BMJ open

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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002623
Article Type:	Research
Date Submitted by the Author:	21-Jan-2013
Complete List of Authors:	Strohacker, Kelley McCaffery, Jeanne wing, rena
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	Immunology < BASIC SCIENCES, EPIDEMIOLOGY, Public health < INFECTIOUS DISEASES

Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A Cohort Study of Middle Aged Adults Living in the United States

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Running Title:

BMI, Physical Activity and Inflammation

Key Words: MIDUS, Intracellular Adhesion Molecule-1, Fibrinogen, C-Reactive Protein

Word Count: 3199

ARTICLE SUMMARY

Article focus

- Systemic inflammation is related to the progression of cardiovascular disease.
- Independent of obesity, physical activity is inversely related to concentrations of well-established inflammatory biomarkers, such as C-reactive protein (CRP) or interleukin-6 (IL-6).
- This article evaluates interactive effects of body mass index and physical activity on established inflammatory markers, CRP, IL-6, and emerging inflammatory markers, fibrinogen, soluble intracellular adhesion molecule (sICAM)-1, soluble E-selectin, and IL-6 soluble receptor.

Key messages

- Interactive effects of body mass index and physical activity were observed for CRP, such that regular physical activity reported by overweight individuals was related to significantly lower CRP levels compared to those reported no regular activity.
- Independent of BMI, regular physical activity was related to lower IL-6, with a trend for lower fibrinogen
- Physical activity had no independent effect on circulating markers related to endothelial inflammation, such as sICAM-1 or sE-selectin.

Strengths and limitations

- 1255 adults from the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study were analyzed. Statistical analyses were adjusted for age, sex, smoking, and relevant medication use. A strength of this paper is categorizing physical activity levels based on national recommendations. This data may be used to determine appropriate levels of physical activity necessary for reducing inflammation in overweight and obese adults. However, cross-sectional data is limited, as causal inferences cannot be obtained. A second limitation is that the sample was predominantly comprised of non-Hispanic white individuals, therefore findings may not extend to all ethnicities.

ABSTRACT

Objectives. Determine whether body mass index (BMI) and physical activity (PA) above, at or below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP), fibrinogen, IL-6 soluble receptor (sr), soluble (s) E-selectin and soluble intracellular adhesion molecule (sICAM)-1. **Design.** Archived cohort data (N=1255, age 54.5±11.7y, BMI 29.8±6.6kg/m^2) from the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study were analyzed for concentrations of inflammatory markers using general linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m^2. Analyses were adjusted for age, sex, smoking and relevant medication use. **Setting.** Respondents reported to three centers to complete questionnaires and provide blood samples. **Participants.** Participants were eligible if they were currently enrolled in the parent MIDUS study (N=1255, 57% female, 93% non-hispanic white, average age 54.5y). **Primary Outcome Measures.** Concentration of IL-6, CRP, fibrinogen, IL-6sr, sE-selectin and sICAM. **Results.** Significant interactions were found between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was similar to obese when no PA was reported, but was similar to normal weight when any level of regular PA was reported. sICAM-1 was differentially lower in obese individuals who reported >1000 MMW compared to obese individuals reporting less exercise. **Conclusion.** Levels of CRP and sICAM-1 depended on exercise and BMI levels, suggesting that regular exercise may buffer weight-associated elevations in CRP in overweight individuals while higher levels of exercise may be necessary to reduce sICAM-1 or CRP in obese individuals. **Trial Registry.** N/A.

INTRODUCTION

Obesity paired with low physical activity is well known to increase morbidity and mortality related to cardiovascular disease (CVD)(1). It is less clear, however, whether the benefits of higher levels of physical activity differ among normal weight, overweight, and obese individuals.

Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of inflammatory markers(7, 8), while physical activity appears to have anti-inflammatory effects(9, 10). It is unclear whether the effects of physical activity depend on the degree of obesity

The purpose of our study was to disentangle the relative contributions of BMI and physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr, CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were determined using values put forth by the Physical Activity Guidelines for Americans, which states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week) produces substantial health benefits for adults(11). We hypothesized that BMI and MMW at or above the 500-1000 MMW guidelines would interact, such that the impact of MMW on inflammatory markers would differ by degrees of overweight or obesity.

MATERIALS AND METHODS

Design and Sample. This study was a cross-sectional analysis of archived data (BMI, self-reported physical activity and inflammatory biomarker concentrations) from 1255 respondents aged 25 to 74 who provided consent (as approved by The University of Wisconsin Madison Health Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study(12). The purpose of the Biomarker Project was to add comprehensive biological assessments on a subsample of the parent MIDUS study to further understand age-related differences in physical and mental health. Those who agreed to participate stayed overnight at one of three General Clinical Research Centers: University of California Los Angeles, University of Wisconsin-Madison and Georgetown University. Upon arrival, each respondent provided a detailed medical history (including physical activity levels) and provided all prescription, over-the-counter, and alternative medications to be inventoried by project staff. Following an overnight stay, morning fasting blood samples were obtained. Cohorts were assessed between July 2004 and May 2009 as a follow up to MIDUS I respondents that were previously surveyed by the MacArthur Midlife Research Network between 1995 and 1996. Based on the sample of 1255 participants, 80% power was achieved to detect small effects of 0.08 or greater with alpha level at 0.05 for a two-tailed test(13, 14).

Anthropometrics. Height was measured in centimeters and recorded to the nearest millimeter. Weight was measured in kilograms and recorded to the nearest decimal place. BMI was calculated by dividing body mass in kilograms by height in meters squared.

Categorizing BMI and MMW. BMI categories were organized into 3 groups: normal weight (BMI ≤24.9 kg/m^2), overweight (BMI≥25-29.9) and obese (BMI≥30).

The MMW variable was calculated using data provided in the medical history form. The form first described 3 types of regular physical activity(12):

Vigorous: *Which causes your heart to beat so rapidly you can feel it in your chest and you perform it long enough to work up a good sweat and breathe heavily (e.g., competitive sports, running, vigorous swimming, high intensity aerobics, digging in the garden, or lifting heavy objects).*

Moderate: *Which causes your heart rate to increase slightly and you typically work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming, low intensity aerobics or golfing without a power cart, brisk walking, mowing the lawn with a walking lawnmower).*

Light: *Which requires little physical effort (e.g., light housekeeping like dusting or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).*

Keeping these definitions in mind, participants were asked if they engaged in regular physical activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants answered "yes", they entered up to 7 types of seasonal and/or non-seasonal exercise or activity along with the frequency, duration and intensity.

MMW were calculated in a 2-step process. Step 1: subjects who reported no physical activity (for whom no MMW calculations could be made) were designated as the no regular exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity

reported. Four groups reflecting participation in physical activity and whether or not their participation was below, at or above USDHHS guidelines were created: NRE (reported no regular physical activity), below recommended (reported >500 MMW), recommended (reported 500-1000 MMW) and above recommended (reported >1000 MMW).

Blood Collection, Processing and Assays. Participants were asked to avoid strenuous activity the day of blood collection. Venous blood samples were collected in 10 mL serum separator vacutainers following a 12-h overnight fast and processed at a General Clinical Research Center using standardized procedures. Briefly, following collection, vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-Selectin and sICAM-1).

IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison, Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600, R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV% values for IL-6sr were 5.9-5.7% and 2.0%, respectively.

Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay; R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay,

R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%, respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer with a particle enhanced immunonepolometric assay. Intra-assay and inter-assay precision for CRP was 2.3-4.4% and 2.1-5.7%, respectively.

Statistical Analyses. All variables were assessed for normality and non-normal data were log transformed. To determine the relative impact of MMW and BMI on inflammatory markers, general linear models were performed. For each outcome, the categorical MMW and BMI factors were entered as independent factors with an interaction term. If the interaction term was not significant, the interaction term was dropped and the model was re-fit. All analyses presented in the results were adjusted for confounding variables that are known to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering, corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive). Race was initially included as a covariate; however, approximately 200 data points were lost in the analyses due to incomplete racial data. As race was not found to be a predictor of our dependent variables, with the exception of sICAM-1, race was excluded as a covariate to increase samples size in all analyses excluding sICAM-1. In an exploratory analysis, we examined whether the relative effects of BMI and MMW on the inflammatory markers differed by sex in 3-way interaction models. As none of the interactions approached statistical significance, sex was included as a covariate in the models. All statistical analyses were performed with SPSS v. 17 (Chicago, IL) and significance was set at $P < 0.05$.

RESULTS

Subject Characteristics. Table 1 presents anthropometric characteristics and plasma levels of inflammatory biomarkers in all subjects (N=1255). On average, subjects were 92.6% non-Hispanic white, 56.8% female, middle-aged and overweight. Of all the respondents, 14.9% were currently smoking, 27.8% were taking cholesterol lowering medication, 12.1% were taking corticosteroids, 10.4% were taking anti-diabetic medication, 14.2% were taking antidepressant medication, 7.3% were taking hormone replacement and 2.5% reported contraceptive use. The percentage of participants with missing data for each variable are as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6% for fibrinogen, 1.2% for sE-selectin, and 1.0%for IL-6sr.

CRP (Figure 1, Panel A). We found a significant interaction between BMI and MMW for CRP concentration (F=3.022, P=0.006). In post hoc comparisons, CRP levels were higher in overweight and obese subjects compared to normal weight subjects among those who reported no regular exercise (P's<0.001). However, among subjects who reported any amount of regular exercise (<500, 500-1000 or >1000 MMW), CRP levels were significantly greater only in obese subjects compared to both normal weight and overweight subjects (P's <0.01). These results suggest that regular exercise may mitigate the association between weight and CRP in overweight individuals. In obese individuals, CRP tended to be lower in those reporting >1000 MMW compared to those reporting no regular exercise (P=0.053), suggesting the high levels of activity only may mitigate elevations in CRP levels in obese individuals

We also found main effects of BMI (F=130.873 P<0.001) and MMW (F=11.576, P<0.001) for CRP. CRP was significantly greater with each increasing BMI category, in a dose-dependent manner (P's<0.001). Compared to participants who reported no regular exercise,

CRP was significantly lower in those who reported 500-1000 and >1000 MMW (P's <0.01), with a trend for lower CRP in those who reported <500 MMW of regular exercise (P=0.078).

sICAM-1 (Figure 1, Panel B). We found a significant interaction between BMI and MMW for sICAM-1 concentration (F=2.701, P=0.013). Levels of sICAM-1 were significantly lower in obese subjects who reported >1000 MMW compared to obese subjects who reported no regular exercise (P=0.014) and <500 MMW (P=0.026) and tended to be lower than levels in obese subjects who reported 500-1000 MMW (P=0.079), again suggesting that high levels of physical activity could mitigate the increased sICAM-1 associated with obesity. No differences in sICAM-1 by MMW were observed among normal weight or overweight individuals.

We also observed a main effect of BMI (F=6.060, P=0.002), such that sICAM-1 levels in obese participants were significantly higher than levels found in both normal weight and overweight participants (P's<0.01). No significant main effect of MMW was found for sICAM-1 (F=0.931, P=0.425).

IL-6 (Figure 1, Panel C). Both BMI and MMW had independent effects on circulating concentrations of IL-6 (BMI: F=60.150, P<0.001, MMW: F=10.680, P<0.001), with no significant interaction (F=1.21, P=0.297). We found a dose-dependent effect of BMI, such that higher BMI levels were associated with significantly greater IL-6 (P's<0.001). Independent of BMI, IL-6 was significantly lower in subjects who reported regular exercise (<500 MMW, 500-1000 MMW and >1000 MMW) compared to those who reported no regular exercise (P's <0.01) with no difference between levels of MMW.

Fibrinogen (Figure 1, Panel D). BMI significantly contributed to circulating levels of fibrinogen (F=42.385, P<0.001), such that dose-dependent increases were observed for all BMI

levels (P's<0.01). While we observed a trend for lower fibrinogen with regular physical activity, similar to that of IL-6, the effect did not reach statistical significance (F=2.187, P=0.088). We observed no significant interaction between BMI and MMW for fibrinogen (F=1.680, P=0.122).

sE-Selectin (Figure 1, Panel E). BMI significantly contributed to circulating levels of sE-selectin (F=28.253, P<0.001) with no significant contribution by MMW (F=0.207, P=0.892). Dose-dependent increases in sE-selectin were also observed across BMI levels (P's<0.01). We observed no significant interaction between BMI and MMW for sE-selectin (F=0.570, P=0.755).

IL-6sr (Figure 1, Panel F). No significant main effects for BMI (F=1.783, P=0.169), MMW (F=1.434, P=0.231) or their interaction (F=0.834, P=0.544) were detected for IL-6sr.

DISCUSSION

The current study aimed to delineate the interactive and independent impact of BMI and physical activity on inflammatory markers related to CVD risk. In the cases of CRP and sICAM-1, the effects of BMI and MMW were interactive. Regular physical activity appeared to diminish the effects of higher BMI compared to those who reported no regular physical activity. We found that BMI was strongly and independently related to greater concentrations of both established and emerging inflammatory markers that may increase CVD risk. Independent of BMI, regular physical activity was associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that, although obesity has a clear impact on inflammation, physical activity appears to mitigate at least some of this effect. Further, obese individuals may need to perform levels of physical activity greater than current recommendations for health in order to mitigate obesity-related inflammation, as trends for lower CRP or sICAM-1 were only apparent in obese participants reporting >1000 MMW.

Low-grade, systemic inflammation is characterized by elevated levels of inflammatory markers, such as cytokines, acute phase proteins or soluble adhesion molecules. IL-6 produced in hypertrophied adipose tissue(15, 16) initiates the acute phase response, marked by the release of hepatic CRP and fibrinogen(17, 18). Inflammatory cytokines (IL-6) and acute phase proteins (CRP, fibrinogen) stimulate the production of chemoattractant proteins and adhesion molecules (sICAM-1 and sE-selectin) in the vasculature, promoting cell accumulation and atherosclerotic plaque formation(19, 20). In epidemiologic studies, higher levels of IL-6 and CRP are associated with increasing numbers clinical risk factors for cardiovascular disease(21-23). Cardiovascular disease risk is also increased with higher levels of cell adhesion molecules(24, 25) and acute phase reactants(22, 26, 27).

Interactions between BMI and MMW suggest that regular physical activity may be able to mitigate the effect of an overweight BMI on CRP. Overweight individuals had CRP levels that were similar to levels observed in obese individuals if they reported no regular exercise (4.05 and 4.83 μg/mL, respectively). CRP levels greater than 3 μg/mL are typically associated with high CVD risk(28). In overweight subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it below [<500], within [500-1000] or above [>1000] USDHHS MMW recommendations), CRP levels were lower and not significantly different from CRP levels found in normal weight participants. This suggests that increasing physical activity level to a minimum of 3 days per week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.

Obese individuals may require a higher level of regular physical activity in order to lower inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1 compared to lean and overweight subjects, those who reported >1000 MMW (above the USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than obese subjects reporting no regular physical activity. Taken together, we may speculate that while physical activity levels currently recommended for the general population may reduce particular inflammatory makers in overweight populations, obese populations may require greater levels of physical activity above recommended values to reduce inflammatory markers like CRP and sICAM-1.

As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen, sICAM-1 and sE-selectin, in agreement with previous work(29-32). Independent of BMI effects, our results suggest that physical activity has differentiating independent effects on inflammatory markers. Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and determinants in CArdiovascular disease (MONICA) study(33), the National Health and Nutrition Examination Survey (NHANES III)(34, 35) and the Multi-Ethnic Study of Atherosclerosis (MESA)(36), such that both increased frequency and intensity of physical activity have been related to lower IL-6 and fibrinogen. While similar, our findings add to prior results by standardizing levels of physical activity by using USDHHS recommendations, rather than general tertiles, quartiles, etc.. However, while the USDHHS reports that meeting these recommendations promotes substantial health benefits(11), the impact on specific inflammatory

markers was not addressed. Our results suggest that, regular physical activity at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and possibly fibrinogen, independent of BMI.

