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Walking and inflammatory markers in individuals screened for type 2 diabetes

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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 in Leicester, United Kingdom, between 2005 and 2006.

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 30min on at least 5days/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 intensity physical activity, age, ethnicity, sex, social deprivation and smoking status. 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.

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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 and 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 and 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 and Manson 2005), markers of chronic low-grade inflammation could be a mediating link between levels of physical inactivity and chronic disease. 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 150min/week has been associated with a reduction in the relative risk of developing type 2 diabetes and cardiovascular disease (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

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.

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additional blood samples were taken for the analysis of inflammatory biomarkers. The average age of the participants was 62 ± 9years 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 intensity 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 intensity physical activity across all contexts. It also enables the calculation of metabolic equivalents (MET-hours/week). IPAQ has been shown to correlate reasonably ($p = 0.4$) 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 30min on at least 5days/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 intensity 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-immunoassay 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 0.1kg and 0.5cm 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/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 and 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

Table 1
Characteristics of study participants across activity categories

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				0.89 (medication vs. no medication)
Beta-blockers	52 (13)	27 (14)	25 (12)	
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 [1.3]	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

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].

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 30min on at least 5days a week.

Multivariate statistical analysis found that those who reported walking for at least 30min on at least 5days/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 intensity physical activity, age, ethnicity, sex, social deprivation and smoking status (see Fig. 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 5 days/week for at least 30 min/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 min/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 and 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

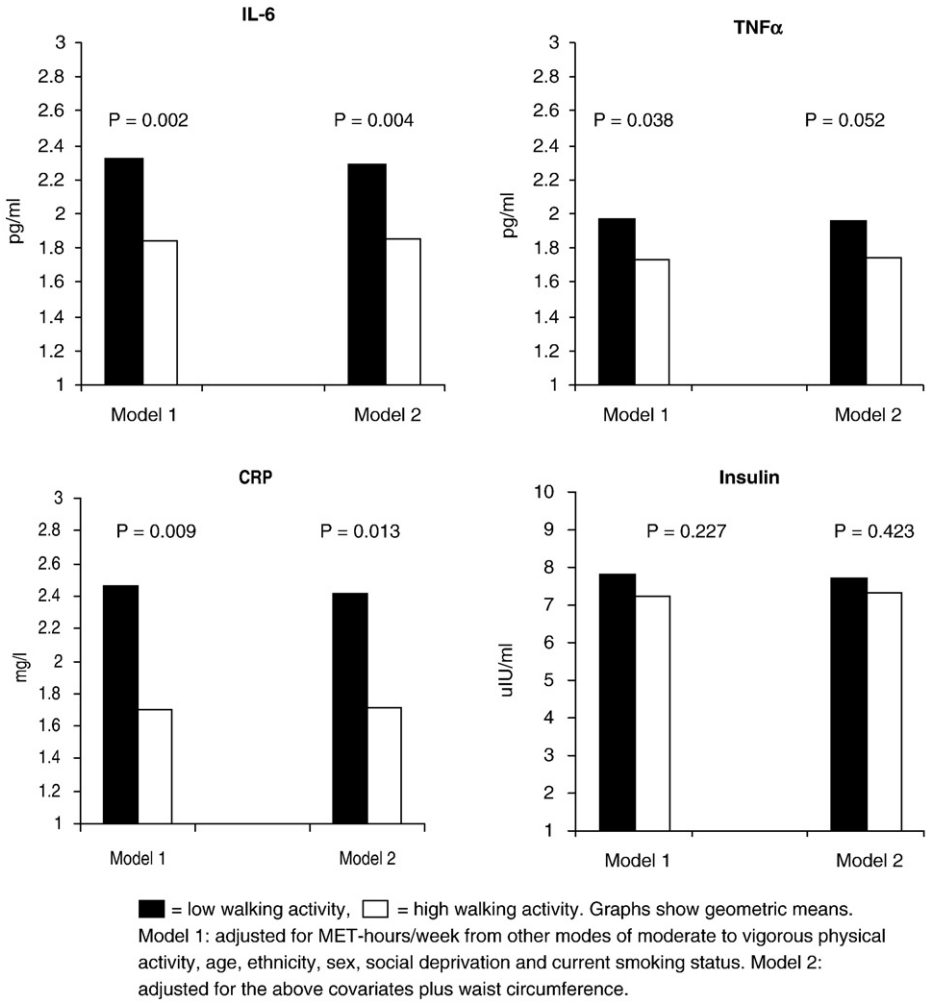


Fig. 1. Association of walking status with circulating interleukin-6 (IL-6), tumor necrosis factor-α (TNFα), high-sensitivity C-reactive protein (CRP) and fasting insulin. Participants were screened for type 2 diabetes in Leicester, United Kingdom, between 2005 and 2006.

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 cytokines (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 and 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 subgroup 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.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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