ASSOCIATION OF LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A_2 AND C-REACTIVE PROTEIN WITH MODERATE-TO-VIGOROUS PHYSICAL ACTIVITY IN MIDDLE-AGED WOMEN

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Coronary heart disease (CHD) is an inflammatory process that is the most common form of cardiovascular disease (CVD). People who are habitually physically active have comparatively lower levels of certain inflammatory biomarkers. C-reactive protein (CRP) is considered the "gold standard" to assess the relation between physical activity and inflammation. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an inflammatory marker that has been shown to be associated with CHD. However, little is known about what association Lp-PLA_2 might have with habitual physical activity.

Purpose: The current investigation examined the association between Lp-PLA_2 and moderate-to-vigorous physical activity (MVPA). A secondary purpose was to examine the association between CRP and MVPA. Finally, this investigation examined the association between Lp-PLA_2 and CRP.

Methods: This investigation was a secondary analysis of data that were previously collected as part of the Epidemiologic Study of Health Risk in Women (ESTHER). Seventy-five females (50.2 ± 10 yrs.) were selected for the current investigation and were assigned to either the ACTIVE or INACTIVE group. The ACTIVE group was comprised of those who reported the highest levels of MVPA (≥ 8.93 hours/week) during the previous 12 months. The INACTIVE group comprised participants who reported no MVPA over the previous 12 months. Blood

samples from each participant were analyzed for levels of Lp-PLA_2 mass, Lp-PLA_2 activity and CRP.

Results: No significant differences were found between the ACTIVE and INACTIVE groups for mean values of either Lp-PLA_2 mass (ACTIVE= 226.4 ± 48.1 ng/ml, INACTIVE=217.6 ± 50.4 ng/ml, $p = 0.44$) or Lp-PLA_2 activity (ACTIVE=133.3 ± 26.4 nmol/min/ml, INACTIVE=136.5 ± 30.5 nmol/min/ml, $p = 0.63$). CRP values were significantly ($p = 0.02$) lower in the ACTIVE group as compared to the INACTIVE group. It was found that neither Lp-PLA_2 mass nor Lp-PLA_2 activity were significantly correlated with CRP values.

Conclusions: Based on the current findings, it can be concluded that Lp-PLA_2 is not associated with MVPA in heterosexual, middle-aged women. Other inflammatory markers that have been found to be associated both with CHD and PA, such as CRP, IL-6, and TNF- α, should continue to be examined.

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1.0 INTRODUCTION

1.1 PURPOSE

The primary purpose of this investigation was to examine the association of moderate to vigorous physical activity (MVPA) with serum levels of a marker that has been linked with increased coronary heart disease (CHD) risk, lipoprotein-associated phospholipase A_2 (Lp-PLA_2), in a sample of 80 middle-aged women. A secondary purpose of this investigation was to examine the association of MVPA with plasma levels of another inflammatory marker related to CHD risk, C-reactive protein (CRP). Finally, this investigation assessed the correlation between serum Lp-PLA_2 and plasma CRP.

1.2 RATIONALE

Research has shown that cardiovascular disease (CVD) is the leading cause of mortality in the United States, accounting for over 700,000 deaths per year (72). The most common form of CVD is CHD, and it is generally accepted that the development of the most common form of CHD, atherosclerosis, is an inflammatory process (36, 64).

The inflammatory process is believed to include a cascade of events containing a number of pathways that influence the development of atherosclerosis, including one involving the A_2 phospholipase enzyme family (51). The Lp-PLA_2 enzyme is an inflammatory marker that is one of the enzymes in the phospholipase A_2 group, and is produced by mast cells, monocytes, and T lymphocytes. Eighty percent of Lp-PLA_2 is transported by low-density lipoproteins (LDL-C),

with the remaining 20% transported by high density lipoproteins (HDL-C) (51). A number of studies have found a strong and consistent relation between CHD risk and serum levels of Lp-PLA_2 in both men and women (27, 31, 48). In addition, most of these studies have found that the relation of Lp-PLA_2 with CHD was independent of the relation of CHD with other inflammatory markers such as CRP (23, 48). While both Lp-PLA_2 mass (the total amount of Lp-PLA_2 present in the blood serum) and Lp-PLA_2 activity (a description of the rate of movement of Lp-PLA_2 in circulation) have been shown to be positively associated with CHD risk, Lp-PLA_2 activity has been reported to have the most consistent association with CHD risk (13, 48, 51,54).

Research has shown that CRP is among the most commonly studied inflammatory markers of CHD risk, and elevated CRP concentration is an early indicator of atherosclerotic-related inflammation (6). Previous investigations have generally shown a positive relation between high plasma levels of CRP and increased risk of CHD (6, 63). The process of CRP-related inflammation is associated with the release of interleuken-6 (IL-6) from many sources, including endothelial tissue (64). As a result of this process, the liver is stimulated by IL-6 to release CRP. CRP is thought to be a marker of the development of CHD through a number of mechanisms, both direct and indirect (43, 64).

Previous studies have shown a reduced risk of CHD for those people who regularly participate in physical activity (PA) (44, 45). In addition, systemic inflammation has been shown to be associated with a variety of factors, including low levels of habitual PA participation (6, 12, 26). The relation between systemic inflammation and PA is somewhat complex. An acute bout of PA is associated with an increased inflammatory response and the magnitude of this inflammatory response tends to be greater for higher intensity PA. In contrast, the acute inflammatory response tends to be blunted following an aerobic PA training program (20, 29).

Long-term participation in PA has been shown to be associated with lower resting levels

of a number of inflammatory markers, among them CRP (63, 66, 69). Plasma CRP has often been used in assessing the relation between coronary artery inflammation and PA (18, 24). It has been shown that those individuals who frequently participate in PA have lower levels of CRP than those individuals who do not frequently engage in PA (20, 63, 66, 69). Specifically, results from the National Health and Nutrition Survey III (NHANES III) showed that of those individuals with CRP levels >85th percentile, 21% were in the lowest PA group, while only 8% were in the highest PA group (20). Further evidence of this inverse relation was demonstrated by Fischer, et al. who found that CRP levels decreased as leisure-time PA increased (17). In this context, the Centers for Disease Control and Prevention defines leisure time PA as **e**xercise, sports, and physically active hobbies done in one's leisure time (53). This is in contrast to PA

done as part of employment (occupational PA), or PA done while performing normal day to day tasks (activities of daily living).

Plasma CRP levels are affected by factors other than coronary artery inflammation, including obesity, cigarette smoking, and hormone therapy (63, 65, 82). Some research has indicated that CRP may be strongly positively associated with body mass index (BMI), but not PA (62). The amount of adipose tissue has been shown to be highly positively correlated with CRP levels. In addition, adipose cells secrete IL-6 and tumor necrosis factor-alpha (TNF-α), both of which are the main stimuli for CRP production in the liver (29). It has also been shown that CRP levels in a cohort of post-menopausal women were nearly twice as high as in a control group of women not taking hormone therapy (64). It is possible that CRP acts more as a marker of systemic inflammation and less as a specific marker of coronary artery inflammation. As a result of such lack of specificity, plasma CRP level may not provide evidence of a clear relation between PA and coronary artery inflammation. Therefore, identification of a different biomarker

of coronary artery inflammation with potentially fewer confounding interactions, such as Lp-PLA_2, would be desirable.

Both Lp-PLA_2 and CRP are markers of coronary artery inflammation. However, previous research has shown that the correlation between Lp-PLA_2 and CRP is equivocal (32, 41, 51). This indicates that Lp-PLA_2 and CRP may be influenced by different mechanisms during coronary artery-related inflammation. Because of the potential mechanistic differences between these biomarkers, it is important to determine if Lp-PLA_2, like CRP, is negatively affected by higher habitual PA levels.

Research has shown Lp-PLA_2 to be an accurate inflammatory predictor of CHD risk (13, 48, 54). What has yet to be clearly elucidated is whether serum Lp-PLA_2 can be affected by habitual moderate-to-vigorous leisure time PA (MVPA). Moderate-to-vigorous physical activity can be defined as PA that requires an energy expenditure between 3 and 9 MET's. It has been reported that a particular genetic variant of Lp-PLA_2 was significantly associated with a reduction in fat mass following a 12 week training program, but the authors of the investigation did not report the mode of PA, nor did they report the duration of PA (85). Another investigation involving selected CHD risk factors found a weak positive correlation between PA and Lp-PLA_2 levels (54). However, the PA levels of the participants in that investigation were not reported, only the correlation between Lp-PLA_2 and PA, ($r = 0.11$, $p<0.05$) (54).

Based on these previous inconclusive results, it is important to investigate the possible association of MVPA with serum levels of Lp-PLA_2. Since previous investigations have found habitual PA to be associated with CRP levels, the current investigation will also examine the association of MVPA with plasma levels of CRP. Finally, given that previous investigations

suggest that Lp-PLA_2 and CRP may be influenced by different mechanisms during coronary artery-related inflammation, the correlation between Lp-PLA_2 and CRP was also assessed.

1.3 RESEARCH AIMS AND HYPOTHESES

1.3.1 Research Aims

(a) To determine the association of MVPA with serum mass and activity of Lp-PLA_2 in a sample of 80 middle-age women.

(b) To determine the association of MVPA with plasma CRP levels in a sample of 80 middle-age women.

(c) To examine the correlation between serum levels of Lp-PLA_2 mass and activity and plasma CRP levels in a sample of 80 middle-age women.

1.3.2 Research Hypotheses

1. It was hypothesized that serum Lp-PLA_2 mass and activity would be significantly lower in the middle-age women who reported greater amounts of MVPA as compared to those who reported lesser amounts of MVPA.
2. It was hypothesized that plasma CRP concentration would be significantly lower in the middle-age women who reported greater amounts of MVPA as compared to those who reported lesser amounts of MVPA.
3. It was hypothesized that serum Lp-PLA_2 levels would not be significantly correlated with plasma CRP levels.

1.4 SIGNIFICANCE

Cardiovascular Disease (CVD) is the leading cause of mortality in the United States, accounting for almost 50% of worldwide deaths (72). The main form of CVD is CHD, and CHD is characterized by an inflammatory response leading to the accumulation of lipids in the coronary arteries that may result in the occlusion of the artery. Habitual PA has been shown to be associated with reduced levels of systemic inflammation and this may help to explain why those individuals who report participating in more PA are at a reduced risk of CHD (3, 7).

Previous research has shown that almost 50% of cardiac events occur in individuals with normal plasma lipid levels (36). Thus, early identification of coronary artery-related inflammation might usefully add to identification of CHD cases. In addition, it is important to determine what relation exists between PA and markers of coronary artery inflammation. A variety of inflammatory biomarkers have been examined to determine their association with CHD risk and their association with PA. These biomarkers include CRP, serum amyloid-A (SAA), fibrinogen, IL-6, and TNF- α. Of these, the most commonly studied marker of inflammation is CRP and this marker has been shown to be positively associated with CHD risk and inversely associated with habitual PA (3, 7, 61).

Recent studies have found Lp-PLA_2 to be an inflammatory biomarker that is strongly and positively associated with CHD risk (13, 48, 54). What has yet to be clearly elucidated is the association of PA with serum levels of Lp-PLA_2. In addition, it is unknown what possible biochemical or behavioral confounders might exist in assessing the association of PA on Lp-PLA_2.

