute bout of physical activity would attenuate the postprandial inflammatory response in YA and OA and (2) OA would experience a greater attenuation from acute exercise than YA. Considering that the mechanisms linking PPI and postprandial lipemia and glycemia remain to be elucidated, an exploratory aim was to assess whether EX had an impact on TG and GLU, which could subsequently modify PPI. The results do not support our hypotheses. A HFM did not increase any of the reported inflammatory markers. Additionally, exercise did not affect the markers of postprandial inflammation that were analyzed, although there was a main effect of age for TNF-α.

IL-6 is the most frequently reported acute phase cytokine. There was no change in IL-6 from baseline to six hours. The decrease in IL-6 at three hours was not expected, but also not unprecedented in the literature. A study by Emerson and colleagues, conducted within several months as the present study and utilizing the same HFM protocol, observed a similar quadratic response for IL-6[11]. This IL-6 response was also reported previously in a study by Teeman and colleagues in which a HFM of 10 kcals/kg BW and 63% fat was used[17]. However, there are conflicting reports on the effect of a HFM on postprandial IL-6. Postprandial increases in IL-6 have been reported in studies using relative and absolute caloric meal sizes[18–20]. Dekker and colleagues measured increased IL-6 over the course of an 8 hour postprandial period after a 9 kcals/kg BW oral fat tolerance test[1]. Increases have also been reported in HFMs with an absolute caloric size as small at 760 kcals with 59% fat[19] to as large as 1,416 kcals with 52% fat[20]. In addition to HFM composition, subject

characteristics have also been investigated in their effect on PPI. Changes in postprandial IL-6 have not been correlated to BMI, age, or HFM caloric load, but have been shown to have a weak negative correlation with the percent fat of a HFM[21]. The HFM fat percentage of the present study falls in between those of studies showing increases in IL-6, thus it is hard to say whether or not the correlation between HFM fat percentage and IL-6 is supported. IL-6 does play a role in glucose regulation by promoting hepatic glucagon secretion[22]. Perhaps increases in postprandial IL-6 are not influenced by the fat percentage of a HFM, but rather the carbohydrate content. HFMs that sacrifice carbohydrate content in exchange for greater fat content may promote increased IL-6 during the postprandial state to mediate gluconeogenesis rather than inflammatory. All HFM studies are conducted after a prolonged fast. If the HFM has only low or moderate carbohydrate content, gluconeogenesis would be likely to occur during an extended postprandial period, as the subjects have to go for hours without another meal. Another proposed mechanisms for the differences in postprandial IL-6 across the literature is based on the diurnal changes in IL-6. IL-6 has been shown to increase in concentration upon awakening. Because the HFM sessions in the present study were all performed in the morning, diurnal changes offer an explanation for the decrease in IL-6 at 3 hours postprandially[23,24]. If baseline measurements in the present study were augmented by diurnal changes, a linear increase in IL-6 might have been observed if baseline measurements were taken further from the time of subject awakening. However, protocols involving morning HFMs are common even in experiments that have reported increases in postprandial IL-6[18–20]. Additionally, baseline IL-6 levels in the present study were not higher than commonly reported in the

literature[19], indicating that the proximity of the HFM to subject awakening likely did not affect baseline measurements.

TNF-α decreased from baseline to 6 hours. TNF-α is one of the most frequently measured inflammatory markers in PPI research, however according to a recent review by Emerson and colleagues, it rarely changes in the postprandial state[16]. There doesn't appear to be a specific HFM fat percentage or caloric load or subject characteristic that consistently elicits an increase in TNF-α, but there is a connection between TNF- α response and IL-6. All of the aforementioned studies that elicited increases in IL-6 also reported increases in TNF-α[18–20], while those with no increase in IL-6 measured decreases in both TNF- α and IL-1β[11,17], including the present study. Although the inflammation response has not been able to be strongly predicted based on meal composition, it appears that the postprandial response of IL-6 and TNF-α are linked. The specific meal composition and subject characteristics needed to promote an increase in TNF-α need further investigation.

Downstream pro-inflammatory markers IL-8 and IL-1β decreased from baseline to 6 hours. These markers are reported infrequently but were measured in the present study in an effort to elucidate more details on PPI cytokine dynamics. Due to the decrease in TNF-α postprandially, it is not a surprise that the downstream markers followed similar trends. IL-10 also decreased at both 3 and 6 hours compared to baseline. This postprandial response of IL-10 was also observed by Teeman and colleagues who speculated that the decrease in IL-6 might be the reason behind the IL-10 response[17]. IL-8, IL-1β, and IL-10 may provide more detailed insight into PPI dynamics and the difference in inflammation between YA and OA. However, in the present study it appears that these downstream

cytokines simply follow suit with their upstream regulators and may not show any independent response to a HFM.

