Factor XIIa and triacylglycerol rich lipoproteins: responses to exercise intervention

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Abstract

Objectives—(a) To determine if factor XIIa (FXIIa) would be sensitive to change from exercise intervention in a group of previously sedentary/low active middle aged men and women; (b) to investigate further the previously reported relation between FXIIa and triacylglycerol (TAG) rich lipoproteins.

Methods—Thirty seven men (mean (SD) age 57 (7) years) and 60 women (mean age 54 (7) years) completed the study. Before the intervention, these subjects were randomly allocated to a group of walkers (n = 81) or controls (n = 16). Before and after an 18 week walking intervention, fasted blood samples were collected and analysed for FXIIa, TAG, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and apolipoprotein (apo) B.

Results—Kruskal-Wallis analysis of data obtained before the intervention showed no significant differences (p>0.4) between the walking and control groups for age, height, body mass, gender, FXIIa, TAG, TC, HDL-C, or apo B, although the women did show significantly lower levels of TAG (p<0.04) and higher HDL-C (p<0.0001) than the men. General linear model analysis of data obtained after the intervention, using the baseline value as a covariate, showed significant reductions (p<0.0001) in FXIIa for the walkers compared with the controls. Pearson product-moment correlations also showed significant relations between the concentrations of FXIIa and TAG, TC, LDL-C, and apo B. **Conclusions**—The findings of this study suggest that FXIIa is sensitive to change from exercise intervention and support previous research showing an association between the concentrations of FXIIa and TAG rich lipoproteins.

(Br J Sports Med 2000;34:289–292)

Keywords: factor XIIa; Hageman factor; exercise; walking; lipoproteins; coronary heart disease

fibrinolysis.⁴ Autoactivation of FXII to FXIIa has been shown to be initiated by the negative charge on the membrane surface of triacylglycerol (TAG) rich lipoprotein particles,^{5,6} which may explain the reported relation between the concentrations of FXIIa and TAG rich lipoproteins.^{5,7} The presence of FXIIa is also believed to be associated with cell damage, and its link with a range of cardiovascular disease risk factors may therefore reflect the exposure of FXII to surfaces not normally exposed to blood, but which have become so as a result of atherothrombotic injury to the vascular endothelium. Consequently, plasma FXIIa concentrations may serve as a marker of severity of the atherosclerotic process,^{1,2} and henceforth any intervention that leads to a reduction in FXIIa levels could be viewed as beneficial in reducing the risk of cardiovascular disease.

Exercise is already advocated as a means of reducing CHD risk, as it has long been apparent that people who are physically active have a lower incidence of heart attack than those who have a sedentary lifestyle.⁸ In addition, it is also widely accepted that a poor blood lipid profile contributes towards a predisposition for CHD,⁹ and regular physical activity can improve this profile by producing increases in concentrations of high density lipoprotein cholesterol (HDL-C),^{10–12} decreases in total cholesterol (TC), TAG, very low density lipoprotein cholesterol, and apolipoprotein (apo) B.^{13–15} These changes appear to be beneficial by preventing or slowing down the process of atherosclerosis,¹⁶ although the exact mechanisms are not clear. However, the effect of exercise training on FXIIa does not appear to have been researched and to date it is not clear whether FXIIa is as sensitive to change from exercise intervention as other blood lipids, lipoproteins, and apo B. Therefore the objectives of this study were to (a) determine whether FXIIa would be sensitive to change in a group of previously sedentary/low active, but otherwise healthy, middle aged men and women participating in 18 weeks of walking intervention, and (b) further investigate the reported relation between FXIIa and TAG rich lipoproteins.

Methods

The study was approved by the ethics committees of Canterbury Christ Church University College and the Kent and Canterbury Hospital NHS Trust. It was carried out in two phases, with the intervention taking place at similar times of the year to limit any seasonal factors that could influence the study. Both phases used an intervention of 18 weeks of brisk

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Accepted for publication 22 February 2000

The thrombotic components of coronary heart disease (CHD) have received increasing attention in recent years. Of particular interest has been the role of the coagulation and fibrinolytic systems, which has suggested an association between factor XIIa (FXIIa) and a number of CHD risk factors.^{1,2} FXIIa is the active form of FXII (Hageman factor) and plays a key role in the early phase of intrinsic coagulation³ and

walking with a separate group of matched controls for each phase. The data from the two phases were amalgamated at the end of the study.

SUBJECT DETAILS AND SELECTION

Volunteers for both phases were recruited through an editorial in local newspapers and radio, which asked for sedentary but otherwise healthy non-smoking people between the ages of 40 and 69. After medical screening and an activity questionnaire, volunteers were excluded from the study if they had a resting blood pressure of either > 160 mm Hg systolic or > 95 mm Hg diastolic, a previous history of cardiovascular disease, were on any medication that could confound the measures that were being recorded, or had a lifestyle that included more than 60 minutes a week of "moderate" intensity exercise such as walking or cycling.

