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The effect of physical activity on haematological predictors of cardiovascular risk – evidence of a dose response

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Abstract. Cardiovascular disease is a major cause of morbidity and mortality in the developed world. Large epidemiological studies have reported a strong association between increases in haematological factors and increased cardiovascular risk. Haematological risk factors predicted cardiovascular disease at least as strongly as traditional risk factors such as blood lipid concentrations. Lifestyle factors such as physical activity level could significantly reduce risk. The aim of this study was to determine the effect of physical activity level on haematological predictors of cardiovascular risk. Healthy subjects (156) were recruited. Physical activity in subjects was assessed by IPAQ physical activity questionnaire. Blood was collected and blood cell counts were determined by automated cell counter; neutrophil elastase was determined by ELISA. Increased levels of physical activity were associated with reduced red cell ($p = 0.001$), white cell ($p = 0.002$) and platelet counts ($p = 0.001$) and with reduced plasma neutrophil elastase concentration ($p = 0.001$). There was a continuous linear relationship between increase in physical activity and decrease in haematological risk factors. Hence, the authors conclude that increased levels of physical activity improve the flow properties of blood and thus reduce the risk of developing cardiovascular disease. Even small increases in activity result in some reduction in cardiovascular risk.

Keywords: Physical activity, cardiovascular disease, haematology

1. Introduction

Cardiovascular diseases, such as myocardial infarction, stroke and peripheral vascular disease, are major causes of morbidity and mortality in the western world. The blood concentration of a wide range of parameters may be used to predict the risk of developing cardiovascular disease. For example, large epidemiological studies have shown that high blood lipid concentrations are associated with increased risk of myocardial infarction [29, 36]. Low plasma cholesterol concentration, and low LDL cholesterol in particular, is associated with decreased risk of coronary heart disease [10, 11], whereas HDL-cholesterol has a protective effect and raised plasma HDL-cholesterol is associated with decreased risk [10]. High plasma cholesterol concentrations lead to accumulation of lipid in the arterial wall and the development of atherosclerotic lesions. Pharmacological intervention with HMG-CoA reductase inhibitors, statins, reduces plasma cholesterol concentration and decreases risk of major vascular events [3, 13], in addition to having endothelial protective effects, as shown in ischemia-reperfusion injury [27]. However, despite widespread monitoring and appropriate reduction of blood lipid concentrations, morbidity and mortality

associated with cardiovascular disease remains high. This suggests that some cardiovascular risk in not
accounted for by traditional risk factors.
Haematological factors are also predictors of cardiovascular risk. Red blood cell and white blood cell count are powerful haematological, long-term predictors of cardiovascular disease [37, 38, 41–43].
These haematological factors may increase cardiovascular risk by increasing resistance to blood flow which increases shear stress at the blood vessel wall and causes damage to the vascular endothelium. Furthermore,
leucocytes produce proteolytic enzymes, such as neutrophil elastase, which may also damage
the endothelium. This damage precipitates inflammation in the vessel wall, recruitment of monocytes, deposition of lipid and atherosclerosis.
These haematological factors predict cardiovascular disease more strongly than plasma lipids [44]. For example, in the Caerphilly collaborative studies individuals in the top fifth of distribution for plasma
cholesterol had a doubling of the relative odds of a myocardial infarction, whereas individuals in the top fifth distribution for white cell count had three times the risk of myocardial infarction [44]. This suggests that a potentially important component in the pathogenesis of vascular disease is currently under-examined and under-treated.
Intervention to reduce these haematological predictors of risk offers the potential to reduce subsequent cardiovascular events. There is some evidence that statins, which are specifically designed to reduce blood cholesterol concentration, may also reduce blood and plasma viscosity [28]. No pharmacological
intervention specifically targeted at a reduction in haematological risk is currently available. Lifestyle interventions offer the potential for reduction in haematological risk. Indeed, high levels of physical activity, particularly in mid-life, are associated with reduced cardiovascular events in later life [18, 39].
However, controversy exists over what level of activity is required to reduce risk [25]. Some studies suggest
that even small increases in activity level produces measurable health benefits [12] whereas others suggest
that high levels of activity are required for any beneficial effects [9, 45]. The UK Department of Health
recommends that individuals undertake at least 30 minutes of at least moderate intensity physical activity
on five or more days of the week, but a substantial proportion of the UK population fail to undertake this
level of activity [15].
The mechanisms by which increased physical activity reduces risk and the level of activity required for benefit is unclear. It has been suggested that the protective effects of exercise on the cardiovascular
system are greater than the effects which can be attributed to the improvements in traditional risk factors
[23]. Those authors estimated that only about forty to sixty percent of the exercise associated reduction
in risk is accounted for by traditional risk factors. The fact that increases in a range of haematological parameters are associated with substantially increased cardiovascular risk, and that exercise may reduce
these parameters, suggests that a haematological mechanism for risk reduction may exist. The aim of this investigation was to determine the effect of physical activity level on haematological predictors of cardiovascular risk and to determine the level of activity required to substantially reduce haematological
predictors of risk. The results of the study can be used to inform development of public health policy.