Inflammation has been shown to increase during the aging process[25]. However, the results from the present study found that TNF-α was the only inflammatory marker to be affected by age. TNF-α was ~23% higher in OA at baseline compared to YA (p=0.015). The decline in postprandial TNF-α was also attenuated by age, by which the decrease in TNF-α postprandially was lesser in OA than YA. Fasting inflammation has been shown to increase with age with OA having higher levels of IL-6 and TNF-α over time. In longitudinal work by Bartlett and colleagues, inflammation markers were collected 10 years apart in 249 subjects. After 10 years, TNF-α and IL-6 were 330% and 130% higher, respectively, demonstrating a large increase in inflammation from the aging process. However, in the two HFM studies that have investigated YA and OA, no differences in TNF-α or IL-6 by age has been found[11,26]. The TNF-α results from the present study do not agree with the findings from the previous HFM studies by Emerson and colleagues[11] and Milan and colleagues[26]. Emerson and colleagues speculated that the amount of dietary fiber between groups may have accounted for differences in TNF- α[11]. Perhaps TNF- α concentrations in OA is determinant on a combination factors such as diet, physical activity level, and intensity. More research needs to be done on the effect aging has on inflammation and the variables that modify age related inflammation.

There was no effect of exercise across time points or age groups for any of the reported inflammation markers. It is likely that there needs to be PPI in the first place in order for exercise to elicit any effect. Exercise is anti-inflammatory[14]. Contracting skeletal muscle releases IL-6 when glycogen is depleted and mediates an anti-inflammatory

cascade by increasing IL-10 and actively inhibits TNF-α and IL-1β activity[27]. Despite our understanding of the capacity of exercise to reduce inflammation, exercise has not consistently attenuated PPI in HFM studies[17,28,29]. Various exercise interventions have been used across the literature in an attempt to elicit an anti-inflammatory effect on PPI. Previous exercise interventions have based exercise duration on different set lengths of time[28,30] or on absolute kcal energy expenditures[29] , all with similar intensities to the present study. Unlike previous interventions, the present study used a relative energy expenditure to the subject's HFM kcals to see if a greater effect of exercise could be elicited. Although various durations and energy expenditures have been used in an attempt to attenuate PPI, the main problem persists that the majority of HFM exercise intervention studies do not use a HFM that produces any PPI to begin with. As previously discussed, the magnitude of PPI is inconsistent in the literature with some studies reporting either an absence[28] or decline[30] in inflammation and others reporting an increase[20]. Until a reliable method of inducing PPI is found, efforts to investigate the effects of exercise on PPI appear to be tedious.

As expected after a HFM, TG and GLU increased in both age groups. OA experienced higher postprandial TG, but not GLU. These results are similar to previous research investigating postprandial TG and GLU in YA and regularly active OA in which active OA experienced greater postprandial TG[31]. Regular physical activity in OA has been shown to attenuate postprandial glycemia, but not lipemia compared to inactive OA[31]. In a study be Emerson and colleagues, active OA experienced similar postprandial GLU levels to active YA, but PPL levels were more similar to inactive OA. The OA in the present study may have had activity levels close to those in Emerson and colleagues' in order to attenuate age related change in GLU, but not TG. Exercise attenuated postprandial

glycemia at 2 hours in both age groups, but there was only a trend in the effect of exercise on TG. The proposed mechanism behind PPI is dependent on elevated postprandial LDL penetrating vascular tissue, however this marker is infrequently reported in HFM studies. In the present study, LDL decreased postprandially from baseline to six hours in both age groups. In the study by Dekker and colleagues in which increases in IL-6 and TNF-α were reported, there was also no change in LDL[18]. The lack of PPI inducing HFM studies that also report on postprandial LDL makes it difficult to draw conclusions on whether or not the proposed mechanism of LDL eliciting PPI is supported. Further muddying the relationship of LDL to PPI, OA experienced elevated LDL over time compared to YA, despite not experiencing a consistent increased PPI response compared to YA. The increase in OA LDL could be a result of the trending increase in TG in OA, however this does not explain the absence of PPI. There is little support for the association between TG and PPI[28,32]. In the present study, TG were not associated with any inflammatory marker. Furthermore, there was also no association between inflammation and GLU. Evidence suggests that TG and GLU are not the mediators of a PPI response in the typical HFM challenge. Further research needs to be done to determine if an alternate metabolic marker is associated with PPI.

Conclusion

Our findings question the direct role that HFMs and a western diet play in chronic inflammatory related diseases. Despite the use of a large HFM that was also high in saturated fats, no PPI was detected in either age group. The growing body of literature that has failed to induce PPI after a HFM increases doubt behind the connection of a single HFM increasing the risk of inflammation related diseases. Investigation into meal timing and multiple HFM spaced throughout the day may lead to answers on how diet and behavior work together to promote inflammation and disease. Further research needs to be done to elucidate the root cause of PPI to be able to consistently produce a PPI state in experimental trials. Additionally, more work needs to be done to determine whether or not exercise truly does have a protective effect in PPI and the proper exercise protocols to generate such effect.

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Table 1. Protocols for incremental VO_{2peak} cycle ergometer test based on subject determined warm up wattage.

	Protocol A	**Protocol B**	**Protocol C**	**Protocol D**
Warm up Wattage	≤ 25	25-50	50-75	≥75
Stage 1 (Watts)	25	25	50	75
Stage 2 (Watts)	35	50	75	100
Additional Stages	10 W increases	25 W increases	25 W increases	25 W increases

$$\frac{bwkg * 12.5\ kcals * 0.75}{absolute\ VO2 * 4.825L\ O2/kcal * 0.65}$$

Figure 1. Determining exercise duration sufficient to burn 75% of the calories from the HFM.

Figure 2. Protocol for the HFM sessions. Time increments are in minutes. P: plasma collected for inflammation markers, G: glucose collected; L: lipids collected. Satiety collected via SLIM scale.