Interestingly, in the MIDUS sample, results suggest that regular exercise may have a more profound impact on lowering classical markers of inflammation and less impact on the inflammatory status of the endothelium. Regular physical activity was independently associated with lower levels of IL-6 and CRP, both classical inflammatory markers related to adipose and systemic inflammation(37). However, regular exercise appeared to have no independent impact on markers of endothelial activation, particularly sE-selectin and sICAM-1. Inverse relationships between physical activity and sICAM-1 or sE-selectin have been reported previously, in drug-treated hypertensive men(38). However, cross-sectional reporting of inverse relationships between physical activity and other markers of atherosclerotic activity, particularly carotid arterial wall thickness, has yielded variable results(39-42). Upon reviewing this literature, Thijssen and colleagues suggest that inverse correlations between arterial wall thickness and physical activity were more likely to be found in studies that utilized specifically-designed instruments to assess physical activity, rather that non-specific questionnaires that obtain information about general exercise behavior(43). Therefore, it is possible that a more objective or validated measure of physical activity utilized may have increased the likelihood of observing significant relationships between physical activity and circulating makers of atherosclerotic activity that were independent of BMI.

Several limitations must be addressed. First, the cross-sectional design does not allow us to infer causal relationships. Prospective and interventional designs are necessary to confirm our findings. Second, the use of self-report physical activity data may reduce accuracy compared to

direct measures of physical activity. However, in addition to being in line with previous studies using self-report physical activity, our findings are also in line with previous studies(44, 45) that demonstrated that higher cardiorespiratory fitness was associated with lower levels of inflammation independent of visceral adiposity or BMI. Furthermore, regular physical activity may have positive health effects independent of fitness, as individuals of similar fitness levels demonstrate reduced risk for coronary heart disease, CVD and stroke with higher levels of physical activity compared to those with lower activity levels and both low physical activity and fitness levels directly increase risk of metabolic disease and type 2 diabetes mellitus(46, 47). Finally, as the sample was predominantly comprised of non-Hispanic white individuals, findings may not extend to all ethnicities. Finally, BMI and physical activity variables are correlated, potentially raising the concern of small sample sizes in specific categories crossing BMI and MMW. However, the minimum category contained 54 individuals (normal weight individuals reporting no exercise).

In summary, our results demonstrate both interactive and independent influences of BMI and levels of physical activity on both established and emerging markers of inflammation. Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular physical activity appears to mitigate the effects of higher BMI on some inflammatory markers, particularly CRP, which is strongly implicated in CVD. More importantly, while any level of regular physical activity may help reduce inflammation in overweight individuals, similar effects in obese individuals may require levels of physical activity that are greater than currently recommended by the USDHHS for general health. It is important that future research aims to elucidate effective exercise levels that can produce anti-inflammatory effects in overweight and obese individuals.

ACKNOWLEDGEMENTS

We thank the staff of the Clinical Research Centers at the University of Wisconsin-Madison, UCLA, and Georgetown University for their effort in conducting the original data collection.

FUNDING

KS was supported through a T32 Training Fellowship (Training in Behavioral and Preventive Medicine; T32 HL076134). The original research was supported by a grant from the National Institute on Aging (P01-AG020166) to conduct a longitudinal follow-up of the MIDUS (Midlife in the U.S.) Investigation.

The original study was supported by the John D. and Catherine T. MacArther Foundation Research Network on Successful Midlife Development and by the following grants: M01-RR023942 (Georgetown), M01-RR00865 (UCLA) from the General Clinical Research Centers Program and 1UL1RR025011 (UW) from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources, National Institutes of Health.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTORSHIP

KS, JMM and RRW each made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation, as well as to drafting and revision for substantial intellectual content. All authors made final approval of the version to be published.

DATA SHARING STATEMENT

Data and documentation for MIDUS studies are available at the Inter-university Consortium for Political and Social Research (ICPSR). http://www.icpsr.umich.edu/icpsrweb/landing.jsp

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FIGURE AND TABLE LEGENDS

Figure 1: Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value, INT=interaction effect P value.

Table 1: Subject Characteristics. BMI = body mass index; CRP = C-reactive protein; IL = interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin = soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.

Demographic Variables	Overall N = 1255 Mean ± SD (N)
Age (years)	54.5 ± 11.7 (1255)
Gender (%)	
Male	43.20 (542)
Female	56.80 (713)
Race (%)	
Non-Hispanic White	92.60 (974)
Hispanic	0.05 (5)
African American	2.60 (27)
Asian/Pacific Islander	0.30 (3)
Native American	1.30 (14)
Other	2.30 (29)
Medication Use (%)	
Cholesterol-Lowering	27.80 (349)
Corticosteroids	12.10 (152)
Anti-Diabetic	10.40 (130)
Antidepressant	14.2 (178)
Hormone Replacement Therapy	7.3 (92)
Oral Contraceptive	2.5 (31)
Currently Smoking	14.90 (187)
BMI (kg/m^2)	29.8 ± 6.6 (1254)
IL-6 (pg/mL)	3.0 ± 3.1 (1243)
IL-6sr (pg/mL)	35184.7 ± 10359.1 (1243)
CRP (μg/mL)	3.0 ± 4.8 (1235)
Fibrinogen (mg/dL)	348.9 ± 87.9 (1235)
sE-Selectin (ng/mL)	43.4 ± 22.7 (1242)
sICAM-1 (ng/mL)	288.6 ± 115.6 (1242)

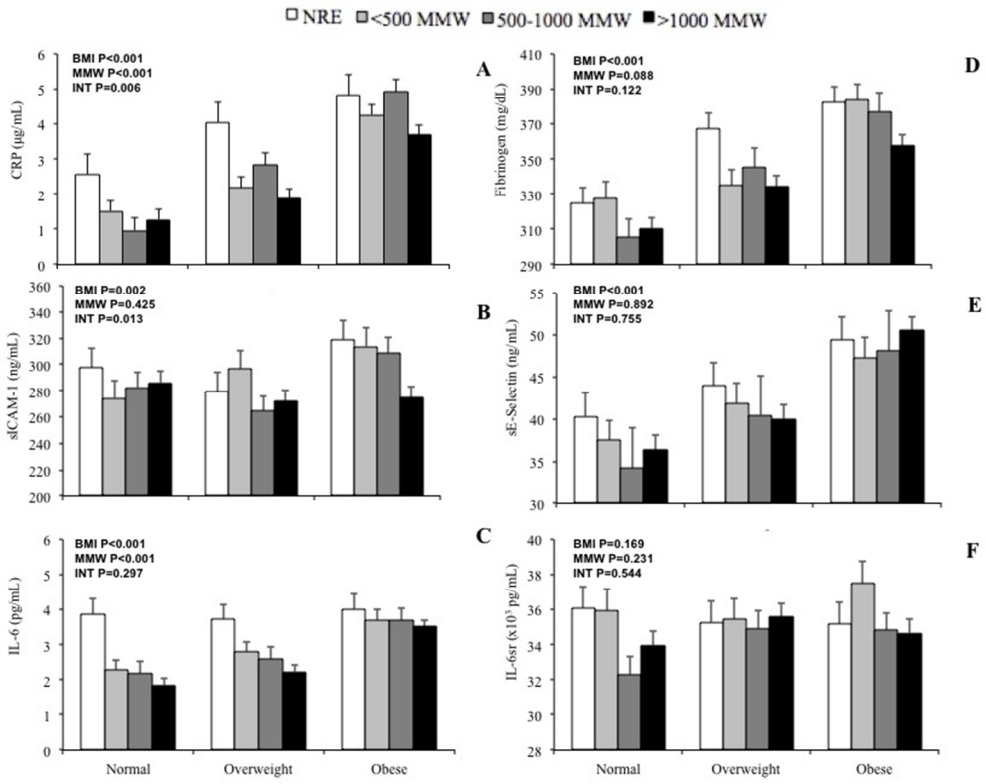

Figure 1: Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value, INT=interaction effect P value.
292x229mm (72 x 72 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any pre-specified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4-7*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	8-10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	8-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ open

Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A Cohort Study of Middle Aged Adults Living in the United States

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002623.R1
Article Type:	Research
Date Submitted by the Author:	18-Mar-2013
Complete List of Authors:	Strohacker, Kelley McCaffery, Jeanne wing, rena
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	Immunology < BASIC SCIENCES, EPIDEMIOLOGY, Public health < INFECTIOUS DISEASES

Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A Cohort Study of Middle Aged Adults Living in the United States

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Running Title:

BMI, Physical Activity and Inflammation

Key Words: MIDUS, Intracellular Adhesion Molecule-1, Fibrinogen, C-Reactive Protein

Word Count: 3331

ABSTRACT

Objectives. Determine whether body mass index (BMI) and physical activity (PA) above, at or below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP), fibrinogen, IL-6 soluble receptor (IL-6sr), soluble E-selectin and soluble intracellular adhesion molecule (sICAM)-1. **Design.** Archived cohort data (N=1254, age 54.5±11.7y, BMI 29.8±6.6kg/m^2) from the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study were analyzed for concentrations of inflammatory markers using general linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m^2. Analyses were adjusted for age, sex, smoking and relevant medication use. **Setting.** Respondents reported to three centers to complete questionnaires and provide blood samples. **Participants.** Participants were men and women currently enrolled in the MIDUS Biomarker Project (N=1254, 93% non-hispanic white, average age 54.5y). **Primary Outcome Measures.** Concentration of serum IL-6, CRP, fibrinogen, IL-6sr, sE-selectin and sICAM. **Results.** Significant interactions were found between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was similar to obese when no PA was reported, but was similar to normal weight when any level of regular PA was reported. sICAM-1 was differentially lower in obese individuals who reported >1000 MMW compared to obese individuals reporting less exercise. **Conclusion.** The association of exercise with CRP and sICAM-1 differed by BMI, suggesting that regular exercise may buffer weight-associated elevations in CRP in overweight individuals while higher levels of exercise may be necessary to reduce sICAM-1 or CRP in obese individuals. **Trial Registry.** N/A.

INTRODUCTION

Obesity paired with low physical activity is well known to increase morbidity and mortality related to cardiovascular disease (CVD)(1). It is less clear, however, whether the benefits of higher levels of physical activity differ among normal weight, overweight, and obese individuals.

Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Circulating Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of inflammatory markers(7, 8). Further, body fat distribution is also an important factor relating to inflammatory status. Accumulation of fat in visceral depots is more strongly associated with low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots(9, 10).

The effects of physical activity on markers of inflammation are more complex and may vary depending on body weight. A number of epidemiological studies have shown an inverse relationship between physical activity and CRP and IL-6, independent of obesity(11-16). Laboratory studies conducted in aerobically trained, typically normal weight, individuals have demonstrated that a single bout of exercise stimulates IL-6 release from skeletal muscle, which promotes anti-inflammatory effects (17-19), as opposed to adipose tissue-derived IL-6 that is associated with pro-inflammatory effects (20). Randomized controlled trials have also been conducted, often in populations that also tend to be overweight or obese, to examine the effects

of aerobic exercise interventions on inflammation and the results are mixed (21). Thus, the contribution of physical activity to inflammation in the context of obesity remains unclear.

The purpose of our study was to disentangle the relative contributions of BMI and physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr, CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were determined using values put forth by the Physical Activity Guidelines for Americans, which states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week) produces substantial health benefits for adults(22). We hypothesized that BMI and MMW would interact, such that greater MMW reported would lessen the impact of obesity on markers of inflammation.

MATERIALS AND METHODS

Design and Sample. This study was a cross-sectional analysis of archived data (BMI, self-reported physical activity and inflammatory biomarker concentrations) from 1254 respondents who provided consent (as approved by The University of Wisconsin Madison Health Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study (23). The Biomarker Project was one of 5 projects within MIDUS II, with the purpose of adding comprehensive biological assessments on a subsample of the MIDUS participants to further understand age-related differences in physical and mental health. Participants were eligible for The Biomarker Project if they were previously enrolled in MIDUS I, which recruited non-institutionalized, English-speaking adults residing in the contiguous United States aged 25-74. The random digit

dialing sample for the parent study was selected from working telephone banks and a list of all individuals between the ages of 25 and 74 years within each household was generated in order to select a random respondent. Those who agreed to participate in the Biomarker Study stayed overnight at one of three General Clinical Research Centers: University of California Los Angeles, University of Wisconsin-Madison and Georgetown University. Upon arrival, each respondent provided a detailed medical history (including physical activity levels) and provided all prescription, over-the-counter, and alternative medications to be inventoried by project staff. Following an overnight stay, morning fasting blood samples were obtained. Cohorts were assessed between July 2004 and May 2009 as a follow up to MIDUS I respondents that were previously surveyed by the MacArthur Midlife Research Network between 1995 and 1996. Based on the sample of 1254 participants, 80% power was achieved to detect small effects of 0.08 or greater with alpha level at 0.05 for a two-tailed test(24, 25).

Anthropometrics. Height was measured in centimeters and recorded to the nearest millimeter. A single measure of WC was taken directly on the skin or over a single layer of light, close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the nearest millimeter. Weight was measured in kilograms and BMI was calculated by dividing body mass in kilograms by height in meters squared. BMI categories were organized into 3 groups: normal weight (BMI ≤24.9 kg/m^2), overweight (BMI≥25-29.9) and obese (BMI≥30).

Categorizing Physical Activity by MET-Minutes per Week (MMW).

The MMW variable was calculated using data provided in the medical history form. The form first described 3 types of regular physical activity(23):

Vigorous: *Which causes your heart to beat so rapidly you can feel it in your chest and you perform it long enough to work up a good sweat and breathe heavily (e.g.,*

competitive sports, running, vigorous swimming, high intensity aerobics, digging in the garden, or lifting heavy objects).

Moderate: *Which causes your heart rate to increase slightly and you typically work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming, low intensity aerobics or golfing without a power cart, brisk walking, mowing the lawn with a walking lawnmower).*

Light: *Which requires little physical effort (e.g., light housekeeping like dusting or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).*

Keeping these definitions in mind, participants were asked if they engaged in regular physical activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants answered "yes", they entered up to 7 types of seasonal and/or non-seasonal exercise or activity along with the frequency, duration and intensity.

MMW were calculated in a 2-step process. Step 1: subjects who reported no physical activity (for whom no MMW calculations could be made) were designated as the no regular exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity reported. Four groups reflecting participation in physical activity and whether or not their participation was below, at or above USDHHS guidelines were created: NRE (reported no regular physical activity), below recommended (reported <500 MMW), recommended (reported 500-1000 MMW) and above recommended (reported >1000 MMW).

Blood Collection, Processing and Assays. Participants were asked to avoid strenuous activity the day of blood collection. Venous blood samples were collected in 10 mL serum

separator vacutainers following a 12-h overnight fast and processed at a General Clinical Research Center using standardized procedures. Blood samples were not collected at any specific point during the menstrual cycle in female participants. Briefly, following collection, vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-Selectin and sICAM-1).

IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison, Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600, R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV% values for IL-6sr were 5.9-5.7% and 2.0%, respectively.

Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay; R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay, R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%, respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer

with a particle enhanced immunonepolometric assay. Intra-assay and inter-assay precision for CRP was 2.3-4.4% and 2.1-5.7%, respectively.

Statistical Analyses. All variables were assessed for normality and non-normal data were log transformed, which included data for CRP, IL-6, IL-6sr, fibrinogen, sE-selectin and sICAM-1. General Linear Models were performed to determine the relationship of MMW and BMI with the inflammatory markers. For each outcome, the ordinal MMW and BMI factors were entered as independent factors with an interaction term. If the interaction term was not significant, the interaction term was dropped and the model was re-fit for main effects only. Pairwise comparisons were assessed using post hoc univariate analyses with a Bonferroni adjustment for multiple comparisons. Covariates for all models included factors that are known to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering, corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive). Race was initially included as a covariate; however, approximately 200 data points were lost in the analyses due to incomplete racial data. As race was not found to be a predictor of our dependent variables, with the exception of sICAM-1, race was excluded as a covariate to increase samples size in all analyses excluding sICAM-1. All statistical analyses were performed with SPSS v. 17 (Chicago, IL) and statistical significance was set $\alpha = 0.05$.

