Original Research
Biomarkers of Inflammation and Longitudinal Evaluation of Lung Function, Physical Activity, and Grip Strength: A Secondary Analysis in the CASCADE Study

David M. MacDonald, MD, MS[1,2] Sarah Samorodnitsky,[2] Eric F. Lock,[2] Vincent Fan,[3] Zijing Chen,[4] Huong Q. Nguyen,[5] Chris H. Wendt[1,2]

[1]Minneapolis VA Health Care System, Minneapolis, Minnesota, United States

[2]University of Minnesota, Minneapolis, Minnesota, United States

[3]VA Puget Sound Health Care System, Seattle, Washington, United States

[4]Tsinghua University, Beijing, China

[5]Kaiser Permanente Southern California, Pasadena, California, United States

Address correspondence to:
David MacDonald, MD, MS
Minneapolis VA Health Care System
Pulmonary, Critical Care, and Sleep (111N)
One Veterans Drive
Minneapolis, MN, USA, 55417
Email: macdo147@umn.edu

Running Head: **Inflammation, Lung Function, and Physical Activity**

Keywords: chronic obstructive pulmonary disease; exercise; hand strength; inflammation; biomarkers

Abbreviations:

This article has an online supplement.

Previous Publication: Portions of these data were presented as a poster at the American Thoracic Society 2023 International Conference, Washington, DC, May 2023.

ABSTRACT

RATIONALE: Physical activity, lung function, and grip strength are associated with exacerbations, hospitalizations, and mortality in people with chronic obstructive pulmonary disease (COPD). We tested whether baseline inflammatory biomarkers were associated with longitudinal outcomes of these physiologic measurements.

METHODS:

The COPD Activity: Serotonin Transporter, Cytokines, and Depression (CASCADE) study was a prospective observational study of individuals with COPD. Fourteen inflammatory biomarkers were measured at baseline. Participants were followed for 2 years. We analyzed associations between baseline biomarkers and FEV_1, physical activity, and grip strength.

We used a hierarchical hypothesis testing procedure to reduce type I error. We used Pearson correlations to test associations between baseline biomarkers and longitudinal changes in the outcomes of interest. We used Fisher's linear discriminant analysis to test if linear combinations of baseline biomarkers predict rapid FEV_1 decline. Finally, we used linear mixed modeling to test associations between baseline biomarkers and outcomes of interest at baseline, year 1, and year 2; models were adjusted for age, smoking status, baseline biomarkers, and FEV_1.

RESULTS: 302 participants (age 67.5 ± 8.5 years, 19.5% female, 28.5% currently smoking) were included. Baseline biomarkers were not associated with longitudinal changes in grip strength, physical activity, or rapid FEV_1 decline. Higher IL-6 and CRP were associated with lower physical activity at baseline and these relationships persisted at year 1 and year 2.

CONCLUSION: Baseline inflammatory biomarkers did not predict changes in lung function or physical activity, but higher inflammatory biomarkers were associated with persistently low levels of physical activity.

INTRODUCTION: Chronic obstructive pulmonary disease (COPD) is characterized by fixed airflow obstruction on spirometry, a history of exposure to noxious stimuli such as cigarette smoke, and chronic respiratory symptoms.[1] COPD is associated with chronic lung and systemic inflammation, and once the inflammatory process is established, it often persists despite smoking cessation or removal of other inciting factors.[2] Lung function decline and respiratory symptoms similarly can progress despite removal of noxious stimuli, and faster lung function decline is also associated with increased mortality and COPD hospitalizations.[3,4] Despite these well-established associations, attempts to identify inflammatory biomarkers that predict lung function decline have been unsuccessful.[5]

Another important prognostic marker in COPD is physical activity. Reduced physical activity is common in people with COPD and is associated with faster lung function decline, increased rates of COPD hospitalizations and rehospitalizations, and higher mortality.[6–9] Associations between systemic inflammation and physical activity among people with COPD are complex. At least one study found that higher physical activity is associated with higher systemic inflammation[10], while others have found that decreased physical activity and hand grip strength are associated with higher systemic inflammation.[11–14] Exercise interventions in COPD have been shown to decrease systemic inflammation.[15] Exercise-physical activity relationships may be also be influenced by body mass index, malnutrition, and duration between the end of physical activity and measurement of inflammation.[16,17]

Reducing lung function decline and increasing physical activity are important targets in people with COPD, but interventions thus far have been only modestly successful. With an objective to

identify potential etiologic and therapeutic pathways, we tested the hypothesis that higher systemic inflammation is associated with faster lung function decline and reductions in physical activity and strength.

METHODS:

A full description of the statistical analysis methods is included in the supplement.

Participants. The COPD Activity: Serotonin Transporter, Cytokines, and Depression (CASCADE) study (NCT01074515) was an observational study of subjects with COPD to study the biologic effects and functional consequences of depression in people with COPD.[18–20] Full inclusion and exclusion criteria have been published previously[19], but briefly, inclusion criterial were: 1) a diagnosis of COPD based upon post-bronchodilator spirometry [forced expiratory volumes in 1-second (FEV_1)/forced vital capacity (FVC) ratio <0.7 and FEV_1 <80% predicted]; 2) greater than 10 pack-years current or past cigarette smoking; 3) no acute exacerbation of COPD in the last 4 weeks, and 4) ability to speak, read, and write in English. Because CASCADE was a study of inflammation, those with other conditions that could cause changes in inflammation were excluded. These conditions included other chronic lung diseases, chronic inflammatory diseases, uncompensated heart failure, autoimmune disease, lung or metastatic cancer, and chronic oral prednisone use. Patients who were unable to walk and could not complete the 6-minute walk test were also excluded. We included all participants with baseline biomarker measurements in this analysis.

Procedures. Institutional review boards at each site approved the study and written informed consent was obtained from each participant. Spirometry was performed according to American Thoracic Society standards using an EasyOne spirometry (ndd Medical Technologies Inc), and post-bronchodilator spirometry was used in this analysis. A six-minute walk test was performed to determine the total distance walked. Physical activity was measured with a Stepwatch 3 Activity Monitor fastened above the right ankle. Participant were asked to wear this for 7 days during waking hours. A valid day was defined as having ≥ 600 minutes of monitor wear time. Grip strength was measured using a hand dynamometer on the dominant hand. The participant was asked to hold the dynamometer at a 90-degree angle from the body and squeeze the grip handle as hard as they can. The measurement was repeated 3 times and the best result was used. Spirometry, physical activity monitoring, and grip strength were performed at baseline and repeated at the year 1 and year 2 follow-up visits.