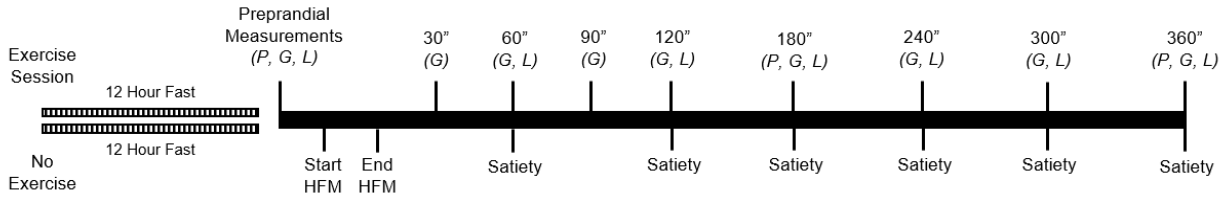

Table 2. Participant characteristics

	YA (7F/5M)			**OA (4F/8M)**			**P value**
Age (years)	23.3	±	3.9	67.6	±	6*	<0.000
Height (cm)	167.5	±	8.1	175.9	±	8.6	0.22
Weight (kg)	71.4	±	16.9	80.7	±	14.7*	0.047
Body mass index (BMI) (kg/m^2)	25.3	±	5.0	26.7	±	4.3	0.454
Body Fat (%)	28.2	±	8.7	33.1	±	6.5	0.158
Absolute VO2peak (L/min)	2.3	±	0.4	2.3	±	0.8	0.993
Relative VO2peak (mL/kg/min)	33.4	±	5.3	28.3	±	6.7*	0.005
MET-minutes per week	3,776	±	3,532	6,157	±	5,456	0.262

* Signifies $p<0.05$ compared to YA.

Table 3. High fat meal (HFM) composition and exercise session calculations for YA and OA. Exercise duration was capped at two hours, there were three OA participants whose calculated exercise duration exceeded the two hour cap.

	YA (7F/5M)	**OA (4F/8M)**	**P value**
HFM Calories	856.8 ± 207.1	968.8 ± 180.8	0.172
HFM Total Fat Calories	488.3 ± 118.1	552.2 ± 103.0	0.172
HFM Saturated Fat Calories	278.4 ± 67	314.8 ± 58.8	0.172
HFM CHO Calories	334.1 ± 80.8	377.8 ± 70.3	0.172
Exercise Energy Expenditure (kcals)	642.6 ± 155.4	726.6 ± 135.6	0.172
Exercise Session Duration (minutes)	88.1 ± 15.1	102.3 ± 18.4	0.053

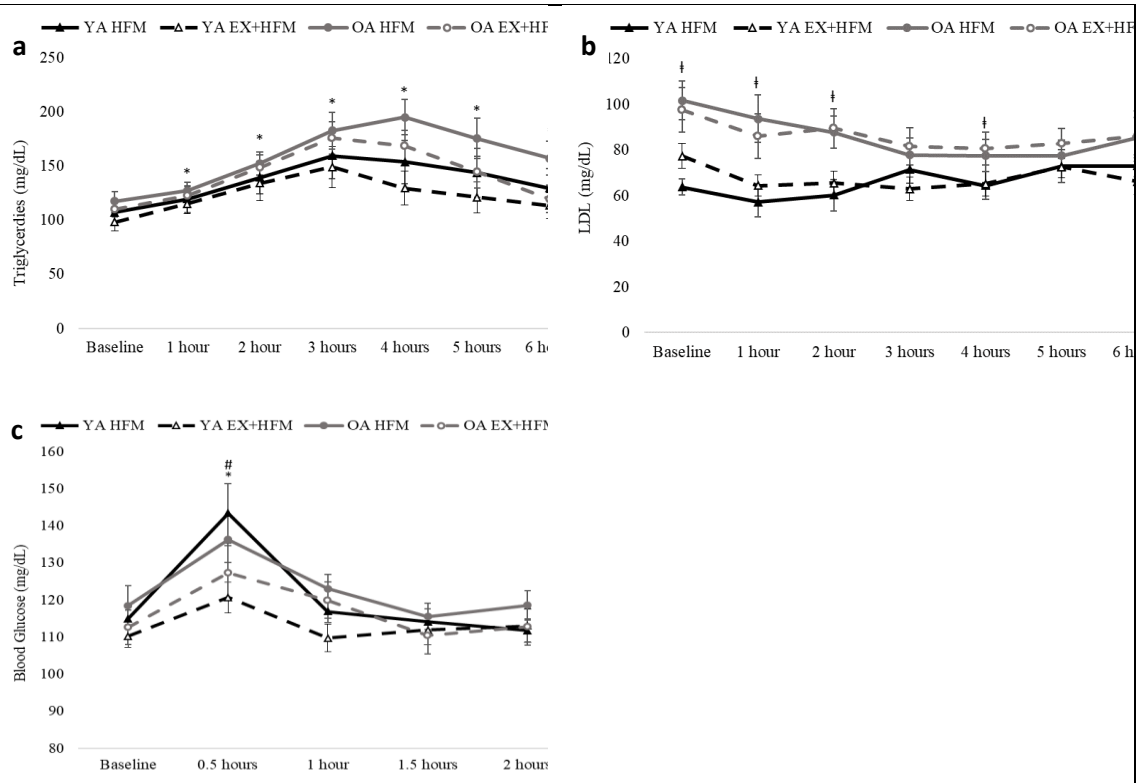

Figure 4. Metabolic response to HFM and EX+HFM in YA and OA. (a) triglycerides, (b) LDL, (c) blood glucose. Dashed lines and empty markers indicated EX+HFM, solid lines and filled markers indicate HFM, triangles indicate YA and circles indicate Older adults. * Indicates within group significance compared to baseline. ǂ Indicates between group significance for age. # Indicates significance by condition. Error bars indicate 95% CI. Significance set at $p<0.05$