Initially, 130 subjects were recruited and randomly allocated to either the exercise or control group. To promote compliance, eight of the subjects were deliberately allocated to the same group as their partner or friend, but were subsequently excluded from the results in the data analysis.¹⁷ After the intervention, 37 men and 60 women were available for reassessment.

COLLECTION AND ANALYSIS OF BLOOD SAMPLES

Blood samples before and after the exercise intervention were collected after an overnight fast using plastic syringes and added to either 105 mmol/l trisodium citrate in plastic tubes (9 parts blood to 1 part citrate for plasma) or glass tubes for serum collection. They were then centrifuged at room temperature for 10 minutes at 2000 *g* and divided into aliquots. Samples for TAG, TC, and HDL-C analysis were analysed immediately while aliquots for all other tests were frozen at -80°C and assayed in convenient batches. Subjects were requested to refrain from alcohol consumption for at least 24 hours before blood sample collection. The samples taken after the intervention were taken at least 24 hours after the last walking session. When providing their initial sample before the intervention, all premenopausal females were asked to fill in a form indicating the stage of the menstrual cycle. Blood samples could then be collected at the same cycle stage after the intervention, thereby limiting the potential effects of this variable on the results.

All blood samples were analysed for plasma concentrations of FXIIa by enzyme linked immunosorbent assay (Shield Diagnostics, Dundee, UK).¹⁸⁻²⁰ TC, TAG, and HDL-C were measured using a Vitros Analyser 700XRC with kits supplied by Ortho-Clinical Diagnostics (Amersham, Bucks, UK). Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula.²¹ Apo B was assayed by rate nephelometry using the Beckman array (Beckman Instruments, High Wycombe, Bucks, UK).

EXERCISE INTERVENTION

Subjects allocated to the exercise group embarked upon an 18 week walking programme, which incorporated a gradual increase in activ-

ity. The programme started with a total of 60 minutes walking a week in the first week, increasing to 200 minutes by the 13th week of the study. The exercise intensity was prescribed to be between 75 and 80% of predicted maximum heart rate.²² Thus, according to Superko,²³ the intensity and duration of the exercise should be effective in producing health related adaptations in blood lipid profiles. To regulate and monitor exercise intensity, 40 heart rate monitors (Polar Favor; Polar Electro, Oy, Finland) were randomly distributed to the walkers, who were also instructed on how to monitor their heart rate manually. This enabled a regression equation to be calculated from the palpated and telemetrically recorded heart rates, thus providing a better indication of actual heart rate while walking when a heart rate monitor was not available.

Subjects completed their walking sessions in a way that fitted into their lifestyle and were also offered the option of supervised walking sessions on a weekly basis. They also completed a diary which detailed the duration and intensity of each walk. Subjects in the control group were asked to maintain their current lifestyle for the duration of the study. All subjects were asked not to make any changes to their regular diet.

STATISTICAL ANALYSIS

A minitab statistical package was used for the statistical analysis of the results, with an alpha value of <0.05 as statistically significant. Kruskal-Wallis non-parametric data analysis was used to assess the differences at baseline between the walkers and controls, and any differences between the sexes. To determine any significant changes observed after intervention, the general linear model was used, with the baseline value as a covariate. Pearson product-moment correlation was used to establish any relations between the measured factors.

Results

WALKING PROGRAMME

From the data collected in the diaries, it was apparent that the walkers ($n = 64$) walked for a total of 2655 (357) minutes for the duration of the 18 week walking programme. Analysis also disclosed that the walkers walked at an intensity of 73.5 (7.2)% of their age predicted heart rate maximum.

FXIIA, BLOOD LIPIDS, LIPOPROTEINS, AND APO B

It was not always possible to collect sufficient data and/or blood samples for all factors on all occasions. Therefore the number of subjects used in the analyses varied between the measured variables.

Baseline data

Kruskal-Wallis analysis of data obtained before the intervention showed no significant differences between the walking ($n = 81$) and control ($n = 16$) groups for age (54.9 (7.7) *v* 56.4 (5.0) years; $p > 0.5$), height (1.67 (0.08) *v* 1.68 (0.09) m; $p > 0.7$), body mass (73.4 (12.8) *v* 73.5 (10.8) kg; $p > 0.9$), FXIIa ($p > 0.8$), TAG,

Table 1 Pearson product-moment correlations between factor XIIa and the measured lipids, lipoproteins, apolipoprotein B, and body mass before and after the walking intervention

	Before intervention			After intervention		
	r	n	p Value	r	n	p Value
TAG (mmol/l)	0.452	80	0.0001	0.348	80	0.002
TC (mmol/l)	0.226	80	0.026	0.235	80	0.036
LDL-C (mmol/l)	0.291	77	0.01	0.207	77	0.071
HDL-C (mmol/l)	-0.098	81	0.338	-0.127	80	0.262
Apo B (g/l)	0.316	77	0.005	0.280	77	0.014
Body mass (kg)	0.213	80	0.036	0.208	80	0.064

TAG, triacylglycerol; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; Apo B, apolipoprotein B.