In an exploratory analysis, we examined whether the relative effects of BMI and MMW on the inflammatory markers differed by sex in 3-way interaction models. As none of the interactions approached statistical significance (data not shown), sex was retained as a covariate in the models.

RESULTS

Subject Characteristics. Table 1 presents anthropometric characteristics and circulating levels of inflammatory biomarkers in all subjects (N=1254). Subjects were 92.6% non-Hispanic white, 56.8% female, and, on average, middle-aged and overweight. Of all the respondents, 14.9% were currently smoked, 27.8% were taking cholesterol lowering medication, 12.1% corticosteroids, 10.4% anti-diabetic medication, 14.2% antidepressant medication, 7.3% hormone replacement and 2.5% oral contraceptives. The percentage of participants with missing data for each variable were as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6% for fibrinogen, 1.2% for sE-selectin, and 1.0%for IL-6sr.

CRP (Figure 1, Panel A). We found a significant interaction between BMI and MMW for CRP concentration (F=3.022, P=0.006). In post hoc comparisons, CRP levels were higher in overweight and obese subjects compared to normal weight subjects among those who reported no regular exercise (P's<0.001). However, among subjects who reported any amount of regular exercise (<500, 500-1000 or >1000 MMW), CRP levels were significantly greater only in obese subjects compared to both normal weight and overweight subjects (P's <0.01). In obese individuals, CRP tended to be lower in those reporting >1000 MMW compared to those reporting no regular exercise (P=0.053).

We also found main effects of BMI (F=130.873 P<0.001) and MMW (F=11.576, P<0.001) on CRP. CRP was significantly greater with each increasing BMI category, in a dose-dependent manner (P's<0.001). Compared to participants who reported no regular exercise, CRP was significantly lower in those who reported 500-1000 and >1000 MMW (P's <0.01), with a trend for lower CRP in those who reported <500 MMW of regular exercise (P=0.078).

sICAM-1 (Figure 1, Panel B). We found a significant interaction between BMI and MMW for sICAM-1 concentration (F=2.701, P=0.013). Levels of sICAM-1 were significantly lower in obese subjects who reported >1000 MMW compared to obese subjects who reported no regular exercise (P=0.014) and <500 MMW (P=0.026) and tended to be lower than levels in obese subjects who reported 500-1000 MMW (P=0.079). No differences in sICAM-1 by MMW were observed among normal weight or overweight individuals.

We also observed a main effect of BMI (F=6.060, P=0.002), such that sICAM-1 levels in obese participants were significantly higher than levels found in both normal weight and overweight participants (P's<0.01). No significant main effect of MMW was found for sICAM-1 (F=0.931, P=0.425).

IL-6 (Figure 1, Panel C). Both BMI and MMW had independent effects on circulating concentrations of IL-6 (BMI: F=60.150, P<0.001, MMW: F=10.680, P<0.001), with no significant interaction (F=1.21, P=0.297). We found a dose-dependent effect of BMI, such that higher BMI levels were associated with significantly greater IL-6 (P's<0.001). Independent of BMI, IL-6 was significantly lower in subjects who reported regular exercise (<500 MMW, 500-1000 MMW and >1000 MMW) compared to those who reported no regular exercise (P's <0.01) with no difference between levels of MMW.

Fibrinogen (Figure 1, Panel D). BMI significantly contributed to circulating levels of fibrinogen (F=42.385, P<0.001), such that dose-dependent increases were observed for all BMI levels (P's<0.01). While we observed a trend for lower fibrinogen with regular physical activity, similar to that of IL-6, the effect did not reach statistical significance (F=2.187, P=0.088). We observed no significant interaction between BMI and MMW for fibrinogen (F=1.680, P=0.122).

sE-Selectin (1, Panel E). BMI significantly contributed to circulating levels of sE-selectin (F=28.253, P<0.001) with no significant contribution by MMW (F=0.207, P=0.892). Dose-dependent increases in sE-selectin were also observed across BMI levels (P's<0.01). We observed no significant interaction between BMI and MMW for sE-selectin (F=0.570, P=0.755).

IL-6sr (Figure 1, Panel F). No significant main effects for BMI (F=1.783, P=0.169), MMW (F=1.434, P=0.231) or their interaction (F=0.834, P=0.544) were detected for IL-6sr.

Waist Circumference (WC) and Inflammatory Markers (Supplemental Figure 1). A secondary analysis was completed using WC and MMW as independent variables and the complete results of these analyses are located in the supplemental information. Briefly, we found a significant interaction between WC and MMW on sICAM-1. In individuals with an at-risk WC (≥102.0 cm for men and ≥88.0 cm for women), sICAM-1 was significantly lower in those reporting 1000+ MMW compared to less than 500 MMW and tended to be lower in those reporting no regular exercise. Overall, main effects were similar to those found for BMI and MMW analyses. Having an at-risk WC was independently related to higher levels of CRP, sICAM-1, IL-6, fibrinogen and sE-selectin. Independent of WC, any level of regular exercise was related to lower levels of CRP, IL-6 with a similar tendency for fibrinogen.

DISCUSSION

The current study aimed to determine whether the impact of BMI and MMW on inflammatory markers varied by level of overweight or obesity. For CRP and s-ICAM-1 regular physical activity appeared to diminish the effects of higher BMI compared to those who reported no regular physical activity. In addition, we found that BMI was strongly and independently related to greater concentrations of both established and emerging inflammatory

markers that may increase CVD risk. Independent of BMI, regular physical activity was also associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that, although obesity has a clear impact on inflammation, physical activity appears to mitigate at least some of this effect.

For example, overweight individuals had CRP levels that were similar to levels observed in obese individuals if they reported no regular exercise (4.05 and 4.83 μg/mL, respectively). CRP levels greater than 3 μg/mL are typically associated with high CVD risk(26). In overweight subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it below [<500], within [500-1000] or above [>1000] USDHHS MMW recommendations), CRP levels were lower and not significantly different from CRP levels found in normal weight participants (). This suggests that increasing physical activity level to a minimum of 3 days per week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.

Obese individuals may require a higher level of regular physical activity in order to lower inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1 compared to lean and overweight subjects, those who reported >1000 MMW (above the USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than obese subjects reporting no regular physical activity. Taken together, we may speculate that while physical activity levels currently recommended for the general population may reduce particular inflammatory makers in overweight populations, obese populations may require greater levels of physical activity above recommended values to reduce inflammatory markers like CRP and sICAM-1.

As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen, sICAM-1 and sE-selectin, in agreement with previous work (27-30). Independent of BMI effects,

our results suggest that physical activity has differentiating effects on inflammatory markers. Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and determinants in CArdiovascular disease (MONICA) study(31), the National Health and Nutrition Examination Survey (NHANES III)(12, 14) and the Multi-Ethnic Study of Atherosclerosis (MESA)(32), such that both increased frequency and intensity of physical activity have been related to lower IL-6 and fibrinogen. Our findings add to these prior results by standardizing levels of physical activity by using USDHHS. Our results suggest that, regular physical activity at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and possibly fibrinogen, independent of BMI.

Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and physical activity was detected for CRP, but not IL-6. While IL-6 and CRP were significantly correlated (r=0.514, see Supplemental Table 1), this correlation suggests that IL-6 levels do not fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing to its substantially longer plasma half-life (37), which may improve our ability to detect interaction effects in CRP compared to IL-6.

Interestingly, our results also suggest that regular exercise may have a more profound impact on lowering classical markers of inflammation and less impact on the inflammatory status of the endothelium. Regular physical activity has reliably been associated with lower levels of IL-6 and CRP, both classical inflammatory markers related to adipose and systemic inflammation(38). However, regular exercise appeared to have no independent impact on

markers of endothelial activation, particularly sE-selectin. Higher levels of exercise were related to lower sICAM-1 in obese individuals only. In one prior study, inverse relationships between physical activity and sICAM-1 and sE-selectin were reported in drug-treated hypertensive men (39). Thus, further research is necessary to understand mechanisms underlying differential associations of exercise with systemic and endothelial inflammation.

Several limitations must be addressed. First, the cross-sectional design does not allow us to infer causal relationships. Prospective and interventional designs are necessary to confirm our findings. No objective measures of physical activity were available in the MIDUS sample. Therefore, the use of self-report physical activity data may have diminished our ability to detect effects. However, in addition to being in line with previous studies using self-report physical activity, our findings are also in line with previous studies(40, 41) that demonstrated that higher cardiorespiratory fitness, as measured by indirect calorimetry, was associated with lower levels of inflammation independent of visceral adiposity or BMI. Another limitation is that the sample was predominantly comprised of non-Hispanic white individuals, suggesting that findings may not extend to all ethnicities. Finally, BMI and physical activity variables are correlated, potentially raising the concern of small sample sizes in specific groups crossed by BMI and MMW. However, the smallest group for analyses still contained 54 individuals (normal weight individuals reporting no exercise).

In summary, our results demonstrate both interactive and independent influences of BMI and levels of physical activity on both established and emerging markers of inflammation. Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular physical activity appears to mitigate the effects of higher BMI on some inflammatory markers, particularly CRP, which is strongly implicated in CVD. Importantly, while any level of regular

physical activity may help reduce inflammation in overweight individuals, similar effects in obese individuals may require levels of physical activity that are greater than currently recommended by the USDHHS for general health.

ACKNOWLEDGEMENTS

We thank the staff of the Clinical Research Centers at the University of Wisconsin-Madison, UCLA, and Georgetown University for their effort in conducting the original data collection.

FUNDING

KS was supported through a T32 Training Fellowship (Training in Behavioral and Preventive Medicine; T32 HL076134). The original research was supported by a grant from the National Institute on Aging (P01-AG020166) to conduct a longitudinal follow-up of the MIDUS (Midlife in the U.S.) Investigation.

The original study was supported by the John D. and Catherine T. MacArther Foundation Research Network on Successful Midlife Development and by the following grants: M01-RR023942 (Georgetown), M01-RR00865 (UCLA) from the General Clinical Research Centers Program and 1UL1RR025011 (UW) from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources, National Institutes of Health.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTORSHIP

KS, JMM and RRW each made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation, as well as to drafting and revision for substantial intellectual content. All authors made final approval of the version to be published.

DATA SHARING STATEMENT

Data and documentation for MIDUS studies are available at the Inter-university Consortium for Political and Social Research (ICPSR). http://www.icpsr.umich.edu/icpsrweb/landing.jsp

ARTICLE SUMMARY

Article focus

- Systemic inflammation is related to the progression of cardiovascular disease.
- Independent of obesity, physical activity is inversely related to concentrations of well-established inflammatory biomarkers, such as C-reactive protein (CRP) or interleukin-6 (IL-6).
- This article evaluates interactive effects of body mass index and physical activity on established inflammatory markers, CRP, IL-6, and emerging inflammatory markers, fibrinogen, soluble intracellular adhesion molecule (sICAM)-1, soluble E-selectin, and IL-6 soluble receptor.

Key messages

- Interactive effects of body mass index and physical activity were observed for CRP, such that regular physical activity reported by overweight individuals was related to significantly lower CRP levels compared to those reported no regular activity.
- Independent of BMI, regular physical activity was related to lower IL-6, with a trend for lower fibrinogen
- Physical activity had no independent effect on circulating markers related to endothelial inflammation, such as sICAM-1 or sE-selectin.

Strengths and limitations

- 1254 adults from the National Survey of Midlife Development in the United States (MIDUS) Biomarker Project were analyzed. Statistical analyses were adjusted for age, sex, smoking, and relevant medication use. A strength of this paper is categorizing physical activity levels based on national recommendations. This data may be used to determine appropriate levels of physical activity necessary for reducing inflammation in overweight and obese adults. However, cross-sectional data is limited, as causal inferences cannot be obtained. A second limitation is that the sample was predominantly comprised of non-Hispanic white individuals, therefore findings may not extend to all ethnicities.

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FIGURE AND TABLE LEGENDS

Figure 1: Inflammatory Markers. Data from 1254 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. The analysis for sICAM-1 was further adjusted for race. Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value, INT=interaction effect P value.

Table 1: Subject Characteristics. BMI = body mass index; CRP = C-reactive protein; IL = interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin = soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.

Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A Cohort Study of Middle Aged Adults Living in the United States

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Running Title:

BMI, Physical Activity and Inflammation

Key Words: MIDUS, Intracellular Adhesion Molecule-1, Fibrinogen, C-Reactive Protein

Word Count: 3331

ABSTRACT

Objectives. Determine whether body mass index (BMI) and physical activity (PA) above, at or below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP), fibrinogen, IL-6 soluble receptor (IL-6sr), soluble E-selectin and soluble intracellular adhesion molecule (sICAM)-1. **Design.** Archived cohort data (N=1254, age 54.5±11.7y, BMI 29.8±6.6kg/m^2) from the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study were analyzed for concentrations of inflammatory markers using general linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m^2. Analyses were adjusted for age, sex, smoking and relevant medication use. **Setting.** Respondents reported to three centers to complete questionnaires and provide blood samples. **Participants.** Participants were men and women currently enrolled in the MIDUS Biomarker Project (N=1254, 93% non-hispanic white, average age 54.5y). **Primary Outcome Measures.** Concentration of serum IL-6, CRP, fibrinogen, IL-6sr, sE-selectin and sICAM. **Results.** Significant interactions were found between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was similar to obese when no PA was reported, but was similar to normal weight when any level of regular PA was reported. sICAM-1 was differentially lower in obese individuals who reported >1000 MMW compared to obese individuals reporting less exercise. **Conclusion.** The association of exercise with CRP and sICAM-1 differed by BMI, suggesting that regular exercise may buffer weight-associated elevations in CRP in overweight individuals while higher levels of exercise may be necessary to reduce sICAM-1 or CRP in obese individuals. **Trial Registry.** N/A.

INTRODUCTION

Obesity paired with low physical activity is well known to increase morbidity and mortality related to cardiovascular disease (CVD)(1). It is less clear, however, whether the benefits of higher levels of physical activity differ among normal weight, overweight, and obese individuals.

Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Circulating Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of inflammatory markers(7, 8). Further, body fat distribution is also an important factor relating to inflammatory status. Accumulation of fat in visceral depots is more strongly associated with low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots(9, 10).

The effects of physical activity on markers of inflammation are more complex and may vary depending on body weight. A number of epidemiological studies have shown an inverse relationship between physical activity and CRP and IL-6, independent of obesity(11-16). Laboratory studies conducted in aerobically trained, typically normal weight, individuals have demonstrated that a single bout of exercise stimulates IL-6 release from skeletal muscle, which promotes anti-inflammatory effects (17-19), as opposed to adipose tissue-derived IL-6 that is associated with pro-inflammatory effects (20). Randomized controlled trials have also been conducted, often in populations that also tend to be overweight or obese, to examine the effects

of aerobic exercise interventions on inflammation and the results are mixed (21). Thus, the contribution of physical activity to inflammation in the context of obesity remains unclear.

The purpose of our study was to disentangle the relative contributions of BMI and physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr, CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were determined using values put forth by the Physical Activity Guidelines for Americans, which states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week) produces substantial health benefits for adults(22). We hypothesized that BMI and MMW would interact, such that greater MMW reported would lessen the impact of obesity on markers of inflammation.

MATERIALS AND METHODS

Design and Sample. This study was a cross-sectional analysis of archived data (BMI, self-reported physical activity and inflammatory biomarker concentrations) from 1254 respondents who provided consent (as approved by The University of Wisconsin Madison Health Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study (23). The Biomarker Project was one of 5 projects within MIDUS II, with the purpose of adding comprehensive biological assessments on a subsample of the MIDUS participants to further understand age-related differences in physical and mental health. Participants were eligible for The Biomarker Project if they were previously enrolled in MIDUS I, which recruited non-institutionalized, English-speaking adults residing in the contiguous United States aged 25-74. The random digit

dialing sample for the parent study was selected from working telephone banks and a list of all individuals between the ages of 25 and 74 years within each household was generated in order to select a random respondent. Those who agreed to participate in the Biomarker Study stayed overnight at one of three General Clinical Research Centers: University of California Los Angeles, University of Wisconsin-Madison and Georgetown University. Upon arrival, each respondent provided a detailed medical history (including physical activity levels) and provided all prescription, over-the-counter, and alternative medications to be inventoried by project staff. Following an overnight stay, morning fasting blood samples were obtained. Cohorts were assessed between July 2004 and May 2009 as a follow up to MIDUS I respondents that were previously surveyed by the MacArthur Midlife Research Network between 1995 and 1996. Based on the sample of 1254 participants, 80% power was achieved to detect small effects of 0.08 or greater with alpha level at 0.05 for a two-tailed test(24, 25).

