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**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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## 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.

54 **ABSTRACT**

55 **Objectives.** Determine whether body mass index (BMI) and physical activity (PA) above, at or  
56 below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity  
57 Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP),  
58 fibrinogen, IL-6 soluble receptor (sr), soluble (s) E-selectin and soluble intracellular adhesion  
59 molecule (sICAM)-1. **Design.** Archived cohort data (N=1255, age 54.5±11.7y, BMI  
60 29.8±6.6kg/m<sup>2</sup>) from the National Survey of Midlife Development in the United States (MIDUS)  
61 Biomarkers Study were analyzed for concentrations of inflammatory markers using general  
62 linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000  
63 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m<sup>2</sup>. Analyses were adjusted for age, sex,  
64 smoking and relevant medication use. **Setting.** Respondents reported to three centers to  
65 complete questionnaires and provide blood samples. **Participants.** Participants were eligible if  
66 they were currently enrolled in the parent MIDUS study (N=1255, 57% female, 93% non-  
67 hispanic white, average age 54.5y). **Primary Outcome Measures.** Concentration of IL-6, CRP,  
68 fibrinogen, IL-6sr, sE-selectin and sICAM. **Results.** Significant interactions were found  
69 between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was  
70 similar to obese when no PA was reported, but was similar to normal weight when any level of  
71 regular PA was reported. sICAM-1 was differentially lower in obese individuals who reported  
72 >1000 MMW compared to obese individuals reporting less exercise. **Conclusion.** Levels of  
73 CRP and sICAM-1 depended on exercise and BMI levels, suggesting that regular exercise may  
74 buffer weight-associated elevations in CRP in overweight individuals while higher levels of  
75 exercise may be necessary to reduce sICAM-1 or CRP in obese individuals. **Trial Registry.**  
76 N/A.

## 77 INTRODUCTION

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

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

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

## 99 MATERIALS AND METHODS

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3 100           **Design and Sample.** This study was a cross-sectional analysis of archived data (BMI,  
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6 101 self-reported physical activity and inflammatory biomarker concentrations) from 1255  
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8 102 respondents aged 25 to 74 who provided consent (as approved by The University of Wisconsin  
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10 103 Madison Health Sciences Institutional Review Board) and were subsequently enrolled in the  
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12 104 National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study(12).  
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14  
15 105 The purpose of the Biomarker Project was to add comprehensive biological assessments on a  
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17 106 subsample of the parent MIDUS study to further understand age-related differences in physical  
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19 107 and mental health. Those who agreed to participate stayed overnight at one of three General  
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21 108 Clinical Research Centers: University of California Los Angeles, University of Wisconsin-  
22  
23 109 Madison and Georgetown University. Upon arrival, each respondent provided a detailed medical  
24  
25 110 history (including physical activity levels) and provided all prescription, over-the-counter, and  
26  
27 111 alternative medications to be inventoried by project staff. Following an overnight stay, morning  
28  
29 112 fasting blood samples were obtained. Cohorts were assessed between July 2004 and May 2009  
30  
31 113 as a follow up to MIDUS I respondents that were previously surveyed by the MacArthur Midlife  
32  
33 114 Research Network between 1995 and 1996. Based on the sample of 1255 participants, 80%  
34  
35 115 power was achieved to detect small effects of 0.08 or greater with alpha level at 0.05 for a two-  
36  
37 116 tailed test(13, 14).

38  
39 117           **Anthropometrics.** Height was measured in centimeters and recorded to the nearest  
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41 118 millimeter. Weight was measured in kilograms and recorded to the nearest decimal place. BMI  
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43 119 was calculated by dividing body mass in kilograms by height in meters squared.  
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3 122 **Categorizing BMI and MMW.** BMI categories were organized into 3 groups: normal  
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6 123 weight (BMI  $\leq 24.9$  kg/m<sup>2</sup>), overweight (BMI  $\geq 25$ -29.9) and obese (BMI  $\geq 30$ ).

7  
8 124 The MMW variable was calculated using data provided in the medical history form. The  
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10 125 form first described 3 types of regular physical activity(12):

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13 126 **Vigorous:** Which causes your heart to beat so rapidly you can feel it in your chest  
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15 127 and you perform it long enough to work up a good sweat and breathe heavily (e.g.,  
16  
17 128 competitive sports, running, vigorous swimming, high intensity aerobics, digging  
18  
19 129 in the garden, or lifting heavy objects).

20 130 **Moderate:** Which causes your heart rate to increase slightly and you typically  
21  
22 131 work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming,  
23  
24 132 low intensity aerobics or golfing without a power cart, brisk walking, mowing the  
25  
26 133 lawn with a walking lawnmower).

27 134 **Light:** Which requires little physical effort (e.g., light housekeeping like dusting  
28  
29 135 or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).

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31  
32 136 Keeping these definitions in mind, participants were asked if they engaged in regular physical  
33  
34 137 activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants  
35  
36 138 answered “yes”, they entered up to 7 types of seasonal and/or non-seasonal exercise or activity  
37  
38 139 along with the frequency, duration and intensity.

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41 140 MMW were calculated in a 2-step process. Step 1: subjects who reported no physical  
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43 141 activity (for whom no MMW calculations could be made) were designated as the no regular  
44  
45 142 exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical  
46  
47 143 activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for  
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49 144 low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity  
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3 145 reported. Four groups reflecting participation in physical activity and whether or not their  
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5 146 participation was below, at or above USDHHS guidelines were created: NRE (reported no  
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8 147 regular physical activity), below recommended (reported >500 MMW), recommended (reported  
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10 148 500-1000 MMW) and above recommended (reported >1000 MMW).

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13 149 **Blood Collection, Processing and Assays.** Participants were asked to avoid strenuous  
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15 150 activity the day of blood collection. Venous blood samples were collected in 10 mL serum  
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17 151 separator vacutainers following a 12-h overnight fast and processed at a General Clinical  
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20 152 Research Center using standardized procedures. Briefly, following collection, vacutainers were  
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22 153 allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for 20-min at 2000-  
23  
24 154 3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab and treated  
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26  
27 155 and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-Selectin and  
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29 156 sICAM-1).

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31  
32 157 IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison,  
33  
34 158 Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600,  
35  
36 159 R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6  
37  
38 160 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-  
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40 161 assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV%  
41  
42 162 values for IL-6sr were 5.9-5.7% and 2.0%, respectively.

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45 163 Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory  
46  
47 164 for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of  
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49 165 sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay;  
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51 166 R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was  
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54 167 completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay,  
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3 168 R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%,  
4  
5 169 respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human  
6  
7 170 Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for  
8  
9 171 fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer  
10  
11 172 with a particle enhanced immunonephelometric assay. Intra-assay and inter-assay precision for  
12  
13 173 CRP was 2.3-4.4% and 2.1-5.7%, respectively.  
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17 174 **Statistical Analyses.** All variables were assessed for normality and non-normal data  
18  
19 175 were log transformed. To determine the relative impact of MMW and BMI on inflammatory  
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21 176 markers, general linear models were performed. For each outcome, the categorical MMW and  
22  
23 177 BMI factors were entered as independent factors with an interaction term. If the interaction term  
24  
25 178 was not significant, the interaction term was dropped and the model was re-fit. All analyses  
26  
27 179 presented in the results were adjusted for confounding variables that are known to affect  
28  
29 180 inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering,  
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31 181 corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive).  
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33 182 Race was initially included as a covariate; however, approximately 200 data points were lost in  
34  
35 183 the analyses due to incomplete racial data. As race was not found to be a predictor of our  
36  
37 184 dependent variables, with the exception of sICAM-1, race was excluded as a covariate to  
38  
39 185 increase sample size in all analyses excluding sICAM-1. In an exploratory analysis, we  
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41 186 examined whether the relative effects of BMI and MMW on the inflammatory markers differed  
42  
43 187 by sex in 3-way interaction models. As none of the interactions approached statistical  
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45 188 significance, sex was included as a covariate in the models. All statistical analyses were  
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47 189 performed with SPSS v. 17 (Chicago, IL) and significance was set at  $P < 0.05$ .  
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## 191 RESULTS

192 **Subject Characteristics.** Table 1 presents anthropometric characteristics and plasma  
193 levels of inflammatory biomarkers in all subjects (N=1255). On average, subjects were 92.6%  
194 non-Hispanic white, 56.8% female, middle-aged and overweight. Of all the respondents, 14.9%  
195 were currently smoking, 27.8% were taking cholesterol lowering medication, 12.1% were taking  
196 corticosteroids, 10.4% were taking anti-diabetic medication, 14.2% were taking antidepressant  
197 medication, 7.3% were taking hormone replacement and 2.5% reported contraceptive use. The  
198 percentage of participants with missing data for each variable are as follows: 1.6% for CRP,  
199 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.

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

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

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3 213 CRP was significantly lower in those who reported 500-1000 and >1000 MMW (P's <0.01),  
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6 214 with a trend for lower CRP in those who reported <500 MMW of regular exercise (P=0.078).  
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11 216 **sICAM-1 (Figure 1, Panel B).** We found a significant interaction between BMI and  
12  
13 217 MMW for sICAM-1 concentration (F=2.701, P=0.013). Levels of sICAM-1 were significantly  
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15 218 lower in obese subjects who reported >1000 MMW compared to obese subjects who reported no  
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17 219 regular exercise (P=0.014) and <500 MMW (P=0.026) and tended to be lower than levels in  
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20 220 obese subjects who reported 500-1000 MMW (P=0.079), again suggesting that high levels of  
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22 221 physical activity could mitigate the increased sICAM-1 associated with obesity. No differences  
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24 222 in sICAM-1 by MMW were observed among normal weight or overweight individuals.  
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27 223 We also observed a main effect of BMI (F=6.060, P=0.002), such that sICAM-1 levels in  
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29 224 obese participants were significantly higher than levels found in both normal weight and  
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31 225 overweight participants (P's<0.01). No significant main effect of MMW was found for sICAM-1  
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34 226 (F=0.931, P=0.425).  
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36 227 **IL-6 (Figure 1, Panel C).** Both BMI and MMW had independent effects on circulating  
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38 228 concentrations of IL-6 (BMI: F=60.150, P<0.001, MMW: F=10.680, P<0.001), with no  
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41 229 significant interaction (F=1.21, P=0.297). We found a dose-dependent effect of BMI, such that  
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43 230 higher BMI levels were associated with significantly greater IL-6 (P's<0.001). Independent of  
44  
45 231 BMI, IL-6 was significantly lower in subjects who reported regular exercise (<500 MMW, 500-  
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47 232 1000 MMW and >1000 MMW) compared to those who reported no regular exercise (P's <0.01)  
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50 233 with no difference between levels of MMW.  
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53 234 **Fibrinogen (Figure 1, Panel D).** BMI significantly contributed to circulating levels of  
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55 235 fibrinogen (F=42.385, P<0.001), such that dose-dependent increases were observed for all BMI  
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3 236 levels ( $P$ 's<0.01). While we observed a trend for lower fibrinogen with regular physical activity,  
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5 237 similar to that of IL-6, the effect did not reach statistical significance ( $F=2.187$ ,  $P=0.088$ ). We  
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8 238 observed no significant interaction between BMI and MMW for fibrinogen ( $F=1.680$ ,  $P=0.122$ ).

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10 239 **sE-Selectin (Figure 1, Panel E).** BMI significantly contributed to circulating levels of  
11  
12 240 sE-selectin ( $F=28.253$ ,  $P<0.001$ ) with no significant contribution by MMW ( $F=0.207$ ,  $P=0.892$ ).  
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15 241 Dose-dependent increases in sE-selectin were also observed across BMI levels ( $P$ 's<0.01). We  
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17 242 observed no significant interaction between BMI and MMW for sE-selectin ( $F=0.570$ ,  $P=0.755$ ).

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20 243 **IL-6sr (Figure 1, Panel F).** No significant main effects for BMI ( $F=1.783$ ,  $P=0.169$ ),  
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22 244 MMW ( $F=1.434$ ,  $P=0.231$ ) or their interaction ( $F=0.834$ ,  $P=0.544$ ) were detected for IL-6sr.  
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## 26 27 246 **DISCUSSION**

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29 247 The current study aimed to delineate the interactive and independent impact of BMI and  
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31 248 physical activity on inflammatory markers related to CVD risk. In the cases of CRP and  
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33 249 sICAM-1, the effects of BMI and MMW were interactive. Regular physical activity appeared to  
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35 250 diminish the effects of higher BMI compared to those who reported no regular physical activity.  
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37 251 We found that BMI was strongly and independently related to greater concentrations of both  
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39 252 established and emerging inflammatory markers that may increase CVD risk. Independent of  
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41 253 BMI, regular physical activity was associated with lower IL-6, with a similar trend for fibrinogen.  
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43 254 These results suggest that, although obesity has a clear impact on inflammation, physical activity  
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45 255 appears to mitigate at least some of this effect. Further, obese individuals may need to perform  
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47 256 levels of physical activity greater than current recommendations for health in order to mitigate  
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49 257 obesity-related inflammation, as trends for lower CRP or sICAM-1 were only apparent in obese  
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51 258 participants reporting >1000 MMW.  
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8 261 Low-grade, systemic inflammation is characterized by elevated levels of inflammatory  
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10 262 markers, such as cytokines, acute phase proteins or soluble adhesion molecules. IL-6 produced  
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12 263 in hypertrophied adipose tissue(15, 16) initiates the acute phase response, marked by the release  
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14 264 of hepatic CRP and fibrinogen(17, 18). Inflammatory cytokines (IL-6) and acute phase proteins  
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16 265 (CRP, fibrinogen) stimulate the production of chemoattractant proteins and adhesion molecules  
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18 266 (sICAM-1 and sE-selectin) in the vasculature, promoting cell accumulation and atherosclerotic  
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20 267 plaque formation(19, 20). In epidemiologic studies, higher levels of IL-6 and CRP are  
21  
22 268 associated with increasing numbers clinical risk factors for cardiovascular disease(21-23).  
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24 269 Cardiovascular disease risk is also increased with higher levels of cell adhesion molecules(24,  
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26 270 25) and acute phase reactants(22, 26, 27).

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28 271 Interactions between BMI and MMW suggest that regular physical activity may be able  
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30 272 to mitigate the effect of an overweight BMI on CRP. Overweight individuals had CRP levels  
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32 273 that were similar to levels observed in obese individuals if they reported no regular exercise  
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34 274 (4.05 and 4.83  $\mu\text{g}/\text{mL}$ , respectively). CRP levels greater than 3  $\mu\text{g}/\text{mL}$  are typically associated  
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36 275 with high CVD risk(28). In overweight subjects who reported regular physical activity of at least  
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38 276 3, 20-minute sessions per week (be it below [ $<500$ ], within [ $500-1000$ ] or above [ $>1000$ ]  
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40 277 USDHHS MMW recommendations), CRP levels were lower and not significantly different from  
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42 278 CRP levels found in normal weight participants. This suggests that increasing physical activity  
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44 279 level to a minimum of 3 days per week, at least 20 minutes per day, may improve CRP profiles  
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46 280 among overweight individuals.

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6 283           Obese individuals may require a higher level of regular physical activity in order to lower  
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8 284 inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1  
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10 285 compared to lean and overweight subjects, those who reported >1000 MMW (above the  
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12 286 USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than  
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15 287 obese subjects reporting no regular physical activity. Taken together, we may speculate that  
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17 288 while physical activity levels currently recommended for the general population may reduce  
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19 289 particular inflammatory makers in overweight populations, obese populations may require  
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21  
22 290 greater levels of physical activity above recommended values to reduce inflammatory markers  
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24  
25 291 like CRP and sICAM-1.

