Article Type

Short report

Title

Walking and Inflammatory Markers in Individuals Screened for Type 2 Diabetes

Short Title

T. Yates et al.: Walking and inflammatory markers

Authors

Thomas Yates, School of Sport and Exercise Sciences, Loughborough University, UK
Melanie Davies, Department of Cardiovascular Sciences, University of Leicester, UK
Emer Brady, Department of Cardiovascular Sciences, University of Leicester, UK
David Webb, Department of Cardiovascular Sciences, University of Leicester, UK
Trish Gorely, School of Sport and Exercise Sciences, Loughborough University, UK
Fiona Bull, School of Sport and Exercise Sciences, Loughborough University, UK
Duncan Talbot, Unilever Corporate Research, Colworth Park, Bedfordshire, UK
Naveed Sattar, Department of Vascular Biochemistry, University of Glasgow, UK
Kamlesh Khunti, Department of Health Sciences, University of Leicester, UK

Corresponding Author

Thomas Yates
School of Sports and Exercise Sciences
Loughborough University
Ashby Road
Loughborough
Leicestershire
LE11 3TU
United Kingdom
Tel: (+44)1509226383
Email address: t.e.yates@lboro.ac.uk

Abstract word Count = 164   **Main text word count = 2184**
Number of Tables = 1   **Number of Figures = 1**

Abstract

Objective: To investigate the association of walking activity with inflammatory markers and fasting insulin in a bi-ethnic population screened for type 2 diabetes <u>in Leicester, United Kingdom, between 2005 and 2006.</u>

Method: Physical activity, adipocytokine, high-sensitivity C-reactive protein and fasting insulin measurements were available for 400 individuals screened for type 2 diabetes. Of the 400 participants, 56% were diagnosed with normal glucose control, 36% with prediabetes and 8% with diabetes.

Results: Multivariate statistical analysis showed that those who reported walking for at least 30 minutes on at least five days per week had lower levels of C-reactive protein, interleukin-6, and tumor necrosis factor-α compared to those who reported lower walking activity levels, after adjustment for other modes of moderate-to-vigorous physical activity, age, ethnicity, sex, social deprivation and smoking staus. Further adjustment for waist circumference attenuated the association of walking with tumor necrosis factor-α.

Conclusion: Walking activity, independent of other forms of physical activity, is associated with lower levels of circulating pro-inflammatory markers.

Key Words: Adipocytokine, C-reactive protein, exercise, inflammation, insulin, interleukin-6, physical activity, tumor necrosis factor-α, walking

Abbreviations: ADDITION, Anglo-Danish-Dutch Study of Intensive Treatment In People with Screen Detected Diabetes in Primary Care; CRP, C-reactive protein; hsCRP, high sensitivity C-reactive protein; IMD, Index of Multiple Deprivation; IPAQ, international physical activity questionnaire; MET-hours/week, metabolic equivalents.

Introduction

Markers of chronic low-grade inflammation, such as tumor necrosis factor-α (TNFα) , interleukin-6 (IL-6), and C-reactive protein (CRP), have been shown to predict the risk of developing type 2 diabetes and cardiovascular disease and are thought to be directly involved in the pathogenesis of these chronic diseases (Libby et al 2002, Pickup & Crook 1998, Xu et al. 2003). TNFα and IL-6 are cytokines which are predominantly secreted from adipose tissue while CRP is the principal downstream mediator of the acute phase response and is secreted by the liver in response to TNFα and IL-6 stimuli (Du Clos 2000). Circulating levels of these markers of chronic low-grade inflammation are therefore largely influenced by levels of adiposity and have been proposed as an important meditating link between obesity and chronic disease (Berg & Scherer 2005). However, recent evidence has shown that levels of physical activity are also inversely and independently associated with TNFα , IL-6, and CRP (Panagiotakos et al. 2005, Pischon et al. 2003, Wannamethee et al. 2002). As levels of physical activity have also consistently been shown to be inversely associated with the risk of developing both type 2 diabetes and cardiovascular disease (Bassuk & Manson 2005), markers of chronic low-grade inflammation could be a mediating link between levels of physical activity and chronic disease risk. However, the effect of walking, independent of more vigorous forms of exercise, on inflammatory markers is not well documented. This is an important limitation as walking has been shown to be the preferred choice of physical activity for the majority of individuals (Crespo et al. 1996), and walking for as little as 150 minutes per week has been associated with reduction in the relative risk of developing type 2 diabetes and cardiovascular diease (Hu et al. 1999, Laaksonen et al. 2005, Manson et al. 2002). The aim of this study is to investigate the effect of walking on key markers of chronic low-grade inflammation associated with the development of type 2 diabetes and cardiovascular disease,

