Published in final edited form as:

Med Sci Sports Exerc. 2011 October ; 43(10): 1884–1890. doi:10.1249/MSS.0b013e31821b4d0a.

Vigorous physical activity, mediating biomarkers, and risk of myocardial infarction

Andrea K. Chomistek, MPH¹, Stephanie E. Chiuve, ScD^{2,3}, Majken K. Jensen, PhD², Nancy R. Cook, ScD^{1,3}, and Eric B. Rimm, ScD^{1,2,4}

¹Department of Epidemiology, Harvard School of Public Health, Boston, MA

²Department of Nutrition, Harvard School of Public Health, Boston, MA

³Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

⁴Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Abstract

Purpose—The effects of physical activity on risk of myocardial infarction (MI) are well documented and may include beneficial changes in blood lipids, inflammatory markers, and insulin sensitivity. The degree to which these and other traditional and nontraditional cardiovascular biomarkers mediate the inverse association between physical activity and risk of MI in men remains unclear.

Methods—We conducted a nested case-control study among 18,225 men in the Health Professionals Follow-up Study followed from 1994 to 2004. A total of 412 men with incident MI were matched 1:2 with control participants on age and smoking status using risk-set sampling. From detailed responses to a modified Paffenbarger physical activity questionnaire, we determined the association between average hours of vigorous-intensity activity (activities requiring METs $\geq$ 6) and MI risk.

Results—For a 3-hour per week increase in vigorous-intensity activity, the multivariate relative risk (RR) of MI was 0.78 (95% CI 0.61, 0.98). In models including pre-existing CVD-related conditions, further adjustment for HDL-C, vitamin D, apolipoprotein B, and hemoglobin A1c attenuated the RR by 70% (95% CI 12%, 127%) to a RR of 0.93 (95% CI 0.72, 1.19).

Conclusions—Participating in three hours per week of vigorous-intensity activity is associated with a 22% lower risk of MI among men. This inverse association can be partially explained by beneficial effects of physical activity on HDL-C, vitamin D, apolipoprotein B, and hemoglobin A1c. While the inverse association attributable to these biomarkers is substantial, future research should explore benefits of exercise beyond these biomarkers of risk.

Corresponding Author: Andrea K. Chomistek, MPH, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, Telephone: 617-909-6034, Fax: 617-566-7805, akaye@hsph.harvard.edu.

CONFLICT OF INTEREST

(Paragraph #33) None. The results of the present study do not constitute endorsement by ACSM.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

exercise; men; epidemiology; coronary heart disease; risk factors

INTRODUCTION

(Paragraph #1) Physical activity is known to be associated with lower risk of cardiovascular disease (CVD). Compared to sedentary individuals, persons reporting regular moderate activity have a 20% lower risk of CVD; at higher amounts or with greater intensity CVD risk reduction is even greater (25).

(Paragraph #2) The effects of physical activity on risk of myocardial infarction (MI) are well documented and may be mediated in part through beneficial changes in blood lipids, inflammatory markers, and insulin sensitivity (7, 13, 19, 25). Strong dose-response associations between exercise intensity and blood lipids—especially HDL-C and triglycerides—have been reported in both observational studies and randomized trials (14). In a randomized trial conducted by Kraus et al, exercise at a caloric equivalent of 20 miles per week and an intensity equivalent to that of jogging at a moderate pace significantly increased HDL-C by 9% and decreased triglycerides by 13%, although had no effect on total LDL-C (13). In National Health and Nutrition Examination Survey (NHANES), C-reactive protein (CRP) was lower with greater levels of leisure time activity (6). After adjusting for CVD risk factors, the odds ratio for elevated CRP concentration (dichotomized at the ≥ 85 th percentile of the sex specific distribution) was 0.53 (95% CI 0.40, 0.71) for participants who engaged in vigorous physical activity three or more times per week compared with sedentary participants. Finally, a meta-analysis of 14 trials of physical activity interventions lasting 8 weeks or longer found that exercise training reduced HbA1c levels by 0.66% on average among middle-aged persons with diabetes (3).

(Paragraph #3) The purpose of this study was to determine the extent to which these and other potential mediating novel biomarkers explain the inverse association between physical activity and risk of CHD among men.

METHODS

Study Population

(Paragraph #4) The Health Professionals Follow-up Study (HPFS) began in 1986, when 51 529 male health professionals aged 40 to 75 years completed the baseline 6-page questionnaire. On biennial, self-administered questionnaires, we collected detailed information on lifestyle characteristics and incident disease.

(Paragraph #5) Between January 1, 1993, and December 31, 1995, a blood sample was requested from all surviving cohort participants; 18 225 provided samples. The men who provided samples were somewhat younger, but were otherwise similar with respect to smoking, physical activity, and diet. Among men without CVD or cancer prior to blood draw, we identified 454 men with incident nonfatal myocardial infarction (MI) ($N = 352$) or fatal coronary heart disease (CHD) ($N = 102$) between the date of blood collection and January 31, 2004. Using risk-set sampling (24), we selected controls [2:1] matched on age (in 5-year increments), smoking (in 5 categories), and month of blood return. For this study, we excluded men ($n = 103$) who indicated at blood draw that they had a physical impairment. Additionally, we excluded men missing physical activity ($n = 1$) or traditional lipid markers ($n = 22$). The final analysis included 412 cases and 827 controls. The study

was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital. All subjects gave informed consent to participate.