There are limitations in the previous research that has examined the relation between PA and CRP, mostly due to the presence of many potential confounding factors such as obesity,

cigarette smoking, and hormone therapy (62, 76). Controlling for all of the possible confounding factors associated with coronary artery inflammation including obesity, cigarette smoking, and hormone therapy reveals a clearer picture of the relation between PA and CRP but this potentially reduces the generalizability of the results. In addition, CRP concentration expresses a picture of systemic inflammation that, while important, may not discriminate sufficiently to allow precise identification of inflammation that is associated exclusively with CHD. As such, a different, and more independent, biomarker of inflammation would be desirable. Serum Lp-PLA_2 may be such a biomarker, as it has been shown to be positively associated with coronary artery-associated inflammation. What needed to be examined was the possible association of PA with Lp-PLA_2. The intent of this study was to provide a clearer picture of whether Lp-PLA_2, an inflammatory biomarker shown to be associated with CHD, is associated with PA. This information could provide more evidence to support the beneficial effect of PA on CHD risk.

2.0 REVIEW OF RELATED LITERATURE

The purpose of this investigation was to determine the association of PA with levels of two inflammatory biomarkers of CHD risk, Lp-PLA_2 and CRP. There are a variety of biomarkers of inflammation associated with CHD, including Lp-PLA_2, CRP, serum amyloid-A (SAA), fibrinogen, IL-6, and tumor necrosis factor-alpha (TNF- α). Each of these biomarkers has been shown to have varying degrees of strength of association with PA. The association between each biomarker and CHD risk will be discussed separately in this chapter. In addition, the association of each biomarker with PA will be discussed. The primary focus of this literature review will be the relation between each of the biomarkers and PA and to demonstrate that Lp-PLA_2 is a potential new biomarker of the relation between inflammation and PA.

2.1 PHYSICAL ACTIVITY AND CORONARY HEART DISEASE

The association between PA and CHD was first examined in the middle of the 20^{th} century (45). This examination of mortality among transit workers found that drivers of buses were more likely to die from heart disease than conductors on buses. A subsequent investigation validated these findings by suggesting that those workers in jobs that required more PA were less likely to die from heart disease than those who worked in comparatively sedentary, office-type positions (44).

A low level of PA is considered one of the main risk factors for CHD, along with dyslipidemia, hypertension, cigarette smoking, abnormal blood glucose, and obesity. It is well documented that habitual PA is associated with substantial reductions in CHD risk. In addition,

the reduction in CHD risk as a result of PA may be accompanied by only minor changes in the other risk factors (24). Thus, the mechanism by which habitual PA contributes to CHD risk reduction may be associated with other, less well known risk factors.

In addition to the main risk factors already mentioned, there are a number of relatively new biomarkers of inflammation that have been found to be associated with CHD risk. Among these are Lp-PLA_2, CRP, SAA, fibrinogen, IL-6, and TNF- α. PA has been shown to be associated with each of these markers, with the strength of association varying depending on the marker. These markers have been used in assessing the risk of CHD due to the inflammatory nature of the CHD process.

2.2 CORONARY HEART DISEASE AND INFLAMMATION

Atherosclerosis is the most common form of CHD and is characterized by a progressive accumulation of lipid deposits and fibrous elements in the blood vessels (68, 77). Atherosclerosis develops from endothelial dysfunction, which in turn arises from injury to the endothelial tissue as result of a variety of factors including dyslipidemia, cigarette smoking, and hypertension (36, 76). The injured endothelial tissue forms a lesion and this lesion attracts macrophages and lymphocytes. This process leads to even greater inflammation and the release of enzymes, cytokines, and various growth factors. The inflammatory response acts via a positive feedback mechanism; as more cells migrate to the injured area, the inflammatory response becomes greater. In the most severe cases, the lesion formed as a result of the injury may impair or preclude blood flow past the obstructed site.

The inflammatory cytokine CRP is thought to be involved in the atherosclerotic process via increased expression of cellular adhesion molecules. Previous research has shown that CRP tends to be inversely related to habitual PA levels for both males and females (6, 12, 33).

2.3 INFLAMMATION AND PHYSICAL ACTIVITY

The mechanisms by which PA influences arterial inflammation are not fully understood. It is known that a single bout of exercise is associated with increases in inflammatory biomarkers, but that habitual exercise is associated with reduced levels of inflammatory biomarkers (6, 24, 33).

In examining the association between coronary artery inflammation and PA, other factors must be accounted for, among them the level of adipose tissue (16, 17). Adipose tissue can act as an endocrine organ by secreting IL-6, an inflammatory biomarker that is an up-stream signaler of CRP production (16, 18). Adipose tissue is responsible for up to 25% of IL-6 production, indicating that increased levels of adipose tissue will lead to increased levels of CRP (17).

2.4 INFLAMMATORY BIOMARKERS

2.4.1 Lipoprotein-Associated Phospholipase A_2

The inflammatory biomarker Lp-PLA_2 is one of the enzymes in the phospholipase A_2 group, and is produced by mast cells, monocytes, and T lymphocytes. It has been shown that

approximately 80% of the transport of Lp-PLA_2 in plasma occurs via binding with LDL-C, with the remaining 20% transported bound to HDL-C (51).

There are believed to be a number of inflammatory pathways that influence the development of CHD, including one involving Lp-PLA_2 (51). This inflammatory pathway (that leads to CHD) begins with Lp-PLA_2 combining with LDL-C. These combined particles can then attach to the walls of the arteries and become oxidized (32, 51). This will typically stimulate a pro-inflammatory response which causes plaque accumulation within the arteries. In addition, this process will cause the production of more macrophages and greater plaque accumulation (75). Packard et al. published the first evidence that baseline levels of Lp-PLA_2 were a strong and independent predictor for CHD (51). The authors examined the association between Lp-$PLA_2$and the risk of coronary events. Levels of Lp-PLA_2 were found to have a strong, positive, and linear association with risk of a coronary event (RR> 2.72 in the highest quintile). Subjects in the lowest quintile of Lp-PLA_2 were found to have half of the risk of a coronary event compared to those in the highest quintile. This risk was found to be independent of other biomarkers, such as CRP. Since publication of the Packard, et al. findings in 2000, a number of other studies have confirmed the relation between CHD risk and levels of Lp-PLA_2 in both men and women (13, 48, 54).

2.4.2 Lipoprotein-Associated Phospholipase A_2 and Physical Activity

There has been limited research examining the association between Lp-PLA_2 and PA. In general these studies have found little or no significant correlation between Lp-PLA_2 and PA (9, 10, 54, 61). In a prospective, case-control study, Rana et al. examined CHD risk in a sample of

1002 cases (healthy men and women, 45-79 years of age) (61). These cases were matched to 1859 controls based on sex, age, and enrollment period. This investigation was based on studying a variety of biomarkers and known risk factors. They categorized LTPA into three levels, "inactive", "moderately inactive", and "active". The authors reported that, overall, Lp- PLA_2 was not different between the three PA groups. However, it was reported that Lp-PLA_2 levels were significantly associated with increasing waist size within the "active" group of females ($p=0.007$) and males ($p=0.03$) (61). No significant association of Lp-PLA_2 with increasing waist size was found for either the "inactive" or "moderately inactive" group. Overall, the main findings showed that Lp-PLA_2 was not significantly associated with PA levels. Rana, et al. also examined CRP in the same investigation and found that CRP was significantly associated with PA levels in both females ($p<0.05$) and males ($p<0.05$) (61). In addition, Lp-PLA_2 was significantly ($p<0.05$) associated with CRP in males, but not in females (61). A methodological weakness of this study was the lack of precision of the PA measure. This study used a questionnaire containing only 2 questions to assess PA. Thus, the instrument may have lacked
the precision necessary to determine the true association between Lp-PLA_2 and PA.

Some research that has examined the association between Lp-PLA_2 and PA has used participants who may not have been sufficiently active to accurately determine an association between the variables. A cross-sectional study by Persson et al. examined Lp-PLA_2 in 5402 participants, 3167 women and 2235 men, aged 45-69 years (54). The authors employed a non-standardized questionnaire that allowed participants to select from among 18 different types of PA, incorporating activities from all four seasons of the year. No significant association between Lp-PLA_2 and PA was found for either males or females (54). However, the authors did not report the level of PA undertaken by the participants. This omission makes it difficult to

determine if the participants were sufficiently active to identify an association between Lp-PLA_2 and PA. It should also be noted that Lp-PLA_2 and CRP were not found to be significantly correlated in this investigation.

A cross-sectional study by Detopoulou et al. examined the association between Lp-PLA_2 and PA in group of 100 participants, 52 men, aged 44 ± 13 years and 48 women aged 43 ± 13 years (10). This study assessed PA via the short version of the International Physical Activity Questionnaire (IPAQ) (10). The IPAQ questionnaire measures leisure time PA, occupational PA, and PA undertaken as part of daily living (56, 57). The IPAQ expresses total PA in terms of MET minutes/week. Detopoulou et al. found no significant association between Lp-PLA_2 and PA, for either males or females (10). The median MET minutes/week was 1046 and 1043 for males and females, respectively (10). A high level of PA is considered to be a score of ≥ 3000 MET minutes/week, and a low PA level is considered to be a score of < 600 MET minutes/week. The reported MET minute values in this study are much closer to the low PA score than the high PA score. This indicates that the participants may not have had sufficiently high PA levels to determine if there was an association between Lp-PLA_2 and PA. In addition, the authors reported no significant correlation between Lp-PLA_2 and CRP.

Daniels et al. conducted a cross-sectional assessment of the association of Lp-PLA_2 and PA (9). The investigation assessed the PA of 1077 males and females with a median age of 72 years (9). The primary objective of the study was to examine how well Lp-PLA_2 levels predicted development of CHD. Participants were categorized into two PA groups, those that exercised ≥ 3 times/week and those who exercised < 3 times/week (9). No other information on PA, such as mode of activity or intensity was obtained. It was reported that Lp-PLA_2 was not significantly associated with PA category. However, Lp-PLA_2 and CRP were found to have a weak but

significant correlation (r = 0.10, p=0.003) (9). Given the lack of reported information on exercise mode or intensity, it is difficult to determine if their truly was any significant association between Lp-PLA_2 and PA.

Research has shown Lp-PLA_2 to be associated with CHD. Given this association, it is important to determine if PA, which has been shown to be inversely associated with CHD risk, is also inversely associated with Lp-PLA_2. Unfortunately, previous investigations have not been able to clearly examine the relation between Lp-PLA_2 and PA. These investigations have been limited by methodological constraints, particularly the use of non-validated or non-standardized measures of PA and research that was not primarily designed to examine the relation between Lp-PLA_2 and PA. By attempting to address some of these limitations, it is hoped that the results of the proposed investigation will present a clearer picture of the association of PA on both Lp-PLA_2 and PA.

2.4.3 C-Reactive Protein

Among the most frequently studied of the inflammatory markers of atherosclerosis is CRP. High levels of CRP have been shown to be an early indicator of atherosclerotic-related inflammation (6, 63). The production of CRP secondary to inflammation is associated with the release of IL-6 from endothelial tissue (64). As a result of this process, the liver is stimulated by IL-6 to release CRP. Research studies have generally shown a positive relation between high serum levels of CRP and increased risk of CHD (68, 71, 74). CRP moves from the liver to the endothelial vessel wall, where it increases expression of vascular cell adhesion molecule-1 (VCAM-1) by the cells of the endothelium (67). CRP has also been shown to bind to the plasma

membrane, increasing LDL-C transit into the macrophages (67). This in turn increases the risk of atherosclerosis.