along with fasting insulin, in a bi-ethnic population screened for type 2 diabetes. Our hypothesis was that walking, at levels that are consistent with the current exercise recommendations, would be independently associated with reduced chronic low-grade inflammation.

Methods

The ADDITION study is a Europe-wide screening and treatment programme for type 2 diabetes (Sandbaek et al. 2008). Between 2005 and 2006, 573 (m = 304, f = 269) individuals screened as part of the Leicester-ADDITION study also consented for a sub-study for which additional blood samples were taken for the analysis of inflammatory biomakers. The average age of the participants was 62 ± 9 years and 24% were from a South Asian ethnic background.

Physical activity

Physical activity was measured using the short last-seven-days self-administered format of the International Physical Activity Questionnaire (IPAQ) (Craig et al. 2003). IPAQ measures the frequency and duration of any moderate-to-vigorous physical activity undertaken for more than 10 continuous minutes across all contexts (e.g. work, home and leisure) over a seven-day period. Importantly, IPAQ distinguishes walking activity from other forms of moderate-to-vigorous physical activity across all contexts. It also enables the calculation of metabolic equivalents (MET-hours/week). The IPAQ questionnaire has been shown to correlate reasonably ($\rho = 0.4$) with accelerometer data in the United Kingdom (Craig et al. 2003). Walking activity categories were formed by distinguishing between those who reported walking for at least 30 minutes on at least five days per week and those who reported walking for less than this.

Biochemical, clinical and demographic measurements

Venous blood samples were collected in the morning following an overnight fast. Participants were not asked to avoid vigorous physical activity before attending. Prediabetes and diabetes were defined according to the American Diabetes Association's 1997 criteria (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Diagnosis of diabetes was confirmed on a repeat oral glucose tolerance test.

All assays were measured by individuals blinded to the patients identity. Plasma glucose was measured using a glucose oxidase method on the Beckman Auto Analyzer (Beckman, High Wycombe, UK). High-sensitivity C-reactive protein (hsCRP) was analysed on an ABX Pentra clinical chemistry analyser using a latex-enhanced immunoturbidimetric assay (Horiba Group, Montpellier, France). TNFα and IL-6 were analysed using quantikine high-sensitivity enzyme linked immunosorbant assays (ELISA) (R&D Systems, Abingdon, UK). Insulin was analysed using a Perkin Elmer time-resolved fluoro-immuno assay on the AutoDELFIA. The intraassay and interassay coefficients of variation for the included assays did not exceed 10%, apart from TNFα, which had a maximum interassay coefficient of variation of 16.7%.

Arterial blood pressure was measured in the sitting position (Omron, Healthcare, Henfield, UK); three measurements were obtained and the average of the last two measurements was used. Body weight (Tanita TBE 611, Tanita, West Drayton, UK), waist circumference (midpoint between the lower costal margin and iliac crest) and height were also measured to the nearest 0.1 kg and 0.5 cm respectively.

Current smoking status and medication history were obtained by an interview-administered protocol. For the purposes of this study participants were defined as non-smokers, past smokers, or current smokers (≥ 1 cigarette per day). Blood pressure and statin medication status was defined by whether or not participants were currently taking these medications.