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27 292           As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen,  
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29 293 sICAM-1 and sE-selectin, in agreement with previous work(29-32). Independent of BMI effects,  
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31 294 our results suggest that physical activity has differentiating independent effects on inflammatory  
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34 295 markers. Individuals reporting no regular physical activity had higher levels of IL-6 with a  
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36 296 tendency for higher fibrinogen, compared to those reporting any level of regular physical activity  
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39 297 (<500, 500-1000 or >1000 MMW). Similar results have been observed in the MONItoring  
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41 298 trends and determinants in CARdiovascular disease (MONICA) study(33), the National Health  
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43 299 and Nutrition Examination Survey (NHANES III)(34, 35) and the Multi-Ethnic Study of  
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46 300 Atherosclerosis (MESA)(36), such that both increased frequency and intensity of physical  
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48 301 activity have been related to lower IL-6 and fibrinogen. While similar, our findings add to prior  
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50 302 results by standardizing levels of physical activity by using USDHHS recommendations, rather  
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53 303 than general tertiles, quartiles, etc.. However, while the USDHHS reports that meeting these  
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55 304 recommendations promotes substantial health benefits(11), the impact on specific inflammatory  
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3 305 markers was not addressed. Our results suggest that, regular physical activity at any level (<500,  
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5 306 500-1000, >1000) appears to be associated with lower levels of IL-6 and possibly fibrinogen,  
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8 307 independent of BMI.  
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11 308 Interestingly, in the MIDUS sample, results suggest that regular exercise may have a  
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13 309 more profound impact on lowering classical markers of inflammation and less impact on the  
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15 310 inflammatory status of the endothelium. Regular physical activity was independently associated  
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17 311 with lower levels of IL-6 and CRP, both classical inflammatory markers related to adipose and  
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19 312 systemic inflammation(37). However, regular exercise appeared to have no independent impact  
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22 313 on markers of endothelial activation, particularly sE-selectin and sICAM-1. Inverse  
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24 314 relationships between physical activity and sICAM-1 or sE-selectin have been reported  
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27 315 previously, in drug-treated hypertensive men(38). However, cross-sectional reporting of inverse  
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29 316 relationships between physical activity and other markers of atherosclerotic activity, particularly  
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31 317 carotid arterial wall thickness, has yielded variable results(39-42). Upon reviewing this literature,  
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34 318 Thijssen and colleagues suggest that inverse correlations between arterial wall thickness and  
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36 319 physical activity were more likely to be found in studies that utilized specifically-designed  
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38 320 instruments to assess physical activity, rather than non-specific questionnaires that obtain  
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40 321 information about general exercise behavior(43). Therefore, it is possible that a more objective  
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42 322 or validated measure of physical activity utilized may have increased the likelihood of observing  
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44 323 significant relationships between physical activity and circulating makers of atherosclerotic  
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46 324 activity that were independent of BMI.  
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50 325 Several limitations must be addressed. First, the cross-sectional design does not allow us  
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52 326 to infer causal relationships. Prospective and interventional designs are necessary to confirm our  
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54 327 findings. Second, the use of self-report physical activity data may reduce accuracy compared to  
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3 328 direct measures of physical activity. However, in addition to being in line with previous studies  
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5 329 using self-report physical activity, our findings are also in line with previous studies(44, 45) that  
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7  
8 330 demonstrated that higher cardiorespiratory fitness was associated with lower levels of  
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10 331 inflammation independent of visceral adiposity or BMI. Furthermore, regular physical activity  
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12 332 may have positive health effects independent of fitness, as individuals of similar fitness levels  
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14 333 demonstrate reduced risk for coronary heart disease, CVD and stroke with higher levels of  
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16 334 physical activity compared to those with lower activity levels and both low physical activity and  
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18 335 fitness levels directly increase risk of metabolic disease and type 2 diabetes mellitus(46, 47).  
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20 336 Finally, as the sample was predominantly comprised of non-Hispanic white individuals, findings  
21  
22 337 may not extend to all ethnicities. Finally, BMI and physical activity variables are correlated,  
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24 338 potentially raising the concern of small sample sizes in specific categories crossing BMI and  
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26 339 MMW. However, the minimum category contained 54 individuals (normal weight individuals  
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28 340 reporting no exercise).

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34 341 In summary, our results demonstrate both interactive and independent influences of BMI  
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36 342 and levels of physical activity on both established and emerging markers of inflammation.  
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38 343 Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular  
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40 344 physical activity appears to mitigate the effects of higher BMI on some inflammatory markers,  
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42 345 particularly CRP, which is strongly implicated in CVD. More importantly, while any level of  
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44 346 regular physical activity may help reduce inflammation in overweight individuals, similar effects  
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46 347 in obese individuals may require levels of physical activity that are greater than currently  
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48 348 recommended by the USDHHS for general health. It is important that future research aims to  
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50 349 elucidate effective exercise levels that can produce anti-inflammatory effects in overweight and  
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52 350 obese individuals.  
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9  
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22  
23 360 in the U.S.) Investigation.24  
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34  
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37 36738  
39 368 **CONFLICTS OF INTEREST**40  
41 369 The authors declare no conflict of interest.42  
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3 374 **CONTRIBUTORSHIP**

4  
5 375 KS, JMM and RRW each made substantial contributions to the conception and design of the  
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7  
8 376 study, data acquisition, analysis and interpretation, as well as to drafting and revision for  
9  
10 377 substantial intellectual content. All authors made final approval of the version to be published.  
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15 379 **DATA SHARING STATEMENT**

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17 380 Data and documentation for MIDUS studies are available at the Inter-university Consortium for  
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19 381 Political and Social Research (ICPSR). <http://www.icpsr.umich.edu/icpsrweb/landing.jsp>  
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27 519 **FIGURE AND TABLE LEGENDS**28  
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31 52132  
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34 522 **Figure 1: Inflammatory Markers.** Data from 1255 men and women in MIDUS. Joint35  
36 523 association of BMI category (normal, overweight and obese) and MMW category (no regular37  
38 524 exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C),39  
40 525 fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex,41  
42 526 smoking and relevant medication use. Error bars represent SEM. BMI=BMI main effect P value,43  
44 527 MMW=MMW main effect P value, INT=interaction effect P value.45  
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3 530 **Table 1: Subject Characteristics.** BMI = body mass index; CRP = C-reactive protein; IL =  
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6 531 interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin =  
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8 532 soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.  
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<b>Demographic Variables</b>	<b>Overall N = 1255 Mean ± SD (N)</b>
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 <sup>2</sup> )	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)

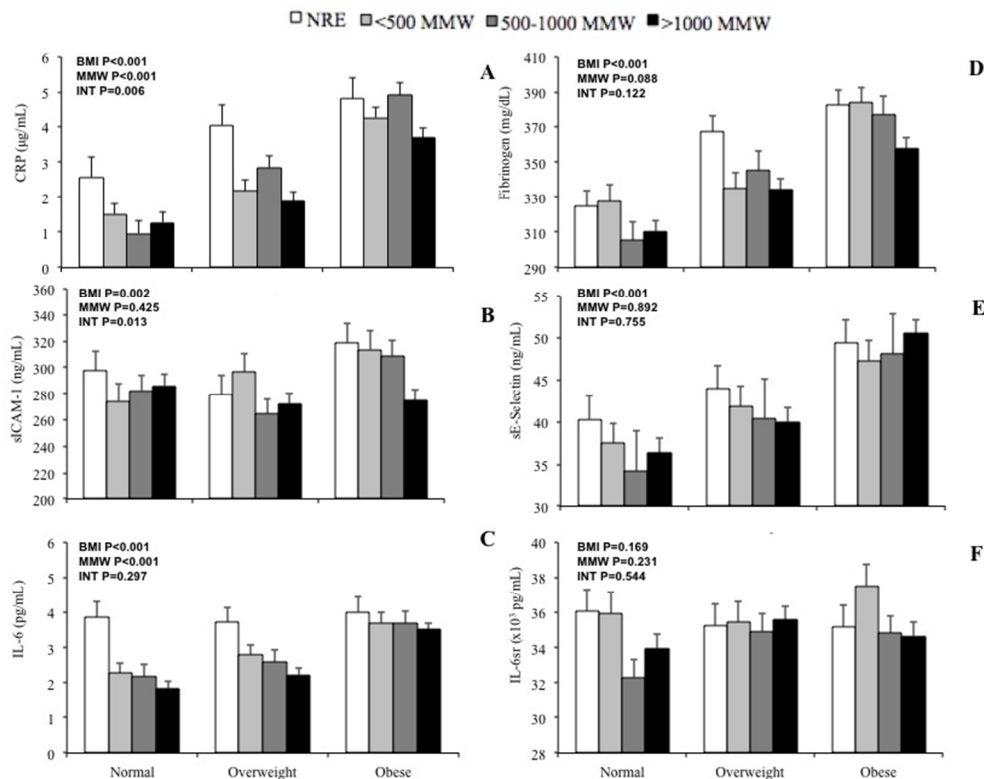


Figure 1: Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These 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)

only

**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
<b>Introduction</b>			
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
<b>Methods</b>			
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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	N/A

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	N/A
<b>Results</b>			
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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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
<b>Discussion</b>			
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
<b>Other information</b>			
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](http://www.strobe-statement.org).



**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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Manuscripts

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3 1 **Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A**  
4 2 **Cohort Study of Middle Aged Adults Living in the United States**  
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3 31 **ABSTRACT**  
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6 32 **Objectives.** Determine whether body mass index (BMI) and physical activity (PA) above, at or  
7  
8 33 below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity  
9  
10 34 Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP),  
11  
12 35 fibrinogen, IL-6 soluble receptor (IL-6sr), soluble E-selectin and soluble intracellular adhesion  
13  
14 36 molecule (sICAM)-1. **Design.** Archived cohort data (N=1254, age 54.5±11.7y, BMI  
15  
16 37 29.8±6.6kg/m<sup>2</sup>) from the National Survey of Midlife Development in the United States (MIDUS)  
17  
18 38 Biomarkers Study were analyzed for concentrations of inflammatory markers using general  
19  
20 39 linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000  
21  
22 40 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m<sup>2</sup>. Analyses were adjusted for age, sex,  
23  
24 41 smoking and relevant medication use. **Setting.** Respondents reported to three centers to  
25  
26 42 complete questionnaires and provide blood samples. **Participants.** Participants were men and  
27  
28 43 women currently enrolled in the MIDUS Biomarker Project (N=1254, 93% non-hispanic white,  
29  
30 44 average age 54.5y). **Primary Outcome Measures.** Concentration of serum IL-6, CRP,  
31  
32 45 fibrinogen, IL-6sr, sE-selectin and sICAM. **Results.** Significant interactions were found  
33  
34 46 between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was  
35  
36 47 similar to obese when no PA was reported, but was similar to normal weight when any level of  
37  
38 48 regular PA was reported. sICAM-1 was differentially lower in obese individuals who reported  
39  
40 49 >1000 MMW compared to obese individuals reporting less exercise. **Conclusion.** The  
41  
42 50 association of exercise with CRP and sICAM-1 differed by BMI, suggesting that regular exercise  
43  
44 51 may buffer weight-associated elevations in CRP in overweight individuals while higher levels of  
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46 52 exercise may be necessary to reduce sICAM-1 or CRP in obese individuals. **Trial Registry.**  
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## 54 INTRODUCTION

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

58 Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase  
59 reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Circulating  
60 Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory  
61 markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and  
62 soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been  
63 associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of  
64 inflammatory markers(7, 8). Further, body fat distribution is also an important factor relating to  
65 inflammatory status. Accumulation of fat in visceral depots is more strongly associated with  
66 low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots(9,  
67 10).

68 The effects of physical activity on markers of inflammation are more complex and may  
69 vary depending on body weight. A number of epidemiological studies have shown an inverse  
70 relationship between physical activity and CRP and IL-6, independent of obesity(11-16).

71 Laboratory studies conducted in aerobically trained, typically normal weight, individuals have  
72 demonstrated that a single bout of exercise stimulates IL-6 release from skeletal muscle, which  
73 promotes anti-inflammatory effects (17-19), as opposed to adipose tissue-derived IL-6 that is  
74 associated with pro-inflammatory effects (20). Randomized controlled trials have also been  
75 conducted, often in populations that also tend to be overweight or obese, to examine the effects

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2  
3 76 of aerobic exercise interventions on inflammation and the results are mixed (21). Thus, the  
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5 77 contribution of physical activity to inflammation in the context of obesity remains unclear.  
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8 The purpose of our study was to disentangle the relative contributions of BMI and  
9  
10 79 physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr,  
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12 80 CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were  
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14 81 determined using values put forth by the Physical Activity Guidelines for Americans, which  
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16 82 states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately  
17  
18 83 equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week)  
19  
20 84 produces substantial health benefits for adults(22). We hypothesized that BMI and MMW would  
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22 85 interact, such that greater MMW reported would lessen the impact of obesity on markers of  
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24 86 inflammation.  
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## 32 88 **MATERIALS AND METHODS**

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34 89 **Design and Sample.** This study was a cross-sectional analysis of archived data (BMI,  
35  
36 90 self-reported physical activity and inflammatory biomarker concentrations) from 1254  
37  
38 91 respondents who provided consent (as approved by The University of Wisconsin Madison Health  
39  
40 92 Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of  
41  
42 93 Midlife Development in the United States (MIDUS) Biomarkers Study (23). The Biomarker  
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44 94 Project was one of 5 projects within MIDUS II, with the purpose of adding comprehensive  
45  
46 95 biological assessments on a subsample of the MIDUS participants to further understand age-  
47  
48 96 related differences in physical and mental health. Participants were eligible for The Biomarker  
49  
50 97 Project if they were previously enrolled in MIDUS I, which recruited non-institutionalized,  
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52 98 English-speaking adults residing in the contiguous United States aged 25-74. The random digit  
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3 99 dialing sample for the parent study was selected from working telephone banks and a list of all  
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6 100 individuals between the ages of 25 and 74 years within each household was generated in order to  
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8 101 select a random respondent. Those who agreed to participate in the Biomarker Study stayed  
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10 102 overnight at one of three General Clinical Research Centers: University of California Los  
11  
12 103 Angeles, University of Wisconsin-Madison and Georgetown University. Upon arrival, each  
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14  
15 104 respondent provided a detailed medical history (including physical activity levels) and provided  
16  
17 105 all prescription, over-the-counter, and alternative medications to be inventoried by project staff.  
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20 106 Following an overnight stay, morning fasting blood samples were obtained. Cohorts were  
21  
22 107 assessed between July 2004 and May 2009 as a follow up to MIDUS I respondents that were  
23  
24 108 previously surveyed by the MacArthur Midlife Research Network between 1995 and 1996.  
25  
26  
27 109 Based on the sample of 1254 participants, 80% power was achieved to detect small effects of  
28  
29 110 0.08 or greater with alpha level at 0.05 for a two-tailed test(24, 25).

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32 111 **Anthropometrics.** Height was measured in centimeters and recorded to the nearest  
33  
34 112 millimeter. A single measure of WC was taken directly on the skin or over a single layer of light,  
35  
36 113 close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the  
37  
38 114 nearest millimeter. Weight was measured in kilograms and BMI was calculated by dividing  
39  
40 115 body mass in kilograms by height in meters squared. BMI categories were organized into 3  
41  
42 116 groups: normal weight ( $BMI \leq 24.9 \text{ kg/m}^2$ ), overweight ( $BMI \geq 25-29.9$ ) and obese ( $BMI \geq 30$ ).

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45  
46 117 **Categorizing Physical Activity by MET-Minutes per Week (MMW).**

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48 118 The MMW variable was calculated using data provided in the medical history form. The  
49  
50 119 form first described 3 types of regular physical activity(23):

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53 120 ***Vigorous:** Which causes your heart to beat so rapidly you can feel it in your chest*  
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55 121 *and you perform it long enough to work up a good sweat and breathe heavily (e.g.,*

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3 122 *competitive sports, running, vigorous swimming, high intensity aerobics, digging*  
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5 123 *in the garden, or lifting heavy objects).*

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8 124 **Moderate:** *Which causes your heart rate to increase slightly and you typically*  
9  
10 125 *work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming,*  
11  
12 126 *low intensity aerobics or golfing without a power cart, brisk walking, mowing the*  
13  
14 127 *lawn with a walking lawnmower).*

15 128 **Light:** *Which requires little physical effort (e.g., light housekeeping like dusting*  
16  
17 129 *or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).*

18  
19  
20 130 Keeping these definitions in mind, participants were asked if they engaged in regular physical  
21  
22 131 activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants  
23  
24 132 answered “yes”, they entered up to 7 types of seasonal and/or non-seasonal exercise or activity  
25  
26 133 along with the frequency, duration and intensity.

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29 134 MMW were calculated in a 2-step process. Step 1: subjects who reported no physical  
30  
31 135 activity (for whom no MMW calculations could be made) were designated as the no regular  
32  
33 136 exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical  
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35 137 activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for  
36  
37 138 low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity  
38  
39 139 reported. Four groups reflecting participation in physical activity and whether or not their  
40  
41 140 participation was below, at or above USDHHS guidelines were created: NRE (reported no  
42  
43 141 regular physical activity), below recommended (reported <500 MMW), recommended (reported  
44  
45 142 500-1000 MMW) and above recommended (reported >1000 MMW).

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48 143 **Blood Collection, Processing and Assays.** Participants were asked to avoid strenuous  
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50 144 activity the day of blood collection. Venous blood samples were collected in 10 mL serum  
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3 145 separator vacutainers following a 12-h overnight fast and processed at a General Clinical  
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5 146 Research Center using standardized procedures. Blood samples were not collected at any  
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8 147 specific point during the menstrual cycle in female participants. Briefly, following collection,  
9  
10 148 vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for  
11  
12 149 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab  
13  
14 150 and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-  
15  
16 151 Selectin and sICAM-1).  
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19  
20 152 IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison,  
21  
22 153 Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600,  
23  
24 154 R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6  
25  
26 155 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-  
27  
28 156 assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV%  
29  
30 157 values for IL-6sr were 5.9-5.7% and 2.0%, respectively.  
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34 158 Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory  
35  
36 159 for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of  
37  
38 160 sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay;  
39  
40 161 R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was  
41  
42 162 completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay,  
43  
44 163 R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%,  
45  
46 164 respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human  
47  
48 165 Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for  
49  
50 166 fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer  
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3 167 with a particle enhanced immunonephelometric assay. Intra-assay and inter-assay precision for  
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5 168 CRP was 2.3-4.4% and 2.1-5.7%, respectively.  
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8 169 **Statistical Analyses.** All variables were assessed for normality and non-normal data  
9  
10 170 were log transformed, which included data for CRP, IL-6, IL-6sr, fibrinogen, sE-selectin and  
11  
12 171 sICAM-1. General Linear Models were performed to determine the relationship of MMW and  
13  
14 172 BMI with the inflammatory markers. For each outcome, the ordinal MMW and BMI factors  
15  
16 173 were entered as independent factors with an interaction term. If the interaction term was not  
17  
18 174 significant, the interaction term was dropped and the model was re-fit for main effects only.  
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20 175 Pairwise comparisons were assessed using post hoc univariate analyses with a Bonferroni  
21  
22 176 adjustment for multiple comparisons. Covariates for all models included factors that are known  
23  
24 177 to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering,  
25  
26 178 corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive).  
27  
28 179 Race was initially included as a covariate; however, approximately 200 data points were lost in  
29  
30 180 the analyses due to incomplete racial data. As race was not found to be a predictor of our  
31  
32 181 dependent variables, with the exception of sICAM-1, race was excluded as a covariate to  
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34 182 increase samples size in all analyses excluding sICAM-1. All statistical analyses were  
35  
36 183 performed with SPSS v. 17 (Chicago, IL) and statistical significance was set  $\alpha = 0.05$ .  
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44 184 In an exploratory analysis, we examined whether the relative effects of BMI and MMW  
45  
46 185 on the inflammatory markers differed by sex in 3-way interaction models. As none of the  
47  
48 186 interactions approached statistical significance (data not shown), sex was retained as a covariate  
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50 187 in the models.  
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## 190 RESULTS

191 **Subject Characteristics.** Table 1 presents anthropometric characteristics and circulating  
192 levels of inflammatory biomarkers in all subjects (N=1254). Subjects were 92.6% non-Hispanic  
193 white, 56.8% female, and, on average, middle-aged and overweight. Of all the respondents,  
194 14.9% were currently smoked, 27.8% were taking cholesterol lowering medication, 12.1%  
195 corticosteroids, 10.4% anti-diabetic medication, 14.2% antidepressant medication, 7.3%  
196 hormone replacement and 2.5% oral contraceptives. The percentage of participants with missing  
197 data for each variable were as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6%  
198 for fibrinogen, 1.2% for sE-selectin, and 1.0% for IL-6sr.