A full description of biomarker procedures has been previously published.[20] Plasma biomarkers included C-reactive protein (CRP) measured using a duoset ELISA (R&D Systems) and interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, interferon, granulocyte macrophage-colony stimulating factor (GM-CSF), and tumor necrosis factor (TNF-α) measured on a Luminex multiplex platform. These 12 biomarkers were chosen for CASCADE based on previous association with COPD and/or depression.[20–23]

Statistical analysis. Biomarkers levels were transformed using a $\log(1+x)$ transformation. After transformation, all biomarkers were centered and scaled to mean 0, standard deviation 1.

The outcomes were: FEV_1, total distance walked, average steps total, average minutes active, average percent time spent inactive, and grip strength. We measured changes in outcomes as the raw change: ($outcome_{year2} - outcome_{baseline}$), and as the percent change: ($outcome_{year2} - outcome_{baseline}$)/$outcome_{baseline}$.

We first implemented an unadjusted analysis for the associations between baseline biomarkers and patient outcomes. We calculated the Pearson correlation between each baseline biomarker and change in each outcome (raw and percent change) and tested the null hypothesis that this correlation is equal to 0. To control the overall false discovery rate (OFDR) across the biomarkers, we used a hierarchical hypothesis testing procedure to test the significance of the correlation between each biomarker and each outcome.[24] We treated each biomarker as a "set" of hypotheses in which 12 hypotheses (raw and percent change in six outcomes) were tested. The null hypotheses were that a given biomarker is not associated with raw or percent change in the outcomes. By controlling the OFDR, we controlled the expected proportion of biomarker sets falsely rejected. We report if we can reject any hypotheses within a biomarker set at the OFDR<0.05 level, and if so, which hypotheses within that set that were rejected and the resulting Pearson correlations.

We also used an adjusted linear mixed modeling analysis to investigate the relationship between baseline biomarkers and the outcomes of interest over time (testing associations between baseline biomarkers and outcomes at baseline, year 1, and year 2 rather than changes in outcomes). We applied the same hierarchical hypothesis testing framework for each biomarker to assess significance. We adjusted for baseline biomarker, time, and smoking status at year of

follow up as fixed effects and adjusted for FEV_1 in the models for non-FEV_1 outcomes. We included an interaction between baseline biomarker and time to capture any change over time of the baseline biomarker's effect on the outcome and included a random intercept for each subject to account for subject-specific variation in the observations.

Finally, in addition to testing associations between individual baseline biomarkers and changes in FEV_1, we also tested if linear combinations of baseline biomarkers predict rapid FEV_1 decline from baseline to year 2 using Fisher's linear discriminant analysis (LDA). We defined rapid FEV_1 decline as an average annual drop in in FEV_1 of 40 milliliters or higher. We used the area under the receiver operating curve (AUROC) under 10-fold cross-validation to evaluate the predictive performance, and a permutation testing approach to assess significance.

RESULTS

Baseline characteristics for all participants (Total), and for participants stratified by rapid FEV_1 decline are shown in **Table 1**. Baseline levels of inflammation have been previously published.[20] Participants had a mean (SD) age of 67.5 (8.5) years, 19.5% were female, and 28.5% were currently smoking. Mean FEV_1 percent predicted was 45.0 (15.8) at baseline and 188 (62.3%) participants were on inhaled corticosteroids. Average annual changes in FEV_1, average minutes active, average steps total, 6-minute walk distance, percent time spent inactive, and grip strength are included in **Supplementary Table S1**.

Baseline biomarkers and longitudinal changes in physical activity and grip strength (Unadjusted). The screening hypothesis was rejected for only 1 biomarker/outcome pair testing associations between baseline biomarkers and longitudinal changes in activity and grip strength. Higher IL-6 was associated with greater percent change in average minutes active (**Figure 1a**), but after removal of 2 outliers this relationship was attenuated (**Figure 1b**).

Baseline biomarkers and associations with physical activity and grip strength at baseline, year 1, and year 2 (Unadjusted and Adjusted). In our linear mixed modeling analysis (adjusted for smoking, time, baseline biomarker, and FEV_1), we rejected two hypotheses for IL-6 and four hypotheses for CRP.

In unadjusted and adjusted analyses, higher baseline IL-6 was associated with lower average minutes active and higher average percent time inactive at baseline and year 1, but results were attenuated at year 2 (Unadjusted analyses: **Figure 2**, Adjusted Analyses: **Table 2**).

In adjusted and adjusted analyses, higher baseline CRP was associated with lower total distance walked, lower average minutes active, lower average steps total, and higher average percent time spent inactive at baseline, year 1, and year 2 (Unadjusted analyses: **Supplementary Figure 1**, Adjusted analyses: **Table 2**).

Baseline biomarkers and FEV_1. The screening hypotheses were not rejected for absolute or percentage change in FEV_1, or for associations between baseline biomarkers and FEV_1 over time (at baseline, year 1, and year 2).

Results from linear discriminant analysis, in which we examined if linear combinations of baseline biomarkers predicted rapid FEV_1 decline are shown in **Figure 3**. The p-value for the area under the receiver operator curve was 1, implying that the predictive performance was not significantly better than random guessing.

DISCUSSION

In this analysis of associations between inflammatory biomarkers and lung function, grip strength, and physical activity we found no strong associations between inflammation and longitudinal changes in these physiologic outcomes.

Our primary goal was to test associations between baseline biomarkers and longitudinal change in lung function. Systemic inflammation has been proposed as a driver of lung function decline in general, smoking related COPD and in other causes for COPD, such as HIV. We found no association between inflammatory biomarkers and several measures of FEV_1 decline. This is consistent with prior studies, most notably the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study which tested associations between baseline biomarkers and longitudinal changes in FEV_1 in 1,793 participants.[5] In ECLIPSE, IL-8 and CRP were associated with baseline FEV_1, but not with longitudinal changes in FEV_1. Higher levels of the pneumoprotein club cell secretory protein (CCSP) were associated with slower decline in FEV_1 in ECLIPSE, but pneumoproteins were not measured in CASCADE. ECLIPSE analyzed a total of five inflammatory biomarkers (CRP, IL-6, IL-8, TNF-α, and fibrinogen) and found that four of these (CRP, IL-6, IL-8, and TNF-α;) were not associated with longitudinal lung function

decline. We analyzed an additional eight biomarkers not analyzed in ECLIPSE that included IL-4 and IL-13 which are involved in allergic inflammation, and IL-10, a key anti-inflammatory mediator.[25,26] Our results in this study are also consistent with our prior results analyzing associations between systemic inflammation and lung function decline in people living with HIV, where we found that higher inflammatory biomarkers (including IL-6 and CRP) were not associated with faster lung function decline.[27,28] Our data provide further evidence that inflammatory biomarkers measured in the blood do not predict lung function decline and suggest that alternative sources for markers of lung function decline such as bronchoalveolar lavage fluid[29] or physiologic/imaging[30] measurements should be pursued.