Social deprivation was determined by assigning an Index of Multiple Deprivation (IMD) score to each participant's postcode (Office for the Deputy Prime Minister 2004). IMDs are publicly available continuous measures of compound social and material deprivation, and are calculated using a variety of data including current income, employment, health, education, and housing.

Statistical analysis

Variables are presented as mean value ± standard deviation. Chi-squared tests were used to analyse categorical variables, independent t-tests were used to analyse normally distributed continuous variables and Mann-Whitney tests were used to analyse non-parametric variables. Multivariate analysis of variance was used to analyse the associations between walking activity categories and hsCRP, IL-6, TNFα, and insulin. Due to their skewed distribution, all dependant variables in the multivariate analysis were log-transformed. Multivariate analysis models were adjusted for non-walking physical activity levels and measured demographic variables (age, sex, ethnicity and social deprivation). Further adjustment was carried out for medication and smoking status if the inclusion of these factors as covariates in the multivariate analysis changed the coefficient for walking status by 10% or more for any of the included dependant variables (Maldonado & Greenland 1993). In addition, further adjustment was also made for waist circumference in order to establish the independent effect of walking activity

status. All analyses were two sided; p <0.05 was considered significant. Analysis was carried out on SPSS 12.0 for Windows (SPSS, Chicago, USA).

Results

Complete physical activity data was available for 400 (70%) participants. Of these participants, 142 (36%) had prediabetes, 33 (8%) had diabetes and 15 (4%) had a previous history of myocardial infarction, stroke or angina. Those that completed the questionnaire were more likely to have normal glucose control and were more likely to come from less deprived areas than non-completers; no significant differences in inflammatory markers, age or ethnicity was observed between completers and non-completers. Data is reported for the subset of 400 participants who completed the physical activity questionnaire.

There was no significant difference between groups in the incidence of diabetes, prediabetes or those with a history of myocardial infarction, stroke or angina. Table 1 shows the characteristics of the study participants overall and according to their walking status. Compared to White Europeans, those from a South Asian ethnic background were less likely to report walking for 30 minutes on at least 5 days a week.

Multivariate statistical analysis found that those who reported walking for at least 30 minutes on at least 5 days per week had lower IL-6, hsCRP and TNFα levels compared to those who reported lower walking activity, after adjustment for other modes of moderate-to-vigorous physical activity, age, ethnicity, sex, social deprivation and smoking staus (see Figure 1). Further adjustment for waist

circumference attenuated the association of walking with TNFα, although the association with IL-6 and hsCRP remained significant. There was no association between fasting insulin and walking status. All results were unaffected by the inclusion of statin or blood pressure medication status as covariates in the statistical analysis.

Discussion

In this cross-sectional study of individuals screened for type 2 diabetes, walking on at least five days per week for at least 30 minutes per day was associated with lower circulating IL-6, TNFα and hsCRP levels after adjustment for other modes of physical activity, demographic variables and smoking status. Further adjustment for waist circumference attenuated the association of walking categories with TNFα, although the association with IL-6 and hsCRP remained significant.

Although other studies have shown that walking for around 150 minutes per week is associated with a reduced risk of developing type 2 diabetes and cardiovascular disease (Hu et al. 1999, Laaksonen et al. 2005, Manson et al. 2002), to our knowledge this is the first study to investigate the effect of walking on inflammatory markers after adjustment for other forms of physical activity. As chronic low-grade inflammation is thought to play an important role in the pathogenesis of type 2 diabetes and cardiovascular disease (Libby et al, 2002, Pickup & Crook 1998, Xu et al. 2003), this study suggests that increased walking activity may reduce the risk of developing a debilitating chronic disease through reduced systemic inflammation. This is clinically important as, for the majority of individuals, walking is the most accessible form of physical activity. The findings reported in this study are consistent with other studies which have shown that overall levels of moderate-to-vigorous intensity physical activity are inversely associated with markers of chronic low-grade inflammation (Panagiotakos et al. 2005, Pischon et al. 2003, Wannamethee et al. 2002).