($p > 0.8$), TC ($p > 0.4$), LDL-C ($p > 0.8$), HDL-C ($p > 0.5$), or apo B ($p > 0.8$) (all values mean (SD)).

To establish whether there was any effect of sex on the measured factors, Kruskal-Wallis was again used. This showed no significant effect (37 men and 60 women) for FXIIa ($p > 0.7$), TC ($p > 0.5$), LDL-C ($p > 0.2$), or apo B ($p > 0.9$). However, the women did show significantly lower levels of TAG (1.15 (0.65) *v* 1.44 (0.60) mmol/l for 49 women and 32 men respectively; $p < 0.04$) and higher HDL-C (1.61 (0.39) *v* 1.26 (0.39) mmol/l for 60 women and 37 men respectively; $p < 0.0001$; values mean (SD)).

Baseline Pearson product-moment correlations showed positive relations between FXIIa and TAG ($r = 0.452$, $p < 0.0001$), TC ($r = 0.226$, $p < 0.03$), LDL-C ($r = 0.291$, $p < 0.01$), apo B ($r = 0.316$, $p < 0.005$), and body mass ($r = 0.231$, $p = 0.036$) (table 1).

After the intervention

To assess whether the measured variables had changed as a result of the exercise intervention, the data were compared using the general linear model, with the baseline data as covariate. Analysis showed a significant amount of change between the walkers and controls in FXIIa ($p < 0.0001$), with borderline

Table 2 Factor XIIa (FXIIa), TAG, TC, LDL-C, HDL-C, and apo B values for the walkers and controls before and after the walking intervention

	n	Before	After	Mean difference	p Value
FXIIa (ng/ml)					
Walkers	64	1.70 (0.6)	1.55 (0.7)	-0.15	0.0001
Controls	16	1.63 (0.7)	2.14 (1.0)	+0.51	
TAG (mmol/l)					
Female walkers	43	1.16 (0.6)	1.28 (0.8)	+0.12	0.631
Female controls	6	1.10 (0.3)	1.13 (0.5)	+0.03	
Male walkers	22	1.52 (0.7)	1.39 (1.0)	-0.13	
Male controls	10	1.26 (0.3)	1.22 (0.4)	-0.04	
TC (mmol/l)					
Walkers	65	5.88 (1.1)	5.78 (1.1)	-0.10	0.062
Controls	16	5.76 (0.9)	5.94 (0.7)	+0.18	
LDL-C (mmol/l)					
Walkers	62	3.56 (0.8)	3.49 (0.8)	-0.07	0.060
Controls	16	3.53 (0.7)	3.69 (0.7)	+0.16	
HDL-C (mmol/l)					
Female walkers	43	1.65 (0.4)	1.66 (0.3)	+0.01	0.280
Female controls	6	1.86 (0.5)	1.91 (0.6)	+0.05	
Male walkers	22	1.27 (0.4)	1.30 (0.3)	+0.03	
Male controls	10	1.36 (0.3)	1.35 (0.3)	-0.01	
Apo B (g/l)					
Walkers	62	1.83 (0.6)	1.80 (0.5)	-0.03	0.212
Controls	16	1.82 (0.4)	1.90 (0.5)	+0.08	

Values are mean (SD). p value was determined using the general linear model, with the values obtained before the intervention as covariate.

TAG, triacylglycerol; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; Apo B, apolipoprotein B.

reductions in TC ($p = 0.062$) and LDL-C ($p = 0.06$) (table 2).

Pearson product-moment correlations disclosed significant positive correlations between FXIIa and TAG ($r = 0.348$, $p < 0.002$), TC ($r = 0.235$, $p < 0.04$), and apo B ($r = 0.280$, $p < 0.02$) after the intervention (table 1).

Discussion

It was apparent from the findings of this study that subjects with greater concentrations of FXIIa also possessed greater concentrations of TAG, TC, LDL-C, and apo B (table 1); this supports the findings of previous research showing an association between FXIIa and TAG rich lipoproteins.^{3 6 24}

The statistically significant reduction in FXIIa observed in the walking group, compared with the controls, would suggest that FXIIa is sensitive to change from exercise intervention. Whether this is directly related to the training induced reductions in TC, TAG, very low density lipoprotein cholesterol, and apo B, which have been reported in previous studies,¹³⁻¹⁵ or some other mechanism remains to be elucidated and consequently warrants further investigation. Furthermore, should subsequent research support the initial findings of Miller *et al*² and FXIIa is confirmed as a valid indicator of CHD risk and atherosclerotic damage, the results of this study provide additional evidence for the antiatherosclerotic properties of moderate exercise.

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Take home message

Regular bouts of moderate intensity walking (about 150–200 minutes a week) can reduce the blood concentrations of FXIIa (the active form of FXII, Hageman factor) in previously sedentary/low active adults. Given the role of FXIIa in the coagulation and fibrinolytic systems of the body and consequently a possible link with the thrombotic components of CHD, the findings of this study suggest that this is an area that merits further research. In particular, investigations are needed into whether high concentrations of FXIIa are indeed linked to CHD and if so the relative contribution of exercise in reducing this risk factor.