We also tested associations between inflammatory biomarkers and physical activity outcomes. Like our lung function analysis, we found no associations between baseline inflammatory biomarkers and longitudinal changes in physical activity or strength. We did find that higher concentrations of inflammatory biomarkers were associated with lower physical activity at baseline, and that association persisted over time, with higher systemic inflammation predicting lower physical activity up to two years later. Prior studies analyzing the randomized effects of exercise on systemic inflammation found that physical activity leads to decreased systemic inflammation.[15] Physical activity may also be particularly beneficial in people with COPD with increased systemic inflammation. In a study of 385 participants with COPD, physical activity was not associated with all-cause mortality among all participants, or in those without elevated CRP; however, in those with an elevated CRP, each 60 minute increase in physical activity was associated with a 31% reduction (HR 0.69, 95% CI 0.51 to 0.93) in all-cause mortality over 78 months of follow up.[31] These findings suggest that it is important to encourage patients with

COPD to increase physical activity, and highlights the importance of interventions to improve physical activity such as pulmonary rehabilitation. Associations between inflammatory biomarkers and activity may vary around the time of an acute exacerbation of COPD. For example, among 50 people with COPD experiencing an acute exacerbation, higher CRP was associated with a greater decrease in 6-minute walk distance at day 3 of the exacerbation.[32] We did not have biomarker measurements at the time of exacerbation and were not able to test if higher inflammatory biomarker concentrations at the time of exacerbation led to longitudinal changes in activity.

Our study has several limitations. Previous studies that found an association between baseline biomarkers and longitudinal changes in outcomes were much larger, and we were underpowered to detect small effects that can be seen in biomarker studies. The small sample size also limited our ability to test associations between biomarkers and clinically important outcomes such as exacerbations or mortality. Metabolites and biomarkers present in lung samples are not necessarily found in peripheral blood samples,[29] and CASCADE did not collect samples from other sites such as bronchoalveolar lavage fluid or perform chest imaging and we were unable to test if these other potentially important markers predicted lung function or physical activity changes.

Our study also has several strengths. We were able to test associations between baseline biomarkers and longitudinal changes in a variety of important physiologic outcomes in more than 300 well characterized participants over a period of two years. We also used a hierarchical hypothesis testing framework that limited the overall false discovery rate (the rate that

biomarkers deemed significant are truly null), an important consideration in studies testing multiple biomarkers.

In conclusion, baseline biomarkers of inflammation were not associated with longitudinal changes in lung function, physical activity, or grip strength. These data provide further evidence that systemic inflammation does not drive lung function decline in COPD, and do not support targeting systemic inflammation for improvement of lung function, physical activity, or strength. Future studies should investigate non-inflammatory pathways, and alternative samples such as bronchoalveolar lavage fluid.

Authors contributions
Conceived the study: DMM, CWH, VF
Designed the study: DMM, CWH, VF, EFL, SS
Obtained funding: VF and HQN
Acquired the data: PL and HQN
Performed the primary statistical analysis: EFL and SS
Drafted the manuscript: DMM
Critically revised the manuscript for important intellectual content and approved the final manuscript: all authors
Take responsibility for the integrity of the data and the accuracy of the data analysis: all authors

Competing Interests Statement: The authors have nothing to declare.

Disclaimer: The views expressed in this article are those of the authors and do not reflect the views of the United States Government, the Department of Veterans Affairs, the National Heart, Lung, and Blood Institute, or any of the authors' affiliated academic institutions.

REFERENCES

1. Singh D, Agusti A, Anzueto A, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: the GOLD science committee report 2019. *Eur Respir J*. 2019;53(5):1900164. doi:10.1183/13993003.00164-2019

2. King PT. Inflammation in chronic obstructive pulmonary disease and its role in cardiovascular disease and lung cancer. *Clin Transl Med*. 2015;4(1):68. doi:10.1186/s40169-015-0068-z

3. Oelsner EC, Balte PP, Bhatt SP, et al. Lung function decline in former smokers and low-intensity current smokers: a secondary data analysis of the NHLBI Pooled Cohorts Study. *Lancet Respir Med*. 2020;8(1):34-44. doi:10.1016/S2213-2600(19)30276-0

4. Mannino DM, Reichert MM, Davis KJ. Lung Function Decline and Outcomes in an Adult Population. *Am J Respir Crit Care Med*. 2006;173(9):985-990. doi:10.1164/rccm.200508-1344OC

5. Vestbo J, Edwards LD, Scanlon PD, et al. Changes in Forced Expiratory Volume in 1 Second over Time in COPD. *N Engl J Med*. 2011;365(13):1184-1192. doi:10.1056/NEJMoa1105482

6. Garcia-Aymerich J, Lange P, Benet M, Schnohr P, Anto JM. Regular physical activity reduces hospital admission and mortality in chronic obstructive pulmonary disease: a population based cohort study. *Thorax*. 2006;61(9):772-778. doi:10.1136/thx.2006.060145

7. Pitta F, Troosters T, Spruit MA, Probst VS, Decramer M, Gosselink R. Characteristics of Physical Activities in Daily Life in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2005;171(9):972-977. doi:10.1164/rccm.200407-855OC

8. Garcia-Aymerich J, Lange P, Benet M, Schnohr P, Antó JM. Regular Physical Activity Modifies Smoking-related Lung Function Decline and Reduces Risk of Chronic Obstructive Pulmonary Disease: A Population-based Cohort Study. *Am J Respir Crit Care Med*. 2007;175(5):458-463. doi:10.1164/rccm.200607-896OC

9. Garcia-Aymerich J. Risk factors of readmission to hospital for a COPD exacerbation: a prospective study. *Thorax*. 2003;58(2):100-105. doi:10.1136/thorax.58.2.100

10. Kawagoshi A, Iwakura M, Furukawa Y, Sugawara K, Takahashi H, Shioya T. The association between systemic inflammation and the time spent in posture and movement during daytime in patients with chronic obstructive pulmonary disease and lower weight. *J Phys Ther Sci*. 2020;32(12):804-809. doi:10.1589/jpts.32.804

11. Byun MK, Cho EN, Chang J, Ahn CM, Kim HJ. Sarcopenia correlates with systemic inflammation in COPD. *Int J Chron Obstruct Pulmon Dis*. 2017;Volume 12:669-675. doi:10.2147/COPD.S130790