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

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

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3 212 **sICAM-1 (Figure 1, Panel B).** We found a significant interaction between BMI and  
4  
5 213 MMW for sICAM-1 concentration ( $F=2.701$ ,  $P=0.013$ ). Levels of sICAM-1 were significantly  
6  
7 214 lower in obese subjects who reported  $>1000$  MMW compared to obese subjects who reported no  
8  
9 215 regular exercise ( $P=0.014$ ) and  $<500$  MMW ( $P=0.026$ ) and tended to be lower than levels in  
10  
11 216 obese subjects who reported 500-1000 MMW ( $P=0.079$ ). No differences in sICAM-1 by MMW  
12  
13 217 were observed among normal weight or overweight individuals.  
14  
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16  
17 218 We also observed a main effect of BMI ( $F=6.060$ ,  $P=0.002$ ), such that sICAM-1 levels in  
18  
19 219 obese participants were significantly higher than levels found in both normal weight and  
20  
21 220 overweight participants ( $P's<0.01$ ). No significant main effect of MMW was found for sICAM-1  
22  
23 221 ( $F=0.931$ ,  $P=0.425$ ).  
24  
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26  
27 222 **IL-6 (Figure 1, Panel C).** Both BMI and MMW had independent effects on circulating  
28  
29 223 concentrations of IL-6 (BMI:  $F=60.150$ ,  $P<0.001$ , MMW:  $F=10.680$ ,  $P<0.001$ ), with no  
30  
31 224 significant interaction ( $F=1.21$ ,  $P=0.297$ ). We found a dose-dependent effect of BMI, such that  
32  
33 225 higher BMI levels were associated with significantly greater IL-6 ( $P's<0.001$ ). Independent of  
34  
35 226 BMI, IL-6 was significantly lower in subjects who reported regular exercise ( $<500$  MMW, 500-  
36  
37 227 1000 MMW and  $>1000$  MMW) compared to those who reported no regular exercise ( $P's <0.01$ )  
38  
39 228 with no difference between levels of MMW.  
40  
41  
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43 229 **Fibrinogen (Figure 1, Panel D).** BMI significantly contributed to circulating levels of  
44  
45 230 fibrinogen ( $F=42.385$ ,  $P<0.001$ ), such that dose-dependent increases were observed for all BMI  
46  
47 231 levels ( $P's<0.01$ ). While we observed a trend for lower fibrinogen with regular physical activity,  
48  
49 232 similar to that of IL-6, the effect did not reach statistical significance ( $F=2.187$ ,  $P=0.088$ ). We  
50  
51 233 observed no significant interaction between BMI and MMW for fibrinogen ( $F=1.680$ ,  $P=0.122$ ).  
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3 234 **sE-Selectin ( 1, Panel E).** BMI significantly contributed to circulating levels of sE-  
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5 235 selectin (F=28.253, P<0.001) with no significant contribution by MMW (F=0.207, P=0.892).  
6  
7  
8 236 Dose-dependent increases in sE-selectin were also observed across BMI levels (P's<0.01). We  
9  
10 237 observed no significant interaction between BMI and MMW for sE-selectin (F=0.570, P=0.755).

11  
12 238 **IL-6sr (Figure 1, Panel F).** No significant main effects for BMI (F=1.783, P=0.169),  
13  
14  
15 239 MMW (F=1.434, P=0.231) or their interaction (F=0.834, P=0.544) were detected for IL-6sr.  
16

17 240 **Waist Circumference (WC) and Inflammatory Markers (Supplemental Figure 1).** A  
18  
19  
20 241 secondary analysis was completed using WC and MMW as independent variables and the  
21  
22 242 complete results of these analyses are located in the supplemental information. Briefly, we  
23  
24 243 found a significant interaction between WC and MMW on sICAM-1. In individuals with an at-  
25  
26 244 risk WC ( $\geq 102.0$  cm for men and  $\geq 88.0$  cm for women), sICAM-1 was significantly lower in  
27  
28  
29 245 those reporting 1000+ MMW compared to less than 500 MMW and tended to be lower in those  
30  
31 246 reporting no regular exercise. Overall, main effects were similar to those found for BMI and  
32  
33 247 MMW analyses. Having an at-risk WC was independently related to higher levels of CRP,  
34  
35 248 sICAM-1, IL-6, fibrinogen and sE-selectin. Independent of WC, any level of regular exercise  
36  
37 249 was related to lower levels of CRP, IL-6 with a similar tendency for fibrinogen.  
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## 42 43 251 **DISCUSSION**

44  
45 252 The current study aimed to determine whether the impact of BMI and MMW on  
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47 253 inflammatory markers varied by level of overweight or obesity. For CRP and s-ICAM-1  
48  
49 254 regular physical activity appeared to diminish the effects of higher BMI compared to those who  
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51 255 reported no regular physical activity. In addition, we found that BMI was strongly and  
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53 256 independently related to greater concentrations of both established and emerging inflammatory  
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3 257 markers that may increase CVD risk. Independent of BMI, regular physical activity was also  
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5 258 associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that,  
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8 259 although obesity has a clear impact on inflammation, physical activity appears to mitigate at least  
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10 260 some of this effect.

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12  
13 261 For example, overweight individuals had CRP levels that were similar to levels observed  
14  
15 262 in obese individuals if they reported no regular exercise (4.05 and 4.83  $\mu\text{g/mL}$ , respectively).  
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17  
18 263 CRP levels greater than 3  $\mu\text{g/mL}$  are typically associated with high CVD risk(26). In overweight  
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20 264 subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it  
21  
22 265 below [ $<500$ ], within [ $500-1000$ ] or above [ $>1000$ ] USDHHS MMW recommendations), CRP  
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25 266 levels were lower and not significantly different from CRP levels found in normal weight  
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27 267 participants (). This suggests that increasing physical activity level to a minimum of 3 days per  
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30 268 week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.

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32 269 Obese individuals may require a higher level of regular physical activity in order to lower  
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34 270 inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1  
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36  
37 271 compared to lean and overweight subjects, those who reported  $>1000$  MMW (above the  
38  
39 272 USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than  
40  
41 273 obese subjects reporting no regular physical activity. Taken together, we may speculate that  
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43  
44 274 while physical activity levels currently recommended for the general population may reduce  
45  
46 275 particular inflammatory makers in overweight populations, obese populations may require  
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48  
49 276 greater levels of physical activity above recommended values to reduce inflammatory markers  
50  
51 277 like CRP and sICAM-1.

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53 278 As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen,  
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56 279 sICAM-1 and sE-selectin, in agreement with previous work (27-30). Independent of BMI effects,  
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3 280 our results suggest that physical activity has differentiating effects on inflammatory markers.  
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6 281 Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for  
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8 282 higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-  
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10 283 1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and  
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12 284 determinants in CARDiovascular disease (MONICA) study(31), the National Health and Nutrition  
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14 285 Examination Survey (NHANES III)(12, 14) and the Multi-Ethnic Study of Atherosclerosis  
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16 286 (MESA)(32), such that both increased frequency and intensity of physical activity have been  
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18 287 related to lower IL-6 and fibrinogen. Our findings add to these prior results by standardizing  
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20 288 levels of physical activity by using USDHHS. Our results suggest that, regular physical activity  
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22 289 at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and  
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24 290 possibly fibrinogen, independent of BMI.  
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29 291 Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase  
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31 292 response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and  
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33 293 physical activity was detected for CRP, but not IL-6. While IL-6 and CRP were significantly  
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35 294 correlated ( $r=0.514$ , see Supplemental Table 1), this correlation suggests that IL-6 levels do not  
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37 295 fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing  
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39 296 to its substantially longer plasma half-life (37), which may improve our ability to detect  
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41 297 interaction effects in CRP compared to IL-6.  
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46 298 Interestingly, our results also suggest that regular exercise may have a more profound  
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48 299 impact on lowering classical markers of inflammation and less impact on the inflammatory status  
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50 300 of the endothelium. Regular physical activity has reliably been associated with lower levels of  
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52 301 IL-6 and CRP, both classical inflammatory markers related to adipose and systemic  
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54 302 inflammation(38). However, regular exercise appeared to have no independent impact on  
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3 303 markers of endothelial activation, particularly sE-selectin. Higher levels of exercise were related  
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5 304 to lower sICAM-1 in obese individuals only. In one prior study, inverse relationships between  
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8 305 physical activity and sICAM-1 and sE-selectin were reported in drug-treated hypertensive men  
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10 306 (39). Thus, further research is necessary to understand mechanisms underlying differential  
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12 307 associations of exercise with systemic and endothelial inflammation.

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14  
15 308 Several limitations must be addressed. First, the cross-sectional design does not allow us  
16  
17 309 to infer causal relationships. Prospective and interventional designs are necessary to confirm our  
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19 310 findings. No objective measures of physical activity were available in the MIDUS sample.  
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21 311 Therefore, the use of self-report physical activity data may have diminished our ability to detect  
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23 312 effects. However, in addition to being in line with previous studies using self-report physical  
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25 313 activity, our findings are also in line with previous studies(40, 41) that demonstrated that higher  
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27 314 cardiorespiratory fitness, as measured by indirect calorimetry, was associated with lower levels  
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29 315 of inflammation independent of visceral adiposity or BMI. Another limitation is that the sample  
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31 316 was predominantly comprised of non-Hispanic white individuals, suggesting that findings may  
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33 317 not extend to all ethnicities. Finally, BMI and physical activity variables are correlated,  
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35 318 potentially raising the concern of small sample sizes in specific groups crossed by BMI and  
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37 319 MMW. However, the smallest group for analyses still contained 54 individuals (normal weight  
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39 320 individuals reporting no exercise).

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41 321 In summary, our results demonstrate both interactive and independent influences of BMI  
42  
43 322 and levels of physical activity on both established and emerging markers of inflammation.  
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45 323 Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular  
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47 324 physical activity appears to mitigate the effects of higher BMI on some inflammatory markers,  
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49 325 particularly CRP, which is strongly implicated in CVD. Importantly, while any level of regular  
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326 physical activity may help reduce inflammation in overweight individuals, similar effects in  
327 obese individuals may require levels of physical activity that are greater than currently  
328 recommended by the USDHHS for general health.

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For peer review only

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22 357 in the U.S.) Investigation.  
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41 365 **CONFLICTS OF INTEREST**  
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43 366 The authors declare no conflict of interest.  
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48 368 **CONTRIBUTORSHIP**  
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50 369 KS, JMM and RRW each made substantial contributions to the conception and design of the  
51  
52 370 study, data acquisition, analysis and interpretation, as well as to drafting and revision for  
53  
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55 371 substantial intellectual content. All authors made final approval of the version to be published.  
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6 373 **DATA SHARING STATEMENT**  
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8 374 Data and documentation for MIDUS studies are available at the Inter-university Consortium for  
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10 375 Political and Social Research (ICPSR). <http://www.icpsr.umich.edu/icpsrweb/landing.jsp>  
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## 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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3 507 **FIGURE AND TABLE LEGENDS**  
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10 510 **Figure 1: Inflammatory Markers.** Data from 1254 men and women in MIDUS. Joint  
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12 511 association of BMI category (normal, overweight and obese) and MMW category (no regular  
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14 512 exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C),  
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16 513 fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex,  
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18 514 smoking and relevant medication use. The analysis for sICAM-1 was further adjusted for race.  
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20 515 Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value,  
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22 516 INT=interaction effect P value.  
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31 519 **Table 1: Subject Characteristics.** BMI = body mass index; CRP = C-reactive protein; IL =  
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33 520 interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin =  
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35 521 soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.  
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3 1 **Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A**  
4 2 **Cohort Study of Middle Aged Adults Living in the United States**  
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56 26 Running Title:

57 27 BMI, Physical Activity and Inflammation  
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54 29 **Key Words:** MIDUS, Intracellular Adhesion Molecule-1, Fibrinogen, C-Reactive Protein

56 30 **Word Count: 3331**

31 **ABSTRACT**

32 **Objectives.** Determine whether body mass index (BMI) and physical activity (PA) above, at or  
33 below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity  
34 Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP),  
35 fibrinogen, IL-6 soluble receptor (IL-6sr), soluble E-selectin and soluble intracellular adhesion  
36 molecule (sICAM)-1. **Design.** Archived cohort data (N=1254, age 54.5±11.7y, BMI  
37 29.8±6.6kg/m<sup>2</sup>) from the National Survey of Midlife Development in the United States (MIDUS)  
38 Biomarkers Study were analyzed for concentrations of inflammatory markers using general  
39 linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000  
40 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m<sup>2</sup>. Analyses were adjusted for age, sex,  
41 smoking and relevant medication use. **Setting.** Respondents reported to three centers to  
42 complete questionnaires and provide blood samples. **Participants.** Participants were men and  
43 women currently enrolled in the MIDUS Biomarker Project (N=1254, 93% non-hispanic white,  
44 average age 54.5y). **Primary Outcome Measures.** Concentration of serum IL-6, CRP,  
45 fibrinogen, IL-6sr, sE-selectin and sICAM. **Results.** Significant interactions were found  
46 between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was  
47 similar to obese when no PA was reported, but was similar to normal weight when any level of  
48 regular PA was reported. sICAM-1 was differentially lower in obese individuals who reported  
49 >1000 MMW compared to obese individuals reporting less exercise. **Conclusion.** The  
50 association of exercise with CRP and sICAM-1 differed by BMI, suggesting that regular exercise  
51 may buffer weight-associated elevations in CRP in overweight individuals while higher levels of  
52 exercise may be necessary to reduce sICAM-1 or CRP in obese individuals. **Trial Registry.**  
53 N/A.

## 54 INTRODUCTION

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

58 Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase  
59 reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Circulating  
60 Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory  
61 markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and  
62 soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been  
63 associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of  
64 inflammatory markers(7, 8). Further, body fat distribution is also an important factor relating to  
65 inflammatory status. Accumulation of fat in visceral depots is more strongly associated with  
66 low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots(9,  
67 10).

68 The effects of physical activity on markers of inflammation are more complex and may  
69 vary depending on body weight. A number of epidemiological studies have shown an inverse  
70 relationship between physical activity and CRP and IL-6, independent of obesity(11-16).

71 Laboratory studies conducted in aerobically trained, typically normal weight, individuals have  
72 demonstrated that a single bout of exercise stimulates IL-6 release from skeletal muscle, which  
73 promotes anti-inflammatory effects (17-19), as opposed to adipose tissue-derived IL-6 that is  
74 associated with pro-inflammatory effects (20). Randomized controlled trials have also been  
75 conducted, often in populations that also tend to be overweight or obese, to examine the effects



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3 76 of aerobic exercise interventions on inflammation and the results are mixed (21). Thus, the  
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5 77 contribution of physical activity to inflammation in the context of obesity remains unclear.  
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8 78 The purpose of our study was to disentangle the relative contributions of BMI and  
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10 79 physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr,  
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12 80 CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were  
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14 81 determined using values put forth by the Physical Activity Guidelines for Americans, which  
15  
16 82 states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately  
17  
18 83 equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week)  
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20 84 produces substantial health benefits for adults(22). We hypothesized that BMI and MMW would  
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22 85 interact, such that greater MMW reported would lessen the impact of obesity on markers of  
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24 86 inflammation.  
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## 32 88 MATERIALS AND METHODS

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34 89 **Design and Sample.** This study was a cross-sectional analysis of archived data (BMI,  
35  
36 90 self-reported physical activity and inflammatory biomarker concentrations) from 1254  
37  
38 91 respondents who provided consent (as approved by The University of Wisconsin Madison Health  
39  
40 92 Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of  
41  
42 93 Midlife Development in the United States (MIDUS) Biomarkers Study (23). The Biomarker  
43  
44 94 Project was one of 5 projects within MIDUS II, with the purpose of adding comprehensive  
45  
46 95 biological assessments on a subsample of the MIDUS participants to further understand age-  
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48 96 related differences in physical and mental health. Participants were eligible for The Biomarker  
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50 97 Project if they were previously enrolled in MIDUS I, which recruited non-institutionalized,  
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52 98 English-speaking adults residing in the contiguous United States aged 25-74. The random digit  
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3 99 dialing sample for the parent study was selected from working telephone banks and a list of all  
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6 100 individuals between the ages of 25 and 74 years within each household was generated in order to  
7  
8 101 select a random respondent. Those who agreed to participate in the Biomarker Study stayed  
9  
10 102 overnight at one of three General Clinical Research Centers: University of California Los  
11  
12 103 Angeles, University of Wisconsin-Madison and Georgetown University. Upon arrival, each  
13  
14 104 respondent provided a detailed medical history (including physical activity levels) and provided  
15  
16 105 all prescription, over-the-counter, and alternative medications to be inventoried by project staff.  
17  
18 106 Following an overnight stay, morning fasting blood samples were obtained. Cohorts were  
19  
20 107 assessed between July 2004 and May 2009 as a follow up to MIDUS I respondents that were  
21  
22 108 previously surveyed by the MacArthur Midlife Research Network between 1995 and 1996.  
23  
24 109 Based on the sample of 1254 participants, 80% power was achieved to detect small effects of  
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26 110 0.08 or greater with alpha level at 0.05 for a two-tailed test(24, 25).