Anthropometrics. Height was measured in centimeters and recorded to the nearest millimeter. A single measure of WC was taken directly on the skin or over a single layer of light, close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the nearest millimeter. Weight was measured in kilograms and BMI was calculated by dividing body mass in kilograms by height in meters squared. BMI categories were organized into 3 groups: normal weight (BMI ≤24.9 kg/m^2), overweight (BMI≥25-29.9) and obese (BMI≥30).

Categorizing Physical Activity by MET-Minutes per Week (MMW).

The MMW variable was calculated using data provided in the medical history form. The form first described 3 types of regular physical activity(23):

Vigorous: *Which causes your heart to beat so rapidly you can feel it in your chest and you perform it long enough to work up a good sweat and breathe heavily (e.g.,*

competitive sports, running, vigorous swimming, high intensity aerobics, digging in the garden, or lifting heavy objects).

Moderate: *Which causes your heart rate to increase slightly and you typically work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming, low intensity aerobics or golfing without a power cart, brisk walking, mowing the lawn with a walking lawnmower).*

Light: *Which requires little physical effort (e.g., light housekeeping like dusting or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).*

Keeping these definitions in mind, participants were asked if they engaged in regular physical activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants answered "yes", they entered up to 7 types of seasonal and/or non-seasonal exercise or activity along with the frequency, duration and intensity.

MMW were calculated in a 2-step process. Step 1: subjects who reported no physical activity (for whom no MMW calculations could be made) were designated as the no regular exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity reported. Four groups reflecting participation in physical activity and whether or not their participation was below, at or above USDHHS guidelines were created: NRE (reported no regular physical activity), below recommended (reported <500 MMW), recommended (reported 500-1000 MMW) and above recommended (reported >1000 MMW).

Blood Collection, Processing and Assays. Participants were asked to avoid strenuous activity the day of blood collection. Venous blood samples were collected in 10 mL serum

separator vacutainers following a 12-h overnight fast and processed at a General Clinical Research Center using standardized procedures. Blood samples were not collected at any specific point during the menstrual cycle in female participants. Briefly, following collection, vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-Selectin and sICAM-1).

IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison, Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600, R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV% values for IL-6sr were 5.9-5.7% and 2.0%, respectively.

Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay; R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay, R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%, respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer

with a particle enhanced immunonepolometric assay. Intra-assay and inter-assay precision for CRP was 2.3-4.4% and 2.1-5.7%, respectively.

Statistical Analyses. All variables were assessed for normality and non-normal data were log transformed, which included data for CRP, IL-6, IL-6sr, fibrinogen, sE-selectin and sICAM-1. General Linear Models were performed to determine the relationship of MMW and BMI with the inflammatory markers. For each outcome, the ordinal MMW and BMI factors were entered as independent factors with an interaction term. If the interaction term was not significant, the interaction term was dropped and the model was re-fit for main effects only. Pairwise comparisons were assessed using post hoc univariate analyses with a Bonferroni adjustment for multiple comparisons. Covariates for all models included factors that are known to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering, corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive). Race was initially included as a covariate; however, approximately 200 data points were lost in the analyses due to incomplete racial data. As race was not found to be a predictor of our dependent variables, with the exception of sICAM-1, race was excluded as a covariate to increase samples size in all analyses excluding sICAM-1. All statistical analyses were performed with SPSS v. 17 (Chicago, IL) and statistical significance was set $\alpha = 0.05$.

In an exploratory analysis, we examined whether the relative effects of BMI and MMW on the inflammatory markers differed by sex in 3-way interaction models. As none of the interactions approached statistical significance (data not shown), sex was retained as a covariate in the models.

RESULTS

Subject Characteristics. Table 1 presents anthropometric characteristics and circulating levels of inflammatory biomarkers in all subjects (N=1254). Subjects were 92.6% non-Hispanic white, 56.8% female, and, on average, middle-aged and overweight. Of all the respondents, 14.9% were currently smoked, 27.8% were taking cholesterol lowering medication, 12.1% corticosteroids, 10.4% anti-diabetic medication, 14.2% antidepressant medication, 7.3% hormone replacement and 2.5% oral contraceptives. The percentage of participants with missing data for each variable were as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6% for fibrinogen, 1.2% for sE-selectin, and 1.0%for IL-6sr.

CRP (Figure 1, Panel A). We found a significant interaction between BMI and MMW for CRP concentration (F=3.022, P=0.006). In post hoc comparisons, CRP levels were higher in overweight and obese subjects compared to normal weight subjects among those who reported no regular exercise (P's<0.001). However, among subjects who reported any amount of regular exercise (<500, 500-1000 or >1000 MMW), CRP levels were significantly greater only in obese subjects compared to both normal weight and overweight subjects (P's <0.01). In obese individuals, CRP tended to be lower in those reporting >1000 MMW compared to those reporting no regular exercise (P=0.053).

We also found main effects of BMI (F=130.873 P<0.001) and MMW (F=11.576, P<0.001) on CRP. CRP was significantly greater with each increasing BMI category, in a dose-dependent manner (P's<0.001). Compared to participants who reported no regular exercise, CRP was significantly lower in those who reported 500-1000 and >1000 MMW (P's <0.01), with a trend for lower CRP in those who reported <500 MMW of regular exercise (P=0.078).

sICAM-1 (Figure 1, Panel B). We found a significant interaction between BMI and MMW for sICAM-1 concentration (F=2.701, P=0.013). Levels of sICAM-1 were significantly lower in obese subjects who reported >1000 MMW compared to obese subjects who reported no regular exercise (P=0.014) and <500 MMW (P=0.026) and tended to be lower than levels in obese subjects who reported 500-1000 MMW (P=0.079). No differences in sICAM-1 by MMW were observed among normal weight or overweight individuals.

We also observed a main effect of BMI (F=6.060, P=0.002), such that sICAM-1 levels in obese participants were significantly higher than levels found in both normal weight and overweight participants (P's<0.01). No significant main effect of MMW was found for sICAM-1 (F=0.931, P=0.425).

IL-6 (Figure 1, Panel C). Both BMI and MMW had independent effects on circulating concentrations of IL-6 (BMI: F=60.150, P<0.001, MMW: F=10.680, P<0.001), with no significant interaction (F=1.21, P=0.297). We found a dose-dependent effect of BMI, such that higher BMI levels were associated with significantly greater IL-6 (P's<0.001). Independent of BMI, IL-6 was significantly lower in subjects who reported regular exercise (<500 MMW, 500-1000 MMW and >1000 MMW) compared to those who reported no regular exercise (P's <0.01) with no difference between levels of MMW.

Fibrinogen (Figure 1, Panel D). BMI significantly contributed to circulating levels of fibrinogen (F=42.385, P<0.001), such that dose-dependent increases were observed for all BMI levels (P's<0.01). While we observed a trend for lower fibrinogen with regular physical activity, similar to that of IL-6, the effect did not reach statistical significance (F=2.187, P=0.088). We observed no significant interaction between BMI and MMW for fibrinogen (F=1.680, P=0.122).

sE-Selectin (1, Panel E). BMI significantly contributed to circulating levels of sE-selectin (F=28.253, P<0.001) with no significant contribution by MMW (F=0.207, P=0.892). Dose-dependent increases in sE-selectin were also observed across BMI levels (P's<0.01). We observed no significant interaction between BMI and MMW for sE-selectin (F=0.570, P=0.755).

IL-6sr (Figure 1, Panel F). No significant main effects for BMI (F=1.783, P=0.169), MMW (F=1.434, P=0.231) or their interaction (F=0.834, P=0.544) were detected for IL-6sr.

Waist Circumference (WC) and Inflammatory Markers (Supplemental Figure 1). A secondary analysis was completed using WC and MMW as independent variables and the complete results of these analyses are located in the supplemental information. Briefly, we found a significant interaction between WC and MMW on sICAM-1. In individuals with an at-risk WC (≥102.0 cm for men and ≥88.0 cm for women), sICAM-1 was significantly lower in those reporting 1000+ MMW compared to less than 500 MMW and tended to be lower in those reporting no regular exercise. Overall, main effects were similar to those found for BMI and MMW analyses. Having an at-risk WC was independently related to higher levels of CRP, sICAM-1, IL-6, fibrinogen and sE-selectin. Independent of WC, any level of regular exercise was related to lower levels of CRP, IL-6 with a similar tendency for fibrinogen.

DISCUSSION

The current study aimed to determine whether the impact of BMI and MMW on inflammatory markers varied by level of overweight or obesity. For CRP and s-ICAM-1 regular physical activity appeared to diminish the effects of higher BMI compared to those who reported no regular physical activity. In addition, we found that BMI was strongly and independently related to greater concentrations of both established and emerging inflammatory

markers that may increase CVD risk. Independent of BMI, regular physical activity was also associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that, although obesity has a clear impact on inflammation, physical activity appears to mitigate at least some of this effect.

For example, overweight individuals had CRP levels that were similar to levels observed in obese individuals if they reported no regular exercise (4.05 and 4.83 μg/mL, respectively). CRP levels greater than 3 μg/mL are typically associated with high CVD risk(26). In overweight subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it below [<500], within [500-1000] or above [>1000] USDHHS MMW recommendations), CRP levels were lower and not significantly different from CRP levels found in normal weight participants (). This suggests that increasing physical activity level to a minimum of 3 days per week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.

Obese individuals may require a higher level of regular physical activity in order to lower inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1 compared to lean and overweight subjects, those who reported >1000 MMW (above the USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than obese subjects reporting no regular physical activity. Taken together, we may speculate that while physical activity levels currently recommended for the general population may reduce particular inflammatory makers in overweight populations, obese populations may require greater levels of physical activity above recommended values to reduce inflammatory markers like CRP and sICAM-1.

As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen, sICAM-1 and sE-selectin, in agreement with previous work (27-30). Independent of BMI effects,

our results suggest that physical activity has differentiating effects on inflammatory markers. Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and determinants in CArdiovascular disease (MONICA) study(31), the National Health and Nutrition Examination Survey (NHANES III)(12, 14) and the Multi-Ethnic Study of Atherosclerosis (MESA)(32), such that both increased frequency and intensity of physical activity have been related to lower IL-6 and fibrinogen. Our findings add to these prior results by standardizing levels of physical activity by using USDHHS. Our results suggest that, regular physical activity at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and possibly fibrinogen, independent of BMI.

Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and physical activity was detected for CRP, but not IL-6. While IL-6 and CRP were significantly correlated (r=0.514, see Supplemental Table 1), this correlation suggests that IL-6 levels do not fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing to its substantially longer plasma half-life (37), which may improve our ability to detect interaction effects in CRP compared to IL-6.

Interestingly, our results also suggest that regular exercise may have a more profound impact on lowering classical markers of inflammation and less impact on the inflammatory status of the endothelium. Regular physical activity has reliably been associated with lower levels of IL-6 and CRP, both classical inflammatory markers related to adipose and systemic inflammation(38). However, regular exercise appeared to have no independent impact on

markers of endothelial activation, particularly sE-selectin. Higher levels of exercise were related to lower sICAM-1 in obese individuals only. In one prior study, inverse relationships between physical activity and sICAM-1 and sE-selectin were reported in drug-treated hypertensive men (39). Thus, further research is necessary to understand mechanisms underlying differential associations of exercise with systemic and endothelial inflammation.

Several limitations must be addressed. First, the cross-sectional design does not allow us to infer causal relationships. Prospective and interventional designs are necessary to confirm our findings. No objective measures of physical activity were available in the MIDUS sample. Therefore, the use of self-report physical activity data may have diminished our ability to detect effects. However, in addition to being in line with previous studies using self-report physical activity, our findings are also in line with previous studies(40, 41) that demonstrated that higher cardiorespiratory fitness, as measured by indirect calorimetry, was associated with lower levels of inflammation independent of visceral adiposity or BMI. Another limitation is that the sample was predominantly comprised of non-Hispanic white individuals, suggesting that findings may not extend to all ethnicities. Finally, BMI and physical activity variables are correlated, potentially raising the concern of small sample sizes in specific groups crossed by BMI and MMW. However, the smallest group for analyses still contained 54 individuals (normal weight individuals reporting no exercise).

In summary, our results demonstrate both interactive and independent influences of BMI and levels of physical activity on both established and emerging markers of inflammation. Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular physical activity appears to mitigate the effects of higher BMI on some inflammatory markers, particularly CRP, which is strongly implicated in CVD. Importantly, while any level of regular

physical activity may help reduce inflammation in overweight individuals, similar effects in obese individuals may require levels of physical activity that are greater than currently recommended by the USDHHS for general health.

ACKNOWLEDGEMENTS

We thank the staff of the Clinical Research Centers at the University of Wisconsin-Madison, UCLA, and Georgetown University for their effort in conducting the original data collection.

FUNDING

KS was supported through a T32 Training Fellowship (Training in Behavioral and Preventive Medicine; T32 HL076134). The original research was supported by a grant from the National Institute on Aging (P01-AG020166) to conduct a longitudinal follow-up of the MIDUS (Midlife in the U.S.) Investigation.

The original study was supported by the John D. and Catherine T. MacArther Foundation Research Network on Successful Midlife Development and by the following grants: M01-RR023942 (Georgetown), M01-RR00865 (UCLA) from the General Clinical Research Centers Program and 1UL1RR025011 (UW) from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources, National Institutes of Health.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTORSHIP

KS, JMM and RRW each made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation, as well as to drafting and revision for substantial intellectual content. All authors made final approval of the version to be published.

DATA SHARING STATEMENT

Data and documentation for MIDUS studies are available at the Inter-university Consortium for Political and Social Research (ICPSR). http://www.icpsr.umich.edu/icpsrweb/landing.jsp

ARTICLE SUMMARY

Article focus

- Systemic inflammation is related to the progression of cardiovascular disease.
- Independent of obesity, physical activity is inversely related to concentrations of well-established inflammatory biomarkers, such as C-reactive protein (CRP) or interleukin-6 (IL-6).
- This article evaluates interactive effects of body mass index and physical activity on established inflammatory markers, CRP, IL-6, and emerging inflammatory markers, fibrinogen, soluble intracellular adhesion molecule (sICAM)-1, soluble E-selectin, and IL-6 soluble receptor.

Key messages

- Interactive effects of body mass index and physical activity were observed for CRP, such that regular physical activity reported by overweight individuals was related to significantly lower CRP levels compared to those reported no regular activity.
- Independent of BMI, regular physical activity was related to lower IL-6, with a trend for lower fibrinogen
- Physical activity had no independent effect on circulating markers related to endothelial inflammation, such as sICAM-1 or sE-selectin.

Strengths and limitations

- 1254 adults from the National Survey of Midlife Development in the United States (MIDUS) Biomarker Project were analyzed. Statistical analyses were adjusted for age, sex, smoking, and relevant medication use. A strength of this paper is categorizing physical activity levels based on national recommendations. This data may be used to determine appropriate levels of physical activity necessary for reducing inflammation in overweight and obese adults. However, cross-sectional data is limited, as causal inferences cannot be obtained. A second limitation is that the sample was predominantly comprised of non-Hispanic white individuals, therefore findings may not extend to all ethnicities.

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FIGURE AND TABLE LEGENDS

Figure 1: Inflammatory Markers. Data from 1254 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. The analysis for sICAM-1 was further adjusted for race. Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value, INT=interaction effect P value.