12. Moy ML, Teylan M, Weston NA, Gagnon DR, Danilack VA, Garshick E. Daily Step Count Is Associated With Plasma C-Reactive Protein and IL-6 in a US Cohort With COPD. *Chest*. 2014;145(3):542-550. doi:10.1378/chest.13-1052

13. Loprinzi PD, Walker JF, Lee H. Association between Physical Activity and Inflammatory Markers among U.S. Adults with Chronic Obstructive Pulmonary Disease. *Am J Health Promot*. 2014;29(2):81-88. doi:10.4278/ajhp.130510-QUAN-235

14. Yende S. Inflammatory markers are associated with ventilatory limitation and muscle dysfunction in obstructive lung disease in well functioning elderly subjects. *Thorax*. 2005;61(1):10-16. doi:10.1136/thx.2004.034181

15. Wang CH, Chou PC, Joa WC, et al. Mobile-phone-based home exercise training program decreases systemic inflammation in COPD: a pilot study. *BMC Pulm Med*. 2014;14(1):142. doi:10.1186/1471-2466-14-142

16. Rodrigues F, Papoila AL, Ligeiro D, Gomes MJM, Trindade H. Acute exercise amplifies inflammation in obese patients with COPD. *Rev Port Pneumol Engl Ed*. 2016;22(6):315-322. doi:10.1016/j.rppnen.2016.05.005

17. Davidson WJ, Verity WS, Traves SL, Leigh R, Ford GT, Eves ND. Effect of incremental exercise on airway and systemic inflammation in patients with COPD. *J Appl Physiol*. 2012;112(12):2049-2056. doi:10.1152/japplphysiol.01615.2011

18. Chen Z, Fan VS, Belza B, Pike K, Nguyen HQ. Association between Social Support and Self-Care Behaviors in Adults with Chronic Obstructive Pulmonary Disease. *Ann Am Thorac Soc*. 2017;14(9):1419-1427. doi:10.1513/AnnalsATS.201701-026OC

19. Nguyen HQ, Fan VS, Herting J, et al. Patients With COPD With Higher Levels of Anxiety Are More Physically Active. *Chest*. 2013;144(1):145-151. doi:10.1378/chest.12-1873

20. Nguyen HQ, Herting JR, Pike KC, et al. Symptom profiles and inflammatory markers in moderate to severe COPD. *BMC Pulm Med*. 2016;16(1):173. doi:10.1186/s12890-016-0330-1

21. Lee CH, Giuliani F. The Role of Inflammation in Depression and Fatigue. *Front Immunol*. 2019;10:1696. doi:10.3389/fimmu.2019.01696

22. Choudhury G, Rabinovich R, MacNee W. Comorbidities and systemic effects of chronic obstructive pulmonary disease. *Clin Chest Med*. 2014;35(1):101-130. doi:10.1016/j.ccm.2013.10.007

23. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Respir J*. 2009;33(5):1165-1185. doi:10.1183/09031936.00128008

24. Li Y, Ghosh D. A two-step hierarchical hypothesis set testing framework, with applications to gene expression data on ordered categories. *BMC Bioinformatics*. 2014;15(1):108. doi:10.1186/1471-2105-15-108

25. Junttila IS. Tuning the Cytokine Responses: An Update on Interleukin (IL)-4 and IL-13 Receptor Complexes. *Front Immunol*. 2018;9:888. doi:10.3389/fimmu.2018.00888

26. Saraiva M, Vieira P, O'Garra A. Biology and therapeutic potential of interleukin-10. *J Exp Med*. 2020;217(1):e20190418. doi:10.1084/jem.20190418

27. MacDonald DM, Zanotto AD, Collins G, et al. Associations between baseline biomarkers and lung function in HIV-positive individuals. *AIDS*. 2019;33(4):655-664. doi:10.1097/QAD.0000000000002101

28. MacDonald DM, Samorodnitsky S, Wendt CH, et al. Pneumoproteins and biomarkers of inflammation and coagulation do not predict rapid lung function decline in people living with HIV. *Sci Rep*. 2023;13(1):4749. doi:10.1038/s41598-023-29739-x

29. Wendt CH, Samorodnitsky S, Lock EF, et al. The lung and plasma metabolome in HIV-associated obstructive lung disease (OLD). *JAIDS J Acquir Immune Defic Syndr*. 2022;Publish Ahead of Print. doi:10.1097/QAI.0000000000003061

30. Bhatt SP, Soler X, Wang X, et al. Association between Functional Small Airway Disease and FEV_1 Decline in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2016;194(2):178-184. doi:10.1164/rccm.201511-2219OC

31. Loprinzi PD, Walker JF. Increased daily movement associates with reduced mortality among COPD patients having systemic inflammation. *Int J Clin Pract*. 2016;70(3):286-291. doi:10.1111/ijcp.12778

32. Alahmari AD, Kowlessar BS, Patel ARC, et al. Physical activity and exercise capacity in patients with moderate COPD exacerbations. *Eur Respir J*. 2016;48(2):340-349. doi:10.1183/13993003.01105-2015

Table 1: Baseline characteristics of participants. Results are presented as mean (standard deviation) unless otherwise noted.

Characteristic	Total (n=302)	Rapid FEV$_1$ decline[*] (n=122)	No rapid FEV$_1$ decline[*] (n=131)
Age, years	67.5 (8.46)	67.4 (8.30)	67.3 (8.61)
Female, n (%)	59 (19.5%)	18 (14.8%)	31 (23.7%)
FEV$_1$ % predicted	45.0 (15.8)	48.5 (14.7)	43.3 (16.6)
BMI	28.1 (6.07)	27.6 (5.84)	28.7 (5.81)
Currently smoking, n(%)	86 (28.5%)	40 (32.8%)	26 (19.8%)
Medications			
ICS, n (%)	188 (62.3%)	71 (58.2%)	85 (64.9%)
Comorbidities			
Heart disease, n (%)	59 (19.5%)	29 (23.8%)	24 (18.3%)
Type 2 diabetes, n (%)	68 (22.5%)	28 (23.0%)	29 (22.1%)
Charlson Comor. Index	0.901 (1.18)	0.885 (1.23)	0.885 (1.12)
6-minute walk total distance, ft	1090 (371)	1130 (374)	1090 (368)
Average steps total	6000 (3340)	6400 (3440)	5900 (3270)
Average minutes active	260 (104)	274 (102)	256 (107)
% Time spent inactive	70.6 (11.8)	69.1 (11.6)	71.2 (11.8)
Grip strength (kg)	32.3 (9.56)	32.8 (9.24)	32.6 (9.66)

* Rapid FEV$_1$ decline was defined as an average annual drop in FEV$_1$ of 40 mL or higher. Forty-nine samples were missing two consecutive (baseline and year 1 or year 1 and year 2) measurements of FEV1.
BMI, body mass index; FEV1, forced expiratory volume in 1-second; ICS, inhaled corticosteroid.