2. Methods

2.1. Subjects

Ethical approval for the study was obtained from the university research ethics committee. Healthy volunteers (n = 156), with no history of cardiovascular disease, were recruited after informed consent.

2.2. Assessment of physical activity

Subjects completed the long-version of The International Physical Activity Questionnaire (IPAQ). The IPAQ is a global questionnaire of physical activity that attempts to record the states of physical activity and inactivity in a population. The questionnaire assesses the length and intensity of activity undertaken in transport, leisure time, work and household activity and has been fully validated [20]. Briefly, the questionnaire asks the subject to answer questions based on the amount and intensity of physical activity they have undertaken in the last 7 days. Levels of activity were defined in terms of metabolic equivalent task (MET) scores. These metabolic equivalents represent the level of activity in subjects. Upon completion of individual calculations for MET min per week, the total MET score for the whole IPAQ questionnaire paper was calculated. The final total MET score was used as a measure of the level of physical activity of the participant.

2.3. Blood samples and cell counting

Subjects were asked to refrain from vigorous activity for 24 hours prior to blood collection. All blood samples were collected and processed in accordance with current rheological guidelines [4]. Blood samples were collected, by venepuncture of the antecubital vein, into sufficient tri-potassium EDTA to give a final concentration of 1.5 mg/ml. Red blood cell, platelet and five part differential leucocyte counts were performed using a Beckman Coulter AcT 5diff analyser (Beckman Coulter Ltd, High Wycombe, UK). Samples were then centrifuged at 1500 g for 10 minutes (Sanyo Harrier 18/80) and plasma was removed and stored at –80◦C (Sanyo VIP series) until analysis.

2.4. Measurement of plasma concentration of α-1-trypsin neutrophil elastase inhibitor complex

Plasma _-1-trypsin neutrophil elastase inhibior complex was measured by ELISA using sheep antihuman neutrophil elastase and peroxidise-conjugated sheep anti-human _-1-antitrypsin (The Binding Site, Birmingham, UK) and PMN leucocyte elastase calibrator (Merck Ltd, UK).

2.5. Statistical analysis

All statistical analysis was performed using Minitab 14 software (Minitab Ltd., Coventry, UK). Subjects were divided into higher activity (>4000 MET min week^{-1}) and lower activity (<4000 MET min week^{-1}) and comparison between groups determined by two sample T-test. Correlation analysis between variables was performed by calculation of Pearson correlation coefficient and corresponding P values were calculated. This analysis gives an indication of the strength of the association between the two variables but no indication of the linearity of the relationship. Regression analysis was, therefore, performed to determine the effect of increases in physical activity on haematological markers of risk. Runs tests on the residuals of the regression analysis was performed to assess the linearity of the relationships between variables.

3. Results

When subjects were split into two groups of physical activity level the higher level activity group had

lower levels of haematological cardiovascular risk markers. Data, summarised in Table 1, indicate that
increased physical activity results in a decrease in haematological markers of cardiovascular risk for all
parameters measured and reaches statistical significance for total white blood cell count, total red cell count, total platelet count and _-1-tripsin inhibitor complex concentration.
Further analysis of the data collected indicated that statistically significant correlations were measured between all parameters and the level of physical activity quantified by calculation of METs (min week–1)
from IPAQ questionnaires. This data is summarised in Table 2.

Table 1
Comparison of haematological predictors of cardiovascular risk between lower activity (<4000 MET min week–1) and higher activity (>4000 MET min week –1).