2.4.4 C-Reactive Protein and Physical Activity

Plasma CRP has often been used in assessing the relation between systemic inflammation and PA. It has also been shown to be the inflammatory biomarker that has the strongest association with PA (63, 64, 68, 82). Individuals who reported frequent participation in LTPA were found to have lower levels of CRP than those subjects who did not engage in frequent LTPA (63, 66, 69). Further evidence of the inverse relation between CRP and PA was reported in a cross-sectional investigation by Fischer, et al (18). The investigation employed eighty-four healthy men and women (mean age 57 ± 11 years old, average BMI of 26 ± 4.3 kg/m^2) who had no history of CHD or any other inflammatory disease and no recent use of a prescription anti-inflammatory medication. The authors reported a significant inverse trend across all PA levels, demonstrating that CRP levels decreased as levels of LTPA increased (18). Herter, et al reported that a twelve-month weight-loss intervention with a combination of aerobic and resistance exercise resulted in a significant ($p=0.01$) negative correlation between moderate- to-vigorous LTPA hrs. /week and median CRP values (24). The Herter study was comprised of
522 males and females, age ranging from 40-65 years and who were classified as overweight or obese based on BMI values (24).

In an investigation by Koenig, et al., that was comprised of 934 healthy men ages 45 to 64 years, participants in the group with the highest level of LTPA (≥ 3 times/week) had CRP

levels that were 37% lower than subjects in the group with the lowest level of LTPA (≤ 1 time/week) (31).

Results of some research suggest a dose-response association of PA on CRP levels. Specifically, results from the 3rd National Health and Nutrition Examination (NHANES III) study showed that, of those individuals with elevated CRP levels, 21% were sedentary , 17% reported light levels of LTPA, 13% reported moderate levels of LTPA, and 8% reported vigorous levels of LTPA (20). In addition to the CRP values being highest in the group with lowest levels of reported LTPA, these results showed a significant trend ($p<0.001$) of increasing CRP across the PA groups (20). A study by Thompson, et al. examined whether a 24-week aerobic exercise program changed IL-6, CRP, soluble intercellular adhesion molecule-1 (sICAM-1), and alanine transaminase (ALT) as a function of PA participation. The authors reported significant reductions in resting levels of IL-6 as result of "moderate" intensity PA (74). Conversely, ALT required "vigorous" intensity PA to show significant reductions in resting levels (74). This suggests that light intensity PA might not provide a sufficient stimulus to reduce resting values of certain inflammatory markers.

Overall, most cross-sectional and longitudinal investigations have shown a significant, inverse association between CRP and PA (80, 81). Some studies, however, have found little or no association between CRP and PA (62). In a longitudinal investigation, Rawson, et al. longitudinally examined CRP levels over a 1-year period and found no association between CRP levels and reported PA (62). This study was comprised of 109 participants (62 male, 47 female) with a mean age of 49 years, who were not taking cholesterol lowering medication. PA was assessed by twenty-four hour recall (via a phone interview) with separate categories used for leisure time PA, occupational PA, and household PA (62). A possible explanation for the lack of

association between CRP and PA may be found in the low overall levels of PA reported by the participants. This could indicate that the participants were not sufficiently active to cause a significant gradient effect of CRP levels across varying levels of PA participation.

2.4.5 Serum Amyloid-A

SAA is a protein produced primarily by the liver and varies with plasma levels of HDL- C. In addition to hepatic production, adipose tissue is also a major source of SAA and this is theorized to be a factor in the chronic inflammatory response that is associated with obesity (35). Research has shown that SSA is active during the early period of inflammation, defined as the acute phase of systemic inflammation (30, 66). It has been demonstrated that SAA levels may increase by more than 1000 times during the systemic inflammatory process (78). This elevated activity of SSA comes from increased migration of immune cells to the site of inflammation.
This is followed by increased expression of the active isoforms of SAA, which are regulated by other inflammatory cytokines, such as IL-6 and TNF- α (60).

Elevated SSA levels have been shown to be associated with an increased risk of CHD in both males and females (64). Upon entering blood, SAA binds to HDL-C and thus may be related to CHD via alteration in cholesterol transport (78). This alteration occurs due to SSA displacing apolipoprotein A-1 (apoA-I) on the HDL-C molecule (35). High serum cholesterol levels have been shown to be strongly associated with acute coronary syndrome (ACS), a series of conditions related to myocardial ischemia (30). ACS patients have been shown to have a ten-fold increase in the plasma concentrations of SAA as compared to healthy patients (30).

2.4.6 Serum Amyloid-A and Physical Activity

Research has shown SAA to be inversely associated with PA levels (31, 59, 83). A cross-sectional assessment of 1514 men and 1528 women without clinical evidence of cardiovascular disease found that those participants in the highest tertile of self-reported PA had SAA values that were approximately 22% lower than the sedentary participants in the investigation (59). The investigators characterized PA by intensity (light, moderate, and high) depending on type of activity performed and defined sedentary as not participating in any physical activities (59). Another cross-sectional assessment of 892 male participants failed to find a significant association between leisure time physical activity and SAA levels ($p=0.58$) (81).

Wegge, et al. examined the effects of a combined diet and exercise intervention on SAA levels in a group of twenty women, all of whom were post-menopausal and ranged in age from 50-79 years (80). In addition, all participants had more than one risk factor for CHD. In addition to a low-fat, high fiber diet, the participants undertook prescribed daily aerobic exercise (primarily walking). Results showed that SAA levels decreased by approximately 37% following the two-week diet and exercise intervention (80).

The mechanism underlying the relation between SAA and PA is in part due to the influence on SAA levels of IL-6 and TNF-α, both of which are lower in individuals who have higher levels of PA (6, 55).

2.4.7 Fibrinogen

Fibrinogen, a precursor of fibrin, is a coagulation protein. As with most plasma proteins, fibrinogen is synthesized by hepatocytes and is an important factor in platelet aggregation (25). In addition to its importance in blood coagulation, fibrinogen is also an acute-phase reactant found in the early stages of CHD inflammation (25). During the coagulation process, thrombin and calcium act to convert the insoluble fibrinogen to loose threads of fibrin (75).

As a coagulation protein, fibrinogen is involved in the blood clotting process. Increased fibrinogen levels in the acute vascular inflammation phase are associated with increased risk of blood clotting and thus, an increased risk of CHD (25). In this context, a number of studies have shown that participants with high concentrations of fibrinogen were at an increased risk of CHD (14, 25, 70). Studies have shown that fibrinogen may also be a mediator for other CHD risk factors such as cigarette smoking and obesity (70).

2.4.8 Fibrinogen and Physical Activity

Research findings suggest that an inverse association exists between fibrinogen levels and PA (19, 42, 59). Folsom, et al., examined a group of 12,000 men and women, ranging in age from 45-64 years old, who had no previous history of CHD (19). The authors reported that fibrinogen levels were lower in those participants who were classified as being physically active. As such, it was theorized that high fibrinogen levels could be reduced via changes in lifestyle involving increased PA participation (19). A cross-sectional study of 3042 people (1514 men, 1528 women) compared fibrinogen levels between those who were classified as having

metabolic syndrome and those who did not have metabolic syndrome (59). The authors reported that that those participants who were physically active had fibrinogen levels that were 15% lower than those subjects who were sedentary (59). The investigators characterized PA by intensity (light, moderate, and high) depending on type of activity performed and defined sedentary as not participating in any physical activities (59). Similarly, a study by Mora, et al. found a significant association ($p<0.001$) between fibrinogen and increasing tertiles of PA in a sample drawn from the Women's Health Study (42). This investigation was comprised of 27,055 female health professionals who were not diagnosed with CHD and were at least 45 years old (mean age of 54 years) (42). Physical activity was calculated based on the average time spent on 8 separate groups of activities and also on the number of flights of stairs climbed on a daily basis (42).

2.4.9 Tumor Necrosis Factor- α

An inflammatory cytokine that is important in the atherosclerotic process is TNF-α (84). Release of TNF-α occurs from a variety of sources, including the immune system, muscles, and adipose tissue (7). Studies have shown that TNF-α stimulates expression of adhesion molecules via increased production of SSA (68). In addition, high levels of TNF-α have been shown to cause a breakdown of the extracellular matrix in the endothelial walls (59, 68).
Activity of TNF-α is also influenced by IL-6, which is produced by muscle cells. In this relation, IL-6 functions primarily as an anti-inflammatory agent, acting to suppress production of TNF-α (55).

Levels of TNF-α have been shown to stimulate production of plasminogen activator inhibitor-1, which itself is linked to CHD (21). High levels of TNF-α have been shown to cause

production of excess amounts of reactive oxygen species, which results in degradation of endothelial tissue (86). In addition, high levels of TNF-α are associated with many chronic secondary infections, such as dental infections (28). Although the mechanisms are not clear, these secondary infections are also known to be linked to CHD (28).

2.4.10 Tumor Necrosis Factor-α and Physical Activity

The available research suggests an inverse association between TNF-α levels and PA (8, 59). Using a cross-sectional design, Pitsavos, et al. examined the association between PA and selected inflammatory biomarkers, including TNF-α (59). The authors found that those participants in the highest PA tertile had TNF-α values that were 15% lower than the sedentary participants (59). In a separate analysis using data from a subgroup of the same cohort, the strength of the association between TNF-α and PA was found to not differ between those who were classified as having metabolic syndrome and those who did not (59). A 12-week intervention study involving elderly nursing home patients showed no change in TNF-α levels following a resistance training program (8). However, the study did find that activity of the TNF- α system at baseline was inversely correlated with changes in muscular strength over the 12
week training period (8). This suggests that those participants with higher baseline levels of TNF-α achieved lower gains in muscular strength consequent to the intervention.

2.4.11 Interleukin-6

IL-6 is an inflammatory cytokine secreted primarily by macrophages and lymphocytes, but also by muscle tissue (7). Its production is driven by secretion of IL-1 and also TNF-α (84).

IL-6 is considered one of the main factors in the inflammatory response, given that it stimulates production of other inflammatory cytokines from macrophages, and also acts to stimulate hepatic production of CRP (17, 84).

High levels of IL-6 are associated with increased risk of future cardiac events (64). In addition to stimulating production of inflammatory agents, IL-6 also stimulates expression of adhesion molecules and chemokines, both prominent factors in the initial stages of endothelial dysfunction. Recent research suggests that IL-6 may have an anti-inflammatory association when released from muscle (39). This form of IL-6 suppresses TNF-α production and thus acts to inhibit systemic inflammation (39). IL-6 is also active in adipose tissue, with adipose tissue accounting for up to 25% of all IL-6 production (16).

2.4.12 Interleukin-6 and Physical Activity

Northoff, et al. were among the first to examine the association between IL-6 levels and PA (47). They found that while plasma concentrations of many cytokines were associated with exercise, IL-6 had the strongest association with exercise (47). IL-6 is believed to be the first of a series of cytokines released into the circulation during exercise (16, 55). Another important effect of IL-6 released from muscle is believed to be its down regulation of TNF-α production. This function indicates that IL-6 may be associated with both pro-inflammatory and anti-inflammatory conditions (16). Studies suggest that there is an inverse association between IL-6 levels and PA levels (18, 30, 52). Fischer, et al. found that subjects who reported participating in >4 h/week of leisure time PA had significantly ($p=0.017$) lower levels of IL-6 as compared to sedentary participants (18). Pitsavos, et al. found that those subjects in the upper tertile of PA had IL-6 values that were 30% lower than those subjects classified as sedentary (59). It has also

been suggested that PA intensity is a factor in the association between IL-6 and PA (50). A significant, negative ($p<0.05$) association has been reported between plasma IL-6 levels and exercise training intensity in a group of long-distance runners (50). The mechanism for this association is thought to be due to the role that IL-6 plays in system homeostasis. As exercise intensity increases, maintaining systemic homeostasis becomes more difficult and IL-6 concentration increase (17, 74).