This study further emphasises the clinical importance of promoting walking activity to levels that are consistent with the current physical activity recommendations in sedentary populations, particularly in those identified with an increased risk of developing a chronic disease. However, there is little evidence that traditional methods of promoting health behaviour change in at-risk populations, such as established diabetes prevention programmes (Carnethon 2007, Yates et al. 2007), have been successful at promoting clinically significant increases in physical activity. Therefore there is a continuing need to develop and test innovative strategies for promoting physical activity, in particular walking activity, in health care and community settings.

The exact mechanisms linking physical activity to reduced inflammation have not been well described. However several studies have shown that exercise training does not affect cytokine production from adipose tissue (Klimcakova et al. 2006, Polak et al. 2006), although it may alter cytokine production from mononuclear cells, another important source of elevated cytokine levels (Smith et al. 1999). It has been hypothesised that the release of myokines (cytokines released from muscle, such as IL-6) from exercising muscle may, when performed regularly, cause adaptations to the immune system resulting in lower levels of cytokines being released from mononuclear cells (You & Nicklas 2008), which in turn would reduce the production of CRP from the liver. However, as the interactive effects of exercise, muscle, body fat, and markers of chronic low-grade inflammation in the development of metabolic and vascular dysfunction are poorly defined (Telford 2007), more research is needed to quantify the overall significance of the findings from this study.

The finding that walking activity status was not associated with fasting insulin levels is in contrast to other studies (Houmard et al. 2004, Mayer-Davis et al. 1998). However, studies in individuals with type 2 diabetes have shown that ambulatory activity is generally accumulated at a low intensity and that exercise intensity, but not exercise volume, predict glycaemic control (Boulé et al. 2003, Johnson et al. 2005). Therefore, given that almost half the participants in this study had prediabetes or diabetes, it is possible that participants accumulated their walked activity at an intensity lower than that needed to improve insulin sensitivity.

The main limitations of this study are that it was not possible to determine causality and the high percentage of missing physical activity data. The small sample size precluded meaningful sub-group analysis, which given the heterogeneity of the study sample is another important limitation. Furthermore, although we investigated the confounding effects of some important determinants, such as smoking and medication status, we cannot discount the influence of other factors in our results, such as dietary status. Given the specific limitations associated with self-reported measures of physical activity we also acknowledge that the use of self-reported walking activity status in this study is another limitation. However, it is not yet feasible to objectively and accurately measure walking activity mode and bout length in large populations; pedometer and accelerometer data typically give a measure of overall ambulatory activity which, in addition to walking, can be accumulated by many other modes of activity such as running, house work and gardening.

Conclusions: This study suggests that greater walking is associated with lower inflammatory markers independent of a range of confounders and of other forms of physical activity.

References

Bassuk SS, Manson JE, 2005. Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease, J Appl Physiol 99, 1193-1204.

Berg AH, Scherer PE, 2005. Adipose tissue, inflammation, and cardiovascular disease. Circ Res 96, 939-949.

Boulé NG, Kenny GP, Haddad E, Wells GA, Sigal RJ, 2003. Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. Diabetologia 46, 1071-1081.

Carnethon MR, 2007, Can we out-run the diabetes epidemic? Diabetologia 50 1113-1115.

Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P, 2003. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 35, 1381-1395.

Crespo CJ, Keteyian SJ, Heath GW, Sempos CT, 1996. Leisure-time physical activity among US adults. Results from the Third National Health and Nutrition Examination Survey. Arch Intern Med 156,. 93-98.

Du Clos TW, 2000. Function of C-reactive protein. Ann Med 32, 274-278.