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29 111 **Anthropometrics.** Height was measured in centimeters and recorded to the nearest  
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31 112 millimeter. A single measure of WC was taken directly on the skin or over a single layer of light,  
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33 113 close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the  
34  
35 114 nearest millimeter. Weight was measured in kilograms and BMI was calculated by dividing  
36  
37 115 body mass in kilograms by height in meters squared. BMI categories were organized into 3  
38  
39 116 groups: normal weight ( $BMI \leq 24.9 \text{ kg/m}^2$ ), overweight ( $BMI \geq 25-29.9$ ) and obese ( $BMI \geq 30$ ).

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

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

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

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3 122 *competitive sports, running, vigorous swimming, high intensity aerobics, digging*  
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5 123 *in the garden, or lifting heavy objects).*

7  
8 124 **Moderate:** *Which causes your heart rate to increase slightly and you typically*  
9  
10 125 *work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming,*  
11  
12 126 *low intensity aerobics or golfing without a power cart, brisk walking, mowing the*  
13  
14 127 *lawn with a walking lawnmower).*

15  
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17 128 **Light:** *Which requires little physical effort (e.g., light housekeeping like dusting*  
18  
19 129 *or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).*

20  
21  
22 130 Keeping these definitions in mind, participants were asked if they engaged in regular physical  
23  
24 131 activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants  
25  
26 132 answered “yes”, they entered up to 7 types of seasonal and/or non-seasonal exercise or activity  
27  
28 133 along with the frequency, duration and intensity.

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31 134 MMW were calculated in a 2-step process. Step 1: subjects who reported no physical  
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33 135 activity (for whom no MMW calculations could be made) were designated as the no regular  
34  
35 136 exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical  
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37 137 activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for  
38  
39 138 low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity  
40  
41 139 reported. Four groups reflecting participation in physical activity and whether or not their  
42  
43 140 participation was below, at or above USDHHS guidelines were created: NRE (reported no  
44  
45 141 regular physical activity), below recommended (reported <500 MMW), recommended (reported  
46  
47 142 500-1000 MMW) and above recommended (reported >1000 MMW).

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51 143 **Blood Collection, Processing and Assays.** Participants were asked to avoid strenuous  
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53 144 activity the day of blood collection. Venous blood samples were collected in 10 mL serum

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3 145 separator vacutainers following a 12-h overnight fast and processed at a General Clinical  
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5 146 Research Center using standardized procedures. Blood samples were not collected at any  
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8 147 specific point during the menstrual cycle in female participants. Briefly, following collection,  
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10 148 vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for  
11  
12 149 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab  
13  
14 150 and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-  
15  
16 151 Selectin and sICAM-1).

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20 152 IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison,  
21  
22 153 Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600,  
23  
24 154 R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6  
25  
26 155 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-  
27  
28 156 assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV%  
29  
30 157 values for IL-6sr were 5.9-5.7% and 2.0%, respectively.

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34 158 Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory  
35  
36 159 for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of  
37  
38 160 sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay;  
39  
40 161 R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was  
41  
42 162 completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay,  
43  
44 163 R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%,  
45  
46 164 respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human  
47  
48 165 Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for  
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50 166 fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer  
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3 167 with a particle enhanced immunonephelometric assay. Intra-assay and inter-assay precision for  
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5 168 CRP was 2.3-4.4% and 2.1-5.7%, respectively.  
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8 169 **Statistical Analyses.** All variables were assessed for normality and non-normal data  
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10 170 were log transformed, which included data for CRP, IL-6, IL-6sr, fibrinogen, sE-selectin and  
11  
12 171 sICAM-1. General Linear Models were performed to determine the relationship of MMW and  
13  
14 172 BMI with the inflammatory markers. For each outcome, the ordinal MMW and BMI factors  
15  
16 173 were entered as independent factors with an interaction term. If the interaction term was not  
17  
18 174 significant, the interaction term was dropped and the model was re-fit for main effects only.  
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20 175 Pairwise comparisons were assessed using post hoc univariate analyses with a Bonferroni  
21  
22 176 adjustment for multiple comparisons. Covariates for all models included factors that are known  
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24 177 to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering,  
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26 178 corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive).  
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28 179 Race was initially included as a covariate; however, approximately 200 data points were lost in  
29  
30 180 the analyses due to incomplete racial data. As race was not found to be a predictor of our  
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32 181 dependent variables, with the exception of sICAM-1, race was excluded as a covariate to  
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34 182 increase samples size in all analyses excluding sICAM-1. All statistical analyses were  
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36 183 performed with SPSS v. 17 (Chicago, IL) and statistical significance was set  $\alpha = 0.05$ .  
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44 184 In an exploratory analysis, we examined whether the relative effects of BMI and MMW  
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46 185 on the inflammatory markers differed by sex in 3-way interaction models. As none of the  
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48 186 interactions approached statistical significance (data not shown), sex was retained as a covariate  
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50 187 in the models.  
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## 190 RESULTS

191 **Subject Characteristics.** Table 1 presents anthropometric characteristics and circulating  
192 levels of inflammatory biomarkers in all subjects (N=1254). Subjects were 92.6% non-Hispanic  
193 white, 56.8% female, and, on average, middle-aged and overweight. Of all the respondents,  
194 14.9% were currently smoked, 27.8% were taking cholesterol lowering medication, 12.1%  
195 corticosteroids, 10.4% anti-diabetic medication, 14.2% antidepressant medication, 7.3%  
196 hormone replacement and 2.5% oral contraceptives. The percentage of participants with missing  
197 data for each variable were as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6%  
198 for fibrinogen, 1.2% for sE-selectin, and 1.0% for IL-6sr.

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

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

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3 212 **sICAM-1 (Figure 1, Panel B).** We found a significant interaction between BMI and  
4  
5 213 MMW for sICAM-1 concentration ( $F=2.701$ ,  $P=0.013$ ). Levels of sICAM-1 were significantly  
6  
7 214 lower in obese subjects who reported  $>1000$  MMW compared to obese subjects who reported no  
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9 215 regular exercise ( $P=0.014$ ) and  $<500$  MMW ( $P=0.026$ ) and tended to be lower than levels in  
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11 216 obese subjects who reported 500-1000 MMW ( $P=0.079$ ). No differences in sICAM-1 by MMW  
12  
13 217 were observed among normal weight or overweight individuals.  
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16  
17 218 We also observed a main effect of BMI ( $F=6.060$ ,  $P=0.002$ ), such that sICAM-1 levels in  
18  
19 219 obese participants were significantly higher than levels found in both normal weight and  
20  
21 220 overweight participants ( $P's<0.01$ ). No significant main effect of MMW was found for sICAM-1  
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23 221 ( $F=0.931$ ,  $P=0.425$ ).  
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27 222 **IL-6 (Figure 1, Panel C).** Both BMI and MMW had independent effects on circulating  
28  
29 223 concentrations of IL-6 (BMI:  $F=60.150$ ,  $P<0.001$ , MMW:  $F=10.680$ ,  $P<0.001$ ), with no  
30  
31 224 significant interaction ( $F=1.21$ ,  $P=0.297$ ). We found a dose-dependent effect of BMI, such that  
32  
33 225 higher BMI levels were associated with significantly greater IL-6 ( $P's<0.001$ ). Independent of  
34  
35 226 BMI, IL-6 was significantly lower in subjects who reported regular exercise ( $<500$  MMW, 500-  
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37 227 1000 MMW and  $>1000$  MMW) compared to those who reported no regular exercise ( $P's <0.01$ )  
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39 228 with no difference between levels of MMW.  
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43 229 **Fibrinogen (Figure 1, Panel D).** BMI significantly contributed to circulating levels of  
44  
45 230 fibrinogen ( $F=42.385$ ,  $P<0.001$ ), such that dose-dependent increases were observed for all BMI  
46  
47 231 levels ( $P's<0.01$ ). While we observed a trend for lower fibrinogen with regular physical activity,  
48  
49 232 similar to that of IL-6, the effect did not reach statistical significance ( $F=2.187$ ,  $P=0.088$ ). We  
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51 233 observed no significant interaction between BMI and MMW for fibrinogen ( $F=1.680$ ,  $P=0.122$ ).  
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3 234 **sE-Selectin ( 1, Panel E).** BMI significantly contributed to circulating levels of sE-  
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5 235 selectin ( $F=28.253$ ,  $P<0.001$ ) with no significant contribution by MMW ( $F=0.207$ ,  $P=0.892$ ).  
6  
7  
8 236 Dose-dependent increases in sE-selectin were also observed across BMI levels ( $P$ 's $<0.01$ ). We  
9  
10 237 observed no significant interaction between BMI and MMW for sE-selectin ( $F=0.570$ ,  $P=0.755$ ).

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12 238 **IL-6sr (Figure 1, Panel F).** No significant main effects for BMI ( $F=1.783$ ,  $P=0.169$ ),  
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15 239 MMW ( $F=1.434$ ,  $P=0.231$ ) or their interaction ( $F=0.834$ ,  $P=0.544$ ) were detected for IL-6sr.  
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17 240 **Waist Circumference (WC) and Inflammatory Markers (Supplemental Figure 1).** A  
18  
19 241 secondary analysis was completed using WC and MMW as independent variables and the  
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21 242 complete results of these analyses are located in the supplemental information. Briefly, we  
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23 243 found a significant interaction between WC and MMW on sICAM-1. In individuals with an at-  
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25 244 risk WC ( $\geq 102.0$  cm for men and  $\geq 88.0$  cm for women), sICAM-1 was significantly lower in  
26  
27 245 those reporting 1000+ MMW compared to less than 500 MMW and tended to be lower in those  
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29 246 reporting no regular exercise. Overall, main effects were similar to those found for BMI and  
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31 247 MMW analyses. Having an at-risk WC was independently related to higher levels of CRP,  
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33 248 sICAM-1, IL-6, fibrinogen and sE-selectin. Independent of WC, any level of regular exercise  
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35 249 was related to lower levels of CRP, IL-6 with a similar tendency for fibrinogen.  
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## 43 251 **DISCUSSION**

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45 252 The current study aimed to determine whether the impact of BMI and MMW on  
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47 253 inflammatory markers varied by level of overweight or obesity. For CRP and s-ICAM-1  
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49 254 regular physical activity appeared to diminish the effects of higher BMI compared to those who  
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51 255 reported no regular physical activity. In addition, we found that BMI was strongly and  
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53 256 independently related to greater concentrations of both established and emerging inflammatory  
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3 257 markers that may increase CVD risk. Independent of BMI, regular physical activity was also  
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5 258 associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that,  
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8 259 although obesity has a clear impact on inflammation, physical activity appears to mitigate at least  
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10 260 some of this effect.

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13 261 For example, overweight individuals had CRP levels that were similar to levels observed  
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15 262 in obese individuals if they reported no regular exercise (4.05 and 4.83  $\mu\text{g/mL}$ , respectively).  
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18 263 CRP levels greater than 3  $\mu\text{g/mL}$  are typically associated with high CVD risk(26). In overweight  
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20 264 subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it  
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22 265 below [ $<500$ ], within [ $500-1000$ ] or above [ $>1000$ ] USDHHS MMW recommendations), CRP  
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25 266 levels were lower and not significantly different from CRP levels found in normal weight  
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27 267 participants (). This suggests that increasing physical activity level to a minimum of 3 days per  
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30 268 week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.

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32 269 Obese individuals may require a higher level of regular physical activity in order to lower  
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34 270 inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1  
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37 271 compared to lean and overweight subjects, those who reported  $>1000$  MMW (above the  
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39 272 USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than  
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41 273 obese subjects reporting no regular physical activity. Taken together, we may speculate that  
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44 274 while physical activity levels currently recommended for the general population may reduce  
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46 275 particular inflammatory makers in overweight populations, obese populations may require  
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49 276 greater levels of physical activity above recommended values to reduce inflammatory markers  
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51 277 like CRP and sICAM-1.

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53 278 As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen,  
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56 279 sICAM-1 and sE-selectin, in agreement with previous work (27-30). Independent of BMI effects,  
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3 280 our results suggest that physical activity has differentiating effects on inflammatory markers.  
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6 281 Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for  
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8 282 higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-  
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10 283 1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and  
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12 284 determinants in Cardiovascular disease (MONICA) study(31), the National Health and Nutrition  
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14 285 Examination Survey (NHANES III)(12, 14) and the Multi-Ethnic Study of Atherosclerosis  
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16 286 (MESA)(32), such that both increased frequency and intensity of physical activity have been  
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18 287 related to lower IL-6 and fibrinogen. Our findings add to these prior results by standardizing  
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20 288 levels of physical activity by using USDHHS. Our results suggest that, regular physical activity  
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22 289 at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and  
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24 290 possibly fibrinogen, independent of BMI.  
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29 291 Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase  
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31 292 response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and  
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33 293 physical activity was detected for CRP, but not IL-6. While IL-6 and CRP were significantly  
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35 294 correlated ( $r=0.514$ , see Supplemental Table 1), this correlation suggests that IL-6 levels do not  
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37 295 fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing  
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39 296 to its substantially longer plasma half-life (37), which may improve our ability to detect  
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41 297 interaction effects in CRP compared to IL-6.  
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46 298 Interestingly, our results also suggest that regular exercise may have a more profound  
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48 299 impact on lowering classical markers of inflammation and less impact on the inflammatory status  
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50 300 of the endothelium. Regular physical activity has reliably been associated with lower levels of  
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52 301 IL-6 and CRP, both classical inflammatory markers related to adipose and systemic  
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54 302 inflammation(38). However, regular exercise appeared to have no independent impact on  
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3 303 markers of endothelial activation, particularly sE-selectin. Higher levels of exercise were related  
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5 304 to lower sICAM-1 in obese individuals only. In one prior study, inverse relationships between  
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8 305 physical activity and sICAM-1 and sE-selectin were reported in drug-treated hypertensive men  
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10 306 (39). Thus, further research is necessary to understand mechanisms underlying differential  
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12 307 associations of exercise with systemic and endothelial inflammation.  
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15 308 Several limitations must be addressed. First, the cross-sectional design does not allow us  
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17 309 to infer causal relationships. Prospective and interventional designs are necessary to confirm our  
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19 310 findings. No objective measures of physical activity were available in the MIDUS sample.  
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21 311 Therefore, the use of self-report physical activity data may have diminished our ability to detect  
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23 312 effects. However, in addition to being in line with previous studies using self-report physical  
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25 313 activity, our findings are also in line with previous studies(40, 41) that demonstrated that higher  
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27 314 cardiorespiratory fitness, as measured by indirect calorimetry, was associated with lower levels  
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29 315 of inflammation independent of visceral adiposity or BMI. Another limitation is that the sample  
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31 316 was predominantly comprised of non-Hispanic white individuals, suggesting that findings may  
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33 317 not extend to all ethnicities. Finally, BMI and physical activity variables are correlated,  
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35 318 potentially raising the concern of small sample sizes in specific groups crossed by BMI and  
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37 319 MMW. However, the smallest group for analyses still contained 54 individuals (normal weight  
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39 320 individuals reporting no exercise).  
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46 321 In summary, our results demonstrate both interactive and independent influences of BMI  
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48 322 and levels of physical activity on both established and emerging markers of inflammation.  
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50 323 Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular  
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52 324 physical activity appears to mitigate the effects of higher BMI on some inflammatory markers,  
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54 325 particularly CRP, which is strongly implicated in CVD. Importantly, while any level of regular  
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3 326 physical activity may help reduce inflammation in overweight individuals, similar effects in  
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6 327 obese individuals may require levels of physical activity that are greater than currently  
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8 328 recommended by the USDHHS for general health.  
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6  
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10 352

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12  
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21  
22 357 in the U.S.) Investigation.  
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41 365 **CONFLICTS OF INTEREST**  
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43 366 The authors declare no conflict of interest.  
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48 368 **CONTRIBUTORSHIP**  
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50 369 KS, JMM and RRW each made substantial contributions to the conception and design of the  
51  
52  
53 370 study, data acquisition, analysis and interpretation, as well as to drafting and revision for  
54  
55 371 substantial intellectual content. All authors made final approval of the version to be published.  
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6 373 **DATA SHARING STATEMENT**7  
8 374 Data and documentation for MIDUS studies are available at the Inter-university Consortium for9  
10 375 Political and Social Research (ICPSR). <http://www.icpsr.umich.edu/icpsrweb/landing.jsp>11  
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15 377**ARTICLE SUMMARY****Article focus**16  
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- 18 • Systemic inflammation is related to the progression of  
19 cardiovascular disease.
- 20 379 • Independent of obesity, physical activity is inversely related to  
21 concentrations of well-established inflammatory biomarkers,  
22 380 such as C-reactive protein (CRP) or interleukin-6 (IL-6).
- 23 381 • This article evaluates interactive effects of body mass index and  
24 physical activity on established inflammatory markers, CRP,  
25 382 IL-6, and emerging inflammatory markers, fibrinogen, soluble  
26 intracellular adhesion molecule (sICAM)-1, soluble E-selectin,  
27 383 and IL-6 soluble receptor.