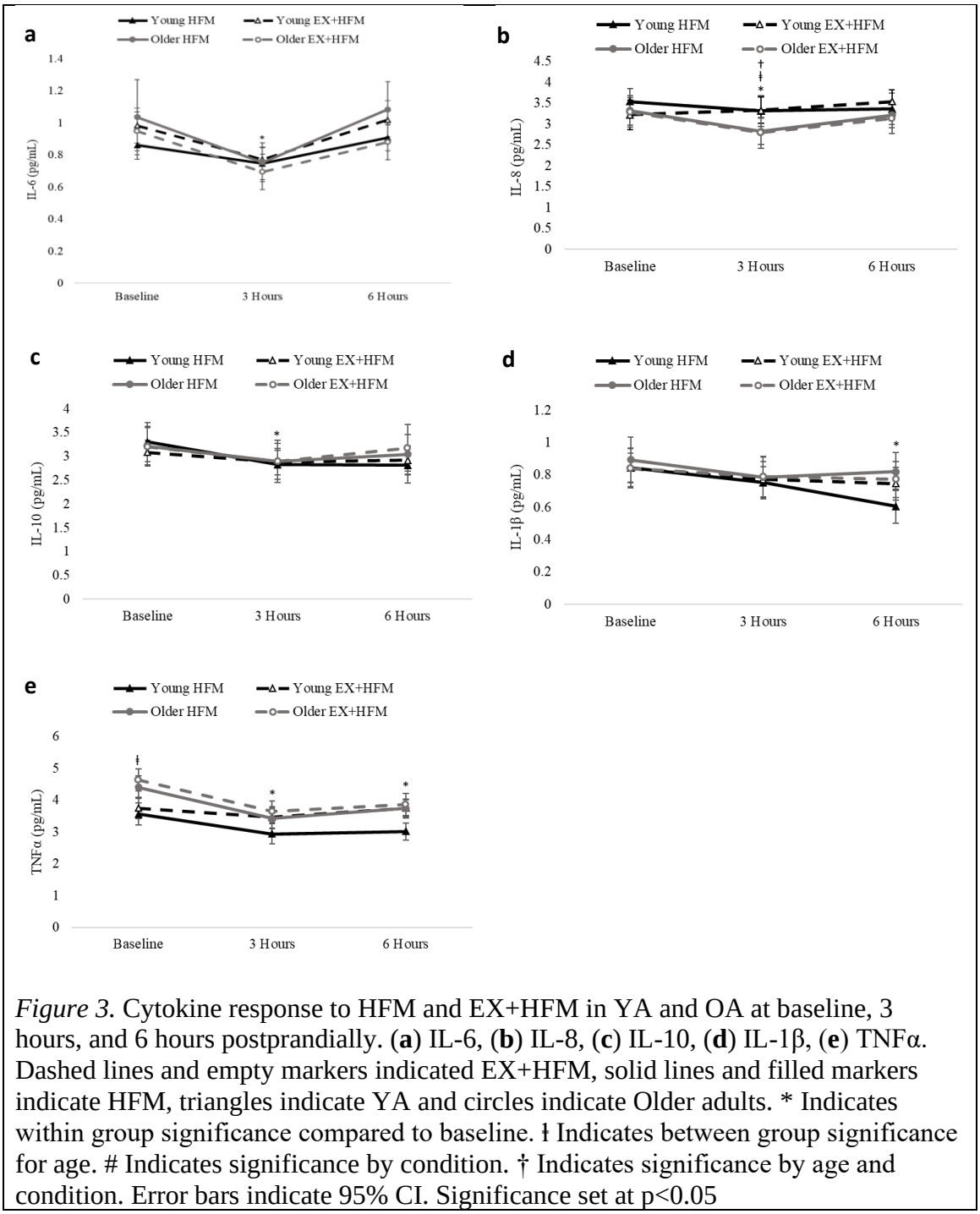

Figure 3. Cytokine response to HFM and EX+HFM in YA and OA at baseline, 3 hours, and 6 hours postprandially. (**a**) IL-6, (**b**) IL-8, (**c**) IL-10, (**d**) IL-1β, (**e**) TNFα. Dashed lines and empty markers indicated EX+HFM, solid lines and filled markers indicate HFM, triangles indicate YA and circles indicate Older adults. * Indicates within group significance compared to baseline. ǂ Indicates between group significance for age. # Indicates significance by condition. † Indicates significance by age and condition. Error bars indicate 95% CI. Significance set at $p<0.05$

Appendices

Does acute preprandial exercise modify postprandial inflammation after a high fat meal in young and older adults?