Table 1: Subject Characteristics. BMI = body mass index; CRP = C-reactive protein; IL = interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin = soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any pre-specified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4-7*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	8-10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	8-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Demographic Variables	BMI < 25 N=298	BMI 25-29.9 N=440	BMI ≥30 N=516	Overall N = 1254
			Mean ± SD	
Age (years)	54.6 ± 12.8	56.4 ± 11.7	54.6 ± 11.2	54.5 ± 11.7
Gender (%)				
Male	31.2	52.7	42.1	43.20
Female	68.8	47.3	57.9	56.80
Race (%)				
Non-Hispanic White	94.0	94.0	90.3	92.60
Hispanic	0.4	0.8	0.3	0.05
African American	1.9	1.6	4.0	2.60
Asian/Pacific Islander	0.7	0.3	0.0	0.30
Native American	1.1	0.8	2.0	1.30
Other	1.9	2.6	3.5	2.30
Medication Use (%)				
Cholesterol-Lowering	13.1	32.3	32.6	27.80
Corticosteroids	12.8	12.5	11.4	12.10
Anti-Diabetic	4.7	8.4	15.3	10.40
Antidepressant	14.4	13.4	16.9	14.2
Hormone Replacement Therapy	9.4	8.6	5.0	7.3
Oral Contraceptive	3.7	3.4	1.0	2.5
Currently Smoking	17.8	14.1	14.0	14.90
BMI (kg/m^2)	22.7 ± 1.8	27.4 ± 1.4	35.9 ± 5.7	29.8 ± 6.6
IL-6 (pg/mL)	2.4 ± 3.1	2.7 ± 2.48	3.7 ± 3.2	3.0 ± 3.1
IL-6sr (pg/mL)	34473.1 ± 10861.9	35337.4 ± 10065.1	35475.7 ± 10325.7	35184.7 ± 10359.1
CRP (μg/mL)	1.5 ± 2.5	2.5 ± 4.0	4.4 ± 5.9	3.0 ± 4.8
Fibrinogen (mg/dL)	315.8 ± 75.9	343.2 ± 82.1	373 ± 92.1	348.9 ± 87.9
sE-Selectin (ng/mL)	36.9 ±19.6	41.2 ± 20.6	49.1 ± 24.7	43.4 ± 22.7
sICAM-1 (ng/mL)	284.8 ± 122.0	276.2 ± 99.9	301.4 ± 123.1	288.6 ± 115.6

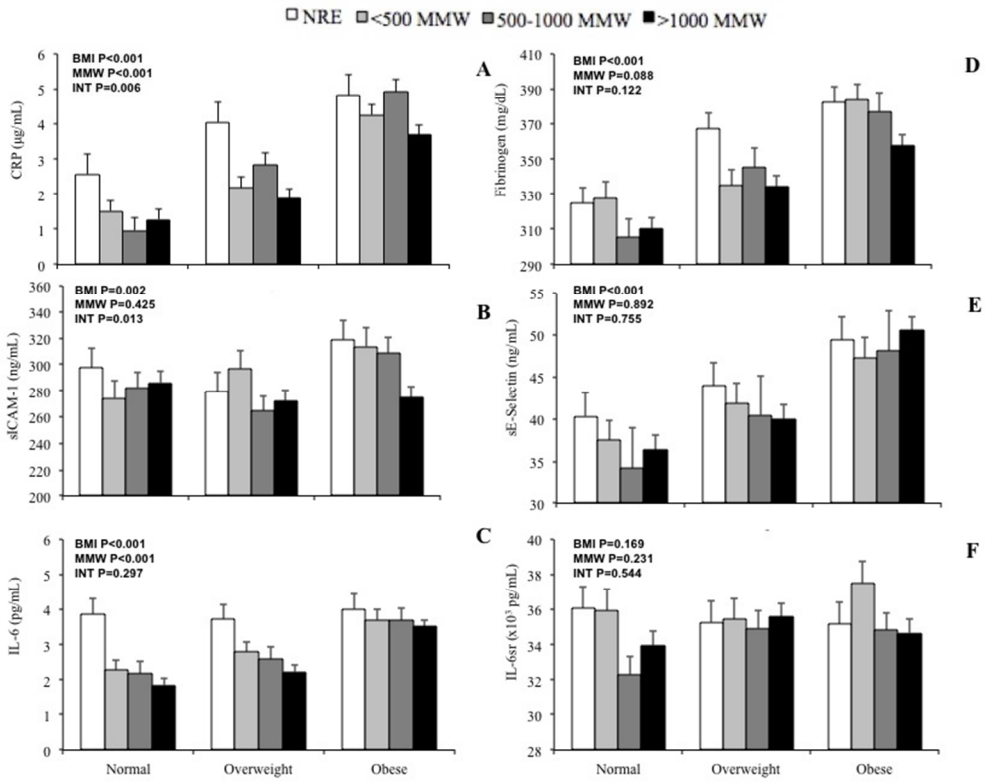


Figure 1: Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value, INT=interaction effect P value.
292x229mm (72 x 72 DPI)

Waist Circumference (WC) and MET-Minutes per Week (MMW)

CRP (Supplementary Figure 1, panel A). We found no significant interaction effect between WC and MMW for CRP (F=1.426, P=0.234). We found significant main effects for WC (F=159.669, P<0.001) and MMW (F=9.766, P<0.001) on circulating CRP. CRP levels were lower in participants who reported a normal waist circumference and any level of regular exercise (<500, 500-1000, and >1000 MMW), compared to those with an at-risk waist circumference (P's<0.001) and those no regular exercise (P's<0.05).

sICAM-1 (Supplementary Figure 1, panel B). We found a significant interaction effect between WC and MMW for sICAM-1 (F=4.846, P=0.002). While sICAM-1 levels were not significantly difference across MMW categories in individuals with a normal WC (P's>0.05), in individuals with an at-risk WC, sICAM-1 was significantly lower in those reporting 1000+ MMW compared to less than 500 MMW (P=0.007) and tended to be lower in those reporting no regular exercise (P=0.072). Similar to BMI, waist circumference independently contributed to sICAM-1 (F=26.841,, P<0.001), such that values were greater in subjects with an at-risk WC compared to those with a normal WC (P <0.001). No effect of MMW was observed (F=1.055, P=0.367) for sICAM-1.

IL-6 (Supplementary Figure 1, panel C). We found no significant interaction effect between WC and MMW for IL-6 (F=1.282, P=0.217). We found significant main effects for waist circumference (F=84.441, P<0.001) and MMW (F=10.255, P<0.001), such that IL-6 levels were lower in participants who reported an normal waist circumference and any level of regular exercise (<500, 500-1000, and >1000 MMW), compared to those with an at risk waist circumference (P's<0.001) and those reporting no regular exercise (P's<0.05).

Fibrinogen (Supplementary Figure 1, panel D). We found no significant interaction effect between WC and MMW for fibrinogen (F=2.019, P=0.110). Waist circumference also impacted fibrinogen (F=38.960, P<0.001), such that values were greater in subjects with an at-risk waist circumference compared to those with a normal waist circumference (P's <0.001). The effect of MMW on fibrinogen bordered on statistical significance (F=2.245, P=0.081), such that values were lower with in individuals who reported greater MMW.

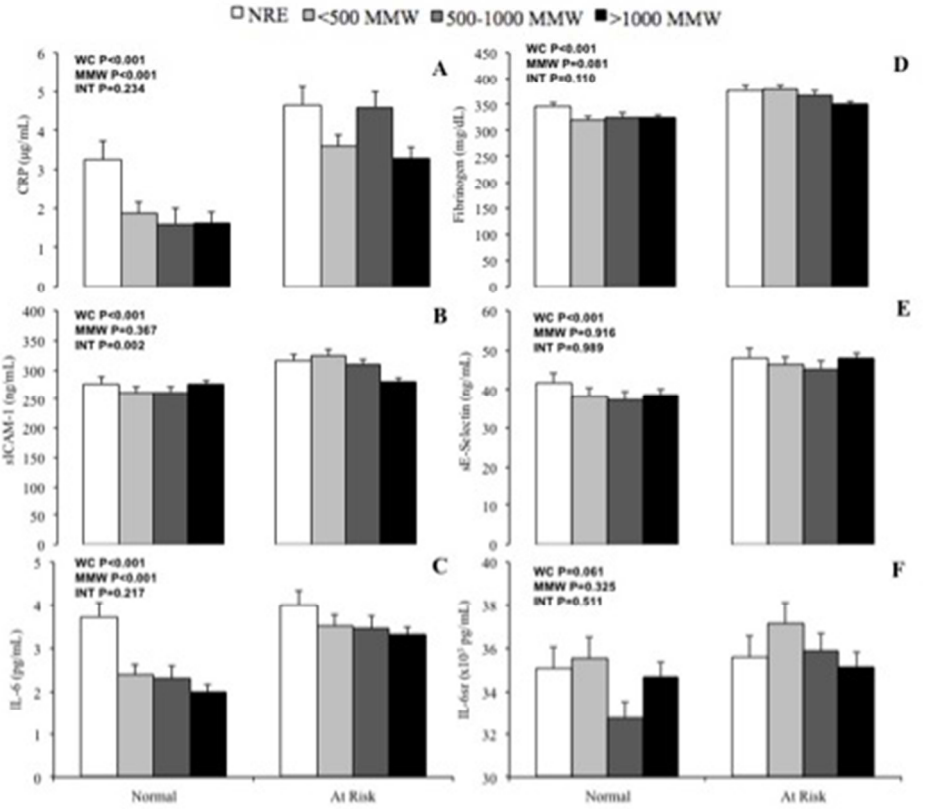
sE-Selectin (Supplementary Figure 1, panel E). We found no significant interaction between WC and MMW for sE-Selectin (F=0.041, P=0.989). Waist circumference also independently contributed to sE-selectin (F=40.967, P<0.001), such that values were greater in subjects with an at-risk waist circumference compared to those with a normal waist circumference (P <0.001). No effect of MMW was observed for sE-selectin (F=0.172, P=0.916).

IL-6sr (Supplementary Figure 1, panel F). We found no significant interaction effect between WC and MMW for IL-6sr (F=0.769, P=0.511). Like BMI, we found no main effects for waist circumference (F=3.505, P=0.061) or MMW on IL-6sr (F=1.158, P=0.325).

Interrelationship of Inflammatory Markers. Correlations between all inflammatory markers are shown in Supplementary Table 1.

Figure Legend

Supplemental Figure 1. Waist Circumference (WC), MET Minutes per Week (MMW) and Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of WC category (normal [(≥102.0 cm for men and ≥88.0 cm for women], at risk [(>102.0 cm for men and >88.0 cm for women]) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. Error bars represent SEM. WC=WC main effect, MMW=MMW main effect, INT=interaction effect.



Supplemental Figure 1. Waist Circumference (WC), MET Minutes per Week (MMW) and Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of WC category (normal [(≥102.0 cm for men and ≥88.0 cm for women], at risk [(>102.0 cm for men and >88.0 cm for women]) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. Error bars represent SEM. WC=WC main effect, MMW=MMW main effect, INT=interaction effect.
165x147mm (72 x 72 DPI)

	IL-6	IL-6sr	Fibrinogen	CRP	sE-Selectin
IL-6					
IL-6sr	0.037				
Fibrinogen	0.417**	0.017			
CRP	0.514**	0.053	0.513**		
sE-Selectin	0.213**	0.035	0.104**	0.156**	
sICAM-1	0.134**	0.140**	0.092**	0.144**	0.041

Supplemental Table 1. Correlations of Inflammatory Biomarkers. IL-6 = Interleukin-6, IL-6sr = IL-6 soluble receptor, CRP = C-reactive protein, sE-Selectin = soluble E-Selectin and sICAM-1 = soluble intracellular adhesion molecule – 1. * denotes significance at $p \leq 0.05$, ** denotes significance at $p \leq 0.001$.

BMJ open

Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A Cohort Study of Middle Aged Adults Living in the United States

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002623.R2
Article Type:	Research
Date Submitted by the Author:	08-Apr-2013
Complete List of Authors:	Strohacker, Kelley McCaffery, Jeanne wing, rena
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	Immunology < BASIC SCIENCES, EPIDEMIOLOGY, Public health < INFECTIOUS DISEASES

Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A Cohort Study of Middle Aged Adults Living in the United States

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Running Title:

BMI, Physical Activity and Inflammation

Key Words: MIDUS, Intracellular Adhesion Molecule-1, Fibrinogen, C-Reactive Protein

Word Count: 3931

ARTICLE SUMMARY

Article focus

- Systemic inflammation is related to the progression of cardiovascular disease.
- Independent of obesity, physical activity is inversely related to concentrations of well-established inflammatory biomarkers, such as C-reactive protein (CRP) or interleukin-6 (IL-6).
- This article evaluates interactive effects of body mass index and physical activity on established inflammatory markers, CRP, IL-6, and emerging inflammatory markers, fibrinogen, soluble intracellular adhesion molecule (sICAM)-1, soluble E-selectin, and IL-6 soluble receptor.

Key messages

- Interactive effects of body mass index and physical activity were observed for CRP, such that regular physical activity reported by overweight individuals was related to significantly lower CRP levels compared to those reported no regular activity.
- Independent of BMI, regular physical activity was related to lower IL-6, with a trend for lower fibrinogen
- Physical activity had no independent effect on circulating markers related to endothelial inflammation, such as sICAM-1 or sE-selectin.

Strengths and limitations

- 1254 adults from the National Survey of Midlife Development in the United States (MIDUS) Biomarker Project were analyzed. Statistical analyses were adjusted for age, sex, smoking, and relevant medication use. A strength of this paper is categorizing physical activity levels based on national recommendations. This data may be used to determine appropriate levels of physical activity necessary for reducing inflammation in overweight and obese adults. However, cross-sectional data is limited, as causal inferences cannot be obtained. A second limitation is that the sample was predominantly comprised of non-Hispanic white individuals, therefore findings may not extend to all ethnicities.

ABSTRACT

Objectives. Determine whether body mass index (BMI) and physical activity (PA) above, at or below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP), fibrinogen, IL-6 soluble receptor (IL-6sr), soluble E-selectin and soluble intracellular adhesion molecule (sICAM)-1.

Design. Archived cohort data (N=1254, age 54.5±11.7y, BMI 29.8±6.6kg/m^2) from the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study were analyzed for concentrations of inflammatory markers using general linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m^2. Analyses were adjusted for age, sex, smoking and relevant medication use.

Setting. Respondents reported to three centers to complete questionnaires and provide blood samples.

Participants. Participants were men and women currently enrolled in the MIDUS Biomarker Project (N=1254, 93% non-hispanic white, average age 54.5y).

Primary Outcome Measures. Concentration of serum IL-6, CRP, fibrinogen, IL-6sr, sE-selectin and sICAM.

Results. Significant interactions were found between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was similar to obese when no PA was reported, but was similar to normal weight when any level of regular PA was reported. sICAM-1 was

differentially lower in obese individuals who reported >1000 MMW compared to obese individuals reporting less exercise.

Conclusion. The association of exercise with CRP and sICAM-1 differed by BMI, suggesting that regular exercise may buffer weight-associated elevations in CRP in overweight individuals while higher levels of exercise may be necessary to reduce sICAM-1 or CRP in obese individuals.

Trial Registry. N/A.

INTRODUCTION

Obesity paired with low physical activity is well known to increase morbidity and mortality related to cardiovascular disease (CVD)(1). It is less clear, however, whether the benefits of higher levels of physical activity differ among normal weight, overweight, and obese individuals.

Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Circulating Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of inflammatory markers(7, 8). Further, body fat distribution is also an important factor relating to inflammatory status. Accumulation of fat in visceral depots is more strongly associated with low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots(9, 10).

The effects of physical activity on markers of inflammation are more complex and may vary depending on body weight. A number of epidemiological studies have shown an inverse relationship between physical activity and CRP and IL-6, independent of obesity(11-16). Laboratory studies conducted in aerobically trained, typically normal weight, individuals have demonstrated that a single bout of exercise stimulates IL-6 release from skeletal muscle, which promotes anti-inflammatory effects (17-19), as opposed to adipose tissue-derived IL-6 that is associated with pro-inflammatory effects (20). Randomized controlled trials have also been conducted, often in populations that also tend to be overweight or obese, to examine the effects of aerobic exercise interventions on inflammation and the results are mixed (21). Thus, the contribution of physical activity to inflammation in the context of obesity remains unclear.