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31**Key messages**

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- 33 • Interactive effects of body mass index and physical activity  
34 385 were observed for CRP, such that regular physical activity  
35 reported by overweight individuals was related to significantly  
36 386 lower CRP levels compared to those reported no regular  
37 activity.
- 38 • Independent of BMI, regular physical activity was related to  
39 387 lower IL-6, with a trend for lower fibrinogen
- 40 • Physical activity had no independent effect on circulating  
41 388 markers related to endothelial inflammation, such as sICAM-1  
42 or sE-selectin.

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44 389**Strengths and limitations**45  
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- 47 • 1254 adults from the National Survey of Midlife Development  
48 391 in the United States (MIDUS) Biomarker Project were  
49 analyzed. Statistical analyses were adjusted for age, sex,  
50 392 smoking, and relevant medication use. A strength of this paper  
51 is categorizing physical activity levels based on national  
52 393 recommendations. This data may be used to determine  
53 appropriate levels of physical activity necessary for reducing  
54 394 inflammation in overweight and obese adults. However, cross-  
55 sectional data is limited, as causal inferences cannot be  
56 obtained. A second limitation is that the sample was  
57 predominantly comprised of non-Hispanic white individuals,  
58 therefore findings may not extend to all ethnicities.

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3 507 **FIGURE AND TABLE LEGENDS**  
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10 **Figure 1: Inflammatory Markers.** Data from 1254 men and women in MIDUS. Joint  
11 association of BMI category (normal, overweight and obese) and MMW category (no regular  
12 exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C),  
13 fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex,  
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20 smoking and relevant medication use. **The analysis for sICAM-1 was further adjusted for race.**  
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22 Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value,  
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519 **Table 1: Subject Characteristics.** BMI = body mass index; CRP = C-reactive protein; IL =  
520 interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin =  
521 soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.  
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**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
<b>Introduction</b>			
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
<b>Methods</b>			
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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	N/A

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	N/A
<b>Results</b>			
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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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
<b>Discussion</b>			
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
<b>Other information</b>			
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](http://www.strobe-statement.org).

Demographic	BMI < 25 N=298	BMI 25-29.9 N=440	BMI ≥30 N=516	Overall N = 1254
Variables	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 <sup>2</sup> )	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

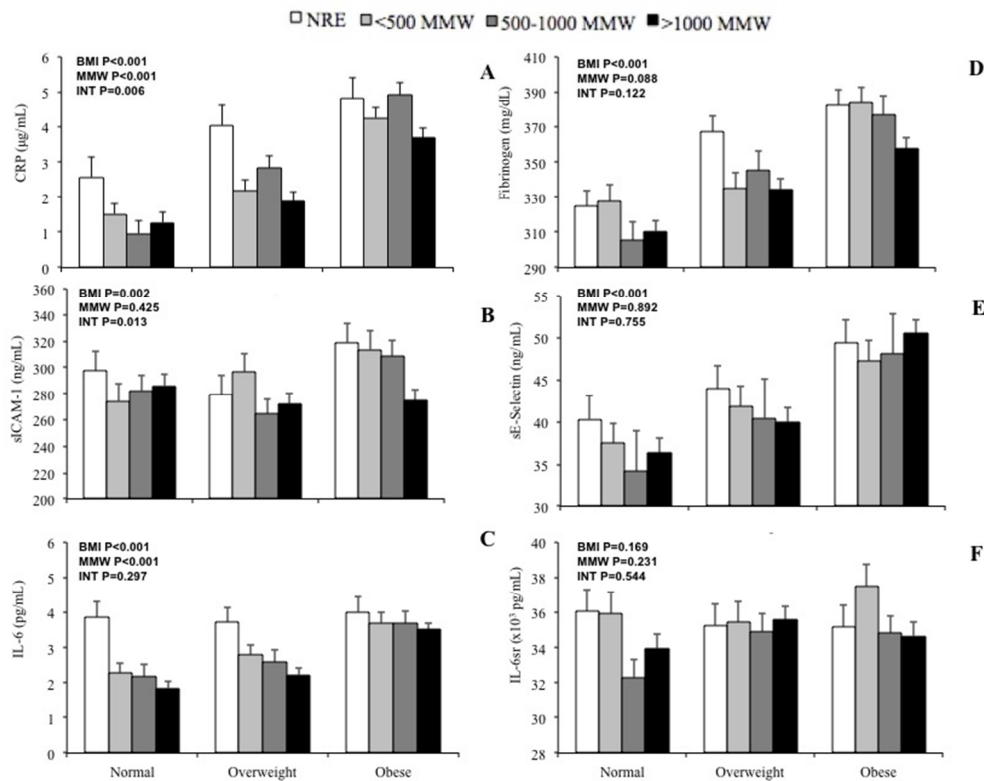


Figure 1: Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These 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)

only



### **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 ( $P's<0.05$ ).

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*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 ( $F=0.172$ ,  $P=0.916$ ).

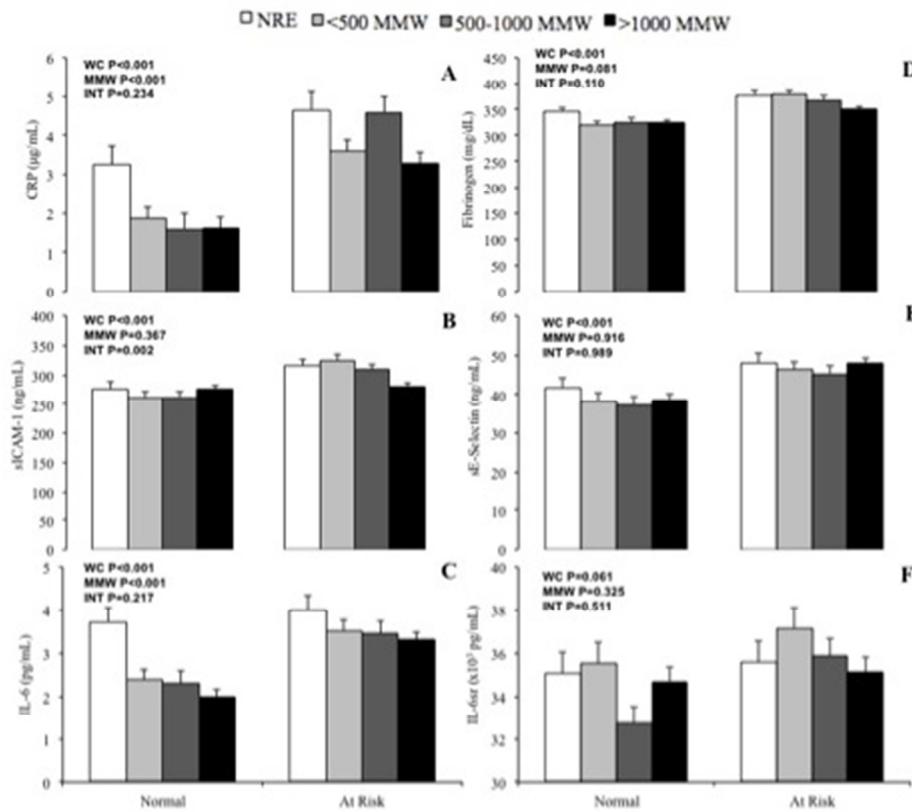
*IL-6sr (Supplementary Figure 1, panel F).* We found no significant interaction effect between WC and MMW for IL-6sr ( $F=0.769$ ,  $P=0.511$ ). Like BMI, we found no main effects for waist circumference ( $F=3.505$ ,  $P=0.061$ ) or MMW on IL-6sr ( $F=1.158$ ,  $P=0.325$ ).

*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 [ $\geq 102.0$  cm for men and  $\geq 88.0$  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 [ $\geq 102.0$  cm for men and  $\geq 88.0$  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  $p \leq 0.001$ .



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

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2013-002623.R2
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Complete List of Authors:	Strohacker, Kelley McCaffery, Jeanne wing, rena
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Keywords:	Immunology < BASIC SCIENCES, EPIDEMIOLOGY, Public health < INFECTIOUS DISEASES

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Manuscripts

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3 1 **Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A**  
4 2 **Cohort Study of Middle Aged Adults Living in the United States**  
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8 5

9 6 Kelley Strohacker, Ph.D.<sup>a</sup>, Rena R. Wing, Ph.D.<sup>a</sup>, and Jeanne M. McCaffery, Ph.D.<sup>a</sup>  
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46 26 Running Title:  
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49 27 BMI, Physical Activity and Inflammation  
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52  
53  
54 29 **Key Words:** MIDUS, Intracellular Adhesion Molecule-1, Fibrinogen, C-Reactive Protein  
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56 30 **Word Count: 3931**  
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<b>ARTICLE SUMMARY</b>	32
<b>Article focus</b>	33
<ul style="list-style-type: none"> <li>• Systemic inflammation is related to the progression of cardiovascular disease.</li> </ul>	34
<ul style="list-style-type: none"> <li>• 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).</li> </ul>	35
<ul style="list-style-type: none"> <li>• 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.</li> </ul>	36
	37
	38
<b>Key messages</b>	39
<ul style="list-style-type: none"> <li>• 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.</li> </ul>	40
	41
<ul style="list-style-type: none"> <li>• Independent of BMI, regular physical activity was related to lower IL-6, with a trend for lower fibrinogen</li> </ul>	42
<ul style="list-style-type: none"> <li>• Physical activity had no independent effect on circulating markers related to endothelial inflammation, such as sICAM-1 or sE-selectin.</li> </ul>	43
	44
<b>Strengths and limitations</b>	45
<ul style="list-style-type: none"> <li>• 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.</li> </ul>	46
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6 55**ABSTRACT**

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8 **Objectives.** Determine whether body mass index (BMI) and physical activity (PA) above, at or  
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10 below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity  
11  
12 Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP),  
13  
14 fibrinogen, IL-6 soluble receptor (IL-6sr), soluble E-selectin and soluble intracellular adhesion  
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16 molecule (sICAM)-1.  
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20 **Design.** Archived cohort data (N=1254, age 54.5±11.7y, BMI 29.8±6.6kg/m<sup>2</sup>) from the  
21  
22 National Survey of Midlife Development in the United States (MIDUS) Biomarkers Study were  
23  
24 analyzed for concentrations of inflammatory markers using general linear models. MMW was  
25  
26 defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000 MMW and BMI was  
27  
28 defined as <25, 25-29.9, ≥30 kg/m<sup>2</sup>. Analyses were adjusted for age, sex, smoking and relevant  
29  
30 medication use.  
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34 **Setting.** Respondents reported to three centers to complete questionnaires and provide blood  
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36 samples.  
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39 **Participants.** Participants were men and women currently enrolled in the MIDUS Biomarker  
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41 Project (N=1254, 93% non-hispanic white, average age 54.5y).  
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44 **Primary Outcome Measures.** Concentration of serum IL-6, CRP, fibrinogen, IL-6sr, sE-  
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46 selectin and sICAM.  
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49 **Results.** Significant interactions were found between BMI and MMW for CRP and sICAM-1  
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51 (P's<0.05). CRP in overweight individuals was similar to obese when no PA was reported, but  
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53 was similar to normal weight when any level of regular PA was reported. sICAM-1 was  
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3 76 differentially lower in obese individuals who reported >1000 MMW compared to obese  
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6 77 individuals reporting less exercise.  
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8 **Conclusion.** The association of exercise with CRP and sICAM-1 differed by BMI, suggesting  
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10 79 that regular exercise may buffer weight-associated elevations in CRP in overweight individuals  
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12 80 while higher levels of exercise may be necessary to reduce sICAM-1 or CRP in obese  
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15 81 individuals.

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17 82 **Trial Registry.** N/A.  
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## 21 22 84 **INTRODUCTION**

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24 85 Obesity paired with low physical activity is well known to increase morbidity and mortality  
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26 86 related to cardiovascular disease (CVD)(1). It is less clear, however, whether the benefits of  
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29 87 higher levels of physical activity differ among normal weight, overweight, and obese individuals.  
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32 88 Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase  
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34 89 reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Circulating  
35  
36 90 Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory  
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38 91 markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and  
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40 92 soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been  
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43 93 associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of  
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45 94 inflammatory markers(7, 8). Further, body fat distribution is also an important factor relating to  
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47 95 inflammatory status. Accumulation of fat in visceral depots is more strongly associated with  
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49 96 low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots(9,  
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3 98 The effects of physical activity on markers of inflammation are more complex and may  
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5 99 vary depending on body weight. A number of epidemiological studies have shown an inverse  
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8 100 relationship between physical activity and CRP and IL-6, independent of obesity(11-16).  
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10 101 Laboratory studies conducted in aerobically trained, typically normal weight, individuals have  
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12 102 demonstrated that a single bout of exercise stimulates IL-6 release from skeletal muscle, which  
13  
14 103 promotes anti-inflammatory effects (17-19), as opposed to adipose tissue-derived IL-6 that is  
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16 104 associated with pro-inflammatory effects (20). Randomized controlled trials have also been  
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18 105 conducted, often in populations that also tend to be overweight or obese, to examine the effects  
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20 106 of aerobic exercise interventions on inflammation and the results are mixed (21). Thus, the  
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22 107 contribution of physical activity to inflammation in the context of obesity remains unclear.  
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27 108 The purpose of our study was to disentangle the relative contributions of BMI and  
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29 109 physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr,  
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31 110 CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were  
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33 111 determined using values put forth by the Physical Activity Guidelines for Americans, which  
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35 112 states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately  
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37 113 equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week)  
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39 114 produces substantial health benefits for adults(22). We hypothesized that BMI and MMW would  
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41 115 interact, such that greater MMW reported would lessen the impact of obesity on markers of  
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43 116 inflammation.  
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## 49 118 **MATERIALS AND METHODS**

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53 119 **Design and Sample.** This study was a cross-sectional analysis of archived data (BMI,  
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55 120 self-reported physical activity and inflammatory biomarker concentrations) from 1254  
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3 121 respondents who provided consent (as approved by The University of Wisconsin Madison Health  
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5 122 Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of  
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7 123 Midlife Development in the United States (MIDUS) Biomarkers Study (23). The Biomarker  
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9 124 Project was one of 5 projects within MIDUS II, with the purpose of adding comprehensive  
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11 125 biological assessments on a subsample of the MIDUS participants to further understand age-  
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13 126 related differences in physical and mental health. Participants were eligible for The Biomarker  
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15 127 Project if they were previously enrolled in MIDUS and MIDUS II, which recruited non-  
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17 128 institutionalized, English-speaking adults residing in the contiguous United States aged 25-74.  
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19 129 Exclusion criteria included non-participation in MIDUS and MIDUS II and unwillingness to  
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21 130 travel to specified sites for biomarker assessment. The random digit dialing sample for the  
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23 131 parent study was selected from working telephone banks and a list of all individuals between the  
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25 132 ages of 25 and 74 years within each household was generated in order to select a random  
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27 133 respondent. Those who agreed to participate in the Biomarker Study stayed overnight at one of  
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29 134 three General Clinical Research Centers: University of California Los Angeles, University of  
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31 135 Wisconsin-Madison and Georgetown University. Upon arrival, each respondent provided a  
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33 136 detailed medical history (including physical activity levels) and provided all prescription, over-  
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35 137 the-counter, and alternative medications to be inventoried by project staff. Following an  
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37 138 overnight stay, morning fasting blood samples were obtained. Cohorts were assessed between  
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39 139 July 2004 and May 2009 as a follow up to MIDUS I respondents that were previously surveyed  
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41 140 by the MacArthur Midlife Research Network between 1995 and 1996. Based on the MIDUS  
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43 141 Biomarker Project sample of 1254 participants, 80% power was estimated to detect small effect  
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45 142 sizes ( $\delta=0.08$  and higher) with an alpha level at 0.05 for a two-tailed test (24, 25).  
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3 143        **Anthropometrics.** Height was measured in centimeters and recorded to the nearest  
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6 144 millimeter. A single measure of WC was taken directly on the skin or over a single layer of light,  
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8 145 close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the  
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10 146 nearest millimeter. Weight was measured in kilograms and BMI was calculated by dividing  
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12 147 body mass in kilograms by height in meters squared. BMI categories were organized into 3  
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15 148 groups: normal weight (BMI  $\leq 24.9$  kg/m<sup>2</sup>), overweight (BMI  $\geq 25-29.9$ ) and obese (BMI  $\geq 30$ ).

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18 149        **Categorizing Physical Activity by MET-Minutes per Week (MMW).**

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20 150        The MMW variable was calculated using data provided in the medical history form. The  
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22 151 form first described 3 types of regular physical activity(23):

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25 152        **Vigorous:** Which causes your heart to beat so rapidly you can feel it in your chest  
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27 153 and you perform it long enough to work up a good sweat and breathe heavily (e.g.,  
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29 154 competitive sports, running, vigorous swimming, high intensity aerobics, digging  
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31 155 in the garden, or lifting heavy objects).

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34 156        **Moderate:** Which causes your heart rate to increase slightly and you typically  
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36 157 work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming,  
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38 158 low intensity aerobics or golfing without a power cart, brisk walking, mowing the  
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40 159 lawn with a walking lawnmower).