Houmard JA, Tanner CJ, Slentz CA, Duscha BD, McCartney JS, Kraus WE, 2004. Effect of the volume and intensity of exercise training on insulin sensitivity. Int J Obes 96, 101-106.

Hu, FB, Sigal RJ, Rich-Edwards JW, Colditz GA, Solomon CG, Willett WC, Speizer, FE, Manson JE, 1999. Walking compared with vigorous physical activity and risk of type 2 diabetes in women: a prospective study. J Amer Med Assoc 282,. 1433-1439.

Johnson ST, Tudor-Locke C, McCargar LJ, Bell RC, 2005, Measuring habitual walking speed of people with type 2 diabetes: are they meeting recommendations? Diabetes care 28, 1503-1504.

Klimcakova E, Polak J, Moro C, Hejnova J, Majercik M, Viguerie N, Berlan M, Langin D, Stich V, 2006. Dynamic strength training improves insulin sensitivity without altering plasma levels and gene expression of adipokines in subcutaneous adipose tissue in obese men. J Clin Endocrinol Metab 91, 5107-5112.

Laaksonen DE, Lindström J, Lakka TA, Eriksson JG, Niskanen L, Wikström K, Aunola S, Keinänen-Kiukaanniemi S, Laakso M, Valle TT, Ilanne-Parikka P, Louheranta A, Hämäläinen H, Rastas M, Salminen V, Cepaitis Z, Hakumäki M,

Kaikkonen H, Härkönen P, Sundvall J, Tuomilehto J, Uusitupa M, 2005. Physical activity in the prevention of type 2 diabetes: the Finnish diabetes prevention study. Diabetes 54, 158-165.

Libby P, Ridker PM, Maseri A, 2002. Inflammation and atherosclerosis. Circulation 105, 1135-1143.

Maldonado G, Greenland S, 1993. Simulation study of confounder-selection strategies. Am J Epidemiol 138, 923-936.

Manson JE, Greenland P, LaCroix AZ, Stefanick ML, Mouton CP, Oberman A, Perri, MG, Sheps DS, Pettinger MB, Siscovick DS, 2002. Walking compared with vigorous exercise for the prevention of cardiovascular events in women. N Engl J Med 347, 716-725.

Mayer-Davis EJ, D'Agostino R, Karter AJ, Haffner SM, Rewers MJ, Saad M, Bergman RN, 1998. Intensity and Amount of Physical Activity in Relation to Insulin Sensitivity: The Insulin Resistance Atherosclerosis Study. J Amer Med Assoc 279, 669-674.

Office for the Deputy Prime Minister, 2004. *English Index of Multiple Deprivation 2004*, Office for the Deputy Prime Minister, London.

Panagiotakos DB, Pitsavos C, Chrysohoou C, Kavouras S, Stefanadis C, 2005. The associations between leisure-time physical activity and inflammatory and coagulation markers related to cardiovascular disease: the ATTICA Study. Prev Med,. 40, 432-437.

Pickup JC, Crook MA, 1998. Is type II diabetes mellitus a disease of the innate immune system?. Diabetologia 41, 1241-1248.

Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Rimm EB, 2003, Leisure-time physical activity and reduced plasma levels of obesity-related inflammatory markers. Obes Res 11, 1055-1064.

Polak J, Klimcakova E, Moro C, Viguerie N, Berlan M, Hejnova J, Richterova B, Kraus I, Langin D, Stich V, 2006. Effect of aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosis factor alpha in obese women. Metabolism 55, 1375-1381.

Sandbaek A, Griffin SJ, Rutten G, Davies M, Stol R, Khunti K, Borch-Johnsen K, Wareham NJ, Lauritzen T, 2008. Stepwise screening for diabetes indentifies people with high but modifiable coronary heart disease risk. The ADDITION study. Diabetologia Online First, DOI 10.1007/s00125-008-1013-0.

Smith JK, Dykes R, Douglas JE, Krishnaswamy G, Berk S, 1999. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease, JAMA 281, 1722-1727.