A recent study involving 406 males and females who underwent a combined diet and PA intervention found that moderate-to-vigorous PA predicted decreases in IL-6 at the one-year follow-up as compared to baseline levels (24). In addition, the intervention group demonstrated significant ($p=0.06$) reductions in IL-6 levels after a one-year follow-up as compared to baseline levels (24). Unfortunately, no measurements were taken between the baseline and 12 month measures, thus making it impossible to determine when the IL-6 levels were reduced.

2.5 SUMMARY

In summary, it has been shown that PA is associated with systemic inflammation, and that this association tends to be inverse. While many inflammatory biomarkers have been shown to be associated with PA, the association is strongest with CRP (18, 26, 40, 80). Unfortunately, from a perspective of assessing CHD risk, CRP levels are influenced by many factors in addition to PA (62, 76). There is a need to determine whether there are other inflammatory biomarkers of CHD risk that have fewer confounding factors. One such candidate marker is Lp-PLA_2. The identification of such an inflammatory biomarker could present a clearer picture of the association between PA and vascular inflammation. This could lead to a better understanding of

how the risk of CHD is mediated by the association between vascular inflammation and PA. Previous research suggests that the association between inflammatory markers and PA may have an intensity threshold (24, 74). A study by Thompson, et al. examined whether a 24-week aerobic exercise program changed inflammatory biomarkers as a function of PA participation. The authors reported significant reductions in resting levels of some inflammatory biomarkers as result of "moderate" intensity PA (74). Conversely, other inflammatory biomarkers required "vigorous" intensity PA to show significant reductions in resting levels (74). This suggests that light intensity PA might not provide a sufficient stimulus to reduce resting values of certain inflammatory markers. Following this line of reasoning, the current investigation examined only PA intensity levels of at least moderate intensity when determining the possible association of PA with the inflammatory biomarkers Lp-PLA_2 and CRP.

3.0 METHODS

The primary purpose of this investigation was to examine the association of moderate-to-vigorous PA (MVPA) with serum levels of Lp-PLA_2, an inflammatory marker that has been linked with increased CHD risk, in a sample of middle-aged women. A secondary purpose of this investigation was to examine the association of MVPA with plasma levels of another inflammatory marker of CHD risk, CRP. Finally, this investigation examined the correlation between Lp-PLA_2 levels and CRP levels.

This investigation was primarily a secondary analysis of data that have previously been collected as part of the Epidemiologic Study of Health Risk in Women (ESTHER).

3.1 PRIMARY INVESTIGATION-ESTHER

3.1.1 Participants

The participants in the current investigation were drawn from the recently completed (2007) ESTHER study at the University of Pittsburgh. The primary purpose of ESTHER was to identify possible differences in CHD risk factors between lesbian women and heterosexual women, to determine if the pattern of CHD risk factors differed between lesbian women and heterosexual women, and to determine if lesbian women are at increased risk of CHD compared to heterosexual women. Similar recruitment strategies were employed for both the heterosexual and lesbian women in the ESTHER study. Study advertisements were placed in bookstores and local newspapers. Flyers were distributed at women focused events. Additional efforts were employed to recruit racial minority women thru "snowball" recruitment. These efforts included:

news and radio advertisements, health events, lesbian, gay, bisexual and transgender (LGBT) events and socials, and the University of Pittsburgh broadcast phone-message system.

Written informed consent was obtained from all participants in the ESTHER study and all procedures were approved by the University of Pittsburgh Institutional Review Board (IRB).

3.1.2 Inclusion/Exclusion Criteria

Eligibility for the ESTHER study required individuals to be women of at least 35 years of age with no self-reported history of CHD. Women were excluded if they had ever been diagnosed with angina pectoris, had sustained a myocardial infarction, or had undergone surgical intervention for CHD.

3.1.3 ESTHER Clinic Procedures

The first clinic visit for the ESTHER study occurred at the Magee Women's Hospital Clinical Research Center (Craft Ave., Pittsburgh, PA). On arrival at the clinic, the participant was met by the study nurse or research assistant, who reviewed the research procedures and when necessary assisted the participant with signing the informed consent. The clinic visit was approximately 3 hours in length and involved the following: 1) review and signing of the informed consent, 2) resting heart rate (radial pulse) and blood pressure measurement, 3) fasting blood sample, 4) light snack, 5) anthropometric measurements**,** including bioelectrical impedance analysis (BIA), and (6) completion of questionnaires. The participants were also scheduled for their second clinic visit at this time. At the second clinic visit, the participant was

greeted by the study research assistant, the measurement procedures were reviewed and the participant's height and weight were measured and recorded. If necessary, the participant underwent a urine pregnancy test prior to having a dual energy x-ray absorptiometry (DXA) scan to assess percent body fat.

The blood sample collected at the first study visit was analyzed for biochemical markers known to be associated with CHD risk. Participants had been instructed to fast for 8 hours prior to venipuncture withdrawal of 40 ml of blood for measurement of serum total cholesterol, HDL-C, LDL-C, triglycerides, and CRP. Participants were also asked for permission to store their blood samples for future analyses of new biochemical factors and hormones that might be related to increased CHD risk. Only blood samples from participants who consented to this request were used in the present secondary analysis of the data. All of the blood assays for the variables listed above were conducted at the Heinz Nutrition Laboratory (University of Pittsburgh, Department of Epidemiology, Graduate School of Public Health), which is certified by the Centers for Disease Control and Prevention (CDC) for lipid analyses and is also certified under the Clinical Laboratory Improvement Amendments (CLIA).

3.1.4 ESTHER Physical Activity Measures

Physical activity was assessed using the Modifiable Activity Questionnaire (MAQ). This questionnaire has been used to determine physical activity levels in a variety of groups in the United States and in other countries (56, 57). The MAQ includes sections to assess leisure time PA, occupational PA, and activities of daily living. The MAQ has been shown to be both reliable and valid through comparisons with activity monitors, fitness (field) testing, and the doubly-

labeled water technique in adults and adolescents (46). The leisure activity component of the questionnaire requests that the individual identify all appropriate leisure-time physical activities in which she participated from a comprehensive list. Estimates of frequency and duration are then obtained for each identified activity (46).

3.1.5 ESTHER Physiological Measures

All clinical research technicians who participated in collection of physiological measures underwent training sessions and were re-certified every 6 months. The training sessions focused on appropriate procedures to determine blood pressure, heart rate, and anthropometric measurements. Resting blood pressure (mm Hg) was measured using the standard MRFIT protocol, utilizing a standard sphygmomanometer. Two measurements were recorded after the participant had been seated and resting quietly for five minutes. If the difference between the first two measures was greater than 5 mm Hg for either systolic blood pressure (SBP) or diastolic blood pressure (DBP, a third measure was taken and recorded. The average of all recorded measures was calculated and used in the analysis. Resting heart rate (HR; beats/min) was measured via the radial pulse after a five minute rest period. As with the BP measurement, two HR measurements were taken and recorded. The average of the first two measurements was used unless a difference of ≥5 beats/min was recorded, in which case a third measurement was obtained. A saliva sample was collected and analyzed for cotinine, an objective measure of exposure to cigarette smoke**.** Standardized anthropometric measurements were performed, including height, weight, anatomical circumference, and sagittal diameter. Two measures were recorded for each variable and if a specified difference between the two measures was observed,

a third measure was conducted. The average of the measures was used in the analysis. Percent body fat was estimated using leg-to-leg bioelectrical impedance analysis (BIA, Tanita Corp., Arlington Heights, IL). Participants who had implanted devices such as cardiac defibrillators did not participate in the BIA measure. Scans by DXA of the whole body, hip, and lumbar spine (L1-L4) were used to assess total body, hip and spine bone mineral density (BMD), respectively. Body composition (total body and regional lean body mass and fat mass) was also assessed by DXA. The DXA scans were performed at the Health Studies Office (130 N. Bellefield Ave.) using a Hologic QDR4500A DXA system (Hologic Medical, Bedford, MA). Urinalysis was conducted prior to DXA scanning to exclude pregnancy in all women reporting menses in the past 12 months. Women known to be pregnant or lactating at the time of the clinic visit or who tested positive during the urine pregnancy test were excluded from the DXA scanning.

3.2 CURRENT INVESTIGATION

3.2.1 Inclusion Criteria

Potential participants for the current study were drawn from those women in the ESTHER study who consented to have their blood stored for future analyses and identified themselves as heterosexual. As the current investigation was a secondary analysis of the existing ESTHER data set, it was considered important to identify the subject groups *a priori* and present them as part of the overview proposal.

Participants were placed into one of two groups, ACTIVE or INACTIVE, based on reported average weekly moderate-to-vigorous PA (MVPA) as measured by the MAQ. Since the MAQ includes activities of varying intensities, it was necessary to exclude all activities that were not of at least a moderate intensity. Using the Compendium of Physical Activity, all activities listed in the MAQ with an energy expenditure of less than 3 MET's were excluded when calculating each participants average weekly MVPA (2). The 3 MET value was chosen because this value represents the lower cut-point for moderate intensity PA (53).

A total of 377 women from the parent ESTHER study gave permission to have their blood stored for future investigations and also identified themselves as heterosexual. Available funding allowed for blood samples from 80 women to be analyzed. These 80 women were selected from the group of 377 women that comprised the eligible ESTHER sample. The 80 women selected to participate were placed in either the ACTIVE or the INACTIVE group based on reported weekly MVPA. In the interest of finding differences between groups based on differences in MVPA, it was decided to select women from the group of 377 who were at opposite ends of the PA spectrum. Thus, the ACTIVE group was comprised of the women who reported comparatively high levels of MVPA (range: 8.93 hrs/week to 17.65 hrs/week) and the INACTIVE group was comprised of those women who reported no MVPA (0 hrs/week).

Of the 377 eligible women, a total of 41 reported participating in zero hours per week of MVPA. One participant from this group was excluded at random, leaving a total of 40 women. These 40 women were categorized as the INACTIVE group. The ACTIVE group was comprised of the 40 women who reported the greatest number of hours of average weekly MVPA. Two women reported average weekly MVPA of 25.8 and 27.1 hours, respectively. Given these unreasonably high values and the fact that these two values were over two standard

deviations from the mean of the remaining 40 participants with the highest MVPA hours, it was decided to exclude these two potential participants. After these two exclusions, the remaining 40 participants with the greatest number of hours of average weekly MVPA were categorized as the ACTIVE group.

3.2.2 Exclusion Criteria

Women in the ESTHER study who did not consent to have their blood stored for future analyses or who identified themselves as being lesbian were not included in the current investigation. Previous research has suggested that lesbian women may be more likely to participate in vigorous intensity physical activity than their heterosexual counterpart (1). In the current investigation this could have led to lesbian women being over-represented in the ACTIVE group and under-represented in the INACTIVE group, thus creating a potential selection bias between the ACTIVE and INACTIVE groups. Given this possibility and the relatively small sizes of the PA groups, it was decided *a priori* to exclude women from the current investigation who identified themselves as lesbian.

3.2.3 Assessment Procedures: Dependent Variables

The dependent variables for the present investigation are Lp-PLA_2 and CRP. The blood samples from the ESTHER investigation are currently stored at -70^{o}C in a locked freezer at the University of Pittsburgh, School of Dental Medicine. The frozen serum vials of the 40 women assigned to the ACTIVE group and the 40 serum vials of the women assigned to the INACTIVE

group were identified and removed from storage. The vials were then packed in dry ice and shipped to the diaDexus company (San Francisco, CA) for analysis of Lp-PLA_2 mass and Lp-PLA_2 activity. The 80 vials were de-identified prior to shipment. Enzyme activity of Lp-PLA_2 was measured and the results reported in units of nmol/min/Ml. This measure indicates how active the Lp-PLA_2 enzyme is in the blood. Enzyme mass of Lp-PLA_2 was also measured and the results were reported in units of ng/ml. This measure indicates the amount of Lp-PLA_2 present in a specific volume of blood. Given the exploratory nature of this investigation in regards to assessing the possible association between PA and Lp-PLA_2, both Lp-PLA_2 mass and Lp-PLA_2 activity data were collected for analysis.