Consent to Participate in Research

Identification of Investigators & Purpose of the Study

You are being asked to participate in a research study conducted by Drs. Stephanie Kurti and Elizabeth Edwards from James Madison University. To participate, you must be in good health and able to exercise (per the physical activity readiness-questionnaire and medical records). The purpose of this study is to determine whether being active impacts how your body responds to a high-fat meal. Previous research tells us that this response after a meal is actually a more accurate prediction of your disease risk than fasting levels. We also want to see if how active you are on a regular basis changes how your body responds to a high-fat meal. This study will contribute to the knowledge of both practitioners and clinicians, and may provide an important public health message in promoting exercise as a way to affect how your body responds to high-fat meals, without the need for prescription drugs. Should you decide to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction.

Research Procedures

This study consists of a series of surveys, a body composition scan, and fitness test that will be administered to individual participants in the Human Performance Laboratory at James Madison University. There will be one acute exercise session and two meal sessions. The total time required for participation in the research study is outlined on the following page (time required section). The specific procedures during each visit are included here:

Initial visit: If you are older, you will have already have had to receive medical clearance from your physician to participate in this study prior to your initial visit. On the first visit to the laboratory, you will be briefed on the study and asked to complete a series of questionnaires, including the international physical activity questionnaire, the physical activity readiness-questionnaire plus, and a medical history form. If you'd like to see any of these questionnaires prior to consenting, please ask a researcher and we'll be happy to provide one for you.

On this first day, you'll also undergo a few tests. First, we'll assess your height and weight. Then we'll perform a DEXA scan to assess your body composition scan (how much of your body is fat vs. lean mass). Finally, you'll perform an exercise test on a cycle ergometer. The exercise test is a test to assess your peak aerobic capacity, which is the best measure of cardiorespiratory fitness. The exercise bout lasts approximately 12 to 15 minutes until you need to stop or cannot maintain the required cadence. Before and after the exercise testing, standard pulmonary function tests (PFTs) will be performed, which

will be repeated at 2 minutes and 20 minutes after the bout of exercise. These tests are noninvasive and commonly used to assess the capacity of the pulmonary system. An example of these tests are a breathing maneuver simply requires you to maximally inhale, then forcefully exhale and hold for 6 seconds, and then to maximally inhale again.

Following the measurements and exercise testing, you will be given an accelerometer to wear for one week and will return to the laboratory for your second testing session after the one-week period. Your next visit will either be a meal testing session with exercise the night before or a meal testing session without exercise the night before.

Meal testing sessions: Your HFM testing sessions will be after a 12 hour fast. We'll place an indwelling intravenous catheter inserted into a forearm vein to allow for repeated blood draws. You will be asked to perform several experimental measurements prior to consumption of a single high-fat meal (Marie Callender's Chocolate Satin Pie, 63% fat, 12 kcal/kg body weight). The meals contain dairy and gluten. These same measurements will be performed every hour until 6 hours after the HFM. These include the following: Pulmonary function testing, exhaled nitric oxide, exhaled breath condensate, blood draws for glucose, triglycerides, and inflammatory and angiogenic markers.

Acute exercise session: On the day that you're completing the exercise, you'll come to the lab 12 hours prior to your meal testing session. At this session, you will exercise long enough to expend 75% of the calories that will be consumed in the HFM session. You will exercise on a cycle ergometer at a moderate intensity at 65% of your VO_{2peak} (which will be based on the cycle test from the first day). Throughout this exercise session, heart rate data will be monitored continuously.

Blood sample Storage: After collection, your blood will be stored in a deep freezer on JMU's campus until all samples have been collected. At that point, the blood will either be analyzed here at JMU or shipped off to partner labs (e.g. UVA or EVE Technologies) for analysis there. Any blood shipped to partner sites will be destroyed or returned after those analyses are complete.

Time Required

Participation in this study will require about 18 hours of your time over the course of 4 separate visits. Upon completion of the study, you will be compensated $75.00 upon completion of the study. The visits are outlined below:

Baseline Testing: On the first visit to the laboratory, completing the required forms and questionnaires, performing the acute exercise test, and undergoing the body composition scan will take approximately 2 hours.

HFM Visits: Two HFM sessions will each be ~7 hours in length. During this time, an intravenous catheter will be inserted into the median cubital vein of the forearm and blood samples will be obtained before the HFM and every hour post-meal for 6 hours. Approximately 10ml of blood will be obtained during each time-point post-HFM. The catheter will be kept patent by a slow, continuous flow (1 drop/second) of saline solution. The catheter will remain in the antecubital vein of the participant for the remainder of the measurement period in order to prevent clotting in the catheter (thus allowing a single venipuncture for multiple blood samples). The following variables will be analyzed from these samples: triglycerides, glucose, insulin, angiogenic and inflammatory markers.

Acute exercise session: This session will involve an acute bout of exercise that should last between 30-45 minutes, however may vary from person to person. The exercise bout will not last longer than 90 minutes in any individual.

Risks

Participation in this study does have some risks, although they are small. The risk of any serious event during this study is very small. Possible risks include:

Catheter: You may experience discomfort with the placement of the catheter. The catheter is plastic tubing and allows the arm to be moved throughout the 6-hour testing session. If excessive discomfort exists, the catheter can be taken out at any time during the study.

Exercise bout: Both the baseline exercise test and the exercise bout may be associated with some discomfort and soreness on the following days. This is a normal physiological response and is not dangerous. During the exercise test, you will have a mouthpiece in for the duration of the test. This may be uncomfortable and you may withdraw from the study at any time. Supervision will be provided by research assistants trained in CPR and AED to minimize any potential risk from the exercise bout. Also, your heart rate, ventilatory and metabolic data will be continually monitored throughout the entire exercise bout. If any aberrant responses are recorded, investigators will ask you to discontinue the exercise.