Assessment of Biomarkers

(*Paragraph #6*) Participants underwent local phlebotomy. Blood samples were collected and returned via overnight courier to the HPFS blood storage and processing facility as described elsewhere (7). The biomarkers in this sample were measured using standard methods (7) and generally showed excellent stability and reproducibility during simulated transport and storage (20). Candidate biomarkers assessed in all men included HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides, total cholesterol, apolipoprotein B (Apo B), hemoglobin A1c (HbA1c), high-sensitivity C-reactive protein (CRP) (21), fatty acid binding protein 4 (aP2), lipoprotein-associated phospholipase A2 (Lp-PLA2) and 25-hydroxyvitamin D (25[OH]D). We used the triglyceride:HDL-C ratio as a measure of insulin resistance (17). A subset of biomarkers, which included soluble tumor necrosis factor receptors 1 and 2 (sTNFR1 and sTNFR2), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM), soluble vascular cell adhesion molecule-1 (sVCAM), lipoprotein(a), fibrinogen, adiponectin (23), and oxidized LDL (oxLDL), were only assessed among the cases and controls accrued from 1994-2000 (n = 731).

Assessment of Physical Activity

(*Paragraph #7*) Leisure-time physical activity was assessed by questionnaire every two years since baseline through a series of questions on average total time per week spent on each of ten activities over the previous year. Walking pace, categorized as casual (<2 miles/hour), normal (2-2.9 miles/hour), brisk (3-3.9 miles/hour), or striding (≥ 4 miles/hour), was also assessed. A metabolic equivalent task (MET) score was assigned to each activity on the basis of its energy cost (1). Vigorous activities (MET values ≥ 6) were jogging, running, bicycling, swimming, tennis, squash/racquetball, and calisthenics/rowing. Moderate activities ($3 \leq \text{METs} < 6$) were brisk walking, heavy outdoor work, and weightlifting. The validity and reproducibility of the physical activity measurement have been reported in detail elsewhere (4). Briefly, correlations between four 1-week diaries and the questionnaire for total activity and vigorous activity were 0.65 and 0.58, respectively.

(*Paragraph #8*) We chose to focus on vigorous activity due to its stronger associations with CHD risk (14, 25). To represent long-term levels of physical activity more accurately and to reduce measurement error (12), we calculated the average number of hours of vigorous activity per week from the 1990, 1992, and 1994 questionnaires. We categorized participants into quintiles based on the distribution in controls.

Assessment of Nonfatal MI and Fatal CHD

(*Paragraph #9*) Self-reported MIs were confirmed according to World Health Organization criteria that included symptoms plus either diagnostic ECG changes or elevated cardiac enzymes (26). Fatal CHD was confirmed by hospital records or an autopsy or by CHD listed as the cause of death on the death certificate, if it is listed as an underlying and most plausible cause of death and if evidence of previous CHD was available. We included sudden cardiac deaths, defined as death within one hour of symptom onset with no previous serious illness and no other plausible cause.

Statistical Analysis

(*Paragraph #10*) All analyses were performed using SAS statistical software, version 9.1 (SAS Institute Inc, Cary, North Carolina). We calculated odd ratios as unbiased estimates of

incidence rate ratios from conditional logistic regression models, stratifying on the matching factors. Because some of the matched sets of cases and controls were broken after exclusions, we additionally used unconditional logistic regression for some analyses to maximize power. Similar results were obtained for both, thus conditional logistic regression results are presented. In multivariable analyses, we adjusted for moderate activity (five categories), batch (case before or after 2002), fasting status (< 8 hours, ≥ 8 hours), parental history of MI at or before age 60 years (yes/no), alcohol consumption (five categories), aspirin use (yes/no), vitamin E supplement use (yes/no), energy-adjusted intake of polyunsaturated fat, trans fatty acids, omega-3 fatty acids, and fiber (each in quintiles), as well as history of diabetes, hypertension, and hypercholesterolemia diagnosed in 1992 or earlier (to avoid adjusting for an intermediate in the causal pathway.) We used covariate information primarily from 1994, the time of blood draw. Information from previous questionnaires was used when covariate data were missing (less than 6% was missing). Tests for linear trend were performed using the median value for each category of physical activity.

(Paragraph #11) To examine the extent to which various CVD biomarkers contributed to the risk reduction associated with vigorous-intensity physical activity, we initially assigned the median of each physical activity category to construct a continuous variable. We then calculated the rate ratio associated with a three-hour per week increase in vigorous activity which corresponded to the difference between the highest and lowest quintile. We considered the magnitude of change in the regression coefficient for vigorous-intensity activity with and without adjustment for each potential mediator. The percent of physical activity-CHD association explained by the intermediate variable was computed as follows: $(1 - (\beta_{\text{mediator-adjusted model}} / \beta_{\text{multivariable model}})) \times 100\%$. A positive change in the regression coefficient indicates a change in the rate ratio towards the null. The standard error for this estimate was calculated using equation 5 from Lin et al. (16).

(Paragraph #12) As a subset of the biomarkers were only assessed among the cases and controls accrued from 1994-2000, we restricted analyses examining these potential mediators to this group (n = 731).