The purpose of our study was to disentangle the relative contributions of BMI and physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr, CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were determined using values put forth by the Physical Activity Guidelines for Americans, which states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week) produces substantial health benefits for adults(22). We hypothesized that BMI and MMW would interact, such that greater MMW reported would lessen the impact of obesity on markers of inflammation.

MATERIALS AND METHODS

Design and Sample. This study was a cross-sectional analysis of archived data (BMI, self-reported physical activity and inflammatory biomarker concentrations) from 1254

respondents who provided consent (as approved by The University of Wisconsin Madison Health Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study (23). The Biomarker Project was one of 5 projects within MIDUS II, with the purpose of adding comprehensive biological assessments on a subsample of the MIDUS participants to further understand age-related differences in physical and mental health. Participants were eligible for The Biomarker Project if they were previously enrolled in MIDUS and MIDUS II, which recruited non-institutionalized, English-speaking adults residing in the contiguous United States aged 25-74. Exclusion criteria included non-participation in MIDUS and MIDUS II and unwillingness to travel to specified sites for biomarker assessment. The random digit dialing sample for the parent study was selected from working telephone banks and a list of all individuals between the ages of 25 and 74 years within each household was generated in order to select a random respondent. Those who agreed to participate in the Biomarker Study stayed overnight at one of three General Clinical Research Centers: University of California Los Angeles, University of Wisconsin-Madison and Georgetown University. Upon arrival, each respondent provided a detailed medical history (including physical activity levels) and provided all prescription, over-the-counter, and alternative medications to be inventoried by project staff. Following an overnight stay, morning fasting blood samples were obtained. Cohorts were assessed between July 2004 and May 2009 as a follow up to MIDUS I respondents that were previously surveyed by the MacArthur Midlife Research Network between 1995 and 1996. Based on the MIDUS Biomarker Project sample of 1254 participants, 80% power was estimated to detect small effect sizes (delta=0.08 and higher) with an alpha level at 0.05 for a two-tailed test (24, 25).

Anthropometrics. Height was measured in centimeters and recorded to the nearest millimeter. A single measure of WC was taken directly on the skin or over a single layer of light, close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the nearest millimeter. Weight was measured in kilograms and BMI was calculated by dividing body mass in kilograms by height in meters squared. BMI categories were organized into 3 groups: normal weight (BMI ≤24.9 kg/m^2), overweight (BMI≥25-29.9) and obese (BMI≥30).

Categorizing Physical Activity by MET-Minutes per Week (MMW).

The MMW variable was calculated using data provided in the medical history form. The form first described 3 types of regular physical activity(23):

Vigorous: *Which causes your heart to beat so rapidly you can feel it in your chest and you perform it long enough to work up a good sweat and breathe heavily (e.g., competitive sports, running, vigorous swimming, high intensity aerobics, digging in the garden, or lifting heavy objects).*

Moderate: *Which causes your heart rate to increase slightly and you typically work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming, low intensity aerobics or golfing without a power cart, brisk walking, mowing the lawn with a walking lawnmower).*

Light: *Which requires little physical effort (e.g., light housekeeping like dusting or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).*

Keeping these definitions in mind, participants were asked if they engaged in regular physical activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants answered "yes", they entered up to 7 types of seasonal and/or non-seasonal exercise or activity along with the frequency, duration and intensity.

MMW were calculated in a 2-step process. Step 1: subjects who reported no physical activity (for whom no MMW calculations could be made) were designated as the no regular exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity reported. Four groups reflecting participation in physical activity and whether or not their participation was below, at or above USDHHS guidelines were created: NRE (reported no regular physical activity), below recommended (reported <500 MMW), recommended (reported 500-1000 MMW) and above recommended (reported >1000 MMW).

Blood Collection, Processing and Assays. Participants were asked to avoid strenuous activity the day of blood collection. Venous blood samples were collected in 10 mL serum separator vacutainers following a 12-h overnight fast and processed at a General Clinical Research Center using standardized procedures. Blood samples were not collected at any specific point during the menstrual cycle in female participants. Briefly, following collection, vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-Selectin and sICAM-1).

IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison, Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600, R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-

assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV% values for IL-6sr were 5.9-5.7% and 2.0%, respectively.

Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay; R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay, R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%, respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer with a particle enhanced immunonepolometric assay. Intra-assay and inter-assay precision for CRP was 2.3-4.4% and 2.1-5.7%, respectively.

Statistical Analyses. All variables were assessed for normality and non-normal data were log transformed, which included data for CRP, IL-6, IL-6sr, fibrinogen, sE-selectin and sICAM-1. General Linear Models were performed to determine the relationship of MMW and BMI with the inflammatory markers. For each outcome, the ordinal MMW and BMI factors were entered as independent factors with an interaction term. If the interaction term was not significant, the interaction term was dropped and the model was re-fit for main effects only. Pairwise comparisons were assessed using post hoc univariate analyses with a Bonferroni adjustment for multiple comparisons. Covariates for all models included factors that are known to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering, corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive).

Race was initially included as a covariate; however, approximately 200 data points were lost in the analyses due to incomplete racial data. As race was not found to be a predictor of our dependent variables, with the exception of sICAM-1, race was excluded as a covariate to increase samples size in all analyses excluding sICAM-1. All statistical analyses were performed with SPSS v. 17 (Chicago, IL) and statistical significance was set $\alpha = 0.05$.

In an exploratory analysis, we examined whether the relative effects of BMI and MMW on the inflammatory markers differed by sex in 3-way interaction models. As none of the interactions approached statistical significance (data not shown), sex was retained as a covariate in the models.

RESULTS

Subject Characteristics. Table 1 presents anthropometric characteristics and circulating levels of inflammatory biomarkers in all subjects (N=1254). Subjects were 92.6% non-Hispanic white, 56.8% female, and, on average, middle-aged and overweight. Of all the respondents, 14.9% were currently smoked, 27.8% were taking cholesterol lowering medication, 12.1% corticosteroids, 10.4% anti-diabetic medication, 14.2% antidepressant medication, 7.3% hormone replacement and 2.5% oral contraceptives. The percentage of participants with missing data for each variable were as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6% for fibrinogen, 1.2% for sE-selectin, and 1.0%for IL-6sr.

CRP (Figure 1, Panel A). We found a significant interaction between BMI and MMW for CRP concentration (F=3.022, P=0.006). In post hoc comparisons, CRP levels were higher in overweight and obese subjects compared to normal weight subjects among those who reported

no regular exercise (P's<0.001). However, among subjects who reported any amount of regular exercise (<500, 500-1000 or >1000 MMW), CRP levels were significantly greater only in obese subjects compared to both normal weight and overweight subjects (P's <0.01). In obese individuals, CRP tended to be lower in those reporting >1000 MMW compared to those reporting no regular exercise (P=0.053).

We also found main effects of BMI (F=130.873 P<0.001) and MMW (F=11.576, P<0.001) on CRP. CRP was significantly greater with each increasing BMI category, in a dose-dependent manner (P's<0.001). Compared to participants who reported no regular exercise, CRP was significantly lower in those who reported 500-1000 and >1000 MMW (P's <0.01), with a trend for lower CRP in those who reported <500 MMW of regular exercise (P=0.078).

sICAM-1 (Figure 1, Panel B). We found a significant interaction between BMI and MMW for sICAM-1 concentration (F=2.701, P=0.013). Levels of sICAM-1 were significantly lower in obese subjects who reported >1000 MMW compared to obese subjects who reported no regular exercise (P=0.014) and <500 MMW (P=0.026) and tended to be lower than levels in obese subjects who reported 500-1000 MMW (P=0.079). No differences in sICAM-1 by MMW were observed among normal weight or overweight individuals.

We also observed a main effect of BMI (F=6.060, P=0.002), such that sICAM-1 levels in obese participants were significantly higher than levels found in both normal weight and overweight participants (P's<0.01). No significant main effect of MMW was found for sICAM-1 (F=0.931, P=0.425).

IL-6 (Figure 1, Panel C). Both BMI and MMW had independent effects on circulating concentrations of IL-6 (BMI: F=60.150, P<0.001, MMW: F=10.680, P<0.001), with no significant interaction (F=1.21, P=0.297). We found a dose-dependent effect of BMI, such that

higher BMI levels were associated with significantly greater IL-6 (P's<0.001). Independent of BMI, IL-6 was significantly lower in subjects who reported regular exercise (<500 MMW, 500-1000 MMW and >1000 MMW) compared to those who reported no regular exercise (P's <0.01) with no difference between levels of MMW.

Fibrinogen (Figure 1, Panel D). BMI significantly contributed to circulating levels of fibrinogen (F=42.385, P<0.001), such that dose-dependent increases were observed for all BMI levels (P's<0.01). While we observed a trend for lower fibrinogen with regular physical activity, similar to that of IL-6, the effect did not reach statistical significance (F=2.187, P=0.088). We observed no significant interaction between BMI and MMW for fibrinogen (F=1.680, P=0.122).

sE-Selectin (1, Panel E). BMI significantly contributed to circulating levels of sE-selectin (F=28.253, P<0.001) with no significant contribution by MMW (F=0.207, P=0.892). Dose-dependent increases in sE-selectin were also observed across BMI levels (P's<0.01). We observed no significant interaction between BMI and MMW for sE-selectin (F=0.570, P=0.755).

IL-6sr (Figure 1, Panel F). No significant main effects for BMI (F=1.783, P=0.169), MMW (F=1.434, P=0.231) or their interaction (F=0.834, P=0.544) were detected for IL-6sr.

Waist Circumference (WC) and Inflammatory Markers (Supplemental Figure 1). A secondary analysis was completed using WC and MMW as independent variables and the complete results of these analyses are located in the supplemental information. Briefly, we found a significant interaction between WC and MMW on sICAM-1. In individuals with an at-risk WC (≥102.0 cm for men and ≥88.0 cm for women), sICAM-1 was significantly lower in those reporting 1000+ MMW compared to less than 500 MMW and tended to be lower in those reporting no regular exercise. Overall, main effects were similar to those found for BMI and MMW analyses. Having an at-risk WC was independently related to higher levels of CRP,

sICAM-1, IL-6, fibrinogen and sE-selectin. Independent of WC, any level of regular exercise was related to lower levels of CRP, IL-6 with a similar tendency for fibrinogen.

DISCUSSION

The current study aimed to determine whether the impact of BMI and MMW on inflammatory markers varied by level of overweight or obesity. For CRP and s-ICAM-1 regular physical activity appeared to diminish the effects of higher BMI compared to those who reported no regular physical activity. In addition, we found that BMI was strongly and independently related to greater concentrations of both established and emerging inflammatory markers that may increase CVD risk. Independent of BMI, regular physical activity was also associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that, although obesity has a clear impact on inflammation, physical activity appears to mitigate at least some of this effect.

For example, overweight individuals had CRP levels that were similar to levels observed in obese individuals if they reported no regular exercise (4.05 and 4.83 μg/mL, respectively). CRP levels greater than 3 μg/mL are typically associated with high CVD risk(26). In overweight subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it below [<500], within [500-1000] or above [>1000] USDHHS MMW recommendations), CRP levels were lower and not significantly different from CRP levels found in normal weight participants (). This suggests that increasing physical activity level to a minimum of 3 days per week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.

Obese individuals may require a higher level of regular physical activity in order to lower inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1

compared to lean and overweight subjects, those who reported >1000 MMW (above the USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than obese subjects reporting no regular physical activity. Taken together, we may speculate that while physical activity levels currently recommended for the general population may reduce particular inflammatory makers in overweight populations, obese populations may require greater levels of physical activity above recommended values to reduce inflammatory markers like CRP and sICAM-1.

As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen, sICAM-1 and sE-selectin, in agreement with previous work (27-30). Independent of BMI effects, our results suggest that physical activity has differentiating effects on inflammatory markers. Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and determinants in CArdiovascular disease (MONICA) study(31), the National Health and Nutrition Examination Survey (NHANES III)(12, 14) and the Multi-Ethnic Study of Atherosclerosis (MESA)(32), such that both increased frequency and intensity of physical activity have been related to lower IL-6 and fibrinogen. Our findings add to these prior results by standardizing levels of physical activity by using USDHHS. Our results suggest that, regular physical activity at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and possibly fibrinogen, independent of BMI.

Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and physical activity was detected for CRP, but not IL-6. While IL-6 and CRP were significantly

correlated (r=0.514, see Supplemental Table 1), this correlation suggests that IL-6 levels do not fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing to its substantially longer plasma half-life (37), which may improve our ability to detect interaction effects in CRP compared to IL-6.

Interestingly, our results also suggest that regular exercise may have a more profound impact on lowering classical markers of inflammation and less impact on the inflammatory status of the endothelium. Regular physical activity has reliably been associated with lower levels of IL-6 and CRP, both classical inflammatory markers related to adipose and systemic inflammation(38). However, regular exercise appeared to have no independent impact on markers of endothelial activation, particularly sE-selectin. Higher levels of exercise were related to lower sICAM-1 in obese individuals only. In one prior study, inverse relationships between physical activity and sICAM-1 and sE-selectin were reported in drug-treated hypertensive men (39). Thus, further research is necessary to understand mechanisms underlying differential associations of exercise with systemic and endothelial inflammation.

Several limitations must be addressed. First, the cross-sectional design does not allow us to infer causal relationships. Prospective and interventional designs are necessary to confirm our findings. No objective measures of physical activity were available in the MIDUS sample. Therefore, the use of self-report physical activity data may have diminished our ability to detect effects. However, in addition to being in line with previous studies using self-report physical activity, our findings are also in line with previous studies(40, 41) that demonstrated that higher cardiorespiratory fitness, as measured by indirect calorimetry, was associated with lower levels of inflammation independent of visceral adiposity or BMI. Another limitation is that the sample was predominantly comprised of non-Hispanic white individuals, suggesting that findings may

not extend to all ethnicities. Finally, BMI and physical activity variables are correlated, potentially raising the concern of small sample sizes in specific groups crossed by BMI and MMW. However, the smallest group for analyses still contained 54 individuals (normal weight individuals reporting no exercise).

In summary, our results demonstrate both interactive and independent influences of BMI and levels of physical activity on both established and emerging markers of inflammation. Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular physical activity appears to mitigate the effects of higher BMI on some inflammatory markers, particularly CRP, which is strongly implicated in CVD. Importantly, while any level of regular physical activity may help reduce inflammation in overweight individuals, similar effects in obese individuals may require levels of physical activity that are greater than currently recommended by the USDHHS for general health.

ACKNOWLEDGEMENTS

We thank the staff of the Clinical Research Centers at the University of Wisconsin-Madison, UCLA, and Georgetown University for their effort in conducting the original data collection.

FUNDING

KS was supported through a T32 Training Fellowship (Training in Behavioral and Preventive Medicine; T32 HL076134). The original research was supported by a grant from the National Institute on Aging (P01-AG020166) to conduct a longitudinal follow-up of the MIDUS (Midlife in the U.S.) Investigation.

The original study was supported by the John D. and Catherine T. MacArther Foundation Research Network on Successful Midlife Development and by the following grants: M01-RR023942 (Georgetown), M01-RR00865 (UCLA) from the General Clinical Research Centers

Program and 1UL1RR025011 (UW) from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources, National Institutes of Health.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTORSHIP

KS, JMM and RRW each made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation, as well as to drafting and revision for substantial intellectual content. All authors made final approval of the version to be published.

DATA SHARING STATEMENT

Data and documentation for MIDUS studies are available at the Inter-university Consortium for Political and Social Research (ICPSR). http://www.icpsr.umich.edu/icpsrweb/landing.jsp

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FIGURE AND TABLE LEGENDS

Figure 1: Inflammatory Markers. Data from 1254 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. The analysis for sICAM-1 was further adjusted for race. Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value, INT=interaction effect P value.

Table 1: Subject Characteristics. BMI = body mass index; CRP = C-reactive protein; IL = interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin = soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.