High-fat meal: The high-fat meal protocol is of very short duration, and any changes in blood lipids, glucose, or inflammatory markers are unlikely to create any long-term health problems. Additionally, this meal is not out of line with meals consumed on a regular basis by a significant portion of the US population.

DEXA: The DEXA scan entails a low dose of radiation equivalent to approximately one transatlantic flight (0.015 mSv= millisievert). While there is no validated questionnaire to define extensive exposure, increased radiation correlates with increased risk of cancer and consequently, increased risk of death. However, for reference, the annual permissible dose for a radiation worker is 5,000 mrem or 5 rem, - which would be over 3,000 of these scans in one year. The limit for a pregnant worker, the most conservative level given, is 500mrem - over 300x the dose we are using.

Benefits

By participating in this study, you will learn about your current level of valuable blood, body composition, and pulmonary values. Your data will be provided to you upon completion of your participation in the study. If an emergency arises and you must drop out, you may still receive your data. Having these tests done in a lab would cost several hundreds of dollars. Additionally, you will be compensated $75 via a JMU Prepaid gift card for your time during the study.

Society may benefit from more knowledge about how lifestyle affects the inflammatory, lipemic and angiogenic response to a high-fat meal, which is associated with cancer and cardiopulmonary disease development. Additionally, society will learn whether acute exercise is an effective method in older adults to attenuate these deleterious postprandial responses.

Confidentiality

The results of this research will be presented at the American College of Sports Medicine Annual meeting as well as the Experimental Biology annual conference. The results of this project will be coded in such a way that the respondent's identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole. All data will be stored in a secure location accessible only to the JMU researchers. Upon completion of the study, all information that matches up individual respondents with their answers will be destroyed.

Participation & Withdrawal

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

Questions about the Study

If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

Researcher's Name: Dr. Stephanie Kurti
Email Address: kurtisp@jmu.edu
Cell-Phone number: 630-205-6363
Department of Kinesiology
James Madison University
Office Telephone: 540-568-3947

Researcher's Name: Dr. Elizabeth Edwards
E-mail Address: edwardes@jmu.edu
Office Telephone: 540-568-5220
Department of Kinesiology
James Madison University

Questions about Your Rights as a Research Subject

Dr. Taimi Castle
(540) 568-5929
castletl@jmu.edu
Chair, Institutional Review Board
James Madison University

Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

_____
Name of Participant (Printed)

_____ _____
Name of Participant (Signed) Date

_____ _____

Name of Researcher (Signed) Date

Attn. Dr. _____

Your patient needs the following physician approval to participate in our JMU research project.

Physician Approval Form

Patient Name: _____

Project Description:

The purpose of the study is to investigate to determine whether an acute bout of moderate-intensity exercise will attenuate the lipemic response to a high-fat meal in active and inactive older adults. We hypothesize that an acute bout of moderate-intensity exercise will attenuate the lipemic response to a high-fat meal in active and inactive older adults, with a greater attenuation of the lipemic response in the inactive older adults.

When the participant arrives at the laboratory, we will take resting blood pressure and heart rate, and several pulmonary function tests. These will be performed before and after an exercise test. The exercise test is performed on a cycle ergometer, where the intensity will increase every minute by 10-25 watts until volitional exhaustion (it is too hard to turn the pedals). We monitor heart rate, oxygen saturation, and blood pressure throughout the exercise test.

Usually the test lasts ~10 minutes, with only 1 or 2 minutes of very hard work, until the participant wishes to stop the test. All researchers are trained in CPR and our laboratory has an AED present. We have tested ~50 participants thus far, and nobody has had any issues during or after the exercise test to fatigue.

We are using American College of Sports Medicine guidelines to determine whether participants are eligible for participation in the research project. If the individual does not have a previous heart condition, chest pain when they currently exercise, and is not on any heart medications, they are able to participate in the test without medical supervision.

However if they have not been exercising recently we ask for physician approval to participate in the research study. The participant can stop whenever they want during the test if they feel uncomfortable. Maximal oxygen consumption is the best measure of fitness level and overall health, and we will discuss the results with them after their session.

Contact: Hannah Frick (frickh1@jmu.edu: (c) 703-638-8892) or Scott Wisseman (wissemws@dukes.jmu.edu: (c) 703-268-9976) if you have any questions.

Our departmental fax number is: 540-568-3338

I have reviewed the project description and believe the individual is able to complete a maximal exercise test for the research project.

Printed Name: _____ Date: _____

Signed Name: _____

2017 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Additional notes:

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it ***does not limit your current ability*** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

☑ **If you answered NO to all of the questions above, you are cleared for physical activity.**
Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

2017 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm*

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes*

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

2017 PAR-Q+

6. Do you have any Mental Health Problems or Learning Difficulties? *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*

If the above condition(s) is/are present, answer questions 6a-6b — If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. Do you have a Respiratory Disease? *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d — If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. Do you have a Spinal Cord Injury? *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c — If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. Have you had a Stroke? *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c — If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?

If you have other medical conditions, answer questions 10a-10c — If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE:

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2017 PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

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01-01-2017

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ **Yes**

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

Food Intake Record Directions and Tips

You will have to input all the food that you consume in a day into a nutrition database at your first and second testing sessions. *Also, your food consumption should be the same on both days before your high-fat meal session*.