RESULTS

(Paragraph #13) Table 1 shows the characteristics of case and control participants in the HPFS. As expected, traditional CVD risk factors were more common among cases compared to controls. Cases had a lower HDL-C, higher LDL-C, and were also more likely to have a diagnosis of hypertension, hypercholesterolemia, and diabetes.

(Paragraph #14) Men reporting greater vigorous-intensity physical activity had a lower BMI and better biomarker profiles compared to men reporting lesser amounts of activity (Table 2). HDL-C and vitamin D were higher while triglycerides, CRP, Apo B, and HbA1c were lower in men reporting more vigorous activity compared to less activity. An exception was LDL-C, which was fairly constant across categories of physical activity. Although the P_{trend} for Apo B was not statistically significant in the age-adjusted analysis, when additionally adjusted for other potential confounders, vigorous activity was significantly associated with lower levels of Apo B ($P_{\text{trend}} = 0.02$) (data not shown).

(Paragraph #15) There was a significant inverse association between average vigorous activity and risk of MI ($P_{\text{trend}} = 0.03$) (Table 3). In multivariable-adjusted models additionally adjusted for pre-existing hypertension, hypercholesterolemia, and diabetes, the relative risk (RR) of MI was 0.69 (95% CI 0.45, 1.05) for men who reported three hours or more of vigorous activity per week compared to men reporting no vigorous activity. When

treated as a continuous variable, the RR for a 3-hour per week increase in vigorous activity was 0.78 (95% CI 0.61, 0.98). The RRs (95% CI) for the association between moderate physical activity (in quintiles) and risk of MI, adjusted for the same covariates as well as vigorous activity, were 1.13 (0.77, 1.64), 0.96 (0.65, 1.43), 0.76 (0.51, 1.14), and 0.84 (0.56, 1.26) (p for trend = 0.17) (data not shown). Although the inverse association between moderate activity and CHD was not statistically significant within this nested case-control study, we have found a statistically significant inverse association within the whole HPFS cohort (RR for a 1-hour per week increase in moderate activity: 0.97 (95% CI: 0.95, 0.98)).

(Paragraph #16) We added potential mediating biomarkers individually into multivariable models to assess the magnitude to which each biomarker changed the association of vigorous-intensity physical activity with risk of MI (Table 4). When compared to the multivariate RR of MI of 0.78 (95% CI 0.61, 0.98) for each 3 hour per week increase in vigorous activity, adjustment for HDL-C alone attenuated the RR to 0.86 (95% CI 0.67, 1.09). As noted in Table 4, this suggests that approximately 38% (95% CI 3%, 74%) of the inverse association between vigorous-intensity physical activity and CHD is mediated through HDL-C. Similar analyses for triglycerides (22%), vitamin D (21%), Apo B (19%), HbA1c (14%), and CRP (9%) also attenuated the RR. Additionally, in the subset of 731 men in which it was measured, adjustment for adiponectin attenuated the RR by 52% (95% CI -16%, 121%). The smaller sample size in this subgroup of men resulted in wide confidence intervals.

(Paragraph #17) In multivariable analyses, we also adjusted for the combination of biomarkers where the p -value for percent change was 0.15 or less when modeled separately. We excluded adiponectin because it was not measured in all men. Triglycerides and HDL were individually included rather than their ratio. When we adjusted for the combination of HDL-C, triglycerides, vitamin D, Apo B, HbA1c, and CRP, the regression coefficient for the association between vigorous activity and CHD was attenuated by 70% (95% CI 12%, 128%) (Table 4). In this model, triglycerides and CRP were no longer significantly associated with risk of CHD; thus, the most parsimonious model included HDL-C, vitamin D, Apo B, and HbA1c. This final model attenuated the RR of CHD associated with vigorous-intensity activity by 70% (95% CI 12%, 127%) (Figure 1).

(Paragraph #18) We found that BMI only explained 8% (95% CI -4%, 20%) of the inverse association between physical activity and CHD. As an alternative to BMI, we examined waist circumference as a potential mediator of this association. Adjusting for waist circumference attenuated the RR to a similar extent as BMI (11%); however, we did not include waist circumference in our final results because it was only measured in a subset of individuals.

(Paragraph #19) We performed a sensitivity analysis only including men that were fasting at least 8 hours prior to blood draw ($n = 725$). The percent explained was attenuated, but HDL-C, vitamin D, Apo B, and HbA1c still explained 46% (95% CI 8%, 85%) of the inverse association. We also performed the analysis after adjusting for anti-hypertensive and cholesterol-lowering medications and obtained similar results to those presented. Lastly, we performed the analysis excluding cases that occurred in the first two years of follow-up and found a modestly attenuated total percent explained (54%; 95% CI 17%, 89%).

DISCUSSION

(Paragraph #20) In this prospective analysis of middle-aged men, vigorous exercise was associated with a lower risk of CHD. A three hour per week increase in vigorous-intensity physical activity was associated with a 22% (95% CI 2%, 39%) lower risk of MI. In

multivariable models including pre-existing CVD-related conditions, potential biological mediators of effect— HDL-C, vitamin D, Apo B, and hemoglobin A1c —attenuated the relative risk by 70% (95% CI 12%, 127%).