Demographic Variables	BMI < 25 N=298	BMI 25-29.9 N=440	BMI ≥30 N=516	Overall N = 1254
			Mean ± SD	
Age (years)	54.6 ± 12.8	56.4 ± 11.7	54.6 ± 11.2	54.5 ± 11.7
Gender (%)				
Male	31.2	52.7	42.1	43.20
Female	68.8	47.3	57.9	56.80
Race (%)				
Non-Hispanic White	94.0	94.0	90.3	92.60
Hispanic	0.4	0.8	0.3	0.05
African American	1.9	1.6	4.0	2.60
Asian/Pacific Islander	0.7	0.3	0.0	0.30
Native American	1.1	0.8	2.0	1.30
Other	1.9	2.6	3.5	2.30
Medication Use (%)				
Cholesterol-Lowering	13.1	32.3	32.6	27.80
Corticosteroids	12.8	12.5	11.4	12.10
Anti-Diabetic	4.7	8.4	15.3	10.40
Antidepressant	14.4	13.4	16.9	14.2
Hormone Replacement Therapy	9.4	8.6	5.0	7.3
Oral Contraceptive	3.7	3.4	1.0	2.5
Currently Smoking	17.8	14.1	14.0	14.90
BMI (kg/m^2)	22.7 ± 1.8	27.4 ± 1.4	35.9 ± 5.7	29.8 ± 6.6
IL-6 (pg/mL)	2.4 ± 3.1	2.7 ± 2.48	3.7 ± 3.2	3.0 ± 3.1
IL-6sr (pg/mL)	34473.1 ± 10861.9	35337.4 ± 10065.1	35475.7 ± 10325.7	35184.7 ± 10359.1
CRP (μg/mL)	1.5 ± 2.5	2.5 ± 4.0	4.4 ± 5.9	3.0 ± 4.8
Fibrinogen (mg/dL)	315.8 ± 75.9	343.2 ± 82.1	373 ± 92.1	348.9 ± 87.9
sE-Selectin (ng/mL)	36.9 ±19.6	41.2 ± 20.6	49.1 ± 24.7	43.4 ± 22.7
sICAM-1 (ng/mL)	284.8 ± 122.0	276.2 ± 99.9	301.4 ± 123.1	288.6 ± 115.6

Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A Cohort Study of Middle Aged Adults Living in the United States

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Running Title:

BMI, Physical Activity and Inflammation

Key Words: MIDUS, Intracellular Adhesion Molecule-1, Fibrinogen, C-Reactive Protein

Word Count: 3931

ABSTRACT

Objectives. Determine whether body mass index (BMI) and physical activity (PA) above, at or below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP), fibrinogen, IL-6 soluble receptor (IL-6sr), soluble E-selectin and soluble intracellular adhesion molecule (sICAM)-1. **Design.** Archived cohort data (N=1254, age 54.5±11.7y, BMI 29.8±6.6kg/m^2) from the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study were analyzed for concentrations of inflammatory markers using general linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m^2. Analyses were adjusted for age, sex, smoking and relevant medication use. **Setting.** Respondents reported to three centers to complete questionnaires and provide blood samples. **Participants.** Participants were men and women currently enrolled in the MIDUS Biomarker Project (N=1254, 93% non-hispanic white, average age 54.5y). **Primary Outcome Measures.** Concentration of serum IL-6, CRP, fibrinogen, IL-6sr, sE-selectin and sICAM. **Results.** Significant interactions were found between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was similar to obese when no PA was reported, but was similar to normal weight when any level of regular PA was reported. sICAM-1 was differentially lower in obese individuals who reported >1000 MMW compared to obese individuals reporting less exercise. **Conclusion.** The association of exercise with CRP and sICAM-1 differed by BMI, suggesting that regular exercise may buffer weight-associated elevations in CRP in overweight individuals while higher levels of exercise may be necessary to reduce sICAM-1 or CRP in obese individuals. **Trial Registry.** N/A.

INTRODUCTION

Obesity paired with low physical activity is well known to increase morbidity and mortality related to cardiovascular disease (CVD)(1). It is less clear, however, whether the benefits of higher levels of physical activity differ among normal weight, overweight, and obese individuals.

Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Circulating Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of inflammatory markers(7, 8). Further, body fat distribution is also an important factor relating to inflammatory status. Accumulation of fat in visceral depots is more strongly associated with low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots(9, 10).

The effects of physical activity on markers of inflammation are more complex and may vary depending on body weight. A number of epidemiological studies have shown an inverse relationship between physical activity and CRP and IL-6, independent of obesity(11-16). Laboratory studies conducted in aerobically trained, typically normal weight, individuals have demonstrated that a single bout of exercise stimulates IL-6 release from skeletal muscle, which promotes anti-inflammatory effects (17-19), as opposed to adipose tissue-derived IL-6 that is associated with pro-inflammatory effects (20). Randomized controlled trials have also been conducted, often in populations that also tend to be overweight or obese, to examine the effects

of aerobic exercise interventions on inflammation and the results are mixed (21). Thus, the contribution of physical activity to inflammation in the context of obesity remains unclear.

The purpose of our study was to disentangle the relative contributions of BMI and physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr, CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were determined using values put forth by the Physical Activity Guidelines for Americans, which states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week) produces substantial health benefits for adults(22). We hypothesized that BMI and MMW would interact, such that greater MMW reported would lessen the impact of obesity on markers of inflammation.

MATERIALS AND METHODS

Design and Sample. This study was a cross-sectional analysis of archived data (BMI, self-reported physical activity and inflammatory biomarker concentrations) from 1254 respondents who provided consent (as approved by The University of Wisconsin Madison Health Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study (23). The Biomarker Project was one of 5 projects within MIDUS II, with the purpose of adding comprehensive biological assessments on a subsample of the MIDUS participants to further understand age-related differences in physical and mental health. Participants were eligible for The Biomarker Project if they were previously enrolled in MIDUS and MIDUS II, which recruited non-institutionalized, English-speaking adults residing in the contiguous United States aged 25-74.

Exclusion criteria included non-participation in MIDUS and MIDUS II and unwillingness to travel to specified sites for biomarker assessment. The random digit dialing sample for the parent study was selected from working telephone banks and a list of all individuals between the ages of 25 and 74 years within each household was generated in order to select a random respondent. Those who agreed to participate in the Biomarker Study stayed overnight at one of three General Clinical Research Centers: University of California Los Angeles, University of Wisconsin-Madison and Georgetown University. Upon arrival, each respondent provided a detailed medical history (including physical activity levels) and provided all prescription, over-the-counter, and alternative medications to be inventoried by project staff. Following an overnight stay, morning fasting blood samples were obtained. Cohorts were assessed between July 2004 and May 2009 as a follow up to MIDUS I respondents that were previously surveyed by the MacArthur Midlife Research Network between 1995 and 1996. Based on the MIDUS Biomarker Project sample of 1254 participants, 80% power was estimated to detect small effect sizes (delta=0.08 and higher) with an alpha level at 0.05 for a two-tailed test (24, 25).

Anthropometrics. Height was measured in centimeters and recorded to the nearest millimeter. A single measure of WC was taken directly on the skin or over a single layer of light, close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the nearest millimeter. Weight was measured in kilograms and BMI was calculated by dividing body mass in kilograms by height in meters squared. BMI categories were organized into 3 groups: normal weight (BMI ≤24.9 kg/m^2), overweight (BMI≥25-29.9) and obese (BMI≥30).

Categorizing Physical Activity by MET-Minutes per Week (MMW).

The MMW variable was calculated using data provided in the medical history form. The form first described 3 types of regular physical activity(23):

Vigorous: *Which causes your heart to beat so rapidly you can feel it in your chest and you perform it long enough to work up a good sweat and breathe heavily (e.g., competitive sports, running, vigorous swimming, high intensity aerobics, digging in the garden, or lifting heavy objects).*

Moderate: *Which causes your heart rate to increase slightly and you typically work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming, low intensity aerobics or golfing without a power cart, brisk walking, mowing the lawn with a walking lawnmower).*

Light: *Which requires little physical effort (e.g., light housekeeping like dusting or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).*

Keeping these definitions in mind, participants were asked if they engaged in regular physical activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants answered "yes", they entered up to 7 types of seasonal and/or non-seasonal exercise or activity along with the frequency, duration and intensity.

MMW were calculated in a 2-step process. Step 1: subjects who reported no physical activity (for whom no MMW calculations could be made) were designated as the no regular exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity reported. Four groups reflecting participation in physical activity and whether or not their participation was below, at or above USDHHS guidelines were created: NRE (reported no regular physical activity), below recommended (reported <500 MMW), recommended (reported 500-1000 MMW) and above recommended (reported >1000 MMW).

Blood Collection, Processing and Assays. Participants were asked to avoid strenuous activity the day of blood collection. Venous blood samples were collected in 10 mL serum separator vacutainers following a 12-h overnight fast and processed at a General Clinical Research Center using standardized procedures. Blood samples were not collected at any specific point during the menstrual cycle in female participants. Briefly, following collection, vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-Selectin and sICAM-1).

IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison, Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600, R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV% values for IL-6sr were 5.9-5.7% and 2.0%, respectively.

Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay; R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay, R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%, respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for

fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer with a particle enhanced immunonepolometric assay. Intra-assay and inter-assay precision for CRP was 2.3-4.4% and 2.1-5.7%, respectively.

Statistical Analyses. All variables were assessed for normality and non-normal data were log transformed, which included data for CRP, IL-6, IL-6sr, fibrinogen, sE-selectin and sICAM-1. General Linear Models were performed to determine the relationship of MMW and BMI with the inflammatory markers. For each outcome, the ordinal MMW and BMI factors were entered as independent factors with an interaction term. If the interaction term was not significant, the interaction term was dropped and the model was re-fit for main effects only. Pairwise comparisons were assessed using post hoc univariate analyses with a Bonferroni adjustment for multiple comparisons. Covariates for all models included factors that are known to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering, corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive). Race was initially included as a covariate; however, approximately 200 data points were lost in the analyses due to incomplete racial data. As race was not found to be a predictor of our dependent variables, with the exception of sICAM-1, race was excluded as a covariate to increase samples size in all analyses excluding sICAM-1. All statistical analyses were performed with SPSS v. 17 (Chicago, IL) and statistical significance was set $\alpha = 0.05$.

In an exploratory analysis, we examined whether the relative effects of BMI and MMW on the inflammatory markers differed by sex in 3-way interaction models. As none of the interactions approached statistical significance (data not shown), sex was retained as a covariate in the models.

RESULTS

Subject Characteristics. Table 1 presents anthropometric characteristics and circulating levels of inflammatory biomarkers in all subjects (N=1254). Subjects were 92.6% non-Hispanic white, 56.8% female, and, on average, middle-aged and overweight. Of all the respondents, 14.9% were currently smoked, 27.8% were taking cholesterol lowering medication, 12.1% corticosteroids, 10.4% anti-diabetic medication, 14.2% antidepressant medication, 7.3% hormone replacement and 2.5% oral contraceptives. The percentage of participants with missing data for each variable were as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6% for fibrinogen, 1.2% for sE-selectin, and 1.0%for IL-6sr.

CRP (Figure 1, Panel A). We found a significant interaction between BMI and MMW for CRP concentration (F=3.022, P=0.006). In post hoc comparisons, CRP levels were higher in overweight and obese subjects compared to normal weight subjects among those who reported no regular exercise (P's<0.001). However, among subjects who reported any amount of regular exercise (<500, 500-1000 or >1000 MMW), CRP levels were significantly greater only in obese subjects compared to both normal weight and overweight subjects (P's <0.01). In obese individuals, CRP tended to be lower in those reporting >1000 MMW compared to those reporting no regular exercise (P=0.053).

We also found main effects of BMI (F=130.873 P<0.001) and MMW (F=11.576, P<0.001) on CRP. CRP was significantly greater with each increasing BMI category, in a dose-dependent manner (P's<0.001). Compared to participants who reported no regular exercise, CRP was significantly lower in those who reported 500-1000 and >1000 MMW (P's <0.01), with a trend for lower CRP in those who reported <500 MMW of regular exercise (P=0.078).

sICAM-1 (Figure 1, Panel B). We found a significant interaction between BMI and MMW for sICAM-1 concentration (F=2.701, P=0.013). Levels of sICAM-1 were significantly lower in obese subjects who reported >1000 MMW compared to obese subjects who reported no regular exercise (P=0.014) and <500 MMW (P=0.026) and tended to be lower than levels in obese subjects who reported 500-1000 MMW (P=0.079). No differences in sICAM-1 by MMW were observed among normal weight or overweight individuals.

We also observed a main effect of BMI (F=6.060, P=0.002), such that sICAM-1 levels in obese participants were significantly higher than levels found in both normal weight and overweight participants (P's<0.01). No significant main effect of MMW was found for sICAM-1 (F=0.931, P=0.425).

IL-6 (Figure 1, Panel C). Both BMI and MMW had independent effects on circulating concentrations of IL-6 (BMI: F=60.150, P<0.001, MMW: F=10.680, P<0.001), with no significant interaction (F=1.21, P=0.297). We found a dose-dependent effect of BMI, such that higher BMI levels were associated with significantly greater IL-6 (P's<0.001). Independent of BMI, IL-6 was significantly lower in subjects who reported regular exercise (<500 MMW, 500-1000 MMW and >1000 MMW) compared to those who reported no regular exercise (P's <0.01) with no difference between levels of MMW.

Fibrinogen (Figure 1, Panel D). BMI significantly contributed to circulating levels of fibrinogen (F=42.385, P<0.001), such that dose-dependent increases were observed for all BMI levels (P's<0.01). While we observed a trend for lower fibrinogen with regular physical activity, similar to that of IL-6, the effect did not reach statistical significance (F=2.187, P=0.088). We observed no significant interaction between BMI and MMW for fibrinogen (F=1.680, P=0.122).

sE-Selectin (1, Panel E). BMI significantly contributed to circulating levels of sE-selectin (F=28.253, P<0.001) with no significant contribution by MMW (F=0.207, P=0.892). Dose-dependent increases in sE-selectin were also observed across BMI levels (P's<0.01). We observed no significant interaction between BMI and MMW for sE-selectin (F=0.570, P=0.755).

IL-6sr (Figure 1, Panel F). No significant main effects for BMI (F=1.783, P=0.169), MMW (F=1.434, P=0.231) or their interaction (F=0.834, P=0.544) were detected for IL-6sr.

Waist Circumference (WC) and Inflammatory Markers (Supplemental Figure 1). A secondary analysis was completed using WC and MMW as independent variables and the complete results of these analyses are located in the supplemental information. Briefly, we found a significant interaction between WC and MMW on sICAM-1. In individuals with an at-risk WC (≥102.0 cm for men and ≥88.0 cm for women), sICAM-1 was significantly lower in those reporting 1000+ MMW compared to less than 500 MMW and tended to be lower in those reporting no regular exercise. Overall, main effects were similar to those found for BMI and MMW analyses. Having an at-risk WC was independently related to higher levels of CRP, sICAM-1, IL-6, fibrinogen and sE-selectin. Independent of WC, any level of regular exercise was related to lower levels of CRP, IL-6 with a similar tendency for fibrinogen.

DISCUSSION

The current study aimed to determine whether the impact of BMI and MMW on inflammatory markers varied by level of overweight or obesity. For CRP and s-ICAM-1 regular physical activity appeared to diminish the effects of higher BMI compared to those who reported no regular physical activity. In addition, we found that BMI was strongly and independently related to greater concentrations of both established and emerging inflammatory

markers that may increase CVD risk. Independent of BMI, regular physical activity was also associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that, although obesity has a clear impact on inflammation, physical activity appears to mitigate at least some of this effect.

For example, overweight individuals had CRP levels that were similar to levels observed in obese individuals if they reported no regular exercise (4.05 and 4.83 μg/mL, respectively). CRP levels greater than 3 μg/mL are typically associated with high CVD risk(26). In overweight subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it below [<500], within [500-1000] or above [>1000] USDHHS MMW recommendations), CRP levels were lower and not significantly different from CRP levels found in normal weight participants (). This suggests that increasing physical activity level to a minimum of 3 days per week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.