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43 160        **Light:** Which requires little physical effort (e.g., light housekeeping like dusting  
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45 161 or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).

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48 162 Keeping these definitions in mind, participants were asked if they engaged in regular physical  
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50 163 activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants  
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52 164 answered “yes”, they entered up to 7 types of seasonal and/or non-seasonal exercise or activity  
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54 165 along with the frequency, duration and intensity.

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3 166 MMW were calculated in a 2-step process. Step 1: subjects who reported no physical  
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6 167 activity (for whom no MMW calculations could be made) were designated as the no regular  
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8 168 exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical  
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10 169 activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for  
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12 170 low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity  
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14 171 reported. Four groups reflecting participation in physical activity and whether or not their  
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16 172 participation was below, at or above USDHHS guidelines were created: NRE (reported no  
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18 173 regular physical activity), below recommended (reported <500 MMW), recommended (reported  
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20 174 500-1000 MMW) and above recommended (reported >1000 MMW).  
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24 175 **Blood Collection, Processing and Assays.** Participants were asked to avoid strenuous  
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26 176 activity the day of blood collection. Venous blood samples were collected in 10 mL serum  
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28 177 separator vacutainers following a 12-h overnight fast and processed at a General Clinical  
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30 178 Research Center using standardized procedures. Blood samples were not collected at any  
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32 179 specific point during the menstrual cycle in female participants. Briefly, following collection,  
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34 180 vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for  
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36 181 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab  
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38 182 and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-  
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40 183 Selectin and sICAM-1).  
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46 184 IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison,  
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48 185 Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600,  
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50 186 R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6  
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52 187 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-  
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3 188 assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV%  
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5 189 values for IL-6sr were 5.9-5.7% and 2.0%, respectively.  
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8 190 Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory  
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10 191 for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of  
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12 192 sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay;  
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15 193 R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was  
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17 194 completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay,  
18  
19 195 R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%,  
20  
21 196 respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human  
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23 197 Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for  
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25 198 fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer  
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27 199 with a particle enhanced immunonephelometric assay. Intra-assay and inter-assay precision for  
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29 200 CRP was 2.3-4.4% and 2.1-5.7%, respectively.  
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34 201 **Statistical Analyses.** All variables were assessed for normality and non-normal data  
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36 202 were log transformed, which included data for CRP, IL-6, IL-6sr, fibrinogen, sE-selectin and  
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38 203 sICAM-1. General Linear Models were performed to determine the relationship of MMW and  
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40 204 BMI with the inflammatory markers. For each outcome, the ordinal MMW and BMI factors  
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42 205 were entered as independent factors with an interaction term. If the interaction term was not  
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44 206 significant, the interaction term was dropped and the model was re-fit for main effects only.  
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46 207 Pairwise comparisons were assessed using post hoc univariate analyses with a Bonferroni  
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48 208 adjustment for multiple comparisons. Covariates for all models included factors that are known  
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50 209 to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering,  
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52 210 corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive).  
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3 211 Race was initially included as a covariate; however, approximately 200 data points were lost in  
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5 212 the analyses due to incomplete racial data. As race was not found to be a predictor of our  
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7 213 dependent variables, with the exception of sICAM-1, race was excluded as a covariate to  
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9 214 increase samples size in all analyses excluding sICAM-1. All statistical analyses were  
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11 215 performed with SPSS v. 17 (Chicago, IL) and statistical significance was set  $\alpha = 0.05$ .  
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15 216 In an exploratory analysis, we examined whether the relative effects of BMI and MMW  
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17 217 on the inflammatory markers differed by sex in 3-way interaction models. As none of the  
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19 218 interactions approached statistical significance (data not shown), sex was retained as a covariate  
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21 219 in the models.  
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## 222 RESULTS

31 223 **Subject Characteristics.** Table 1 presents anthropometric characteristics and circulating  
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33 224 levels of inflammatory biomarkers in all subjects (N=1254). Subjects were 92.6% non-Hispanic  
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35 225 white, 56.8% female, and, on average, middle-aged and overweight. Of all the respondents,  
36  
37 226 14.9% were currently smoked, 27.8% were taking cholesterol lowering medication, 12.1%  
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39 227 corticosteroids, 10.4% anti-diabetic medication, 14.2% antidepressant medication, 7.3%  
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41 228 hormone replacement and 2.5% oral contraceptives. The percentage of participants with missing  
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43 229 data for each variable were as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6%  
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45 230 for fibrinogen, 1.2% for sE-selectin, and 1.0% for IL-6sr.  
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51 231 **CRP (Figure 1, Panel A).** We found a significant interaction between BMI and MMW  
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53 232 for CRP concentration ( $F=3.022$ ,  $P=0.006$ ). In post hoc comparisons, CRP levels were higher in  
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55 233 overweight and obese subjects compared to normal weight subjects among those who reported  
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3 234 no regular exercise ( $P$ 's<0.001). However, among subjects who reported any amount of regular  
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5 235 exercise (<500, 500-1000 or >1000 MMW), CRP levels were significantly greater only in obese  
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8 236 subjects compared to both normal weight and overweight subjects ( $P$ 's <0.01). In obese  
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10 237 individuals, CRP tended to be lower in those reporting >1000 MMW compared to those  
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13 238 reporting no regular exercise ( $P$ =0.053).

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15 239 We also found main effects of BMI ( $F$ =130.873  $P$ <0.001) and MMW ( $F$ =11.576,  
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17 240  $P$ <0.001) on CRP. CRP was significantly greater with each increasing BMI category, in a dose-  
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20 241 dependent manner ( $P$ 's<0.001). Compared to participants who reported no regular exercise,  
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22 242 CRP was significantly lower in those who reported 500-1000 and >1000 MMW ( $P$ 's <0.01),  
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25 243 with a trend for lower CRP in those who reported <500 MMW of regular exercise ( $P$ =0.078).

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27 244 **sICAM-1 (Figure 1, Panel B).** We found a significant interaction between BMI and  
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29 245 MMW for sICAM-1 concentration ( $F$ =2.701,  $P$ =0.013). Levels of sICAM-1 were significantly  
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32 246 lower in obese subjects who reported >1000 MMW compared to obese subjects who reported no  
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34 247 regular exercise ( $P$ =0.014) and <500 MMW ( $P$ =0.026) and tended to be lower than levels in  
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36 248 obese subjects who reported 500-1000 MMW ( $P$ =0.079). No differences in sICAM-1 by MMW  
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39 249 were observed among normal weight or overweight individuals.

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41 250 We also observed a main effect of BMI ( $F$ =6.060,  $P$ =0.002), such that sICAM-1 levels in  
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43 251 obese participants were significantly higher than levels found in both normal weight and  
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46 252 overweight participants ( $P$ 's<0.01). No significant main effect of MMW was found for sICAM-1  
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48 253 ( $F$ =0.931,  $P$ =0.425).

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50 254 **IL-6 (Figure 1, Panel C).** Both BMI and MMW had independent effects on circulating  
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52 255 concentrations of IL-6 (BMI:  $F$ =60.150,  $P$ <0.001, MMW:  $F$ =10.680,  $P$ <0.001), with no  
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55 256 significant interaction ( $F$ =1.21,  $P$ =0.297). We found a dose-dependent effect of BMI, such that  
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3 257 higher BMI levels were associated with significantly greater IL-6 ( $P$ 's<0.001). Independent of  
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5 258 BMI, IL-6 was significantly lower in subjects who reported regular exercise (<500 MMW, 500-  
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7 259 1000 MMW and >1000 MMW) compared to those who reported no regular exercise ( $P$ 's <0.01)  
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9 260 with no difference between levels of MMW.  
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12 261 **Fibrinogen (Figure 1, Panel D).** BMI significantly contributed to circulating levels of  
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14 262 fibrinogen ( $F=42.385$ ,  $P<0.001$ ), such that dose-dependent increases were observed for all BMI  
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16 263 levels ( $P$ 's<0.01). While we observed a trend for lower fibrinogen with regular physical activity,  
17  
18 264 similar to that of IL-6, the effect did not reach statistical significance ( $F=2.187$ ,  $P=0.088$ ). We  
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20 265 observed no significant interaction between BMI and MMW for fibrinogen ( $F=1.680$ ,  $P=0.122$ ).  
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24 266 **sE-Selectin (1, Panel E).** BMI significantly contributed to circulating levels of sE-  
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26 267 selectin ( $F=28.253$ ,  $P<0.001$ ) with no significant contribution by MMW ( $F=0.207$ ,  $P=0.892$ ).  
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28 268 Dose-dependent increases in sE-selectin were also observed across BMI levels ( $P$ 's<0.01). We  
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30 269 observed no significant interaction between BMI and MMW for sE-selectin ( $F=0.570$ ,  $P=0.755$ ).  
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34 270 **IL-6sr (Figure 1, Panel F).** No significant main effects for BMI ( $F=1.783$ ,  $P=0.169$ ),  
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36 271 MMW ( $F=1.434$ ,  $P=0.231$ ) or their interaction ( $F=0.834$ ,  $P=0.544$ ) were detected for IL-6sr.  
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39 272 **Waist Circumference (WC) and Inflammatory Markers (Supplemental Figure 1).** A  
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41 273 secondary analysis was completed using WC and MMW as independent variables and the  
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43 274 complete results of these analyses are located in the supplemental information. Briefly, we  
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45 275 found a significant interaction between WC and MMW on sICAM-1. In individuals with an at-  
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47 276 risk WC ( $\geq 102.0$  cm for men and  $\geq 88.0$  cm for women), sICAM-1 was significantly lower in  
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49 277 those reporting 1000+ MMW compared to less than 500 MMW and tended to be lower in those  
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51 278 reporting no regular exercise. Overall, main effects were similar to those found for BMI and  
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53 279 MMW analyses. Having an at-risk WC was independently related to higher levels of CRP,  
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3 280 sICAM-1, IL-6, fibrinogen and sE-selectin. Independent of WC, any level of regular exercise  
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6 281 was related to lower levels of CRP, IL-6 with a similar tendency for fibrinogen.  
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10 283 **DISCUSSION**  
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13 284 The current study aimed to determine whether the impact of BMI and MMW on  
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15 285 inflammatory markers varied by level of overweight or obesity. For CRP and s-ICAM-1  
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17 286 regular physical activity appeared to diminish the effects of higher BMI compared to those who  
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19 287 reported no regular physical activity. In addition, we found that BMI was strongly and  
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21 288 independently related to greater concentrations of both established and emerging inflammatory  
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23 289 markers that may increase CVD risk. Independent of BMI, regular physical activity was also  
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25 290 associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that,  
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27 291 although obesity has a clear impact on inflammation, physical activity appears to mitigate at least  
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29 292 some of this effect.  
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34 293 For example, overweight individuals had CRP levels that were similar to levels observed  
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36 294 in obese individuals if they reported no regular exercise (4.05 and 4.83  $\mu\text{g}/\text{mL}$ , respectively).  
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38 295 CRP levels greater than 3  $\mu\text{g}/\text{mL}$  are typically associated with high CVD risk(26). In overweight  
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40 296 subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it  
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42 297 below [ $<500$ ], within [ $500-1000$ ] or above [ $>1000$ ] USDHHS MMW recommendations), CRP  
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44 298 levels were lower and not significantly different from CRP levels found in normal weight  
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46 299 participants (). This suggests that increasing physical activity level to a minimum of 3 days per  
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48 300 week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.  
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52 301 Obese individuals may require a higher level of regular physical activity in order to lower  
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54 302 inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1  
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3 303 compared to lean and overweight subjects, those who reported >1000 MMW (above the  
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5 304 USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than  
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8 305 obese subjects reporting no regular physical activity. Taken together, we may speculate that  
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10 306 while physical activity levels currently recommended for the general population may reduce  
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12 307 particular inflammatory makers in overweight populations, obese populations may require  
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14 308 greater levels of physical activity above recommended values to reduce inflammatory markers  
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16 309 like CRP and sICAM-1.  
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20 310 As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen,  
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22 311 sICAM-1 and sE-selectin, in agreement with previous work (27-30). Independent of BMI effects,  
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24 312 our results suggest that physical activity has differentiating effects on inflammatory markers.  
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26 313 Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for  
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28 314 higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-  
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30 315 1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and  
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32 316 determinants in Cardiovascular disease (MONICA) study(31), the National Health and Nutrition  
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34 317 Examination Survey (NHANES III)(12, 14) and the Multi-Ethnic Study of Atherosclerosis  
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36 318 (MESA)(32), such that both increased frequency and intensity of physical activity have been  
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38 319 related to lower IL-6 and fibrinogen. Our findings add to these prior results by standardizing  
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40 320 levels of physical activity by using USDHHS. Our results suggest that, regular physical activity  
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42 321 at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and  
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44 322 possibly fibrinogen, independent of BMI.  
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50 323 Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase  
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52 324 response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and  
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54 325 physical activity was detected for CRP, but not IL-6. While IL-6 and CRP were significantly  
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3 326 correlated ( $r=0.514$ , see Supplemental Table 1), this correlation suggests that IL-6 levels do not  
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5 327 fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing  
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8 328 to its substantially longer plasma half-life (37), which may improve our ability to detect  
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10 329 interaction effects in CRP compared to IL-6.

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13 330 Interestingly, our results also suggest that regular exercise may have a more profound  
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15 331 impact on lowering classical markers of inflammation and less impact on the inflammatory status  
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17 332 of the endothelium. Regular physical activity has reliably been associated with lower levels of  
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20 333 IL-6 and CRP, both classical inflammatory markers related to adipose and systemic  
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22 334 inflammation(38). However, regular exercise appeared to have no independent impact on  
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25 335 markers of endothelial activation, particularly sE-selectin. Higher levels of exercise were related  
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27 336 to lower sICAM-1 in obese individuals only. In one prior study, inverse relationships between  
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29 337 physical activity and sICAM-1 and sE-selectin were reported in drug-treated hypertensive men  
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32 338 (39). Thus, further research is necessary to understand mechanisms underlying differential  
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34 339 associations of exercise with systemic and endothelial inflammation.

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36 340 Several limitations must be addressed. First, the cross-sectional design does not allow us  
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39 341 to infer causal relationships. Prospective and interventional designs are necessary to confirm our  
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41 342 findings. No objective measures of physical activity were available in the MIDUS sample.  
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43 343 Therefore, the use of self-report physical activity data may have diminished our ability to detect  
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46 344 effects. However, in addition to being in line with previous studies using self-report physical  
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48 345 activity, our findings are also in line with previous studies(40, 41) that demonstrated that higher  
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50 346 cardiorespiratory fitness, as measured by indirect calorimetry, was associated with lower levels  
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53 347 of inflammation independent of visceral adiposity or BMI. Another limitation is that the sample  
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55 348 was predominantly comprised of non-Hispanic white individuals, suggesting that findings may

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3 349 not extend to all ethnicities. Finally, BMI and physical activity variables are correlated,  
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6 350 potentially raising the concern of small sample sizes in specific groups crossed by BMI and  
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8 351 MMW. However, the smallest group for analyses still contained 54 individuals (normal weight  
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10 352 individuals reporting no exercise).

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13 353 In summary, our results demonstrate both interactive and independent influences of BMI  
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15 354 and levels of physical activity on both established and emerging markers of inflammation.  
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17 355 Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular  
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20 356 physical activity appears to mitigate the effects of higher BMI on some inflammatory markers,  
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22 357 particularly CRP, which is strongly implicated in CVD. Importantly, while any level of regular  
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24 358 physical activity may help reduce inflammation in overweight individuals, similar effects in  
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27 359 obese individuals may require levels of physical activity that are greater than currently  
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29 360 recommended by the USDHHS for general health.