(Paragraph #21) Other studies have investigated the potential mediators between physical activity and risk CHD, finding that biomarkers explain 22 – 40% of the inverse association (2, 11, 18, 28). Inflammatory factors have been identified as mediators in all studies, explaining 13 – 28% of the association between exercise and CHD. Hamer et al (11) found that CRP and fibrinogen explained 13.2% of the association between physical activity and total CVD, which is similar to what we found. In the Women's Health Study (18), inflammatory markers explained 20.9%, novel lipids 11.1%, traditional lipids 13.4%, HbA1c 5%, and BMI 6.8%. All risk factors combined explained 35.5% of the risk reduction. A larger proportion of the inverse association between physical activity and CHD risk was explained by the potential mediators we investigated when compared to previous studies. Potential explanations for this difference may be better exercise assessment in our cohort with the repeated measures of activity over 4 years and the greater variability in moderate and vigorous physical activity in this cohort of men.

(Paragraph #22) We also found a modest proportion of the physical activity-CHD association was explained by serum 25-hydroxyvitamin D (25[OH]D), which was not accounted for in previous studies. Although not a traditional cardiovascular risk factor, low levels of serum 25(OH)D have previously been found to be associated with increased risk of MI (8). Additionally, physical activity has been associated with increased levels of serum 25(OH)D likely due to increased sun exposure, a strong determinant of serum 25(OH)D (9).

(Paragraph #23) There are several possible mechanisms by which physical activity decreases risk of CHD. One pathway is through anti-atherogenic effects resulting from improved inflammation, lipid profile, insulin sensitivity, and body fat (3, 6, 7, 13, 19, 22, 25). In the current analysis, we found these pathways explain approximately 45-70% of the decreased risk of CHD associated with physical activity. Additionally, physical activity may induce favorable changes in blood viscosity, blood pressure, and hemostatic factors that decrease risk of thrombogenesis and thrombosis (5, 19). Physical activity also decreases myocardial O₂ demand and improves coronary blood flow and endothelial function which decrease risk of myocardial ischemia. Decreased risk of ventricular arrhythmias results from increased vagal tone and decreased adrenergic activity (9, 15). Future studies are necessary to determine which of the above mechanisms account for the remainder of risk reduction.

(Paragraph #24) Strengths of our study include the prospective design, detailed information on physical activity and covariates, a wide range of cardiovascular biomarkers, long duration of follow-up for the endpoint of MI, and strict criteria for defining coronary events.

(Paragraph #25) Our study also has limitations that should be considered. In a mediator analysis, we must be aware of potential confounders for the main effect association and the association between mediators and CHD. We adjusted for age, smoking, diet, alcohol use, and pre-existing disease. Unmeasured confounding by other variables, particularly biomarkers or genes that may be associated with both mediator and CHD, remains a potential limitation.

(Paragraph #26) Our study population, consisting of male health professionals, is not representative of the general population. Therefore, we cannot necessarily generalize our results to other populations with different educational levels, incomes, or distributions of race and ethnicity. Nonetheless, the relative homogeneity of the cohort in socioeconomic status may actually enhance the internal validity of this study.

(Paragraph #27) We measured biomarkers at a single point in time. One measurement may not reflect true levels over time. To examine the potential impact of measurement error, we used data from men in a reproducibility study to correct for random within-person measurement error in HDL-C (27). After correcting for measurement error, we found that HDL-C explained 50% of the physical activity-CHD association, compared to the uncorrected estimate of 38%.

(Paragraph #28) The mediators determined to explain the greatest proportion of association may be statistical intermediates, but may not be in the causal pathway. The pathway between physical activity and CHD is likely quite complex; thus, the most significant mediators may simply be markers of various pathways and not causal components. Additionally, as we fit the parsimonious model by selecting mediators with the most significant percent change when modeled separately, the 70% attenuation of the PA-CHD association may be optimistic.

(Paragraph #29) Measurement error in physical activity is unlikely to bias our results because data were assessed prospectively; thus, errors in physical activity are likely to be non-differential with respect to subsequent MI status. In addition, reporting error is unlikely to be associated with measurement of biomarkers. Random misclassifications would be expected to lead to underestimation of the true association.

(Paragraph #30) Engaging in regular vigorous-intensity activity is associated with a lower risk of MI among men. This inverse association can be partially explained by the beneficial effects of physical activity on HDL-C, vitamin D, Apo B, and HbA1c. While the inverse association attributable to these biomarkers is substantial, future research should explore benefits of exercise beyond these biomarkers of risk.

Acknowledgments

(Paragraph #31) The authors thank the participants of the HPFS for their continued cooperation and participation, Dr. Walter Willett for his comments and suggestions in the preparation of this manuscript, Lydia Liu and Mathew Pazaris for their helpful statistical assistance, and Kathy Bush and Janine Neville-Golden for their laboratory support.

(Paragraph #32) This study was supported National Institute of Health grants AA11181 and HL35464. A. Chomistek was supported by an institutional training grant (HL07575) from the National Heart, Lung, and Blood Institute.

Sources of funding: This study was supported National Institute of Health grants AA11181 and HL35464. A. Chomistek was supported by an institutional training grant (HL07575) from the National Heart, Lung, and Blood Institute.