Upon arrival at the diaDexus company, the samples were thawed and approximately 1 ml of serum was withdrawn for analysis. The samples were tested by both the mass assay and the activity assay techniques, using a SpectraMax plate reader and a SpectraMax plate washer from the Molecular Devices Corporation (Sunnyvale, CA). Calibrators and controls were run in duplicate, and 30% of the samples were run in double point analysis. Coefficient of variation (CV) for both Lp-PLA_2 mass and activity were calculated on duplicate calibrators. Acceptable CV for controls and samples was less than or equal to 15%.

Following withdrawal of the 1 ml of serum for analysis, the remaining serum was repacked in dry ice and shipped back to Pittsburgh. The remainder of each sample was returned to frozen (-70°C) storage at the University of Pittsburgh, School of Dental Medicine.

The other inflammatory biomarker of interest, CRP, was measured as part of the parent ESTHER study. All CRP analysis was done at the Heinz Laboratory at the University of Pittsburgh. Concentration of CRP was expressed in units of mg/L, indicating the concentration of CRP in a specific volume of blood. Measurement of CRP was done using reagents obtained from Olympus

Diagnostic Division and analyzed on an AU400 from Olympus America, Inc. (Melville, NY). Blanks, controls and standards (0.5 to 20 mg/L) were analyzed simultaneously with all samples. The intra-assay CV was 5.5% and the inter-assay CV was 3.0%. The functional sensitivity of this assay was found to be 0.05 mg/L.

3.3 DATA ANALYSIS

Data analysis was conducted using the SPSS 18.0 for the Windows Statistical Package (SPSS, Inc., Chicago, IL). Initial analyses included descriptive statistics for both the ACTIVE and INACTIVE groups and tests of normality to assess distribution of the data. The main variables that were compared between the ACTIVE and INACTIVE groups were Lp-PLA_2 mass (ng/mL), Lp-PLA_2 activity (mmol/min/ml) and CRP concentration (mg/L).

Lp-PLA_2 mass and Lp-PLA_2 activity were found to be normally distributed and parametric testing was used for the statistical analyses. Independent samples t-tests were used to compare mean values of Lp-PLA_2 mass and Lp-PLA_2 activity between the ACTIVE and INACTIVE groups. Pearson Product Moment tests were used to calculate correlation coefficients between selected variables that have previously been shown to be associated with CVD risk, Lp-PLA_2 mass, and Lp-PLA_2 activity. The CRP data were not normally distributed and were analyzed with non-parametric tests. A Mann-Whitney *U* test was used to compare median values of CRP concentration between the ACTIVE and INACTIVE groups. Spearman's Rank Order Correlation tests were used to calculate correlation coefficients between CRP and

variables that have previously been shown to be associated with CVD risk. Statistical significance for all analyses was set *a priori* at $p \leq 0.05$.

In addition to the univariate analysis, stepwise multivariate regression was used to further examine the possible association of Lp-PLA_2 mass, Lp-PLA_2 activity, and CRP with MVPA, while accounting for the influence of other factors that may be associated with vascular inflammation and PA. Independent variables that were included in the regression models were chosen based on two criteria. First, descriptive variables associated with CVD that differed ($p \leq 0.10$) between the ACTIVE and INACTIVE groups (Tables 1 and 2) were included in the individual regression models. Second, descriptive variables from the current investigation that were correlated ($p \leq 0.10$) with Lp-PLA_2 mass, Lp-PLA_2 activity (Table 4) or CRP values (Table 5) for the entire sample were also included in the respective models.

A less stringent alpha level criterion ($p \leq 0.10$) was used to determine which independent variables to include in the models. This was considered appropriate given that the significance for evaluating the final regression models was kept at $p \leq 0.05$, and also given the exploratory nature of the regression procedure for the current investigation.

3.4 POWER ANALYSIS

Power analysis was conducted to determine whether a sample size of 40 participants per group was sufficient to detect significant differences between the ACTIVE and INACTIVE groups for measures of Lp-PLA_2 mass, Lp-PLA_2 activity, and CRP concentration. Conventional definitions of small, medium, and large effects, as proposed by Cohen were used in the power calculation. Using a two-tailed test, an α level of 0.05, and a medium effect size ($d=0.5$), it was determined

that 40 participants per group resulted in a power of 59.8% to detect differences in Lp-PLA_2 mass and activity based on PA group. Using a sample of 35 participants per group resulted in a power of 57.5% to detect differences in Lp-PLA_2 mass and activity based on PA group.

4.0 RESULTS

The primary purpose of this investigation was to examine the association of MVPA with serum levels of a marker that has been linked with increased CHD risk, Lp-PLA_2, in a sample of 80 middle-aged women. A secondary purpose of this investigation was to examine the association of MVPA with plasma levels of another inflammatory marker related to CHD risk, CRP. Finally, this investigation assessed the correlation between serum Lp-PLA_2 and plasma CRP. It was hypothesized that Lp-PLA_2 mass and activity would be significantly lower in the middle-aged women who reported greater amounts of MVPA as compared to those who reported lesser amounts of MVPA. It was also hypothesized that CRP concentration would be significantly lower in the middle-aged women who reported greater amounts of MVPA as compared to those who reported lesser amounts of MVPA. Finally, it was hypothesized that Lp-PLA_2 mass and activity would not be significantly correlated with CRP levels. The present investigation involved a secondary analysis of data that were previously collected as part of ESTHER.

4.1 PARTICIPANT CHARACTERISTICS

Eighty participants from the ESTHER study were chosen for the current investigation. From this initial pool, 40 participants comprised the ACTIVE group and 40 participants comprised the INACTIVE group. Laboratory analysis determined that it was not possible to obtain Lp-PLA_2 mass and activity values for 5 individuals, leaving 75 participants for the final analysis. All of the participants with missing Lp-PLA_2 mass and activity values were from the INACTIVE group. Statistical comparisons of descriptive characteristics were made between the five individuals

from the INACTIVE group with missing Lp-PLA_2 mass and activity values and the remaining 35 individuals from the INACTIVE group. The analyses indicated that the descriptive characteristics of the five individuals with missing data did not differ from the remaining 35 individuals (see appendix). Therefore, for the purpose of statistical analyses, the INACTIVE group was comprised of the 35 participants with complete Lp-PLA_2 data and the ACTIVE group was comprised of 40 participants. Table 1 and Table 2 list the descriptive characteristics of the two activity groups and indicate the p-value for differences between the groups for each variable.

Table 1. Descriptive characteristics of participants (continuous variables).

	ACTIVE Group (n=40)	INACTIVE Group (n=35)	p-value for difference
Age (yr)	50.95 ± 10.25	49.35 ± 10.39	0.505
Height (cm)	63.91 ± 2.79	63.64 ± 2.89	0.677
Body mass (kg)	71.59 ± 15.91	83.74 ± 20.20	0.005*
BMI (kg/m^2)	27.17 ± 6.11	31.59 ± 7.17	0.005*
Body fat (%)	33.20 ± 9.46	39.85 ± 7.87	0.002*
Insulin (pmol/L)	12.67 ± 7.73	16.20 ± 12.46	0.139
Glucose (mmol/L)	94.70 ± 9.18	105.74 ± 41.59	0.132
Total Cholesterol (mmol/L)	203.68 ± 34.3	200.23 ± 47.4	0.617
Triglycerides (mmol/L)	102.88 ± 73.2	109.94 ± 42.05	0.717
HDL-C (mmol/L)	63.73 ± 18.17	51.01 ± 12.04	0.001*
LDL-C (mmol/L)	118.97 ± 28.12	128.11 ± 43.79	0.284

*Values are in means ± SD. * Significant difference between groups as assessed via independent samples t-test.*

Table 2. Descriptive characteristics of participants (categorical variables).

		ACTIVE Group (n=40)	INACTIVE Group (n=35)	p-value for difference
High Blood pressure[1]	Yes	15% (6)	22.9% (8)	0.384
	No	85% (34)	77.1% (27)	
High cholesterol[1]	Yes	17.5% (7)	31.4% (11)	0.159
	No	82.5% (33)	68.6% (24)	
High triglycerides[1]	Yes	10.3% (5)	5.7% (2)	0.475
	No	89.7% (35)	94.3% (33)	
Diabetes[1]	Yes	7.5% (3)	5.7% (2)	0.757
	No	92.5% (37)	94.3% (33)	
Obesity[1]	Yes	12.5% (5)	22.9% (8)	0.237
	No	87.5% (35)	77.1% (27)	
Depression[1]	Yes	20% (8)	34.3% (12)	0.163
	No	80% (32)	65.7% (23)	
Oral contraceptives	Yes	65% (26)	71.4% (25)	0.552
	No	35% (14)	28.6% (10)	
Other female hormones	Yes	37.5% (15)	17.1% (6)	0.050*
	No	62.5% (25)	82.9% (29)	
Hysterectomy	Yes	17.5% (7)	34.3% (12)	0.095
	No	82.5% (33)	65.7% (23)	
Smoke cigarettes	Yes	5% (2)	22.9% (8)	0.041*
	No	95% (38)	77.1% (27)	
Consumed Alcohol in past year	Yes	82.1% (32)	65.6% (21)	0.113
	No	17.9% (7)	34.4% (11)	

*Values are percent with number of participants within each response category in parentheses. 1 = "Ever diagnosed with", * Significant difference between groups assessed via chi-square analysis.*

Table 3. Comparison of INACTIVE group with the sub-set of the five excluded participants

	Excluded Group (n=5)	INACTIVE Group (n=35)	p-value for difference
Age (yr)	50.15 ± 10.25	49.35 ± 10.39	0.715
CRP (mg/L)	2.20 (1.01-3.80)*	2.40 (0.80-4.81)*	0.178
Body mass (kg)	81..89 ± 16.81	83.74 ± 20.20	0.355
BMI (kg/m^2)	32.25 ± 6.99	31.59 ± 7.17	0.511
Body fat (%)	37.20 ± 5.49	39.85 ± 7.87	0.290
Total Cholesterol (mmol/L)	203.87 ± 39.3	200.23 ± 47.4	0.197
HDL-C (mmol/L)	53.73 ± 13.17	51.01 ± 12.04	0.376
LDL-C (mmol/L)	126.77 ± 29.43	128.11 ± 43.79	0.484

*Values are in means ± SD except * [median (IQ range)]*

Differences between activity groups were assessed via t-tests for all continuous variables (Table 1) and chi-square tests for all categorical variables (Table 2). It was found that the ACTIVE group participants had significantly lower body mass, BMI, body fat % and higher HDL-C values than the INACTIVE group participants. Chi-square analysis indicated that a greater percentage of the ACTIVE group participants reported that they had used female hormones (other than oral contraceptives) than the INACTIVE group participants. In addition, it was shown that a lower percentage of the ACTIVE group participants reported smoking cigarettes as compared to the INACTIVE group participants.

Table 3 presents a comparison of the sub-set of the five participants from the INACTIVE group who did not have usable Lp-PLA_2 values with the actual INACTIVE group used in the analysis. No significant differences were found between the five participants with missing Lp-PLA_2 values and the remaining 35 participants from the INACTIVE group who did have usable Lp-PLA_2 values.