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26  
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31  
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10 397 **CONFLICTS OF INTEREST**

11  
12 398 The authors declare no conflict of interest.  
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17 400 **CONTRIBUTORSHIP**

18  
19  
20 401 KS, JMM and RRW each made substantial contributions to the conception and design of the  
21  
22 402 study, data acquisition, analysis and interpretation, as well as to drafting and revision for  
23  
24 403 substantial intellectual content. All authors made final approval of the version to be published.  
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27 404 **DATA SHARING STATEMENT**

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29 405 Data and documentation for MIDUS studies are available at the Inter-university Consortium for  
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31 406 Political and Social Research (ICPSR). <http://www.icpsr.umich.edu/icpsrweb/landing.jsp>  
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22 537 **FIGURE AND TABLE LEGENDS**

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29 540 **Figure 1: Inflammatory Markers.** Data from 1254 men and women in MIDUS. Joint  
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31 541 association of BMI category (normal, overweight and obese) and MMW category (no regular  
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33 542 exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C),  
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35 543 fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex,  
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37 544 smoking and relevant medication use. The analysis for sICAM-1 was further adjusted for race.  
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39 545 Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value,  
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41 546 INT=interaction effect P value.  
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50 549 **Table 1: Subject Characteristics.** BMI = body mass index; CRP = C-reactive protein; IL =  
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52 550 interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin =  
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54 551 soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.  
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<b>Demographic</b>	<b>BMI &lt; 25 N=298</b>	<b>BMI 25-29.9 N=440</b>	<b>BMI ≥30 N=516</b>	<b>Overall N = 1254</b>
<b>Variables</b>	<b>Mean ± SD</b>			
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 <sup>2</sup> )	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

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3 1 **Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A**  
4 2 **Cohort Study of Middle Aged Adults Living in the United States**  
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56 26 Running Title:

57 27 BMI, Physical Activity and Inflammation  
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59 29 **Key Words:** MIDUS, Intracellular Adhesion Molecule-1, Fibrinogen, C-Reactive Protein  
60 30

**Word Count: 3931**



31 **ABSTRACT**

32 **Objectives.** Determine whether body mass index (BMI) and physical activity (PA) above, at or  
33 below MET-minute per week (MMW) levels recommended in the 2008 Physical Activity  
34 Guidelines interact or have additive effects on interleukin (IL)-6, C-reactive protein (CRP),  
35 fibrinogen, IL-6 soluble receptor (IL-6sr), soluble E-selectin and soluble intracellular adhesion  
36 molecule (sICAM)-1. **Design.** Archived cohort data (N=1254, age 54.5±11.7y, BMI  
37 29.8±6.6kg/m<sup>2</sup>) from the National Survey of Midlife Development in the United States (MIDUS)  
38 Biomarkers Study were analyzed for concentrations of inflammatory markers using general  
39 linear models. MMW was defined as no regular exercise, <500 MMW, 500-1000 MMW, >1000  
40 MMW and BMI was defined as <25, 25-29.9, ≥30 kg/m<sup>2</sup>. Analyses were adjusted for age, sex,  
41 smoking and relevant medication use. **Setting.** Respondents reported to three centers to  
42 complete questionnaires and provide blood samples. **Participants.** Participants were men and  
43 women currently enrolled in the MIDUS Biomarker Project (N=1254, 93% non-hispanic white,  
44 average age 54.5y). **Primary Outcome Measures.** Concentration of serum IL-6, CRP,  
45 fibrinogen, IL-6sr, sE-selectin and sICAM. **Results.** Significant interactions were found  
46 between BMI and MMW for CRP and sICAM-1 (P's<0.05). CRP in overweight individuals was  
47 similar to obese when no PA was reported, but was similar to normal weight when any level of  
48 regular PA was reported. sICAM-1 was differentially lower in obese individuals who reported  
49 >1000 MMW compared to obese individuals reporting less exercise. **Conclusion.** The  
50 association of exercise with CRP and sICAM-1 differed by BMI, suggesting that regular exercise  
51 may buffer weight-associated elevations in CRP in overweight individuals while higher levels of  
52 exercise may be necessary to reduce sICAM-1 or CRP in obese individuals. **Trial Registry.**  
53 N/A.

## 54 INTRODUCTION

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

58 Chronic, low-grade inflammation, marked by elevations in cytokines, acute phase  
59 reactants and soluble adhesion molecules, is a developing CVD risk factor(2, 3). Circulating  
60 Interleukin-6 (IL-6) and, C-reactive protein (CRP) are both considered established inflammatory  
61 markers related to CVD(3). Fibrinogen, soluble intracellular adhesion molecule (sICAM-1) and  
62 soluble e-selectin (sE-selectin) also have key roles in the progression of CVD and have been  
63 associated with elevated risk(4-6). Obesity is strongly associated with greater concentrations of  
64 inflammatory markers(7, 8). Further, body fat distribution is also an important factor relating to  
65 inflammatory status. Accumulation of fat in visceral depots is more strongly associated with  
66 low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots(9,  
67 10).

68 The effects of physical activity on markers of inflammation are more complex and may  
69 vary depending on body weight. A number of epidemiological studies have shown an inverse  
70 relationship between physical activity and CRP and IL-6, independent of obesity(11-16).

71 Laboratory studies conducted in aerobically trained, typically normal weight, individuals have  
72 demonstrated that a single bout of exercise stimulates IL-6 release from skeletal muscle, which  
73 promotes anti-inflammatory effects (17-19), as opposed to adipose tissue-derived IL-6 that is  
74 associated with pro-inflammatory effects (20). Randomized controlled trials have also been  
75 conducted, often in populations that also tend to be overweight or obese, to examine the effects

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3 76 of aerobic exercise interventions on inflammation and the results are mixed (21). Thus, the  
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5 77 contribution of physical activity to inflammation in the context of obesity remains unclear.  
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8 78 The purpose of our study was to disentangle the relative contributions of BMI and  
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10 79 physical activity recorded in MET-minutes per week (MMW) to circulating levels of IL-6, IL-6sr,  
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12 80 CRP, sICAM-1 and sE-selectin in middle-aged adults. MMW categories for this study were  
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14 81 determined using values put forth by the Physical Activity Guidelines for Americans, which  
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16 82 states that total weekly physical activity in the range of 500-1000 MET-minutes (approximately  
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18 83 equivalent to 150-300 minutes of moderate or 75-150 minutes of vigorous activity per week)  
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20 84 produces substantial health benefits for adults(22). We hypothesized that BMI and MMW would  
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22 85 interact, such that greater MMW reported would lessen the impact of obesity on markers of  
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24 86 inflammation.  
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## 30 31 88 **MATERIALS AND METHODS**

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34 89 **Design and Sample.** This study was a cross-sectional analysis of archived data (BMI,  
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36 90 self-reported physical activity and inflammatory biomarker concentrations) from 1254  
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38 91 respondents who provided consent (as approved by The University of Wisconsin Madison Health  
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40 92 Sciences Institutional Review Board) and were subsequently enrolled in the National Survey of  
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42 93 Midlife Development in the United States (MIDUS) Biomarkers Study (23). The Biomarker  
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44 94 Project was one of 5 projects within MIDUS II, with the purpose of adding comprehensive  
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46 95 biological assessments on a subsample of the MIDUS participants to further understand age-  
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48 96 related differences in physical and mental health. Participants were eligible for The Biomarker  
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50 97 Project if they were previously enrolled in MIDUS and MIDUS II, which recruited non-  
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52 98 institutionalized, English-speaking adults residing in the contiguous United States aged 25-74.  
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3 99 Exclusion criteria included non-participation in MIDUS and MIDUS II and unwillingness to  
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6 100 travel to specified sites for biomarker assessment. The random digit dialing sample for the  
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8 101 parent study was selected from working telephone banks and a list of all individuals between the  
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10 102 ages of 25 and 74 years within each household was generated in order to select a random  
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12 103 respondent. Those who agreed to participate in the Biomarker Study stayed overnight at one of  
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14 104 three General Clinical Research Centers: University of California Los Angeles, University of  
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16 105 Wisconsin-Madison and Georgetown University. Upon arrival, each respondent provided a  
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18 106 detailed medical history (including physical activity levels) and provided all prescription, over-  
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20 107 the-counter, and alternative medications to be inventoried by project staff. Following an  
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22 108 overnight stay, morning fasting blood samples were obtained. Cohorts were assessed between  
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24 109 July 2004 and May 2009 as a follow up to MIDUS I respondents that were previously surveyed  
25  
26 110 by the MacArthur Midlife Research Network between 1995 and 1996. Based on the MIDUS  
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28 111 Biomarker Project sample of 1254 participants, 80% power was estimated to detect small effect  
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30 112 sizes ( $\delta=0.08$  and higher) with an alpha level at 0.05 for a two-tailed test (24, 25).  
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36 113 **Anthropometrics.** Height was measured in centimeters and recorded to the nearest  
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38 114 millimeter. A single measure of WC was taken directly on the skin or over a single layer of light,  
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40 115 close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the  
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42 116 nearest millimeter. Weight was measured in kilograms and BMI was calculated by dividing  
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44 117 body mass in kilograms by height in meters squared. BMI categories were organized into 3  
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46 118 groups: normal weight ( $BMI \leq 24.9 \text{ kg/m}^2$ ), overweight ( $BMI \geq 25-29.9$ ) and obese ( $BMI \geq 30$ ).  
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50 119 **Categorizing Physical Activity by MET-Minutes per Week (MMW).**

51 120 The MMW variable was calculated using data provided in the medical history form. The  
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53 121 form first described 3 types of regular physical activity(23):  
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3 122 **Vigorous:** Which causes your heart to beat so rapidly you can feel it in your chest  
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5 123 and you perform it long enough to work up a good sweat and breathe heavily (e.g.,  
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8 124 competitive sports, running, vigorous swimming, high intensity aerobics, digging  
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10 125 in the garden, or lifting heavy objects).

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12 126 **Moderate:** Which causes your heart rate to increase slightly and you typically  
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14 127 work up a sweat (e.g., leisurely sports like light tennis, slow or light swimming,  
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16 128 low intensity aerobics or golfing without a power cart, brisk walking, mowing the  
17  
18 129 lawn with a walking lawnmower).

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20 130 **Light:** Which requires little physical effort (e.g., light housekeeping like dusting  
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22 131 or laundry, bowling, archery, easy walking, golfing with a power cart or fishing).

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25 132 Keeping these definitions in mind, participants were asked if they engaged in regular physical  
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27 133 activity of any type for 20 minutes or more at least 3 times per week (yes or no). If participants  
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29 134 answered “yes”, they entered up to 7 types of seasonal and/or non-seasonal exercise or activity  
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31 135 along with the frequency, duration and intensity.

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34 136 MMW were calculated in a 2-step process. Step 1: subjects who reported no physical  
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36 137 activity (for whom no MMW calculations could be made) were designated as the no regular  
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38 138 exercise group (NRE). Step 2: For subjects who indicated that they performed regular physical  
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40 139 activity, total MMW were calculated by multiplying minutes per week by intensity level (1.1 for  
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42 140 low, 3.0 for moderate and 6.0 for vigorous) and summed across each non-seasonal activity  
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44 141 reported. Four groups reflecting participation in physical activity and whether or not their  
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46 142 participation was below, at or above USDHHS guidelines were created: NRE (reported no  
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48 143 regular physical activity), below recommended (reported <500 MMW), recommended (reported  
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50 144 500-1000 MMW) and above recommended (reported >1000 MMW).

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3 145 **Blood Collection, Processing and Assays.** Participants were asked to avoid strenuous  
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6 146 activity the day of blood collection. Venous blood samples were collected in 10 mL serum  
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8 147 separator vacutainers following a 12-h overnight fast and processed at a General Clinical  
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10 148 Research Center using standardized procedures. Blood samples were not collected at any  
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12 149 specific point during the menstrual cycle in female participants. Briefly, following collection,  
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14 150 vacutainers were allowed to stand 15-30-min (2-h maximum) prior to centrifugation at 4°C for  
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16 151 20-min at 2000-3000 rpm. Serum samples were frozen and shipped to the MIDUS Biocore Lab  
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18 152 and treated and/or analyzed for inflammation markers (IL-6, IL-6sr, CRP, fibrinogen, sE-  
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20 153 Selectin and sICAM-1).

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22 154 IL-6 and IL-6sr were assayed in the MIDUS Biocore Laboratory (University of Madison,  
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24 155 Madison WI) using Quantikine® High-sensitivity ELISA kits (cat# HS600B and cat# DR600,  
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26 156 R&D Systems, Minneapolis, MN). Plates were read at 490 and 450 nm, respectively for IL-6  
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28 157 and IL-6sr using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA). Intra-  
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30 158 assay and inter-assay precision (CV%) for IL-6 was approximately 4.1% and 13.0%. CV%  
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32 159 values for IL-6sr were 5.9-5.7% and 2.0%, respectively.

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34 160 Assays for sICAM-1, sE-Selectin, fibrinogen and CRP were performed at the Laboratory  
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36 161 for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Measurement of  
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38 162 sICAM-1 was completed using an ELISA assay (Parameter-Human sICAM-1 Immunoassay;  
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40 163 R&D Systems). Inter-assay precision for sICAM-1 was 5.0%. Measurement of sE-selectin was  
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42 164 completed using a high-sensitivity ELISA assay (Parameter Human sE-Selectin Immunoassay,  
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44 165 R&D Systems). Intra-assay and inter-assay precision for sE-selectin was 4.7-5.0% and 5.7-8.8%,  
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46 166 respectively. Fibrinogen was measured using the BNII nephelometer (N Antiserum to Human  
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48 167 Fibrinogen; Dade Behring Inc., Deerfield, IL). Intra-assay and inter-assay precision for  
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3 168 fibrinogen was 2.7% and 2.6%, respectively. CRP was analyzed using a BNII nephelometer  
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6 169 with a particle enhanced immunonephelometric assay. Intra-assay and inter-assay precision for  
7  
8 170 CRP was 2.3-4.4% and 2.1-5.7%, respectively.  
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10 **Statistical Analyses.** All variables were assessed for normality and non-normal data  
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12 were log transformed, which included data for CRP, IL-6, IL-6sr, fibrinogen, sE-selectin and  
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14 sICAM-1. General Linear Models were performed to determine the relationship of MMW and  
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17 174 BMI with the inflammatory markers. For each outcome, the ordinal MMW and BMI factors  
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19 were entered as independent factors with an interaction term. If the interaction term was not  
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21 significant, the interaction term was dropped and the model was re-fit for main effects only.  
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24 177 Pairwise comparisons were assessed using post hoc univariate analyses with a Bonferroni  
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26 adjustment for multiple comparisons. Covariates for all models included factors that are known  
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28 to affect inflammatory status: age, sex, smoking and relevant medications (cholesterol-lowering,  
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30 179 corticosteroids, anti-diabetic, antidepressant, hormone replacement and hormonal contraceptive).  
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32 180 Race was initially included as a covariate; however, approximately 200 data points were lost in  
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34 181 the analyses due to incomplete racial data. As race was not found to be a predictor of our  
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36 182 dependent variables, with the exception of sICAM-1, race was excluded as a covariate to  
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38 increase sample size in all analyses excluding sICAM-1. All statistical analyses were  
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40 184 performed with SPSS v. 17 (Chicago, IL) and statistical significance was set  $\alpha = 0.05$ .  
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46 186 In an exploratory analysis, we examined whether the relative effects of BMI and MMW  
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48 187 on the inflammatory markers differed by sex in 3-way interaction models. As none of the  
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50 188 interactions approached statistical significance (data not shown), sex was retained as a covariate  
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52 189 in the models.  
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192 **RESULTS**

193 **Subject Characteristics.** Table 1 presents anthropometric characteristics and circulating  
194 levels of inflammatory biomarkers in all subjects (N=1254). Subjects were 92.6% non-Hispanic  
195 white, 56.8% female, and, on average, middle-aged and overweight. Of all the respondents,  
196 14.9% were currently smoked, 27.8% were taking cholesterol lowering medication, 12.1%  
197 corticosteroids, 10.4% anti-diabetic medication, 14.2% antidepressant medication, 7.3%  
198 hormone replacement and 2.5% oral contraceptives. The percentage of participants with missing  
199 data for each variable were as follows: 1.6% for CRP, 1.0% for sICAM-1, 1.0% for IL-6, 1.6%  
200 for fibrinogen, 1.2% for sE-selectin, and 1.0% for IL-6sr.

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

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



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3 214 **sICAM-1 (Figure 1, Panel B).** We found a significant interaction between BMI and  
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5 215 MMW for sICAM-1 concentration ( $F=2.701$ ,  $P=0.013$ ). Levels of sICAM-1 were significantly  
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7 216 lower in obese subjects who reported  $>1000$  MMW compared to obese subjects who reported no  
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9 217 regular exercise ( $P=0.014$ ) and  $<500$  MMW ( $P=0.026$ ) and tended to be lower than levels in  
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11 218 obese subjects who reported 500-1000 MMW ( $P=0.079$ ). No differences in sICAM-1 by MMW  
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13 219 were observed among normal weight or overweight individuals.  
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17 220 We also observed a main effect of BMI ( $F=6.060$ ,  $P=0.002$ ), such that sICAM-1 levels in  
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19 221 obese participants were significantly higher than levels found in both normal weight and  
20  
21 222 overweight participants ( $P's<0.01$ ). No significant main effect of MMW was found for sICAM-1  
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23 223 ( $F=0.931$ ,  $P=0.425$ ).  
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27 224 **IL-6 (Figure 1, Panel C).** Both BMI and MMW had independent effects on circulating  
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29 225 concentrations of IL-6 (BMI:  $F=60.150$ ,  $P<0.001$ , MMW:  $F=10.680$ ,  $P<0.001$ ), with no  
30  
31 226 significant interaction ( $F=1.21$ ,  $P=0.297$ ). We found a dose-dependent effect of BMI, such that  
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33 227 higher BMI levels were associated with significantly greater IL-6 ( $P's<0.001$ ). Independent of  
34  
35 228 BMI, IL-6 was significantly lower in subjects who reported regular exercise ( $<500$  MMW, 500-  
36  
37 229 1000 MMW and  $>1000$  MMW) compared to those who reported no regular exercise ( $P's <0.01$ )  
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39 230 with no difference between levels of MMW.  
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43 231 **Fibrinogen (Figure 1, Panel D).** BMI significantly contributed to circulating levels of  
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45 232 fibrinogen ( $F=42.385$ ,  $P<0.001$ ), such that dose-dependent increases were observed for all BMI  
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47 233 levels ( $P's<0.01$ ). While we observed a trend for lower fibrinogen with regular physical activity,  
48  
49 234 similar to that of IL-6, the effect did not reach statistical significance ( $F=2.187$ ,  $P=0.088$ ). We  
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51 235 observed no significant interaction between BMI and MMW for fibrinogen ( $F=1.680$ ,  $P=0.122$ ).  
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3 236 **sE-Selectin ( 1, Panel E).** BMI significantly contributed to circulating levels of sE-  
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6 237 selectin (F=28.253, P<0.001) with no significant contribution by MMW (F=0.207, P=0.892).  
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8 238 Dose-dependent increases in sE-selectin were also observed across BMI levels (P's<0.01). We  
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10 239 observed no significant interaction between BMI and MMW for sE-selectin (F=0.570, P=0.755).

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13 240 **IL-6sr (Figure 1, Panel F).** No significant main effects for BMI (F=1.783, P=0.169),  
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15 241 MMW (F=1.434, P=0.231) or their interaction (F=0.834, P=0.544) were detected for IL-6sr.