REFERENCES

1. Ainsworth BE, Haskell WL, Leon AS, Jacobs DR Jr, Montoye HJ, Sallis JF, Paffenbarger RS Jr. Compendium of physical activities: classification of energy costs of human physical activities. *Medicine and Science in Sports and Exercise*. 1993; 25:71–80. [PubMed: 8292105]
2. Boekholdt SM, Sandhu MS, Day NE, Luben R, Bingham SA, Peters RJ, Wareham NJ, Khaw KT. Physical activity, C-reactive protein levels and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. *European Journal of Cardiovascular Prevention & Rehabilitation*. 2006; 13:970–976. [PubMed: 17143130]
3. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *The Journal of the American Medical Association*. 2001; 286:1218–27.

4. Chasan-Taber S, Rimm EB, Stampfer MJ, Spiegelman D, Colditz GA, Giovannucci E, Ascherio A, Willett WC. Reproducibility and validity of a self administered physical activity questionnaire for male health professionals. *Epidemiology*. 1996; 7:81–6. [PubMed: 8664406]
5. Cornelissen VA, Fagard RH. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. *Hypertension*. 2005; 46(4):667–75. [PubMed: 16157788]
6. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. *Epidemiology*. 2002; 13:561–8. [PubMed: 12192226]
7. Fung TT, Hu FB, Yu J, Chu NF, Spiegelman D, Tofler GH, Willett WC, Rimm EB. Leisure-time physical activity, television watching, and plasma biomarkers of obesity and cardiovascular disease risk. *American Journal of Epidemiology*. 2000; 152:1171–8. [PubMed: 11130623]
8. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-Hydroxyvitamin D and risk of myocardial infarction in men. *Archives of Internal Medicine*. 2008; 168(11):1174–1180. [PubMed: 18541825]
9. Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, Willett WC. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *Journal of the National Cancer Institute*. 2006; 98:451–459. [PubMed: 16595781]
10. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, Schoene N, Schuler G. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *The New England Journal of Medicine*. 2000; 342:454–60. [PubMed: 10675425]
11. Hamer M, Stamatakis E. Physical activity and risk of cardiovascular disease events: inflammatory and metabolic mechanisms. *Medicine and Science in Sports and Exercise*. 2009; 41:1206–1211. [PubMed: 19461547]
12. Hu FB, Stampfer MJ, Colditz GA, Ascherio A, Rexrode KM, Willett WC, Manson JE. Physical activity and risk of stroke in women. *The Journal of the American Medical Association*. 2000; 283:2961–7.
13. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, Bales CW, Henes S, Samsa GP, Otvos JD, Kulkarni KR, Slentz CA. Effects of the amount and intensity of exercise on plasma lipoproteins. *The New England Journal of Medicine*. 2002; 347:1483–92. [PubMed: 12421890]
14. Lee, IM. *Epidemiologic Methods in Physical Activity Studies*. Oxford University Press; Oxford: 2009. p. 328
15. Leung FP, Yung LM, Laher I, Yao X, Chen ZY, Huang Y. Exercise, vascular wall and cardiovascular diseases: an update (Part 1). *Sports Med*. 2008; 38(12):1009–24. [PubMed: 19026018]
16. Lin DY, Fleming TR, De Gruttola V. Estimating the proportion of treatment effect explained by a surrogate marker. *Statistics in Medicine*. 1997; 16:1515–1527. [PubMed: 9249922]
17. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Annals of Internal Medicine*. 2003; 139:802–809. [PubMed: 14623617]
18. Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation*. 2007; 116:2110–2118. [PubMed: 17967770]
19. Mora S, Lee IM, Buring JE, Ridker PM. Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. *The Journal of the American Medical Association*. 2006; 295:1412–9.
20. Pai JK, Curhan GC, Cannuscio CC, Rifai N, Ridker PM, Rimm EB. Stability of novel plasma markers associated with cardiovascular disease: processing within 36 hours of specimen collection. *Clinical Chemistry*. 2002; 48:1781–1784. [PubMed: 12324497]
21. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshupura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB. Inflammatory markers and the risk of coronary heart disease in men and women. *The New England Journal of Medicine*. 2004; 351:2599–2610. [PubMed: 15602020]
22. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Rimm EB. Leisure-time physical activity and reduced plasma levels of obesity-related inflammatory markers. *Obesity Research*. 2003; 11(9): 1055–1064. [PubMed: 12972675]

23. Pischon T, Hotamisligil GS, Rimm EB. Adiponectin: stability in plasma over 36 hours and within-person variation over 1 year. *Clinical Chemistry*. 2003; 49:650–652. [PubMed: 12651819]
24. Prentice RL, Breslow NE. Retrospective studies and failure time models. *Biometrika*. 1978; 65:153–158.
25. Physical Activity Guidelines Advisory Committee. Physical Activity Guidelines Advisory Committee Report, 2008. U.S.Department of Health and Human Services; Washington, DC: 2008. p. 683
26. Rose, GA.; Blackburn, H.; Gillum, R.; Prineas, RJ. Cardiovascular Survey Methods: WHO Monograph Series No. 56. World Health Organization; Geneva, Switzerland: 1982. p. 162-165.
27. Rosner B, Spiegelman D, Willett WC. Correction of logistic regression relative risk estimates and confidence intervals for random within-person measurement error. *American Journal of Epidemiology*. 1992; 136(11):1400–1413. [PubMed: 1488967]
28. Wennberg P, Wensley F, Johansson L, Boman K, Di Angelantonio E, Rumley A, Lowe G, Hallmans G, Jansson JH. Reduced risk of myocardial infarction related to active commuting: inflammatory and haemostatic effects are potential major mediating mechanisms. *European Journal of Cardiovascular Prevention & Rehabilitation*. 2010; 17:56–62. [PubMed: 19820396]