Table 4 presents the mean Lp-PLA_2 mass and activity values and the median CRP values for the entire sample. Correlations for variables eventually included in the regression models are presented in Table 4 for Lp-PLA_2 mass and Lp-PLA_2 activity and Table 5 for CRP.

Table 4. Mean Lp-PLA_2 mass and Lp-PLA_2 activity and median CRP values for entire sample (n=75)

Lp-PLA_2 mass (ng/ml)	Lp-PLA_2 activity (nmol/min/ml)	CRP (mg/L)
222.29 ± 49.03	134.77 ± 28.23	1.40 (0.60-3.7)

Values are in means ± SD (Lp-PLA_2 mass and Lp-PLA_2 activity) and median scores (CRP; interquartile range)

Table 5. Independent variables for the regression models and their correlations with Lp-PLA_2 mass and Lp-PLA_2 activity using the total sample (N=75).

	Lp-PLA_2 mass (ng/ml) *Correlation*	Lp-PLA_2 activity (nmol/min/ml) *Correlation*
Lp-PLA_2 mass (ng/ml)	1.0 ---	0.589* <0.001
Lp-PLA_2 activity (nmol/min/ml)	0.589* <0.001	1.0 ---
Triglycerides (mmol/L)	0.030 0.890	0.232* 0.045
Total Cholesterol (mmol/L)	0.246* 0.033	0.367* 0.001
HDL-C (mmol/L)	0.012 0.920	-0.356* 0.002
LDL-C (mmol/L)	0.280* 0.016	0.533* <0.001

**Significant correlation (2-tailed) between variables assessed via Pearson Product Moment Correlation.*

Table 6. Independent variables for the regression model and their correlations with CRP using the total sample (N=75).

	CRP (mg/L) *Correlation*
BMI (kg/m^2)	0.598* < 0.001
Percent Body Fat	0.649* < 0.001
Body mass (kg)	0.585* < 0.001
HDL-C (mmol/L)	-0.415* < 0.001
Triglycerides (mmol/L)	0.425* < 0.001
Insulin (pmol/L)	0.536* < 0.001

**Significant correlation (2-tailed) between variables assessed via Spearman Rank Order Correlation.*

4.2 LP-PLA_2 AND MVPA: BETWEEN GROUP COMPARISONS

4.2.1 Comparison of Lp-PLA_2 between MVPA Groups

Results of independent samples t-tests showed no significant differences in either Lp-PLA_2 mass (p=0.44) or Lp-PLA_2 activity (p=0.63) between the ACTIVE and INACTIVE groups (Table 7).

Table 7. Comparison of Lp-PLA_2 mass and Lp-PLA_2 activity between MVPA groups

	ACTIVE Group (n=40)	INACTIVE Group (n=35)	t-value	p-value for between group differences
Lp-PLA_2 mass (ng/ml)	226.41 ± 48.07	217.57 ± 50.38	-0.777	0.440
Lp-PLA_2 activity (nmol/min/ml)	133.27 ± 26.43	136.48 ± 30.45	0.488	0.627

Values are in means ± SD.

4.2.2 Lp-PLA_2 and MVPA Regression Models: Exploratory Analyses

Stepwise multiple regression analysis was used to further examine the possible association between Lp-PLA_2 and MVPA. Models were developed to separately predict Lp-PLA_2 mass and Lp-PLA_2 activity while accounting for the influence of other factors that may be associated with inflammation and PA. The entire sample was used in the development of the models. In the regression models, Lp-PLA_2 mass and Lp-PLA_2 activity served as the dependent variables. The independent variables that were included in the models were selected based on the criteria presented in section 3.3 and are listed in Tables 1, 2, and 4. Based on these selection criteria, the primary pool of independent variables for the regression model for Lp-PLA_2 mass included, body mass, BMI, percent body fat, use of female hormones, current smoking status, total cholesterol, LDL-C, and MVPA group (ACTIVE or INACTIVE). Independent variables included in the regression model for Lp-PLA_2 activity were, body mass, BMI, percent body fat, use of female hormones, current smoking status, triglycerides, total cholesterol, HDL-C, LDL-C, and MVPA group (ACTIVE or INACTIVE).

4.2.3 Lp-PLA$_2$ mass and MVPA Regression Model

Only LDL-C was included in the final stepwise regression model to predict Lp-PLA$_2$ mass (Table 8). The variance explained by the remaining independent variables was not significant. The final model found that LDL-C was a significant ($F_{1, 71}$=6.041, p=0.016, R^2=0.078), predictor of Lp-PLA$_2$ mass. This model explained 7.8% of the variance in Lp-PLA$_2$ mass: Lp-PLA$_2$ mass (ng/ml) = 175.799 + LDL-C (mmol/L) (0.377).

Table 8. Stepwise regression model to predict Lp-PLA$_2$ mass

	Independent Variable	Partial Correlation	t-value	p-value	r	R^2
Model 1	LDL-C (mmol/L)	0.280	2.458	0.016	0.280	0.078
Excluded Variables	BMI (kg/m^2)	-0.069	-0.580	0.564		
	Percent body fat	-0.057	-0.477	0.635		
	Body mass (kg)	-0.052	-0.438	0.663		
	Other female hormone use	-0.111	-0.938	0.351		
	Cigarette smoking	0.044	0.367	0.714		
	MVPA group	0.132	1.116	0.268		
	Total cholesterol (mmol/L)	-0.032	-0.272	0.787		

Predictors in the Model: (Constant), LDL-C

4.2.4 Lp-PLA$_2$ activity and MVPA Regression Model

Both LDL-C and HDL-C were included in the final stepwise regression model to predict Lp-PLA$_2$ activity (Table 9). The remaining independent variables did not significantly explain the

variance in Lp-PLA_2 activity. The initial model found that LDL-C was a significant ($F_{1, 71}$=21.596, p<0.05, R^2=0.23) predictor of Lp-PLA_2 activity, explaining 23% of the variance. The final model found that LDL-C and HDL-C together were significant ($F_{2, 70}$=15.678, p<0.05, R^2=0.309) predictors of Lp-PLA_2 activity. This model explained 31% of the variance in Lp-PLA_2 activity:

Lp-PLA_2 activity (nmol/min/ml) = 88.611 + LDL-C (mmol/L) (0.336) + HDL-C (mmol/L) - (0.472).

Table 9. Stepwise regression models to predict Lp-PLA_2 activity

	Variable	Correlation	t-value	p-value	r	R^2
Model 1	LDL-C (mmol/L)	0.483	4.647	<0.001	0.483	0.233
Model 2	LDL-C (mmol/L)	0.457	4.301	<0.001		
	HDL-C (mmol/L)	-0.315	-2.778	0.007	0.556	0.309
Excluded Variables (model 1)	BMI (kg/m^2)	0.046	0.385	0.701		
	Percent body fat	0.011	0.094	0.925		
	Body mass (kg)	0.037	0.310	0.757		
	Other female hormone use	-0.263	-2.283	0.025		
	Cigarette smoking	0.127	1.067	0.289		
	Triglycerides (mmol/L)	0.072	0.607	0.546		
	HDL-C (mmol/L)	-0.315	-2.778	0.007		
	MVPA Group	0.004	0.037	0.258		
	Total Cholesterol (mmol/L)	-0.231	-1.988	0.920		
Excluded Variables (model 2)	BMI (kg/m^2)	-0.140	-1.174	0.244		
	Percent body fat	-0.156	-1.316	0.193		
	Body mass (kg)	-0.147	-1.232	0.222		
	Other female hormone use	-0.166	-1.394	0.168		
	Cigarette smoking	0.096	0.804	0.424		
	Triglycerides (mmol/L)	-0.039	-0.320	0.750		
	MVPA Group	0.136	1.142	0.258		
	Total Cholesterol (mmol/L)	-0.012	-0.101	0.920		

Predictors in Model 1: (Constant), LDL-C

Predictors in Model 2: (Constant), LDL-C, HDL-C

4.3 CRP AND MVPA: BETWEEN GROUP COMPARISONS

4.3.1 Comparison of CRP between MVPA Groups

Preliminary data analysis found that the CRP values were not normally distributed. As such, non-parametric statistical tests were used for the between group CRP comparisons. Pair-wise comparisons via a Mann-Whitney *U* test indicated that the median values of CRP concentration were significantly (p=0.016) lower in the ACTIVE group than in the INACTIVE group. Table 9 presents the median values of CRP by MVPA group.

Table 10. Comparison of CRP between MVPA groups

	ACTIVE Group (n=40)	INACTIVE Group (n=35)	p-value for difference between groups
Median CRP (mg/L)	1.05 (0.50-2.85)	2.40 (0.80-4.80)	0.016*

Values are median scores (*interquartile range). *Significant difference between groups assessed by Mann-Whitney U test.*

4.3.2 CRP and MVPA Regression Models: Exploratory Analyses

Stepwise multiple regression analysis was used to further examine the possible association between CRP and MVPA. The model was developed to predict CRP concentration while accounting for the influence of other factors that may be associated with inflammation and PA. In the regression analysis, CRP served as the dependent variable. The independent variables that were included in the regression analysis were selected based on the criteria presented in section

3.3 and are listed in Tables 1, 2, and 5. Based on the *a priori* selection criteria, the primary pool of independent variables for the regression model included BMI, percent body fat, body mass, HDL-C, triglycerides, insulin, use of female hormones, current smoking status, and MVPA group (ACTIVE or INACTIVE).

Only body mass was included in the stepwise regression model to predict CRP (Table 10). All other independent variables were excluded from the model. The model found that body mass significantly ($F_{1, 72}$=4705, $p < 0.05$, R^2=0.40) predicted CRP, explaining 40% of the variance in CRP:

$$\text{CRP (mg/L)} = -6.45 + \text{body mass (kg)}\ (0.12).$$

Table 11. Stepwise regression model to predict CRP

	Independent Variable	Partial Correlation	t-value	p-value	r	R^2
Model 1	Body Mass (kg)	0.629	6.859	< 0.001	0.629	0.395
Excluded Variables	BMI (kg/m^2)	-0.015	-0.129	0.897		
	MVPA group	-0.045	-0.379	0.706		
	Percent body fat	0.050	0.419	0.676		
	Insulin (pmol/L)	0.139	1.181	0.242		
	Triglycerides (mmol/L)	0.039	0.329	0.743		
	HDL-C (mmol/L)	-0.031	-0.264	0.793		
	Cigarette smoking	-0.035	-0.297	0.768		

Predictors in the Model: (Constant), Body Mass

4.4 LP-PLA2 AND CRP ASSOCIATION

Results of the Pearson Product Moment correlation showed a significant ($r= 0.59$, $p < 0.05$) association between Lp-PLA_2 mass and Lp-PLA_2 activity. Spearman Rank Order correlation tests found that neither Lp-PLA_2 mass ($r= -0.03$, $p=0.78$) nor Lp-PLA_2 activity ($r= 0.12$, $p=0.30$) were significantly associated with CRP.

4.5 SUMMARY OF RESULTS

This investigation examined whether Lp-PLA_2 and CRP levels were associated with MVPA. The median number of weekly hours reported over the past 12 months by the ACTIVE group was found to be 10.9 hours. The INACTIVE group reported no MVPA over the past 12 months. Univariate results found that mean Lp-PLA_2 mass was not significantly different ($p = 0.440$) between the ACTIVE (226.41 ± 48.07 ng/mL) and the INACTIVE (217.57 ± 50.38 ng/mL) groups. In addition, mean Lp-PLA_2 activity did not differ ($p = 0.63$) between the ACTIVE (133.27 ± 26.43 mmol/min/ml) and INACTIVE (136.48 ± 30.45 mmol/min/ml) groups. Stepwise regression analysis found that the only independent variable that significantly contributed to explained variance in Lp-PLA_2 mass was LDL-C, while the final prediction model for Lp-PLA_2 activity included both LDL-C and HDL-C as independent variables.