Table 2: Linear mixed models of associations between IL-6, CRP, and activity outcomes at baseline, year 1, and year 2. Year 1 and year 2 effects incorporate results from biomarker*time interactions to show the changes over time in the effect of the baseline biomarker on the outcome. Effects show the change in outcome for a 1 SD change in log transformed biomarker concentration.

Outcome	Baseline effect (95% CI)	Year 1 effect (95% CI)	Year 2 effect (95% CI)
Baseline IL-6			
Average minutes active	-24.5 (-36.0 to -13.0)	-16.6 (-28.4 to -4.9)	-7.8 (-19.8 to 4.2)
Percent time inactive	2.7 (1.4 to 4.0)	2.1 (0.7 to 3.4)	1.1 (-0.2 to 2.5)
Baseline CRP			
Average minutes active	-24.6 (-36.1 to -13.0)	-19.9 (-31.8 to -7.9)	-16.7 (-29.4 to -4.0)
Percent time inactive	3.0 (1.7 to 4.3)	2.8 (1.5 to 4.2)	2.3 (0.9 to 3.7)
Total distance walked	-64.0 (-102.0 to -25.9)	-67.8 (-107.5 to -28.1)	-83.2 (-125.5 to -40.9)
Total steps	-784 (-1141 to -427)	-676 (-1045 to -307)	-576 (-968 to -185)

Models are adjusted for baseline biomarker, smoking status, and FEV_1 and incorporate biomarker*time interactions.

Figure Legends:

Figure 1: Scatterplots of associations between baseline IL-6 [log(1+x) transformed, and centered and scaled to mean 0, standard deviation 1] and percentage change in average minutes active. Panel a) includes all participants, and panel b) has two visual outliers removed (IL-6 > 4).
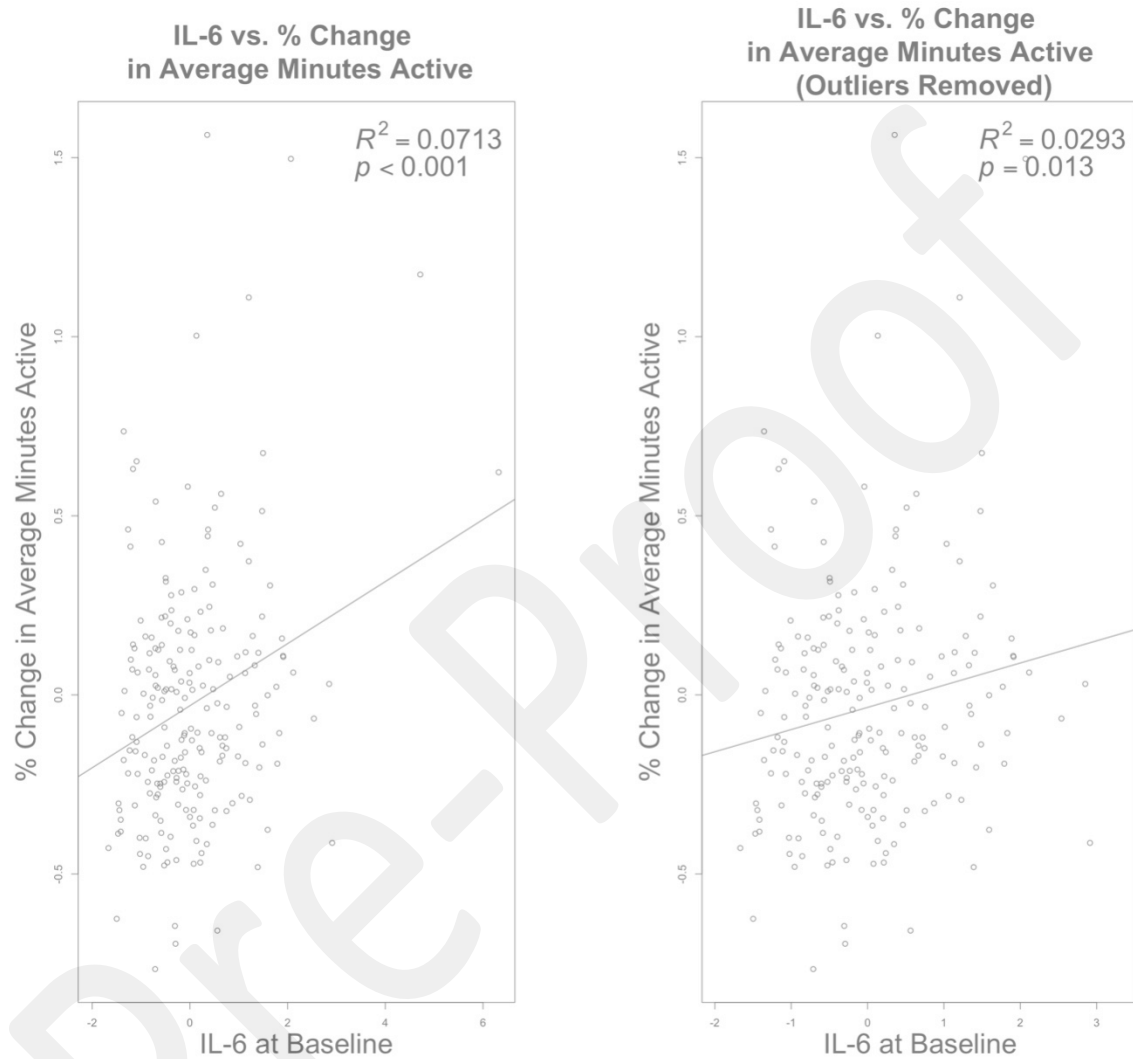
Figure 2: Scatterplots of associations between baseline IL-6 and average minutes active (first row) and average percent time spent inactive (second row). Associations between baseline IL-6 and activity outcomes are shown at baseline (first column), year 1 (second column), and year 2 (3rd column).
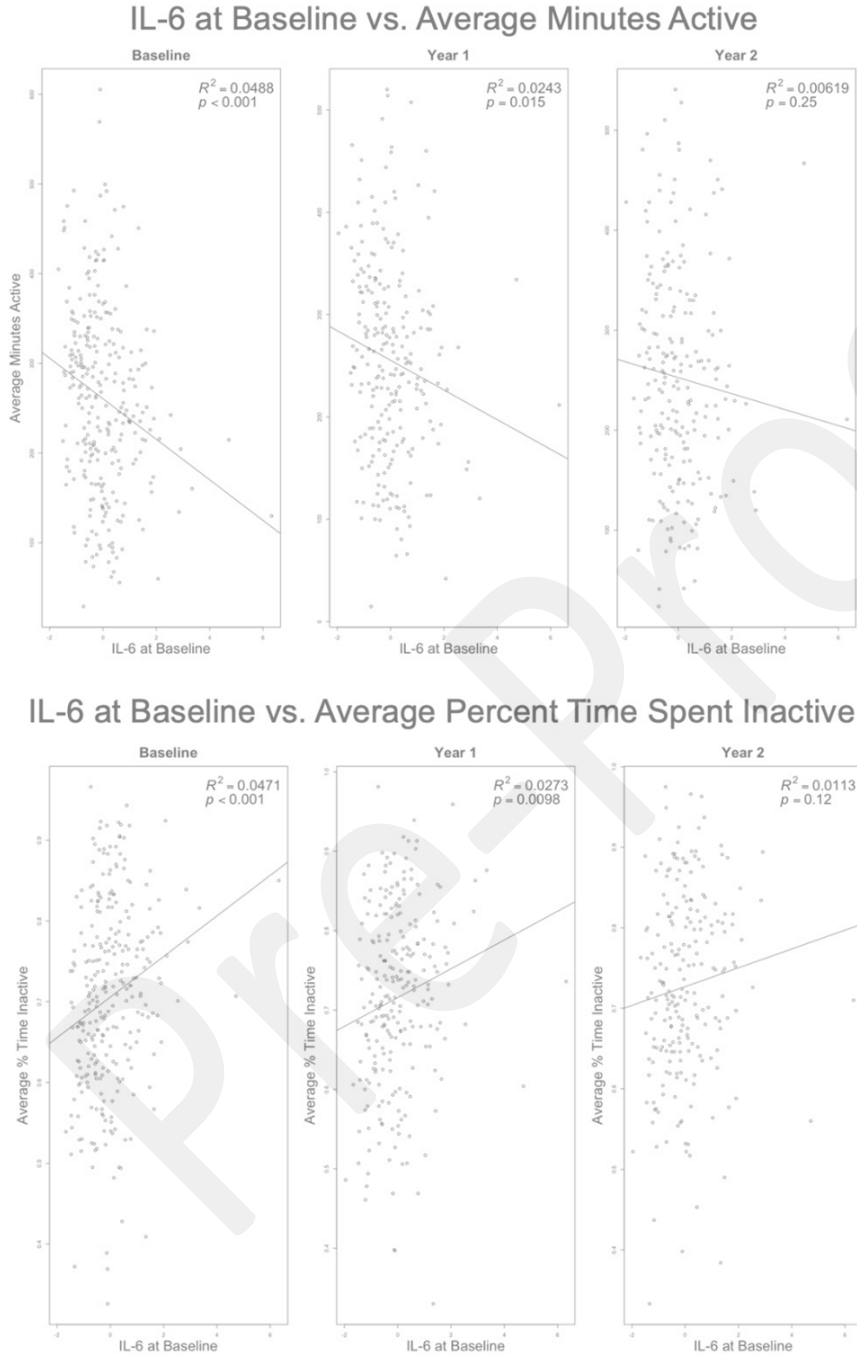
Figure 3: Scores from linear discriminant analysis to predict rapid FEV1 decline on the full training data. The scores represent linear combinations of baseline biomarker expression across the samples. Overlap (dark purple) in the resulting scores illustrates limited capacity for baseline biomarker expression to predict rapid decline in FEV1 over the course of two years (area under the receiver operator curve p-value = 1). Rapid FEV_1 decline was defined as an average annual drop in FEV_1 of 40 milliliters or higher.
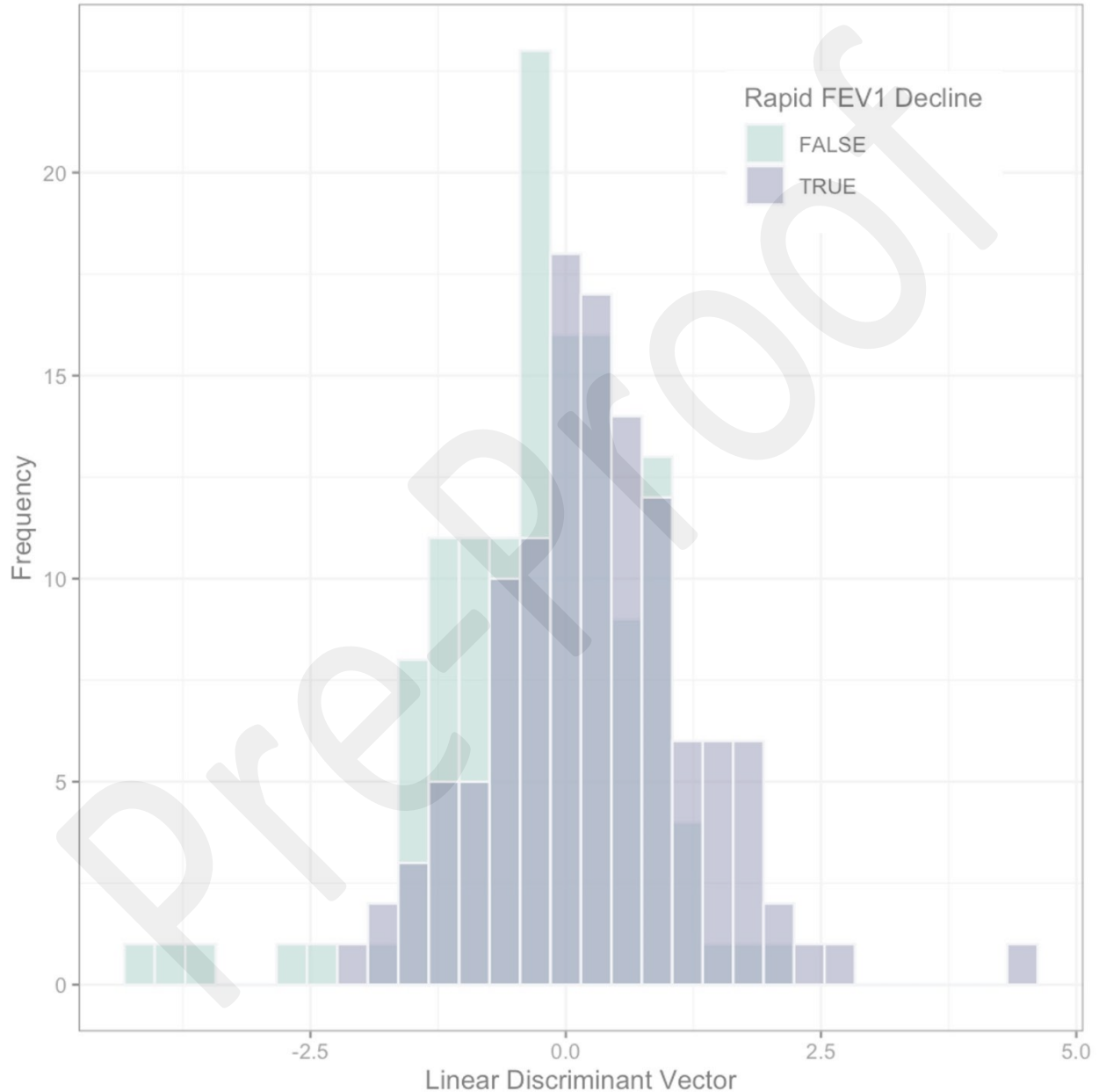
Online Supplement