Figure 1. Main mediators of the association between physical activity and CHD: percent of physical activity-CHD association explained by risk factors

The rate ratios are conditioned on the matching factors of age, smoking status, and date of blood sample return and adjusted for moderate activity, batch, fasting status, parental history of MI at or before age 60 years, aspirin, vitamin E supplement use, intake of polyunsaturated fat, trans fat, EPA+DHA, fiber, alcohol intake, and pre-existing hypertension, diabetes, or hypercholesterolemia. The percent of physical activity effect explained by the intermediate variable was computed as follows: $(1 - (\beta_{\text{mediator-adjusted model}} / \beta_{\text{multivariable model}})) \times 100\%$.

Table 1

Baseline characteristics of 412 incident MI cases and 827 matched controls in the Health Professionals Follow-up Study

Variable	Cases (n = 412)	Controls (n = 827)	P-value *
Average vigorous activity, hrs/week	0.7 (0 – 2.1)	0.8 (0.1 – 2.5)	0.34
Age, y	64.7 (57.3 – 70.8)	64.8 (57.7 – 71.1)	0.65
BMI, kg/m ²	25.8 (24.0 – 28.1)	25.1 (23.3 – 27.3)	0.001
Alcohol, g/d	4.2 (0 – 14.9)	7.0 (0.9 – 18.0)	0.0005
Diabetes, %	8.3%	3.6%	0.002
Hypertension, %	35.7%	28.5%	0.01
Hypercholesterolemia, %	50.0%	41.2%	0.004
Cholesterol-lowering medication, %	8.0%	6.7%	0.36
Biomarkers:			
Cholesterol, mmol/L	5.5 (4.8 – 6.1)	5.2 (4.6 – 5.8)	0.0001
Triglycerides, mmol/L	1.6 (1.1 – 2.3)	1.3 (0.9 – 1.9)	< .0001
HDL-C, mmol/L	1.1 (0.9 – 1.2)	1.2 (1.0 – 1.4)	< .0001
LDL-C, mmol/L	3.5 (2.9 – 4.1)	3.2 (2.7 – 3.8)	0.0003
oxLDL, U/L [†]	73 (61 – 90)	66 (56 – 81)	0.0002
Triglyceride / HDL ratio	3.6 (2.1 – 5.6)	2.6 (1.7 – 4.3)	< .0001
CRP, mg/L	1.3 (0.6 – 2.6)	1.0 (0.5 – 2.0)	0.0003
Fibrinogen, μmol/L [†]	11.9 (10.4 – 13.3)	11.3 (10.1 – 12.8)	0.002
Lp(a), μmol/L [†]	0.5 (0.2 – 1.4)	0.4 (0.2 – 1.3)	0.12
ApoB, g/L	1.0 (0.8 – 1.1)	0.9 (0.8 – 1.0)	< .0001
Adiponectin, pg/mL [†]	14.2 (9.3 – 20.1)	16.6 (11.7 – 22.9)	< .0001
sTNFR1, pg/mL [†]	1378 (1179 – 1702)	1386 (1148 – 1677)	0.89
sTNFR2, pg/mL [†]	2848 (2371 – 3366)	2802 (2335 – 3367)	0.68
sICAM, ng/mL [†]	337 (280 – 396)	322 (282 – 368)	0.05
sVCAM, ng/mL [†]	1328 (1128 – 1553)	1272 (1096 – 1486)	0.04
IL-6, pg/mL [†]	1.8 (1.1 – 2.9)	1.5 (1.0 – 2.7)	0.03
HbA1c, %	5.6 (5.3 – 5.9)	5.5 (5.3 – 5.7)	< .0001
aP2, ng/mL	20.6 (15.5 – 26.1)	19.2 (13.8 – 24.7)	0.02
Lp-PLA2 mass, ng/ml	229 (200 – 251)	226 (201 – 257)	0.54
Lp-PLA2 activity, nmol/ml/min	213 (191 – 241)	206 (182 – 234)	0.0002
25(OH)D, nmol/L	57.7 (45.7 – 69.1)	59.9 (47.9 – 73.6)	0.0006

BMI indicates body mass index; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; oxLDL, oxidized LDL cholesterol; CRP, C-reactive protein; Lp(a), lipoprotein(a); ApoB, apolipoprotein B; sTNFR1 and sTNFR2, soluble tumor necrosis factor receptors 1 and 2; sICAM, soluble intercellular adhesion molecule-1; sVCAM, soluble vascular cell adhesion molecule-1; IL-6, interleukin-6; HbA1c, hemoglobin A1c; aP2, fatty acid binding protein 4; Lp-PLA2, lipoprotein-associated phospholipase A2; and 25(OH)D, 25-hydroxyvitamin D. Data are medians and IQR for all continuous variables

* Hypothesis tests based on means; biomarkers were ln-transformed

[†] Only available in a subset of the population (n = 731)