Telford RD, 2007, Low physical activity and obesity: causes of chronic disease or simply predictors?. Med Sci Sports Exerc 39, 1233-1240.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997, Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes care 20, 1183-1197.

Wannamethee SG, Lowe GD, Whincup PH, Rumley A, Walker M, Lennon L, 2002. Physical activity and hemostatic and inflammatory variables in elderly men. Circulation 105, 1785-1790.

Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H, 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112, 1821-1830.

Yates T, Khunti K, Bull F, Gorely T, & Davies MJ, 2007. The role of physical activity in the management of impaired glucose tolerance: a systematic review. Diabetologia 50, 1116-1126.

You T, Nicklas BJ, 2008. Effects of exercise on adipokines and the metabolic syndrome. Curr Diab Rep 8,. 7-11.

Table 1: Characteristics of study participants across activity categories. Participants were screened for type 2 diabetes in Leicester, United Kingdom, between 2005 and 2006. Categorical results as number (column percentage), continuous parametric results as mean ± SD and continuous non-parametric results as median [interquartile range]

Variable	Total sample (n=400)	Low walking activity (n=191)	High walking activity (n=209)	*P* for difference between groups
Walking activity (MET-hours /week)	15.4 [42.9]	3.3 [9.6]	35.8 [52.0]	_
Moderate to vigorous physical activity (excluding walking activity) (MET-hours /week)	0 [30.6]	0 [8.0]	8.2 [44.0]	<0.01
Sex Male	209 (52)	93 (49)	116 (55)	0.17
Female	191 (48)	98 (51)	93 (45)	
Ethnicity White European	307 (77)	135 (71)	172 (82)	0.01
South Asian	93 (23)	56 (29)	37 (18)	
Index of Multiple Deprivation score	19.2 [17.8]	18.6 [18.3]	21.0 [18.2]	0.23
Age (years)	61.8 ± 9.1	62.2 ± 9.0	61.4 ± 9.1	0.64
Blood Pressure Medication Beta-blockers	52 (13)	27 (14)	25 (12)	0.89 (medication vs. no medication)
Angiotensin converting enzyme inhibitors	24 (6)	10 (5)	14 (7)	
Statin medication	59 (14.8)	32 (16.8)	27 (12.9)	0.27
Systolic Blood Pressure (mmHg)	143 ± 21	143 ± 22	142 ± 19	0.83
Diastolic blood pressure (mmHg)	86 ± 11	87 ± 12	85 ± 11	0.64
Current smokers	45 (11)	16 (8)	29 (14)	0.08
BMI (kg/m^2)	29.1 ± 4.6	29.4 ± 4.4	29.3 ± 4.6	0.91
Waist Circumference (cm)	98.7 ± 12.1	98.8 ± 12.7	98.5 ± 11.3	0.79
Fasting insulin (uIU/ml)	7.6 [6.1]	7.9 [6.5]	7.2 [5.8]	0.05
Tumor necrosis factor-α (pg/ml)	1.9 [1.34]	2.1 [1.4]	1.7 [1.3]	0.01
Interleukin-6 (pg/ml)	1.9 [1.8]	2.2 [2.4]	1.8 [1.4]	0.01
High-sensitivity C-reactive protein (mg/l)	2.3 [4.7]	2.3 [5.1]	2.3 [4.9]	0.16

Figure 1: Association of walking status with circulating interleukin-6 (IL-6), tumor necrosis factor-α (TNFα), high sensitivity C-reactive protein (CRP) and fasting insulin. <u>Participants were screened for type 2 diabetes in Leicester, United Kingdom, between 2005 and 2006.</u>