Values expressed as mean(±SD). p determined by two sample T-test

	Lower activity (n = 73)	Higher activity (n = 83)	p
WBC (×10⁹/L)	6.07 (1.67)	5.61 (1.60)	0.043
RBC (×10¹²/L)	5.24 (0.75)	4.96 (0.51)	0.009
HGB (g/dL)	14.45 (1.25)	14.22 (0.98)	0.227
PLT (×10⁹/L)	271 (65)	231 (60)	0.001
Elastase (ng/ml)	236 (186)	100 (100)	0.001

WBC, total leucocyte count; RBC, total red cell count; HGB, haemoglobin concentration; PLT, total platelet count; Elastase, _-1-trypsin neutrophil elastase inhibitor complex concentration.

Table 2
Pearson product moment correlation coefficient (r) between IPAQ score (MET min week–1) and WBC, total leucocyte count; RBC, total red cell count; HGB, haemoglobin concentration; PLT, total platelet count; Elastase, alpha-1-trypsin neutrophil elastase inhibitor complex concentration. (n = 156)

	Correlation coefficient (r)	p
WBC (×10⁹/L)	–0.243	0.002
RBC (×10¹²/L)	–0.323	<0.001
HGB (g/dL)	–0.194	0.015
PLT (×10⁹/L)	–0.303	<0.001
Elastase (ng/ml)	–0.393	<0.001

Table 3
Runs test of linearity of relationship between IPAQ score (MET min week–1) and haematological markers. A runs test was performed on the residuals of linear regression analysis and the expected number of runs compared to the actual number of runs to determine the randomness of scatter around the regression line. If $p < 0.05$ data are not randomly scattered and relationship between the variables is not linear

	Runs	
	Expected vs. observed	p
WBC (×10⁹/L)	72	77 0.396
RBC (×10¹²/L)	67	77 0.104
HGB (g/dL)	77	77 0.941
PLT (×10⁹/L)	73	79 0.335
Elastase (ng/ml)	85	75 0.085

The nature of the correlation was investigated by linear regression analysis and runs testing of the resulting residuals; data presented in Table 3. The runs test was used to test the randomness of the scatter around the straight line in linear regression. The difference between the expected and observed number of runs in the residuals from linear regression was used to determine the linearity of the data. Where the observed number of runs was significantly different from the expected number of runs the data were not scattered randomly around the regression line and the data was not linear. The relationships

between IPAQ score and white cell count, red cell count, haemoglobin, platelet count and alpha-1-trypsin
neutrophil elastase inhibitor complex concentration were linear. Increased levels of physical activity were associated with a linear decrease in these haematological markers of cardiovascular risk. Even small changes in physical activity were associated with a decrease in risk marker.

4. Discussion

The association between decreased morbidity and mortality from cardiovascular disease has been well documented [18, 39]. The measured decrease in haematological predictors of cardiovascular risk in
subjects with higher levels of physical activity suggests a mechanism for this association.

The decrease in red cell count reported here could reduce cardiovascular risk. Haematocrit has long been
shown to be a risk factor for cardiovascular disease, with various studies reporting that high haematocrits
are associated with increased risk [26, 42]. Specifically, haematocrit was found to be an independent risk
factor for mortality and morbidity in coronary heart disease in the Puerto Rico Health Program [35], and a predictor of mortality from the Northwick Park Heart Study [30]. Elevation of haematocrit is also
associated with changes in the coagulation system and increased thrombotic effects [32]. However, this
situation, which could have detrimental effects if dehydration occurs with exercise, does not seem to be
a problem in athletes who drink sufficiently [40].

A decrease in red blood cell count, and the associated decrease in haemoglobin concentration, will result in a decrease in the bulk viscosity of blood. The viscosity of red cells is about seven times higher
than the viscosity of the suspending medium so an increase in their numbers will increase blood viscosity.

Red blood cells also aggregate and the degree of aggregation increases with an increase in cell number
[5]. This aggregation further increases blood viscosity [2]. This decrease in red blood cell count, with increased activity, will increase blood flow for a given perfusion pressure as shown recently *in vivo* [24],
and will also change the shear stress and shear rate at the vessel wall. These changes in rheological properties could decrease tissue ischaemia and damage to the vascular endothelium during flow. Given
that ischaemia is associated with a range of atherosclerosis-associated inflammatory changes, and that damage to the vascular endothelium is an initial step in atherosclerotic changes [21], the decrease in red
cell count with increased activity could at least in part explain the decrease in risk with increased activity.

A decrease in red cell count may occur in elite athletes during training [16]. This reduction in red cell count is probably at least in part caused by an increase in plasma volume rather than a decrease in total
red cell volume in athletes. Athletes may then attempt to “normalise” their haematocrit by various forms
of blood doping including transfusion, auto transfusion, erythropoietin doping and altitude training [31].