An additional purpose of this investigation was to determine if CRP concentration was associated with MVPA. Univariate analysis found that the median CRP value was lower ($p = 0.02$) in the ACTIVE group (1.05 mg/L) than the INACTIVE (2.40 mg/L) group. Stepwise

regression analysis found that body mass was the only independent variable that significantly contributed to explained variance in the CRP model.

Finally, this investigation sought to determine whether Lp-PLA_2 mass and Lp-PLA_2 activity were correlated with CRP concentration. Results indicated that neither Lp-PLA_2 mass ($r= -0.03$, $p=0.78$) nor Lp-PLA_2 activity ($r= 0.12$, $p=0.30$) were significantly correlated with CRP.

5.0 DISCUSSION

The primary purpose of this investigation was to determine if Lp-PLA_2 was associated with leisure time MVPA. A secondary purpose of the investigation was to determine if CRP was associated with leisure time MVPA. Finally, this investigation examined the association between Lp-PLA_2 and CRP.

It was hypothesized that both Lp-PLA_2 and CRP would be inversely associated with MVPA and that Lp-PLA_2 and CRP would not be significantly associated with each other.

5.1 PRIMARY STUDY: ESTHER

The participants in the current investigation were drawn from the ESTHER study at the University of Pittsburgh. The primary purposes of ESTHER were to, (a) identify possible differences in CHD risk factors between lesbian and heterosexual women, (b) to determine if the pattern of CHD risk factors differed between lesbian women and heterosexual women, and (c) to determine if lesbian women were at increased risk of CHD compared to heterosexual women.

5.2 LP-PLA$_2$ AND MVPA

The current investigation found that Lp-PLA$_2$ mass and Lp-PLA$_2$ activity did not differ between the ACTIVE and INACTIVE groups, when examined in a sample of women 50.2 ± 10.3 years of age. Stepwise, multiple regression was used to further examine other possible associations between Lp-PLA$_2$ and MVPA. Using Lp-PLA$_2$ mass as the dependent variable, it was found that only LDL-C was included in the final stepwise regression model, explaining 8% of the variance. Using Lp-PLA$_2$ activity as the dependent variable, it was found that both LDL-C and HDL-C were included in the final stepwise regression model, explaining 31% of the variance.

It was expected that LDL-C and HDL-C would both be significant predictors in the regression models. This expectation was based on previous findings that that LDL-C and HDL-C are the main transporters of Lp-PLA$_2$ in blood (51). It has been shown that, in humans, approximately 80% of Lp-PLA$_2$ is transported by LDL-C, with the remaining 20% of Lp-PLA$_2$ transported by HDL-C (51). Given the importance of LDL-C and HDL-C in the physiological transport of Lp-PLA$_2$, the appearance of both lipid measures as significant predictors of Lp-PLA$_2$ level was not surprising.

The present findings are similar to those reported by Detopoulou, et al., who found no significant independent association between Lp-PLA$_2$ activity and combined occupational and leisure time PA assessed by a recall questionnaire in a sample of males and females (10). However, the authors did report that a regression model containing LDL-C, participant age, smoking status, and PA level as predictors explained approximately 30% of the variance in Lp-PLA$_2$ activity (10). The statistical power of the model held for the male but not female

participants. The only independent variable that appeared in both the Detopoulou, et al. and present regression models to predict Lp-PLA_2 was LDL-C.

Detopoulou, et al. also reported that % body fat was significantly and positively associated with Lp-PLA_2 activity in males (10). The current investigation involving females did not find a significant association between Lp-PLA_2 activity and % body fat. However, a non-significant trend ($p=0.07$) was observed between Lp-PLA2 activity and body fat > 30%. This is consistent with the findings in males reported by Detopoulou, et al (10). Detopoulou, et al hypothesized that acute phase inflammatory markers, such as IL-6 and TNF-α, may play a role in the increase in Lp-PLA_2 activity that is associated with higher amounts of body fat (10). As IL-6 and TNF-α were not measured as part of ESTHER, we were unable to explore this particular hypothesis in the current investigation but these inflammatory markers should be considered in future studies.

Rana et al. found no association between Lp-PLA_2 activity and combined occupational and leisure time PA levels in a prospective case-control study (61). In evaluating the results of the Rana et al. study, it should be noted that the authors assessed PA levels based on two self-reported survey questions (61). The first of these questions asked subjects to report the number of weekly hours of occupational PA that they performed during the previous 12 months. The second question asked the subjects to report the number of weekly hours of leisure time PA in which they participated during the previous 12 months (61). The survey instrument employed by Rana, et al. provided participants with a limited number of activities to choose from (61). The current investigation, by virtue of its use of a validated, standardized questionnaire that assessed a wide range of PA, was methodologically more sensitive than the procedures used by Rana, et al. (61).

Rana et al., using a female sample, reported a non-significant trend ($p=0.07$) for reductions in Lp-PLA_2 activity as PA increased across *a priori* determined groups, i.e., "inactive", "moderately inactive", "moderately active", and "active" (61). In addition, Rana et al. reported that for the "moderately active" and "active" groups there was a significant increase in Lp-PLA_2 activity as waist size increased (61). Waist size is generally considered a corollary of body fat. Thus, this finding is consistent with Detopoulou, et al., who identified % body fat as a significant predictor of Lp-PLA_2 activity (10). The current findings that identified a non-significant trend for body fat levels > 30% to be a predictor of Lp-PLA_2 also fit into this general response milieu.

Results from a prospective cohort study by Persson, et al. indicated that neither Lp-PLA_2 mass nor Lp-PLA_2 activity were associated with leisure time PA in a combined sample of males and females (54). Persson, et al. assessed PA using a self-report questionnaire that recorded the number of minutes per week of participation in 18 different physical activities (54). A graded (i.e., low to high) intensity coefficient was assigned to each of the activities.

Persson, et al. found LDL-C and HDL-C to be the strongest predictors of Lp-PLA_2 activity, with LDL-C explaining 22% of the variance in the regression model (54). In the same model, the combined effects of LDL-C, Sex, and HDL-C explained 32% of the variance in Lp-PLA_2 activity (54). This is similar to the findings of the current investigation, where a regression model containing LDL-C and HDL-C as independent variables explained 31% of the variance in Lp-PLA_2 activity. Persson, et al. also found that LDL-C was the strongest predictor of Lp-PLA_2 mass, explaining 12% of the variance in the regression model (54). This finding is generally consistent with those of the current investigation where LDL-C explained 8% of the variance in Lp-PLA_2 mass.

Daniels, et al., using a prospective cohort research design, found that Lp-PLA_2 mass was not significantly correlated with MVPA in a sample of males and females (9). It is important to mention that the authors used a simple, two-category instrument that assessed frequency of PA ($\geq$ 3 times/ week or < 3 times/week) with no mention of either the mode or the intensity of the PA (9). In addition, Daniels did not present PA levels by sex. As such, it was not possible to examine a possible association between Lp-PLA_2 mass and MVPA separately for females and males (9).

Daniels, et al. did find that Lp-PLA_2 mass was significantly correlated with, among other factors, LDL-C (r = 0.37) (9). This is consistent with the current findings of a significant correlation between Lp-PLA_2 mass and LDL-C (r = 0.28). The results did not differ by sex. Somewhat paradoxically, Daniels, et al. reported a significant negative correlation (r = -0.27) between Lp-PLA_2 mass and HDL-C (9). This was a somewhat unexpected finding, given that HDL-C is an important transporter of Lp-PLA_2. In the current investigation, no significant correlation (r = 0.12) was found between Lp-PLA_2 mass and HDL-C.

The lack of a significant association between either Lp-PLA_2 mass or Lp-PLA_2 activity and MVPA is not consistent with the first hypothesis of this investigation. There are a few possible explanations for this finding.

First, previous longitudinal studies have reported significant reductions in systemic inflammation (as measured by CRP) as a result of PA interventions (5, 26, 33). The length of the PA intervention in these studies varied but the minimum participation time necessary to elicit a significant reduction in CRP levels was two months (49). The current investigation employed a self-report questionnaire to determine participants average minutes per day and days per week of MVPA over the previous 12 months. It may be the case that the proximity of the MVPA

participation to the time when the questionnaire was actually completed was too small to elicit a significant reduction in cardiovascular-specific inflammation (as measured by Lp-PLA_2). In addition, the activities used to form the composite measure of MVPA in the current investigation may not have been specific enough to identify the higher intensity aerobic activities that are necessary to lower resting measures of vascular inflammation, i.e., Lp-PLA_2.

Second, the influence of PA on inflammatory markers, such as Lp-PLA_2 and CRP, may be mediated by changes in certain health-related behaviors that often accompany the actual PA intervention. It has been suggested that the primary behavioral changes linked to increased participation in PA may induce secondary health-related behavioral changes. As an example, reductions in tobacco use and certain dietary changes are associated with reductions in CRP values (15, 38). Given such interactive responsiveness, it may be possible for PA-mediated reduction in tissue inflammation to be achieved by influencing one or more of these health-related behaviors. In effect, these multiple behavioral pathways may help facilitate reductions in resting inflammatory markers. Lp-PLA_2 has not been as widely researched as CRP, particularly with respect to how it may be associated with PA and other health-related behaviors. Future investigations examining the effects of PA on changes in Lp-PLA_2 should also consider the effects of other health-related behaviors on Lp-PLA_2.

Given the results of the current investigation, it can be concluded that Lp-PLA_2 is not influenced by MVPA in this sample of middle-aged, healthy heterosexual women. This being the case, other inflammatory markers that have been found to be associated both with CHD and PA, such as CRP, IL-6, and TNF-α, should continue to be examined.

The negative findings of this investigation are important given the close association between Lp-PLA_2 and CVD, the leading cause of mortality in the United States. Within the

limitations of the research design, the absence of an association between Lp-PLA_2 and MVPA suggests that cardiovascular inflammation, as measured by Lp-PLA_2, may not be associated with MVPA in heterosexual women of the age and health status that was studied.

5.3 CRP AND MVPA

The current investigation found that the median CRP value was significantly higher for the INACTIVE group as compared to the ACTIVE group. This finding supported the second hypotheses of the investigation. Previous investigations that have reported lower levels of CRP with increased levels of PA have been unable to identify the mechanisms underlying this inverse association. It has been reported that IL-6 levels were reduced following an exercise training intervention (55). IL-6 is a stimulator of CRP production. As such, a reduction in IL-6 subsequent to exercise participation could be a possible mechanism for the lower CRP levels that are observed with higher PA levels.

To further examine the association between CRP and MVPA and identify other possible correlates of CRP, stepwise regression modeling was employed. Using CRP as the dependent variable, it was found that only body mass was included in the final stepwise model, accounting for 40% of the variance. It was expected that body mass would be a significant independent variable in the regression model to predict CRP. This expectation was based on previous findings that greater total body mass is associated with elevated CRP levels (15).

Heilbronn et al. reported a significant correlation between body mass and CRP in a sample of 83 obese women (22). Tchemof et al. also reported a significant association between body mass and CRP in a sample of 61 obese, postmenopausal women (71). Mechanistically,

adipose tissue is a significant producer of the cytokine IL-6, a potent stimulator of hepatic production of CRP. Thus, a greater body mass could be expected to be associated with increased levels of CRP.