IL-6

P = 0.002   P = 0.004

TNFα

P = 0.038   P = 0.052

CRP

P = 0.009   P = 0.013

Insulin

P = 0.227   P = 0.423
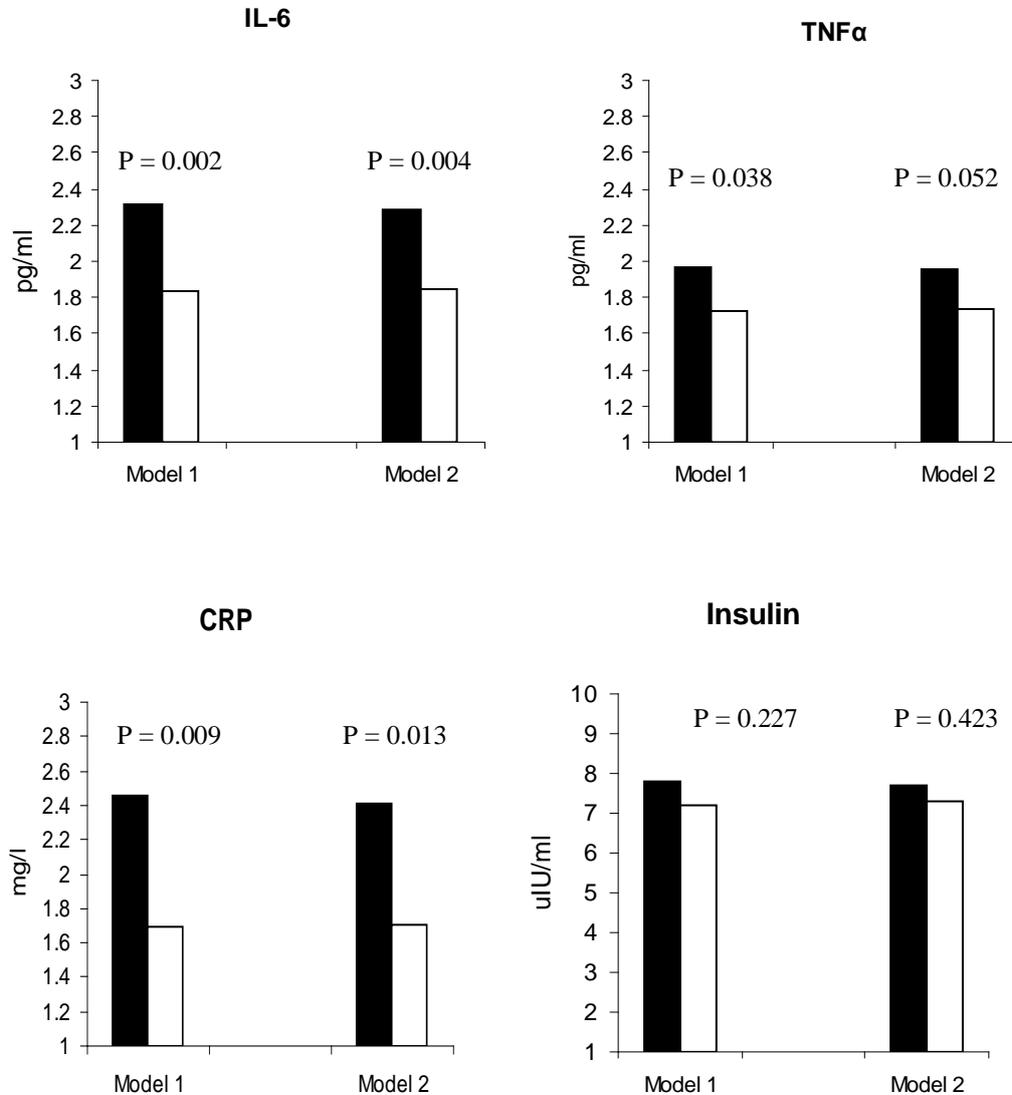
■ = low walking activity, □ = high walking activity. Graphs show geometric means. Model 1: adjusted for MET-hours/week from other modes of moderate to vigorous physical activity, age, ethnicity, sex, social deprivation and current smoking status. Model 2: adjusted for the above covariates plus waist circumference.