Full description of Methods

Data Cleaning: The CASCADE data contained measurements of 14 biomarkers, IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IFN, GMCSF, TNF, and CRP, measured on 302 individuals at baseline, year 1, and year 2. Biomarkers at each timepoint were transformed using a log(1+x) transformation. After transformation, all biomarkers were centered and scaled to mean 0, standard deviation 1.

Statistical Analysis: We sought to identify biomarkers at baseline associated with a change in seven outcomes: forced expiratory volume in 1-second (FEV_1), total distance walked, average step total, average minutes active, grip strength, and percentage of time spent inactive. We collectively refer to total distance walked, average step total, average minutes active, grip strength, and percentage of time spent inactive as our *activity outcomes*.

We measured change as both a raw difference between baseline and year 2, i.e.,

$$FEV1_{year\ 2} - FEV1_{baseline},$$

and as the proportional change from baseline to year 2, i.e.,

$$(FEV1_{year\ 2} - FEV1_{baseline})/FEV1_{baseline}.$$

We calculated the Pearson correlation between each baseline biomarker and change in each outcome (raw and percent change) and tested the null hypothesis that this correlation is equal to 0. To control the overall false discovery rate (OFDR) (Benjamini & Heller, 2008) across the biomarkers, we used a hierarchical hypothesis testing procedure to test the significance of the correlation between each biomarker and each outcome, as described in Li & Ghosh (2014). We treated each biomarker as a "set" of hypotheses in which 14 hypotheses (raw and percent change in seven outcomes) were tested. By controlling the OFDR, we control the expected number of biomarker sets falsely rejected. The steps to our hierarchical hypothesis testing procedure were as follows:

1. Treat each biomarker as a group or set of null hypotheses. These null hypotheses are that a given biomarker is not correlated with raw change or percentage change in the seven outcomes. We index each of the $q = 14$ hypotheses within a set using $j, j = 1, \ldots, 14$.

   Using Procedure 4 defined in (Li & Ghosh, 2014), first test the screening hypothesis for each biomarker, which is whether each biomarker is significantly associated with any outcome. To do this test, calculate the p-value for the association between biomarker $i, i = 1, \ldots, 14$ with each of the outcomes. Then obtain the screening p-values, $p_{(0)}(i)$, by calculating $\min_{1 \leq j \leq q} \left((q + 1 - j)p_{(j)}(i) \right)$ where $p_{(j)}(i)$ is the p-value corresponding to hypothesis j for biomarker set i.

2. Apply the Benjamini-Hochberg FDR correction (Benjamini & Hochberg, 1995) to the screening p-values, $p_{(0)}(1), \ldots, p_{(0)}(14)$. Let R be the number of rejected screening hypotheses at the 0.05 level.

3. For biomarker i, define $R_i = \max\{1 \leq j \leq q : p_{(j)}(i) \leq R\alpha\left(m(q + 1 - j)\right)^{-1}\}$ or $R_i = 0$ if the maximum does not exist.

4. For every i and j such that $p_{(j)}(i) \leq p_{(R_i)}(i)$, reject the corresponding null hypothesis for outcome j in biomarker set i.

We report if we can reject any hypotheses within a biomarker set and if so, which hypotheses within that set that were rejected.

We also tested if baseline biomarkers can predict if an individual experienced a rapid decline in FEV$_1$ from baseline to year 2 using Fisher's linear discriminant analysis (LDA). We defined rapid decline in FEV$_1$ as an average drop of 40 milliliters in FEV$_1$ from baseline to year 1 and year 1 to year 2. We used 10-fold cross validation where we trained the LDA model on 9 out of 10 training folds and predicted rapid decline in FEV$_1$ on the held-out test fold. We used the area under the receiver operating curve (AUROC) to evaluate the predictive performance. We used a permutation testing framework to assess the significance of this AUROC in which we permuted whether each individual experienced a rapid decline in FEV$_1$ across the sample. We then fit the LDA model to predict rapid decline in FEV$_1$ on the permuted samples using 10-fold cross validation. We repeated this 100 times and calculated the permutation p-value as:

$$Permutation\ P - Value\ = \frac{\left(\sum_{i=1}^{100} 1(AUC_i^{perm} > AUC^{true})\right) + 1}{101}$$

Samples were required to have at least two consecutive FEV1 measurements (baseline and year 1 and/or year 1 and year 2) to be included in this analysis. Samples missing any baseline biomarker measurements were not included.

Lastly, we used a linear mixed modeling analysis to investigate the relationship between baseline biomarkers over time and eight outcomes: FEV_1, FEV_1-percent-predicted (FEV_1pp), total distance walked, average step total, average minutes active, grip strength, and percentage of time spent inactive. We considered baseline biomarker, time, and smoking status at each year of follow-up as fixed effects. We also adjusted for FEV_1 in the models for total distance walked, average step total, average minutes active, grip strength, and percentage of time spent inactive. We included an interaction between baseline biomarker and time to capture any change over time of the baseline biomarker's effect on the outcome. We included a random intercept, b_{0i}, for each subject i to account for subject-specific variation in the observations. The model for FEV1 and FEV1pp at time $t, t = baseline, year\ 1, year\ 2$ was as follows:

$$y_{it} = \beta_0 + \beta_1 biomarker_{i0} + \beta_2 1(t = year\ 1) + \beta_3 1(t = year\ 2) + \beta_4 1(t = year\ 1) * biomarker_{i0} + \beta_5 1(t = year\ 2) * biomarker_{i0} + \beta_6 smoking\ status_{it} + b_{i0} + \epsilon_{it}$$

where $1(\cdot)$ represents an indicator function.