We will give you a copy of the food that you have consumed so you can remember what to eat the next time before visiting the laboratory.

Instructions on Use:

- In the first two columns, note the time and location the food or beverage was consumed.
- In the third column, record the food or beverage consumed.
    - Be as detailed as possible in describing the food item. For example not just chicken, but how it was prepared (fried, grilled, baked, etc.) and include any sauces or dressings put on any food item. If you eat a salad, try and write own everything that was in the salad with appropriate portion sizes.
    - Include brand names or restaurant names with the item names whenever possible. Examples: Kraft, General Mills, Campbell's' McDonalds 6 piece McNuggets, etc.
    - Include toppings such as mustard, ketchup, mayo, cream sugar, steak sauce, salsa, dressing, gravy, etc.
    - If you are at a restaurant, it is more than OK to ask the staff or waiter/waitress how something was prepared or about the ingredients in a particular item. Additionally, if you want, you can include the description off the menu.
    - If you have a recipe or a label of a common food you consume, feel free to bring it in. The more detail you can provide, the better analysis we can provide.
- Determine serving size for food and beverage (columns 4-6):
    - *Portion Size: How many?:* How many of this item did you consume?
    - *Portion Size: Food Model:* Estimate the size of your food. Options:
        - Use the *Food Amounts Booklet* as a visual guide.
        - OR if you're cooking at home and you know the measured amount, include the measurements. You can use household measures such as measuring cups and spoons to further help you estimate how much you consumed.
        - OR if a weight from the package is available, include the weight.
        - Notes: Leave this blank unless there is something you would like us to know.
- Remember to include all beverages (even water) and snacks consumed.

- Be as honest as possible! This really helps with the accuracy of the data that is collected. We are simply trying to document what you consumed of the recording period.
- If you have any questions please contact a researcher.
- Thank you again for your participation. Your accuracy not only assists us with our research, but provides us a great tool for understanding the impact on your nutrition on several of the responses to the exercise and acute high-fat feeding sessions.

Food Intake Record

Name of the person you are interviewing _____

Day of the week:_____ Day (circle one) 1 2

Time	*Location*	**DETAILED Description** (Be as detailed as possible in describing the food item. For example not just chicken, but how it's cooked (fried, grilled, baked, etc), and include any sauces or dressings put on any food item. Remember beverages too.)	*Amount Eaten (be specific)*	*Comments*

Is this intake unusual in any way? If so, explain:

ID _____ AC _____ m0

Using Your Activity Monitor

Thank you for agreeing to wear this Activity Monitor. The Activity Monitor is attached to a black belt with a clasping buckle that you will put around your waist like a belt. These instructions are intended to help you get started wearing your Activity Monitor. A phone number is provided at the end of these instructions should you need to contact us.

Checklist

1. **Read all materials in this packet. These instructions contain important information regarding the proper use of your Activity Monitor.**
2. **Begin wearing the monitor when you wake up on the Requested Start Date. The monitor will start working automatically – you DO NOT need to activate it.**
3. **Wear the monitor during all waking hours for the seven requested days.**
4. **Stop wearing the monitor at the end of the day on the Requested End Date.**
5. **For each day you wear the unit, record the exact time of day you put it on, the exact time you took it off, and any time you did not wear the unit on your Accelerometer Log.**
6. **After you have worn the Monitor for seven days, place it back in the padded envelope and mail it back to us with the completed activity log.**

When to Wear the Activity Monitor

- **First, consult the Accelerometer Log form for the actual dates that you should be wearing your Activity Monitor.**
- **The total time you will be wearing the Activity Monitor will be <u>seven days</u>.**
- **You should wear it throughout the <u>entire day</u>. We cannot use the data if the monitor has not been worn during all waking hours. The only times you should NOT wear the monitor are:**

- **While you are sleeping**
- **When you shower, bathe, or swim (water will damage it)**

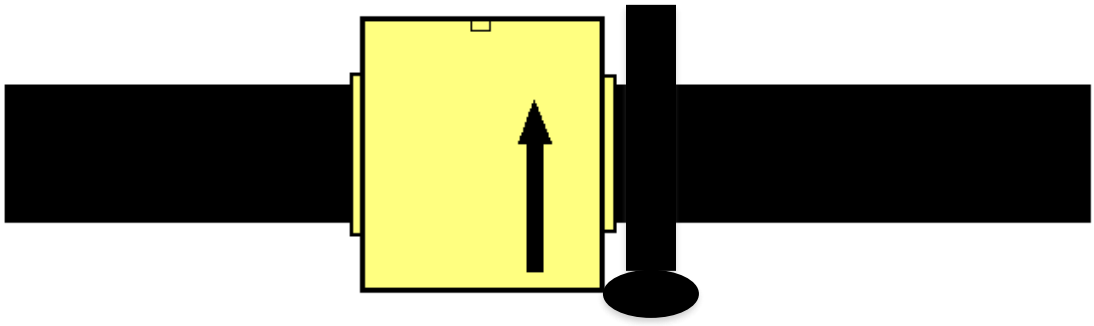
- **We would like you to wear the monitor for the seven days indicated on the Accelerometer Log. (See example below.) However, if you are unable to start wearing the monitor on the requested start date, or you forget to wear it on one of the requested days, you should continue wearing the monitor until you have worn it for seven full days.**

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date	**11/28**	**11/29**	**11/30**	**12/1**	**12/3**	**12/4**	**12/5**
Time of day you put on the unit	6:35 am	7:20 am	7:45 am	7:25 am	5:50 am	6:15 am	6:50 am
Time of day you took off the unit	8:25 pm	9:40 pm	9:25 pm	9:00 pm	8:35 pm	8:15 pm	8:55 pm
Any time you did not wear the unit? (e.g. naps, bathing)	-------	12:30 - 12:50 pm	6:00-6:50 pm	-------	--------	9:15-9:40 am	-------
Please provide comments about problems that occurred while you were wearing the unit.							
I forgot to wear the monitor on 12/2, so I wore it on 12/5 instead.							