Table 2

Age-adjusted mean biomarker levels by quintiles of average vigorous activity among 827 male health professionals, Health Professionals Follow-up Study

	Categories of Average Vigorous Activity, hours/week (1990-1994)					P _{trend} *
	1 (0 hr/wk) (n = 197)	2 (0.01-0.52) (n = 133)	3 (3 0.53-1.49) (n = 166)	4 (1.5-2.99) (n = 155)	5 (≥ 3.0 hrs/wk) (n = 176)	
Average vigorous activity, hrs/wk	0.0	0.3	0.9	2.1	5.6	—
Alcohol, g/d	14.2	14.3	12.2	13.4	11.9	0.16
BMI, kg/m ²	26.3	26.1	25.4	25.1	24.7	<.0001
Diabetes, %	3.7%	5.0%	3.2%	5.1%	1.3%	0.14
Hypertension, %	36%	37%	22%	27%	22%	0.002
Hypercholesterolemia, %	39%	46%	42%	43%	37%	0.30
Cholesterol-lowering medication, %	5%	6%	4%	10%	8%	0.07
Biomarkers:						
Cholesterol, mmol/L	5.13	5.13	5.18	5.18	5.23	0.25
Triglycerides, mmol/L	1.49	1.47	1.41	1.21	1.25	0.0007
HDL-C, mmol/L	1.11	1.09	1.14	1.22	1.22	<.0001
LDL-C, mmol/L	3.08	3.19	3.21	3.21	3.21	0.22
oxLDL, U/L	67	65	70	66	67	0.68
Triglyceride/HDL ratio	3.1	3.1	2.9	2.3	2.3	<.0001
CRP, mg/L	1.59	1.14	0.89	0.93	0.73	<.0001
Fibrinogen, μmol/L [†]	11.7	11.5	11.4	11.0	10.6	<.0001
Lp(a), μmol/L [‡]	0.39	0.35	0.50	0.47	0.47	0.43
ApoB, g/L	0.88	0.90	0.89	0.86	0.86	0.14
Adiponectin, pg/mL [‡]	15.6	15.3	14.7	16.8	16.0	0.42
sTNFR1, pg/mL [‡]	1408	1437	1366	1366	1451	0.39

	Categories of Average Vigorous Activity, hours/week (1990-1994)					<i>P</i> _{trend} [*]
	1 (0 hr/wk) (n = 197)	2 (0.01–0.52) (n = 133)	3 (3 0.53–1.49) (n = 166)	4 (1.5–2.99) (n = 155)	5 (≥ 3.0 hrs/wk) (n = 176)	
sTNFR2, pg/mL [†]	2893	2922	2724	2592	2779	0.20
sICAM, ng/mL [†]	344	324	321	311	308	0.006
sVCAM, ng/mL [†]	1313	1300	1249	1188	1287	0.57
IL-6, pg/mL [†]	1.86	1.63	1.68	1.73	1.56	0.19
HbA _{1c} , %	5.58	5.64	5.53	5.58	5.42	0.0002
ap2, ng/mL	21.1	19.7	19.3	17.1	17.1	<.0001
Lp-PLA2 mass, ng/ml	226	224	228	226	228	0.60
Lp-PLA2 activity, nmol/ml/min	200	206	211	200	204	0.95
25(OH)D, nmol/L	54.9	56.4	57.2	63.6	62.2	<.0001

BMI indicates body mass index; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; oxLDL, oxidized LDL cholesterol; CRP, C-reactive protein; Lp(a), lipoprotein(a); ApoB, apolipoprotein B; sTNFR1 and sTNFR2, soluble tumor necrosis factor receptors 1 and 2; sICAM, soluble intercellular adhesion molecule-1; sVCAM, soluble vascular cell adhesion molecule-1; IL-6, interleukin-6; HbA_{1c}, hemoglobin A_{1c}; ap2, fatty acid binding protein 4; Lp-PLA2, lipoprotein-associated phospholipase A2; and 25[OH]D, 25-hydroxyvitamin D.

Data are means (geometric means for biomarkers)

^{*} Biomarkers were ln-transformed for hypothesis testing

[†] Only available in a subset of the population (n = 731)

Table 3
Rate Ratios for Incident MI According to Categories of Average Vigorous-intensity Physical Activity in 1990-1994

	Vigorous Activity, hours/week					Test for linear trend	
	1 (0 hr/wk) (Lowest)	2 (0.01 – 0.52)	3 (0.53 – 1.49)	4 (1.5 – 2.99)	5 (= 3.0 hrs/wk) (Highest)	RR using quintile medians	p-value
# cases	109	80	79	79	65		
Basic *	1.00	1.04 (0.71, 1.51)	0.92 (0.64, 1.33)	0.96 (0.65, 1.41)	0.68 (0.46, 0.99)	0.92 (0.86, 0.99)	0.03
Multivariable †	1.00	1.11 (0.74, 1.65)	1.01 (0.67, 1.51)	0.97 (0.64, 1.45)	0.69 (0.45, 1.04)	0.92 (0.85, 0.99)	0.03
Multivariable ‡ + pre-existing disease‡	1.00	1.08 (0.72, 1.63)	1.03 (0.69, 1.55)	0.97 (0.64, 1.47)	0.69 (0.45, 1.05)	0.92 (0.85, 0.99)	0.03

* The basic model includes the matching factors of age, smoking status, and date of blood sample return.