In the current investigation, both BMI and percent body fat were lower in the ACTIVE than INACTIVE group. However, neither of these two variables was retained as a significant predictor in the final stepwise regression model. This can be explained partly by the fact that, in the current investigation, body mass, BMI, and percent body fat were all strongly correlated with each other (body mass vs. percent body fat: $r = 0.89$, body mass vs. BMI: $r = 0.94$, percent body fat vs. BMI: $r = 0.90$). Such high inter-correlation between the independent variables, or multicollinearity, can potentially cause errors when interpreting regression results. That is, a high correlation between the independent variables makes it difficult to determine how much each variable is uniquely contributing to the explained variance in the dependent variable. In the current investigation, this is less of a concern given that the three highly correlated independent variables (body mass, BMI, and percent body fat) are all positively associated with total body adiposity. Thus, finding a high degree of correlation between the three variables was not unexpected.

In addition, there are a variety of factors besides PA, one being diet, that may affect resting CRP levels. Studies have reported that diets high in fat and low in carbohydrates are associated with elevated resting CRP levels (15). As the data set in the current investigation did not include dietary information, it was not possible to determine if MVPA was associated with CRP levels independent of nutritional intake.

5.4 LP-PLA2 AND CRP ASSOCIATION

It has been suggested that LP-PLA_2 and CRP are biomarkers of different physiological inflammatory mechanisms. That is, CRP is an indicator of systemic inflammation whereas LP-PLA_2 primarily indicates atherosclerotic inflammation (4). Given these differences, it was hypothesized that the two inflammatory markers would not be significantly correlated in the current investigation.

The current findings supported this hypothesis, in that neither Lp-PLA_2 mass nor Lp-PLA_2 activity were significantly correlated with CRP. Therefore, these findings are consistent with the third research hypothesis of the current investigation.

Packard, et al. were the first to report that high levels of Lp-PLA_2 mass were positively correlated with CHD risk (51). In the same investigation, Packard, et al. also reported that Lp-PLA_2 mass was not significantly correlated with CRP levels (51). A lack of correlation between Lp-PLA_2 (mass and activity) and CRP has been observed in many investigations since the publication of the Packard, et al. study, including a recent study by Miller, et al (41).

An exception to the above findings, however, was reported by Koenig, et al. who found that Lp-PLA_2 was significantly correlated with CRP (32). The significant correlation between LP-PLA_2 and CRP reported by Koenig, et al. may be due to the clinical composition of the study sample. The investigation used participants who had been diagnosed with CHD (32). Previous studies have found CHD to be positively associated with both CRP and LP-PLA_2 (51, 58). If the participants had already been diagnosed with CHD, it would be logical to assume that there was a sufficient stimulus present for increases in systemic and atherosclerotic-specific inflammation.

This increase in total body inflammation would logically lead to a physiologic state where Lp-PLA_2 and CRP would both be elevated and thus, more likely to be correlated.

5.5 LIMITATIONS AND STRENGTHS

Several limitations of the current investigation should be noted. The current sample included only middle-aged, heterosexual females, limiting generalizability of results to the age group that was evaluated. In addition, the sample size of this study was relatively small, limiting our power to detect significant differences between the comparison groups. As noted in the methods section, the small sample size was determined by funding limitations for the Lp-PLA_2 assays, which allowed for a maximum of 80 participants. Valid Lp-PLA_2 assays were subsequently obtained on 75 of the 80 participants. It was determined that all five of the missing Lp-PLA_2 values were from participants in the INACTIVE group. Statistical analysis indicated that the five INACTIVE participants with missing Lp-PLA_2 values did not differ from the other 75 participants with respect to measured demographic characteristics. It was determined that, in order to achieve a significant difference in Lp-PLA_2 activity between groups (i.e., ACTIVE vs. INACTIVE) , the mean Lp-PLA_2 activity for the five INACTIVE participants with missing Lp-PLA_2 data would have to be almost 50% higher than the mean Lp-PLA_2 activity for the remaining 35 INACTIVE participants. In order to achieve a significant difference in Lp-PLA_2 mass between groups (i.e., ACTIVE vs. INACTIVE), the mean LP-PLA_2 mass for the five INACTIVE participants with missing data would have to be approximately 350% higher than the mean Lp-PLA_2 mass for the remaining 35 INACTIVE participants. As a result, it is reasonable

to conclude that the elimination of the five INACTIVE participants with missing Lp-PLA_2 data did not alter the outcome of this investigation.

In the current investigation, MVPA was determined by a self-report questionnaire. The questionnaire asked the participants to recall their average minutes per day and days per week of MVPA over the previous 12 months. While a frequently used tool for PA measurement, self-report questionnaires are not without limitations. Given the potential time delay between actual participation and recall, it is possible that the total amount of PA reported was overestimated. In addition, the questionnaire used in this investigation did not directly measure PA intensity. Intensity in the current investigation was determined based on the PA mode that the participant reported doing. This may have increased the chance of misclassification of the intensity of the activity. Another use of self-report in the current investigation was to assess CHD. The lack of clinical screening for prevalent CHD prior to enrollment was another limitation of the current investigation.

Previous research has shown systemic inflammation, as measured by CRP, to be inversely related with aerobic fitness, independent of PA level (5). Since the association between LP-PLA_2 and PA has not been extensively studied, it is unknown whether it is the actual participation in PA or the aerobic fitness level that may have a stronger association with Lp-PLA_2. Given that maximal oxygen uptake was not measured in the current investigation, there was no way to examine the association between aerobic fitness and Lp-PLA_2.

Another possible limitation of the current investigation pertains to the timing of the MVPA measurements. Participants were asked to recall their MVPA over the previous 12 months. If the participant only recently started to engage in MVPA, there may not have been a

sufficient period of time for the MVPA-induced physiological mechanisms to mediate a reduction in Lp-PLA_2 mass, Lp-PLA_2 activity, or CRP.

The inclusion of only women who identified themselves as heterosexual is another possible limitation of the current investigation. This subject selection process was done to help control for possible differences between the comparison groups as a result of sexual identity. However, data from a comparison lesbian group might have been helpful in explaining the lack of significant differences noted between the ACTIVE and INCACTIVE groups based on Lp-PLA_2 mass and Lp-PLA_2 activity. Additionally, had the data from the lesbian group been included, it could have provided additional prognostic information regarding risk of CHD for lesbians, a group that has been shown to be at an increased risk of CHD (1).

Finally, this study used a cross-sectional design and, therefore, only single time point measures of Lp-PLA_2 and CRP were obtained. The cross-sectional design is useful in helping to generate potential hypotheses but does not provide cause and effect type explanations. No information was available on inflammatory marker levels of the study participants prior to this investigation. If the participants had high levels of inflammation at the time of the assessment as a result of an injury or illness, any reduction in Lp-PLA_2 or CRP values as a result of habitual MVPA might not be noticeable. Despite the limitations of the cross-sectional study design, CRP values were found to be lower in the ACTIVE group as compared to the INACTIVE group. This indicates that the design was likely appropriate to answer research question #2. By extension, the research design was likely appropriate to examine pair-wise differences in Lp-PLA_2 between the ACTIVE and INACTIVE groups to answer research question #1.

This investigation also had significant strengths. It was one of the first studies, to our knowledge, whose main objective was to examine the association between Lp-PLA_2, CRP, and

MVPA in a defined cohort of middle aged, heterosexual females. In addition, this investigation examined both Lp-PLA_2 mass and Lp-PLA_2 activity and their possible association with MVPA. Of the four main studies that have examined the association between Lp-PLA_2 and PA, only Persson et al. compared both Lp-PLA_2 mass and Lp-PLA_2 activity with PA (54). Of the three other main studies, Rana, et al. (61) and Detopoulou, et al. (10) compared only Lp-PLA_2 activity with PA, and Daniels, et al. (9) compared only Lp-PLA_2 mass with PA.

As noted in the Study Limitations, no information was available on inflammatory marker levels of the study participants prior to this investigation. To help adjust for this lack of information on pre-study inflammatory marker levels, we statistically controlled for many factors that have been shown to be associated with inflammation. These factors included: arthritis, asthma, autoimmune disease, cancer, cigarette smoking, diabetes, and hormone use.

5.6 FUTURE RESEARCH DIRECTIONS

As indicated in the Study Limitations section, there are a number of lines of investigation that can be pursued in future research involving Lp-PLA_2. An experimental intervention using graded (i.e., low, moderate, high) PA levels would help to determine if a systematic association exists between Lp-PLA_2 and MVPA. One possible investigation could include four different groups; 3 PA groups and one control group. Both the experimental and the control groups would be comprised of both males and females of varying ages. The experimental groups would be prescribed aerobic PA at different intensities; group #1 exercising at a low intensity, group #2 exercising at a moderate intensity, and group #3 exercising at a high intensity. The control group would perform stretching exercises. Pre-intervention, mid-intervention, and post-intervention

measures from all groups (control and experimental) would include VO_{2max} testing, dietary assessment, and measurement of blood levels of IL-6, TNF-α, CRP, and Lp-PLA_2. In addition, PA participation would be monitored via accelerometers. The accelerometers would be initialed during the baseline clinic visit, downloaded at the study midpoint and again at the end of the study. Participants in the experimental group would be provided with a list of intensity-appropriate activities to participate in. Participants in the control group would be provided with instructions on proper stretching techniques.

The PA intervention study, with measurements taken at the beginning, middle, and at the end of the study, would also help to determine if a cause and effect association exists between Lp-PLA_2 and PA of different intensities. This PA intervention would examine the possible association of Lp-PLA_2 mass and Lp-PLA_2 activity with both PA and aerobic fitness. In addition, the study would examine any possible association between the other biomarkers of inflammation and PA. Finally, this proposed PA intervention would address one of the more serious limitations of the current investigation; the use of a self-reported PA questionnaire. The use of an objective PA measure, such as an accelerometer, would provide a more accurate method of quantifying the total volume and intensity of the PA.

APPENDIX A

PAST YEAR LEISURE-TIME PHYSICAL ACTIVITY QUESTIONNAIRE

PAST YEAR LEISURE-TIME PHYSICAL ACTIVITY ID #:

Now we are going to ask you think about any leisure-time sports or recreational activities you participate in. I want you to think back to _____ (month/year). Make sure you include all individual and team recreational activities or sports that you participated in during the last year from _____ (month/year) through _____ (month/year). Please look at this list and tell me which activities you have participated in at least 10 times in the past year.

1___	Aerobics	10___	Golf	19___	Running	28___	Tai Chi
2___	Basketball	11___	Hiking	20___	Skating (Ice)	29___	Tennis
3___	Bicycling (Mountain)	12___	Horseback Riding	21___	Skating (Roller/Blading)	30___	Volleyball
4___	Bicycling (Stationary)	13___	Kayaking	22___	Skiing (Cross Country)	31___	Walking for Exercise*
5___	Bicycling (Street)*	14___	Kick Boxing	23___	Skiing (Downhill)	32___	Water Aerobics
6___	Bowling	15___	Pilates	24___	Skiing (Water)	33___	Weight Training
7___	Canoeing	16___	Racquetball/Squash	25___	Soccer	34___	Yoga
8___	Cardio-machines	17___	Rock Climbing	26___	Softball		
9___	Dance Class	18___	Rowing	27___	Swimming Laps		

Others: (*Are there any other activities that you did at least 10 times in the past year that were not included in the list I just read?*)

1. _____ 2. _____ 3. _____ 4. _____ 5. _____

(*a) Beginning with _____ (month/year) tell me which months of the past year you participated in _____. (b) Approximately how many days per week did you participate in this activity? (c) On average, how many minutes per day did you participate in this activity?* **[Repeat for each activity.]**

Activity	# of Months	(a) Past Year Activity												(b) Average # Days/Week	(c) Average # Minutes/Day
		Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec		

*** Note: Walking and/or biking to and from work should not be included in this section.**

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