How to Wear the Activity Monitor

The Activity Monitor is already attached to a belt that you will wear around your waist. Following these step-by-step instructions should help you begin wearing your Activity Monitor:

1. **Taking the belt, place it around your waist so that the arrow on the Activity Monitor is facing away from you and is pointed up towards your head, as in the picture below:**



2. **Take both ends of the buckle and connect them so that the belt is attached loosely around your waist.**
3. **You may adjust the tightness of the belt by pulling on the straps to make it tighter or by pulling on the back of the buckle to make it looser. Your belt should be snug, but not uncomfortably tight. (See "Caring for the Activity Monitor" if you need to make alterations to your belt.) You may wear the monitor over or under your clothing.**
4. **The activity monitor should be worn on your <u>left hip</u>. The Activity Monitor should slide into place along the belt with gentle pressure.**

Caring for the Activity Monitor

- **You will probably not need to clean the Activity Monitor. If it gets dirty, however, simply wiping it with a damp (not wet) rag should be sufficient.**
- **If after tightening your belt, there is a lot of excess belt hanging from the buckle, you may wish to trim it back. To do so, you should:**
    - **Make sure to leave about 3-4 inches excess for adjustment purposes.**
    - **Cut the belt evenly with a pair of scissors.**
    - **Seal the cut end of the belt with some clear nail polish to prevent fraying (if necessary).**
- **You may use a safety pin or rubber band to tie up extra belt rather than cutting it if you prefer.**

Other important notes about your activity monitor:

- **This piece of equipment is <u>very expensive</u>, so please take good care of it!**
- **The Activity Monitor is not waterproof, so please avoid getting it wet, as this could cause damage.**

- **The Activity Monitor may or may not flash. Either way, it is not a cause for any concern. Trust us – it's working!**

Returning the Activity Monitor

After you have worn the Activity Monitor for a full week, we ask that you return it to us so that we can collect the data and use it for other participants. When returning the Activity Monitor to us, you should:

- **Remove the Activity Monitor from your waist.**
- **Remember to record your actual end date on the Accelerometer Log and ensure the rest of the form is complete.**
- **Wrap the belt around the Activity Monitor and hold secure with a rubber band.**
- **Place the Activity Monitor back in the padded envelope and mail it back to us with the completed Accelerometer Log.**

Activity Monitor Troubleshooting

If you are experiencing problems with your Activity Monitor or have questions, please contact study coordinator Stephanie Kurti and kurtisp@jmu.edu **or 630-205-6363.**

Frequently Asked Questions

My activity monitor was blinking and then stopped. Is it still working? Yes, some of the monitors blink before they start recording activity but some of our newer versions don't blink at all. Either way, keep wearing your monitor.

I don't want the monitor to show when I wear it. Can I wear it underneath my clothes? Yes, you can wear it over or under your clothes – whatever is most comfortable for you.

My activity monitor seems to ride up and won't stay on my hip. Some people have said they have this issue. It will still record but it's better to have it closer to your hip if possible. You can try tightening it, pinning the band to your pants, or running the band through your belt loops.

I accidentally wore the monitor upside down one day. Do I need to wear it for an extra day? No, as long as you wore the monitor during all of your waking hours you do not need to wear it for an extra day. The data it collects are still accurate even if it's upside down.

I forgot to put on my monitor and then went out for the morning. Should I wear it for the afternoon/evening or just skip today? It is very important that you wear the monitor for the entire day. If you forget to wear it for part of the day, it is best to skip that day and add an extra full day to the end instead. Be sure to record this on your log.

My schedule for the week I'm supposed to wear the monitor is not a normal week for me. Should I still wear it? Yes. The important thing is that you wear it during the time we have specified. If needed you can make note of any special circumstances on your log.

I am going to be traveling when I'm supposed to wear my monitor. Will I be able to go through security at the airport? Yes. You will need to take it off just as you do your jewelry. The x-ray will not harm the device.

ID _____ AC _____ m0

Activity Monitor Log

As a participant in this program, we ask that you wear your Activity Monitor for one week. Begin wearing the monitor on the **Requested Start Date**. Please try to wear the monitor for seven consecutive days, but if you do need to skip a day for any reason, continue wearing it until you have worn it for a full seven days.

Requested Start Date: __/ __/ 18__ When you get up in the morning

Requested End Date: __/ __/ 18__ When you go to bed at night

Please record the actual dates/times you wore the accelerometer in the table below. Please be as precise as possible in reporting the times you put on and removed the monitor.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date							
Time of day you put on the unit							
Time of day you took off the unit							
Any time you did not wear the unit? (e.g. naps, bathing)							
Please provide comments about problems that occurred while you were wearing the unit.							

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