This attempt to increase oxygen delivery to the tissues by doping may actually be counterproductive as an increase in blood viscosity and reduced tissue perfusion could result. Furthermore, this attempt to increase haematocrit would reduce some of the cardiovascular risk reduction effects of exercise and could potentially cause increased cardiac risk.

The decrease in white cell count reported here could reduce cardiovascular risk as it has been reported

that high white cell counts are associated with increased risk [37]. The measured decrease in total white blood cell count with increased activity could have two effects. Firstly, leucocytes deform slowly during flow through the microcirculation [1] where the lumen diameter of the vessel is often smaller than that of the cell. Leucocytes, therefore, have a large effect on flow in the microcirculation [17, 22], and have been shown to block capillaries and decrease capillary flow [8]. Furthermore, leucocytes perform a dual role of immune surveillance and response and may polymerise their cytoskeleton on activation [33, 34] and this further increases the resistance to flow in the microcirculation. The effect of leucocytes on flow in the microcirculation means that a decrease in their numbers could decrease cardiovascular risk by increasing blood flow and decreasing ischaemia. Secondly leucocytes produce a range of inflammatory mediators and proteolytic enzymes, such as neutrophil elastase, which could damage the vascular endothelium and initiate atherosclerosis. Associations between increased production of these mediators is associated with endothelial damage in a range of diseases. For example, in peripheral vascular disease a decrease in tissue oxygenation is associated with an activation of leucocytes [14] and an increase in plasma neutrophil elastase production, which is associated with damage to the vascular endothelium [7]. A similar association between increased elastase production by leucocytes and endothelial damage has been reported following angiography in peripheral vascular disease [6]. Here, it is reported that decreased levels of physical activity are associated with higher plasma concentration of neutrophil elastase and this increased elastase may result in endothelial damage which would promote atherosclerosis. The measured decrease in total white cell count with increased physical activity could, therefore, decrease cardiovascular risk by a combination of flow base and inflammatory mechanisms.

Increased activity was also associated with a decrease in platelet count in whole blood. Although resting platelets have little effect on flow in large vessels or in the microcirculation, it is plausible that changes in their numbers could affect thrombosis, and a decrease in platelet number could reduce the risk of thrombosis and would reduce cardiovascular risk, particularly in subjects with atherosclerosis where plaque rupture and the associated thrombosis results in vessel occlusion and clinical events such as myocardial infarction. However, despite the fact that the risk of thrombosis is increased in patients with essential thrombocythemia [19], there is currently no epidemiologically-based study which support this hypothesis, and in fact, the platelet count was found to be unrelated to the incidence of ischaemic heart disease [30].

Controversy exists over the level of physical activity required to reduce cardiovascular risk. Some studies suggest even small increases in activity reduce risk whereas others suggest high levels of activity are required [12, 25]. The data presented here indicates that for all the risk factors assessed here there is a linear relationship between physical activity and haematological risk factors for cardiovascular risk.

If only high levels of activity reduced haematological factors then the association would not be linear. Analysis of the residuals of the fit of a linear regression to the data indicated that the data is adequately described by a linear model as the residuals are randomly scattered around the line. The data suggests

that even small changes in the level of physical activity will have a beneficial effect in terms of risk reduction. This data has important implications for heath policy. The UK Department of Health currently
recommends that individuals undertake at least 30 minutes of at least moderate activity five times a week
[15]. Public health interventions in the UK, therefore, currently aim to attain this level of activity in the
population and interventions which increase activity to lower levels may be dismissed by policymakers.
The data presented here suggest that while activity increases to the DOH guidelines will certainly be of
benefit, even small changes in activity will result in risk reduction.
The long form IPAQ questionnaire used here assesses activity in four domains; transport, work, home and leisure. Other studies have less fully assessed physical activity levels and often focus on one domain
such as leisure time or work time [28]. The IPAQ offers a method of calculation of MET and definition
of activity on a continuous scale, whereas, other studies simply categorised individual as high, medium
or low activity levels. Analysis of a continuous scale here allows clear observation that the relationship
between activity and haematological markers of risk is a continuous linear relationship and suggests that
even a small increase in physical activity have benefits.
In summary, increased levels of physical activity are associated with a decrease in a range of haematological
predictors of cardiovascular risk. This association is linear and even small increases in physical activity results in reduction of the risk factors.

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