† The multivariable model adjusts for moderate activity (quintiles), batch (case before or after 2002), fasting status (< 8 hrs, ≥8 hours), parental history of MI at or before age 60 years (yes/no), aspirin (yes/no), vitamin E supplement use (yes/no), intake of polyunsaturated fat, trans fat, EPA+DHA, fiber (each in quintiles), and alcohol use (5 categories).

‡ Pre-existing disease includes a diagnosis of any of the following in 1992 or earlier: hypertension, diabetes, or hypercholesterolemia

Table 4

Comparison of rate ratios for incident MI for a three hour/week increase in vigorous-intensity PA after adjustment for mediating biomarkers* among men (n = 1239)

Model	RR (95% CI)	% change [†] (95% CI)	p-value for % change
Multivariable model [‡]	0.78 (0.61, 0.98)	-----	-----
+Cholesterol, mg/dL	0.77 (0.61, 0.97)	-4.3% (-21.7, 13.0)	0.62
+Triglycerides, mg/dL	0.82 (0.65, 1.04)	22.1% (-2.3, 46.5)	0.08
+HDL-C, mg/dL	0.86 (0.67, 1.09)	38.1% (2.6, 73.5)	0.04
+LDL-C, mg/dL	0.78 (0.61, 0.98)	0.1% (-13.0, 13.3)	0.98
+ Triglyceride / HDL ratio	0.85 (0.67, 1.08)	34.3% (1.6, 67.1)	0.04
+CRP, mg/L	0.80 (0.63, 1.01)	9.2% (-3.3, 21.6)	0.15
+Apo B, mg/dL	0.82 (0.64, 1.04)	19.4% (-7.2, 46.0)	0.15
+HbA1c, %	0.80 (0.63, 1.02)	14.0% (-4.1, 32.0)	0.13
+aP2, ng/mL	0.79 (0.62, 1.00)	6.5% (-2.8, 15.8)	0.17
+Lp-PLA2 mass, ng/mL	0.78 (0.62, 0.99)	1.6% (-1.6, 4.9)	0.33
+Lp-PLA2 activity, nmol/ml/min	0.79 (0.62, 1.00)	6.6% (-7.4, 20.6)	0.36
+ BMI, kg/m ²	0.79 (0.63, 1.00)	8.4% (-3.5, 20.3)	0.16
+ 25(OH)D, ng/mL	0.82 (0.65, 1.04)	21.4% (-0.8, 43.5)	0.06
Multivariable model in subset (n = 731) [‡]	0.81 (0.59, 1.10)	-----	-----
+Fibrinogen, mg/dL	0.82 (0.60, 1.13)	9.0% (-12.1, 30.1)	0.40
+Lp(a), mg/dL	0.79 (0.57, 1.08)	-13.7% (-41.9, 14.4)	0.34
+Adiponectin, pg/mL	0.90 (0.66, 1.24)	52.4% (-15.8, 120.6)	0.13
+sTNFR1, pg/mL	0.81 (0.59, 1.10)	-0.7% (-11.2, 9.8)	0.89
+sTNFR2, pg/mL	0.81 (0.59, 1.10)	0.5% (-4.3, 5.3)	0.85
+sICAM, ng/mL	0.81 (0.60, 1.11)	2.1% (-8.9, 13.1)	0.71
+sVCAM, ng/mL	0.81 (0.59, 1.10)	-1.7% (-14.5, 11.1)	0.80
+IL-6, pg/mL	0.80 (0.59, 1.10)	-4.0% (-16.9, 8.9)	0.55
+ oxLDL, U/L	0.81 (0.59, 1.11)	0.9% (-30.3, 32.1)	0.96
HDL, 25(OH)D, Trig, HbA1c, Apo B, CRP (n = 1239)	0.93 (0.72, 1.19)	70.4% (12.4, 128.4)	0.02
Final parsimonious model [§] (n = 1239)	0.93 (0.72, 1.19)	69.7% (12.1, 127.2)	0.02

HDL-C indicates HDL cholesterol; LDL-C, LDL cholesterol; CRP, C-reactive protein; ApoB, apolipoprotein B; HbA1c, hemoglobin A1c; aP2, fatty acid binding protein 4; Lp-PLA2, lipoprotein-associated phospholipase A2; BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; Lp(a), lipoprotein(a); sTNFR1 and sTNFR2, soluble tumor necrosis factor receptors 1 and 2; sICAM, soluble intercellular adhesion molecule-1; sVCAM, soluble vascular cell adhesion molecule-1; IL-6, interleukin-6; and oxLDL, oxidized LDL cholesterol;

* All biomarkers except BMI are ln-transformed

[†]Percent of physical activity effect explained by intermediate variable: $(1 - (\beta_{\text{mediator-adjusted model}} / \beta_{\text{multivariable model}})) \times 100\%$

[‡]Rate ratios are conditioned on matching factors of age, smoking status, and date of blood sample return and adjusted for moderate activity, batch, fasting status, parental history of MI at or before age 60 years, aspirin, vitamin E supplement use, intake of polyunsaturated fat, trans fat, EPA +DHA, fiber, alcohol, pre-existing hypertension, diabetes, or hypercholesterolemia

[§]Includes HDL, 25(OH)D, HbA1c, Apo B