Obese individuals may require a higher level of regular physical activity in order to lower inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1 compared to lean and overweight subjects, those who reported >1000 MMW (above the USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than obese subjects reporting no regular physical activity. Taken together, we may speculate that while physical activity levels currently recommended for the general population may reduce particular inflammatory makers in overweight populations, obese populations may require greater levels of physical activity above recommended values to reduce inflammatory markers like CRP and sICAM-1.

As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen, sICAM-1 and sE-selectin, in agreement with previous work (27-30). Independent of BMI effects,

our results suggest that physical activity has differentiating effects on inflammatory markers. Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and determinants in CArdiovascular disease (MONICA) study(31), the National Health and Nutrition Examination Survey (NHANES III)(12, 14) and the Multi-Ethnic Study of Atherosclerosis (MESA)(32), such that both increased frequency and intensity of physical activity have been related to lower IL-6 and fibrinogen. Our findings add to these prior results by standardizing levels of physical activity by using USDHHS. Our results suggest that, regular physical activity at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and possibly fibrinogen, independent of BMI.

Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and physical activity was detected for CRP, but not IL-6. While IL-6 and CRP were significantly correlated (r=0.514, see Supplemental Table 1), this correlation suggests that IL-6 levels do not fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing to its substantially longer plasma half-life (37), which may improve our ability to detect interaction effects in CRP compared to IL-6.

Interestingly, our results also suggest that regular exercise may have a more profound impact on lowering classical markers of inflammation and less impact on the inflammatory status of the endothelium. Regular physical activity has reliably been associated with lower levels of IL-6 and CRP, both classical inflammatory markers related to adipose and systemic inflammation(38). However, regular exercise appeared to have no independent impact on

markers of endothelial activation, particularly sE-selectin. Higher levels of exercise were related to lower sICAM-1 in obese individuals only. In one prior study, inverse relationships between physical activity and sICAM-1 and sE-selectin were reported in drug-treated hypertensive men (39). Thus, further research is necessary to understand mechanisms underlying differential associations of exercise with systemic and endothelial inflammation.

Several limitations must be addressed. First, the cross-sectional design does not allow us to infer causal relationships. Prospective and interventional designs are necessary to confirm our findings. No objective measures of physical activity were available in the MIDUS sample. Therefore, the use of self-report physical activity data may have diminished our ability to detect effects. However, in addition to being in line with previous studies using self-report physical activity, our findings are also in line with previous studies(40, 41) that demonstrated that higher cardiorespiratory fitness, as measured by indirect calorimetry, was associated with lower levels of inflammation independent of visceral adiposity or BMI. Another limitation is that the sample was predominantly comprised of non-Hispanic white individuals, suggesting that findings may not extend to all ethnicities. Finally, BMI and physical activity variables are correlated, potentially raising the concern of small sample sizes in specific groups crossed by BMI and MMW. However, the smallest group for analyses still contained 54 individuals (normal weight individuals reporting no exercise).

In summary, our results demonstrate both interactive and independent influences of BMI and levels of physical activity on both established and emerging markers of inflammation. Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular physical activity appears to mitigate the effects of higher BMI on some inflammatory markers, particularly CRP, which is strongly implicated in CVD. Importantly, while any level of regular

physical activity may help reduce inflammation in overweight individuals, similar effects in obese individuals may require levels of physical activity that are greater than currently recommended by the USDHHS for general health.

ACKNOWLEDGEMENTS

We thank the staff of the Clinical Research Centers at the University of Wisconsin-Madison, UCLA, and Georgetown University for their effort in conducting the original data collection.

FUNDING

KS was supported through a T32 Training Fellowship (Training in Behavioral and Preventive Medicine; T32 HL076134). The original research was supported by a grant from the National Institute on Aging (P01-AG020166) to conduct a longitudinal follow-up of the MIDUS (Midlife in the U.S.) Investigation.

The original study was supported by the John D. and Catherine T. MacArther Foundation Research Network on Successful Midlife Development and by the following grants: M01-RR023942 (Georgetown), M01-RR00865 (UCLA) from the General Clinical Research Centers Program and 1UL1RR025011 (UW) from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources, National Institutes of Health.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTORSHIP

KS, JMM and RRW each made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation, as well as to drafting and revision for substantial intellectual content. All authors made final approval of the version to be published.

DATA SHARING STATEMENT

Data and documentation for MIDUS studies are available at the Inter-university Consortium for Political and Social Research (ICPSR). http://www.icpsr.umich.edu/icpsrweb/landing.jsp

ARTICLE SUMMARY

Article focus

- Systemic inflammation is related to the progression of cardiovascular disease.
- Independent of obesity, physical activity is inversely related to concentrations of well-established inflammatory biomarkers, such as C-reactive protein (CRP) or interleukin-6 (IL-6).
- This article evaluates interactive effects of body mass index and physical activity on established inflammatory markers, CRP, IL-6, and emerging inflammatory markers, fibrinogen, soluble intracellular adhesion molecule (sICAM)-1, soluble E-selectin, and IL-6 soluble receptor.

Key messages

- Interactive effects of body mass index and physical activity were observed for CRP, such that regular physical activity reported by overweight individuals was related to significantly lower CRP levels compared to those reported no regular activity.
- Independent of BMI, regular physical activity was related to lower IL-6, with a trend for lower fibrinogen
- Physical activity had no independent effect on circulating markers related to endothelial inflammation, such as sICAM-1 or sE-selectin.

Strengths and limitations

- 1254 adults from the National Survey of Midlife Development in the United States (MIDUS) Biomarker Project were analyzed. Statistical analyses were adjusted for age, sex, smoking, and relevant medication use. A strength of this paper is categorizing physical activity levels based on national recommendations. This data may be used to determine appropriate levels of physical activity necessary for reducing inflammation in overweight and obese adults. However, cross-sectional data is limited, as causal inferences cannot be obtained. A second limitation is that the sample was predominantly comprised of non-Hispanic white individuals, therefore findings may not extend to all ethnicities.

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FIGURE AND TABLE LEGENDS

Figure 1: Inflammatory Markers. Data from 1254 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. The analysis for sICAM-1 was further adjusted for race. Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value, INT=interaction effect P value.

Table 1: Subject Characteristics. BMI = body mass index; CRP = C-reactive protein; IL = interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin = soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any pre-specified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4-7*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	8-10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	8-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

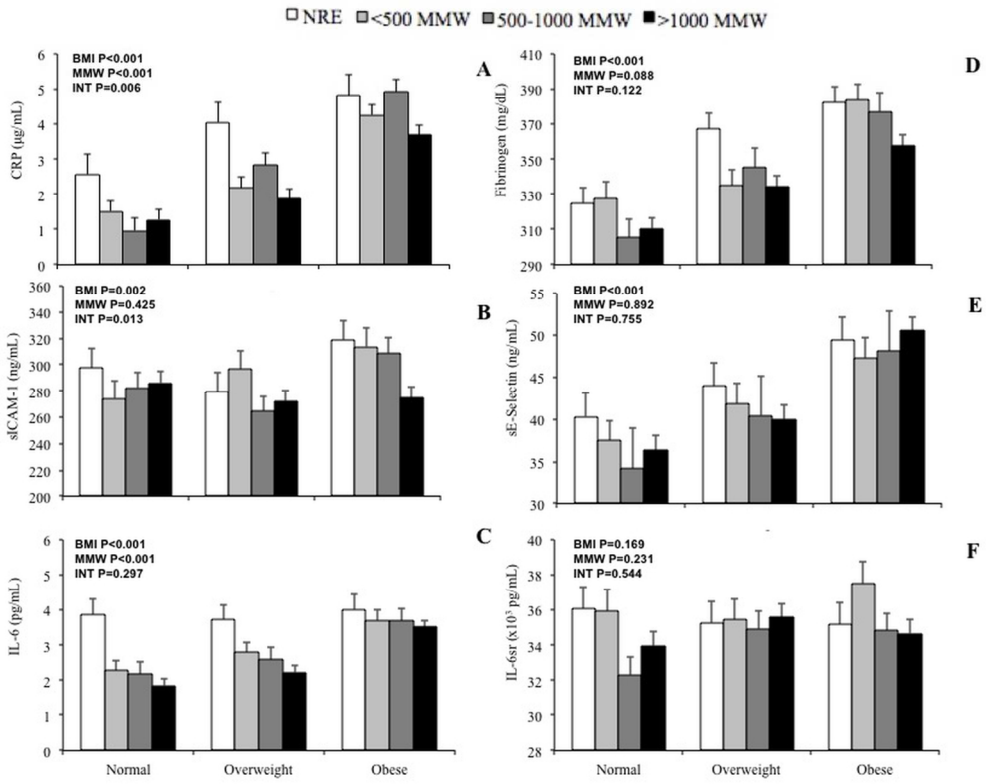

Figure 1: Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value, INT=interaction effect P value.
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Waist Circumference (WC) and MET-Minutes per Week (MMW)

CRP (Supplementary Figure 1, panel A). We found no significant interaction effect between WC and MMW for CRP (F=1.426, P=0.234). We found significant main effects for WC (F=159.669, P<0.001) and MMW (F=9.766, P<0.001) on circulating CRP. CRP levels were lower in participants who reported a normal waist circumference and any level of regular exercise (<500, 500-1000, and >1000 MMW), compared to those with an at-risk waist circumference (P's<0.001) and those no regular exercise (P's<0.05).

sICAM-1 (Supplementary Figure 1, panel B). We found a significant interaction effect between WC and MMW for sICAM-1 (F=4.846, P=0.002). While sICAM-1 levels were not significantly difference across MMW categories in individuals with a normal WC (P's>0.05), in individuals with an at-risk WC, sICAM-1 was significantly lower in those reporting 1000+ MMW compared to less than 500 MMW (P=0.007) and tended to be lower in those reporting no regular exercise (P=0.072). Similar to BMI, waist circumference independently contributed to sICAM-1 (F=26.841,, P<0.001), such that values were greater in subjects with an at-risk WC compared to those with a normal WC (P <0.001). No effect of MMW was observed (F=1.055, P=0.367) for sICAM-1.

IL-6 (Supplementary Figure 1, panel C). We found no significant interaction effect between WC and MMW for IL-6 (F=1.282, P=0.217). We found significant main effects for waist circumference (F=84.441, P<0.001) and MMW (F=10.255, P<0.001), such that IL-6 levels were lower in participants who reported an normal waist circumference and any level of regular exercise (<500, 500-1000, and >1000 MMW), compared to those with an at risk waist circumference (P's<0.001) and those reporting no regular exercise (P's<0.05).

Fibrinogen (Supplementary Figure 1, panel D). We found no significant interaction effect between WC and MMW for fibrinogen (F=2.019, P=0.110). Waist circumference also impacted fibrinogen (F=38.960, P<0.001), such that values were greater in subjects with an at-risk waist circumference compared to those with a normal waist circumference (P's <0.001). The effect of MMW on fibrinogen bordered on statistical significance (F=2.245, P=0.081), such that values were lower with in individuals who reported greater MMW.

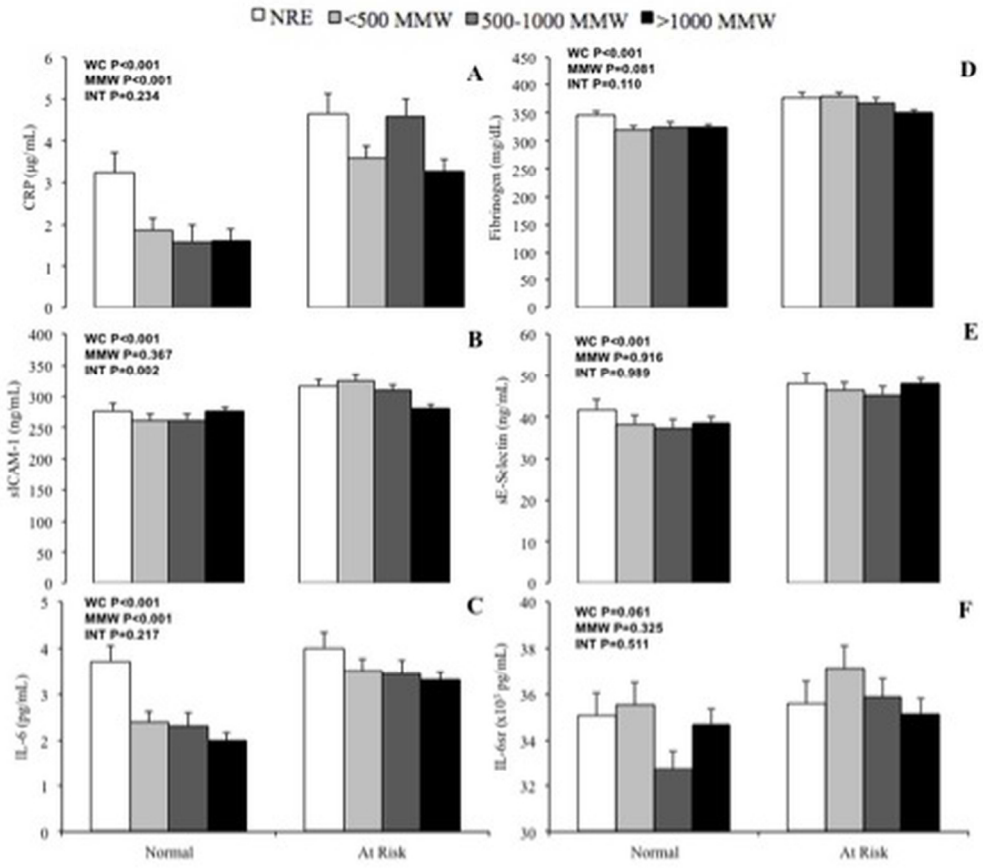
sE-Selectin (Supplementary Figure 1, panel E). We found no significant interaction between WC and MMW for sE-Selectin (F=0.041, P=0.989). Waist circumference also independently contributed to sE-selectin (F=40.967, P<0.001), such that values were greater in subjects with an at-risk waist circumference compared to those with a normal waist circumference (P <0.001). No effect of MMW was observed for sE-selectin (F=0.172, P=0.916).

IL-6sr (Supplementary Figure 1, panel F). We found no significant interaction effect between WC and MMW for IL-6sr (F=0.769, P=0.511). Like BMI, we found no main effects for waist circumference (F=3.505, P=0.061) or MMW on IL-6sr (F=1.158, P=0.325).

Interrelationship of Inflammatory Markers. Correlations between all inflammatory markers are shown in Supplementary Table 1.

Figure Legend

Supplemental Figure 1. Waist Circumference (WC), MET Minutes per Week (MMW) and Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of WC category (normal [(≥102.0 cm for men and ≥88.0 cm for women], at risk [(>102.0 cm for men and >88.0 cm for women]) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. Error bars represent SEM. WC=WC main effect, MMW=MMW main effect, INT=interaction effect.



Supplemental Figure 1. Waist Circumference (WC), MET Minutes per Week (MMW) and Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of WC category (normal [(≥102.0 cm for men and ≥88.0 cm for women], at risk [(>102.0 cm for men and >88.0 cm for women]) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex, smoking and relevant medication use. Error bars represent SEM. WC=WC main effect, MMW=MMW main effect, INT=interaction effect.
100x90mm (300 x 300 DPI)

	IL-6	IL-6sr	Fibrinogen	CRP	sE-Selectin
IL-6					
IL-6sr	0.037				
Fibrinogen	0.417**	0.017			
CRP	0.514**	0.053	0.513**		
sE-Selectin	0.213**	0.035	0.104**	0.156**	
sICAM-1	0.134**	0.140**	0.092**	0.144**	0.041

Supplemental Table 1. Correlations of Inflammatory Biomarkers. IL-6 = Interleukin-6, IL-6sr = IL-6 soluble receptor, CRP = C-reactive protein, sE-Selectin = soluble E-Selectin and sICAM-1 = soluble intracellular adhesion molecule – 1. * denotes significance at $p \leq 0.05$, ** denotes significance at $p \leq 0.001$.