The model for our activity outcomes at time $t, t = baseline, year\ 1, year\ 2$ was as follows:

$$y_{it} = \beta_0 + \beta_1 biomarker_{i0} + \beta_2 1(t = year\ 1) + \beta_3 (t = year\ 2) + \beta_4 1(t = year\ 1) * biomarker_{i0} + \beta_5 1(t = year\ 2) * biomarker_{i0} + \beta_6 smoking\ status_{it} + \beta_7 FEV1_{it} + b_{i0} + \epsilon_{it}$$

We were interested in the significance of the baseline biomarker effect on the outcome. We applied an analogous hierarchical hypothesis testing framework for each biomarker to assess significance, where each biomarker was treated as a set. The null hypotheses within each set were that each baseline biomarker has no effect on the seven outcomes considered in our analysis.

Supplementary Table 1: Mean annual changes in outcomes for all participants (Total) and stratified by rapid FEV_1 decline.

Characteristic	Total (n=302)	Rapid FEV_1 decline[*] (n=122)	No rapid FEV_1 decline[*] (n=131)
FEV_1	-32.8 (141)	-137 (98.9)	64.5 (98.4)
Average minutes active	-9.23 (43.0)	-14.3 (46.0)	-3.84 (38.6)
Average steps total	-335 (1310)	-474 (1540)	-172 (1020)
6-minute walk total distance (ft)	-16.1 (164)	-52.7 (147)	17.6 (171)
% time spent inactive	1.75 (4.40)	2.34 (4.66)	1.17 (4.00)
Grip strength (kg)	-1.25 (3.06)	-1.14 (2.57)	-1.37 (3.45)

[*] Rapid FEV_1 decline was defined as an average annual drop of 40 mL or higher. Forty-nine samples were missing two consecutive (baseline and year 1 or year 1 and year 2) measurements of FEV1.
FEV1, forced expiratory volume in 1-second

Supplementary Figure 1: Scatterplots of associations between baseline CRP and total distance walked (first row), average minutes active (second row), average steps total (3rd row), and average percent time spent inactive (fourth row). Associations between baseline CRP and activity outcomes are shown at baseline (first column), year 1 (second column), and year 2 (3rd column).
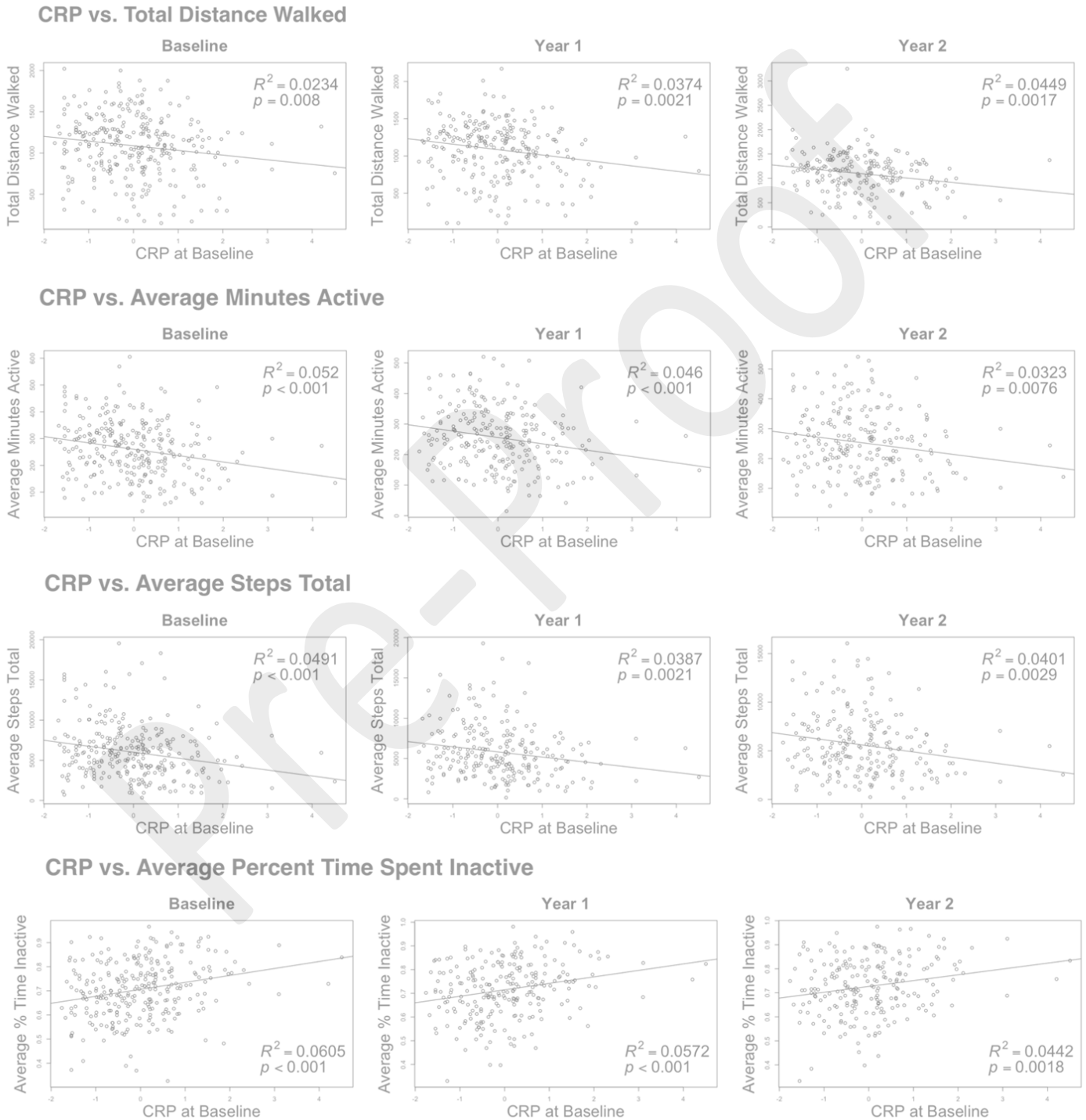
Supplementary References

Benjamini, Y., & Heller, R. (2008). Screening for Partial Conjunction Hypotheses. *Biometrics*, *64*(4), 1215–1222. https://doi.org/10.1111/j.1541-0420.2007.00984.x

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Kawagoshi, A., Iwakura, M., Furukawa, Y., Sugawara, K., Takahashi, H., & Shioya, T. (2020). The association between systemic inflammation and the time spent in posture and movement during daytime in patients with chronic obstructive pulmonary disease and lower weight. *Journal of Physical Therapy Science*, *32*(12), 804–809. https://doi.org/10.1589/jpts.32.804

Li, Y., & Ghosh, D. (2014). A two-step hierarchical hypothesis set testing framework, with applications to gene expression data on ordered categories. *BMC Bioinformatics*, *15*(1), 108. https://doi.org/10.1186/1471-2105-15-108