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17 242 **Waist Circumference (WC) and Inflammatory Markers (Supplemental Figure 1).** A  
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20 243 secondary analysis was completed using WC and MMW as independent variables and the  
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22 244 complete results of these analyses are located in the supplemental information. Briefly, we  
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24 245 found a significant interaction between WC and MMW on sICAM-1. In individuals with an at-  
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26 246 risk WC ( $\geq 102.0$  cm for men and  $\geq 88.0$  cm for women), sICAM-1 was significantly lower in  
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28 247 those reporting 1000+ MMW compared to less than 500 MMW and tended to be lower in those  
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30 248 reporting no regular exercise. Overall, main effects were similar to those found for BMI and  
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32 249 MMW analyses. Having an at-risk WC was independently related to higher levels of CRP,  
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34 250 sICAM-1, IL-6, fibrinogen and sE-selectin. Independent of WC, any level of regular exercise  
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36 251 was related to lower levels of CRP, IL-6 with a similar tendency for fibrinogen.

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## 42 43 253 **DISCUSSION**

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45 254 The current study aimed to determine whether the impact of BMI and MMW on  
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47 255 inflammatory markers varied by level of overweight or obesity. For CRP and s-ICAM-1  
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49 256 regular physical activity appeared to diminish the effects of higher BMI compared to those who  
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51 257 reported no regular physical activity. In addition, we found that BMI was strongly and  
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53 258 independently related to greater concentrations of both established and emerging inflammatory  
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3 259 markers that may increase CVD risk. Independent of BMI, regular physical activity was also  
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5 260 associated with lower IL-6, with a similar trend for fibrinogen. These results suggest that,  
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8 261 although obesity has a clear impact on inflammation, physical activity appears to mitigate at least  
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10 262 some of this effect.

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13 263 For example, overweight individuals had CRP levels that were similar to levels observed  
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15 264 in obese individuals if they reported no regular exercise (4.05 and 4.83  $\mu\text{g/mL}$ , respectively).  
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18 265 CRP levels greater than 3  $\mu\text{g/mL}$  are typically associated with high CVD risk(26). In overweight  
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20 266 subjects who reported regular physical activity of at least 3, 20-minute sessions per week (be it  
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22 267 below [ $<500$ ], within [ $500-1000$ ] or above [ $>1000$ ] USDHHS MMW recommendations), CRP  
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25 268 levels were lower and not significantly different from CRP levels found in normal weight  
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27 269 participants (). This suggests that increasing physical activity level to a minimum of 3 days per  
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30 270 week, at least 20 minutes per day, may improve CRP profiles among overweight individuals.

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32 271 Obese individuals may require a higher level of regular physical activity in order to lower  
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34 272 inflammatory markers. While obese subjects also had greater levels of CRP and sICAM-1  
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37 273 compared to lean and overweight subjects, those who reported  $>1000$  MMW (above the  
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39 274 USDHHS recommendation) had lower levels of sICAM-1 and tended to have lower CRP than  
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41 275 obese subjects reporting no regular physical activity. Taken together, we may speculate that  
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44 276 while physical activity levels currently recommended for the general population may reduce  
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46 277 particular inflammatory makers in overweight populations, obese populations may require  
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49 278 greater levels of physical activity above recommended values to reduce inflammatory markers  
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51 279 like CRP and sICAM-1.

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53 280 As expected, strong main effects of BMI were observed for CRP, IL-6, fibrinogen,  
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56 281 sICAM-1 and sE-selectin, in agreement with previous work (27-30). Independent of BMI effects,  
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3 282 our results suggest that physical activity has differentiating effects on inflammatory markers.  
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6 283 Individuals reporting no regular physical activity had higher levels of IL-6 with a tendency for  
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8 284 higher fibrinogen, compared to those reporting any level of regular physical activity (<500, 500-  
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10 285 1000 or >1000 MMW). Similar results have been observed in the MONItoring trends and  
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12 286 determinants in Cardiovascular disease (MONICA) study(31), the National Health and Nutrition  
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14 287 Examination Survey (NHANES III)(12, 14) and the Multi-Ethnic Study of Atherosclerosis  
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16 288 (MESA)(32), such that both increased frequency and intensity of physical activity have been  
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18 289 related to lower IL-6 and fibrinogen. Our findings add to these prior results by standardizing  
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20 290 levels of physical activity by using USDHHS. Our results suggest that, regular physical activity  
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22 291 at any level (<500, 500-1000, >1000) appears to be associated with lower levels of IL-6 and  
23  
24 292 possibly fibrinogen, independent of BMI.  
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29 293 Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase  
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31 294 response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and  
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33 295 physical activity was detected for CRP, but not IL-6. While IL-6 and CRP were significantly  
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35 296 correlated ( $r=0.514$ , see Supplemental Table 1), this correlation suggests that IL-6 levels do not  
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37 297 fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing  
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39 298 to its substantially longer plasma half-life (37), which may improve our ability to detect  
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41 299 interaction effects in CRP compared to IL-6.  
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45 300 Interestingly, our results also suggest that regular exercise may have a more profound  
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47 301 impact on lowering classical markers of inflammation and less impact on the inflammatory status  
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49 302 of the endothelium. Regular physical activity has reliably been associated with lower levels of  
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51 303 IL-6 and CRP, both classical inflammatory markers related to adipose and systemic  
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53 304 inflammation(38). However, regular exercise appeared to have no independent impact on  
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3 305 markers of endothelial activation, particularly sE-selectin. Higher levels of exercise were related  
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5 306 to lower sICAM-1 in obese individuals only. In one prior study, inverse relationships between  
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8 307 physical activity and sICAM-1 and sE-selectin were reported in drug-treated hypertensive men  
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10 308 (39). Thus, further research is necessary to understand mechanisms underlying differential  
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12 309 associations of exercise with systemic and endothelial inflammation.

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14  
15 310 Several limitations must be addressed. First, the cross-sectional design does not allow us  
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17 311 to infer causal relationships. Prospective and interventional designs are necessary to confirm our  
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19 312 findings. No objective measures of physical activity were available in the MIDUS sample.  
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21 313 Therefore, the use of self-report physical activity data may have diminished our ability to detect  
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23 314 effects. However, in addition to being in line with previous studies using self-report physical  
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25 315 activity, our findings are also in line with previous studies(40, 41) that demonstrated that higher  
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27 316 cardiorespiratory fitness, as measured by indirect calorimetry, was associated with lower levels  
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29 317 of inflammation independent of visceral adiposity or BMI. Another limitation is that the sample  
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31 318 was predominantly comprised of non-Hispanic white individuals, suggesting that findings may  
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33 319 not extend to all ethnicities. Finally, BMI and physical activity variables are correlated,  
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35 320 potentially raising the concern of small sample sizes in specific groups crossed by BMI and  
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37 321 MMW. However, the smallest group for analyses still contained 54 individuals (normal weight  
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39 322 individuals reporting no exercise).

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41 323 In summary, our results demonstrate both interactive and independent influences of BMI  
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43 324 and levels of physical activity on both established and emerging markers of inflammation.  
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45 325 Inflammation is both a consequence of obesity and a mechanism promoting CVD. Regular  
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47 326 physical activity appears to mitigate the effects of higher BMI on some inflammatory markers,  
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49 327 particularly CRP, which is strongly implicated in CVD. Importantly, while any level of regular  
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3 328 physical activity may help reduce inflammation in overweight individuals, similar effects in  
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6 329 obese individuals may require levels of physical activity that are greater than currently  
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8 330 recommended by the USDHHS for general health.  
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19  
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21  
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41 367 **CONFLICTS OF INTEREST**  
42

43 368 The authors declare no conflict of interest.  
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47  
48 370 **CONTRIBUTORSHIP**  
49

50 371 KS, JMM and RRW each made substantial contributions to the conception and design of the  
51  
52 372 study, data acquisition, analysis and interpretation, as well as to drafting and revision for  
53  
54 373 substantial intellectual content. All authors made final approval of the version to be published.  
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3 374 **DATA SHARING STATEMENT**  
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5 375 Data and documentation for MIDUS studies are available at the Inter-university Consortium for  
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8 376 Political and Social Research (ICPSR). <http://www.icpsr.umich.edu/icpsrweb/landing.jsp>  
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13 **ARTICLE SUMMARY**

14 **Article focus**

- 15 379
- 16 • Systemic inflammation is related to the progression of  
17 cardiovascular disease.
  - 18 380 • Independent of obesity, physical activity is inversely related to  
19 concentrations of well-established inflammatory biomarkers,  
20 381 such as C-reactive protein (CRP) or interleukin-6 (IL-6).
  - 21 382 • This article evaluates interactive effects of body mass index and  
22 physical activity on established inflammatory markers, CRP,  
23 383 IL-6, and emerging inflammatory markers, fibrinogen, soluble  
24 intracellular adhesion molecule (sICAM)-1, soluble E-selectin,  
25 384 and IL-6 soluble receptor.

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29 385 **Key messages**

- 30 • Interactive effects of body mass index and physical activity  
31 were observed for CRP, such that regular physical activity  
32 386 reported by overweight individuals was related to significantly  
33 lower CRP levels compared to those reported no regular  
34 387 activity.
- 35 • Independent of BMI, regular physical activity was related to  
36 388 lower IL-6, with a trend for lower fibrinogen
- 37 • Physical activity had no independent effect on circulating  
38 389 markers related to endothelial inflammation, such as sICAM-1  
39 or sE-selectin.  
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43 391 **Strengths and limitations**

- 44 • 1254 adults from the National Survey of Midlife Development  
45 in the United States (MIDUS) Biomarker Project were  
46 392 analyzed. Statistical analyses were adjusted for age, sex,  
47 smoking, and relevant medication use. A strength of this paper  
48 393 is categorizing physical activity levels based on national  
49 recommendations. This data may be used to determine  
50 394 appropriate levels of physical activity necessary for reducing  
51 inflammation in overweight and obese adults. However, cross-  
52 395 sectional data is limited, as causal inferences cannot be  
53 obtained. A second limitation is that the sample was  
54 396 predominantly comprised of non-Hispanic white individuals,  
55 therefore findings may not extend to all ethnicities.  
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3 509 **FIGURE AND TABLE LEGENDS**  
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10 512 **Figure 1: Inflammatory Markers.** Data from 1254 men and women in MIDUS. Joint  
11  
12 513 association of BMI category (normal, overweight and obese) and MMW category (no regular  
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14 514 exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C),  
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16 515 fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These analyses were adjusted for age, sex,  
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18 516 smoking and relevant medication use. The analysis for sICAM-1 was further adjusted for race.  
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20 517 Error bars represent SEM. BMI=BMI main effect P value, MMW=MMW main effect P value,  
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22 518 INT=interaction effect P value.  
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31 521 **Table 1: Subject Characteristics.** BMI = body mass index; CRP = C-reactive protein; IL =  
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33 522 interleukin; IL-6sr = IL-6 soluble receptor; MMW = MET-Minutes per Week; sE-Selectin =  
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35 523 soluble E-Selectin; sICAM-1= soluble intracellular adhesion molecule-1.  
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**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
<b>Introduction</b>			
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
<b>Methods</b>			
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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	N/A

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	N/A
<b>Results</b>			
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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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
<b>Discussion</b>			
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
<b>Other information</b>			
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](http://www.strobe-statement.org).



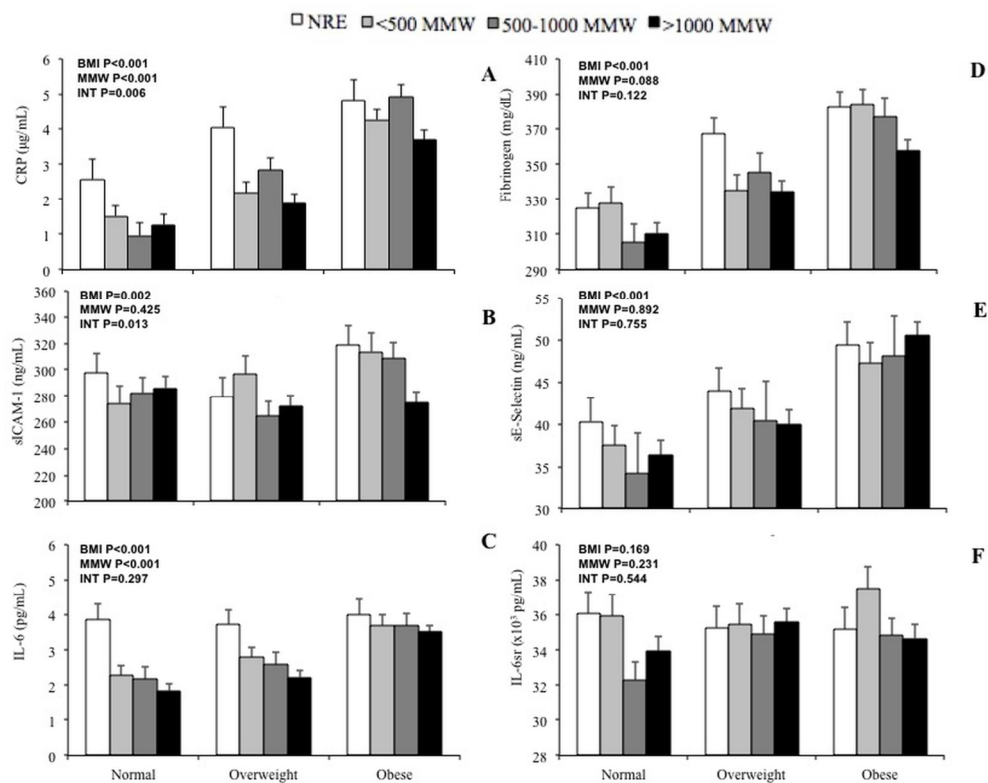


Figure 1: Inflammatory Markers. Data from 1255 men and women in MIDUS. Joint association of BMI category (normal, overweight and obese) and MMW category (no regular exercise, <500 MMW, 500-1000 MMW and >1000 MMW) for CRP (A), sICAM-1 (B), IL-6 (C), fibrinogen (D), sE-Selectin (E) and IL-6sr (F). These 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. 114x90mm (300 x 300 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 ( $P's<0.05$ ).

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*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 ( $F=0.172$ ,  $P=0.916$ ).

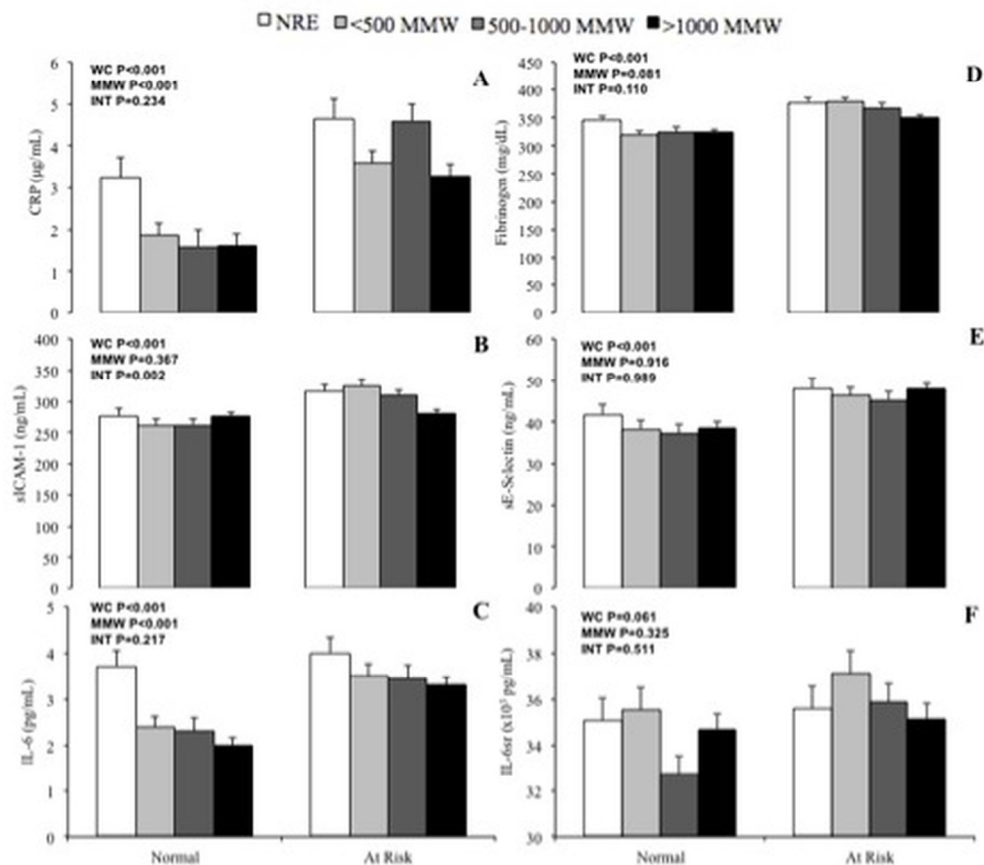
*IL-6sr (Supplementary Figure 1, panel F).* We found no significant interaction effect between WC and MMW for IL-6sr ( $F=0.769$ ,  $P=0.511$ ). Like BMI, we found no main effects for waist circumference ( $F=3.505$ ,  $P=0.061$ ) or MMW on IL-6sr ( $F=1.158$ ,  $P=0.325$ ).

*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 [ $\geq 102.0$  cm for men and  $\geq 88.0$  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 [ $\geq 102.0$  cm for men and  $\geq 88.0$  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  $p \leq 0.001$ .