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ORIGINAL ARTICLE

Oslo Diet and Exercise Study: a one-year randomized intervention trial. Effect on hemostatic variables and other coronary risk factors

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Abstract

The objective of Oslo Diet and Exercise Study has been to compare the single and joint effect of diet and exercise on coronary risk factors with special emphasis on the hemostatic system. The study is an unmasked, randomized, 2x2 factorial intervention trial with one year duration for each participant. The participants were recruited from a screening examination of 40-year-old persons in Oslo. Two-hundred and nineteen sedentary men and women, with body mass index > 24 (kg/m^2), diastolic blood pressure 86-99 mmHg, total cholesterol 5.20-7.74 mmol/L, HDL cholesterol < 1.20 mmol/L, triglycerides > 1.4 mmol/L were randomly allocated to (1) control (n=43), (2) diet (n= 55), (3) exercise (n=54) and (4) diet+exercise (n=67). Participants in the diet group were adviced to reduce weight, increase intake of fish and fish products and reduce total fat intake. Those in the exercise group were given supervised endurance exercise three times/week. Euglobulin clot lysis time, coagulation factor VII, fibrinogen and platelet volume were the main outcome measures. As compared with controls, euglobulin clot lysis time decreased 43 minutes in the combined group (p=0.056), 41 minutes in the diet group and 38 minutes in the exercise group. Coagulation factor VII decreased significantly within the combined group (-8.9%) but did not change significantly for either of the individual interventions. Fibrinogen and platelet volume did not change significantly. Triglycerides decreased 0.75 mmol/L (p<0.0001), 0.39 mmol/L (p<0.40) and 0.40 mmol/L (p<0.04) in the combined, diet, and exercise group respectively. HDL-cholesterol increased 0.12 mmol/L (p<0.0001) and blood pressure decreased -5.9 mmHg systolic and -5.2 mmHg diastolic (p<0.003) in the combined group as compared to control. We conclude that diet and exercise interventions affected lipid concentrations and blood pressure markedly, but hemostatic and fibrinolytic components moderately. The combined intervention was often superior to the single ones in producing clinically important and statistically significant changes.

Introduction

Platelet aggregation, blood coagulation and fibrinolysis play a major role in the pathogenesis of cardiovascular disease (CVD). Thaulow et al. (1) demonstrated that the number of platelets in healthy men correlated to total cardiovascular mortality. Blood platelet size is increased in moderate essential hypertension (2), and platelet size influences the outcome after myocardial infarction (3). Fibrinogen and coagulation factor VII have been found to be strong and indipendent predictors for coronary heart disease (4). Hamsten et al. (5) found that plasminogen activator inhibitor 1 (PAI-1) is an independent risk factor for recurrent myocardial infarction in young patients.

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Observational studies have shown that fibrinogen and coagulation factor VII are associated with body weight, triglycerides, low density lipoprotein (LDL-c) and the level of physical activity (6, 7). This suggests that lifestyle changes such as diet and exercise, might also modify hemostatic CVD risk factors.

Moderate to intensive physical exercise reduces platelet aggregation *in vitro*, among sedentary and moderately overweight men (8). Also, diet intervention with special emphasis on omega-3 fatty acids has been shown to reduce platelet aggregation (9-11). Diet intervention has been associated with reduced factor VII activity (12-14) and might improve fibrinolytic activity as measured by shortening of the euglobulin clot lysis time (ECLT)(15) or by a decrease in PAI-1 and an increase in tissue plasminogen activator (t-PA) antigen (14). In the trial of Folsom et al. (14) plasma fibrinogen level was not affected by dietary intervention in spite of a substantial decrease in body weight.

Furthermore, Stratton et al (16) conclude that intensive exercise enhances resting t-PA activity and reduces fibrinogen concentration and PAI-1 activity in older men (60-82 years). De Geus et al. (17) could not confirm these positive results of exercise on PAI-1 activity in a group of younger men (25-40 years).

To our knowledge, no randomized trial has been reported on the isolated and combined effects of exercise and diet upon hemostatic and fibrinolytic variables. Furthermore, most previous studies have been of short duration. The primary research objective of the Oslo Diet and Exercise Study (ODES) was to test by a randomized 2x2 factorial design intervention trial, whether dietary changes, exercise, or the combination of the two, affect the hemostatic system. Since this system has several main components, four outcome variables were selected: coagulation factor VII, fibrinogen, fibrinolytic activity measured as ECLT, and platelet volume. A series of secondary hypotheses were also tested, such as the effects on other coagulation and fibrinolytic components and activities, lipids and lipoproteins, blood pressure, and anthropometric variables.

Materials and Methods

Recruitment, the intervention program and the laboratory procedures have been described in detail elsewhere (18).

Experimental design and participants

ODES is an unmasked randomized 2x2 factorial trial of one year's duration for each participant. The participants were recruited from a continuous ongoing screening examination for all 40-year old persons in Oslo since 1981. In order to have a good intervention potential, the participants were included based on simultaneous derangement of several coronary risk factors. In both men and women the inclusion criteria were as follows; physically inactive (exercising at most once per week), body mass index (BMI) >24 (kg/m^2), diastolic blood pressure 86-99 mmHg, total serum cholesterol 5.20-7.74 mmol/l, high density lipoprotein cholesterol (HDL-c)< 1.20 mmol/l and fasting serum triglycerides >1.4 mmol/l.

Six-hundred and sixty out of approximately 26000 persons met all the inclusion criteria and were invited to a clinical examination at Ullevål hospital. At the time of inclusion into the trial, risk factor measurements might have taken place up to nine years earlier. None of these individuals had been subject to lifestyle intervention. At baseline only 30% satisfied all the inclusion criteria, mostly due to a slight decrease in diastolic blood pressure from the screening to the baseline measurement. Those with cardiovascular disease, diabetes, or other diseases, or those using drugs that could interfere with the test results, were excluded.

During 1990-91, two-hundred and nineteen participants (198 males and 21 females) aged 41-50, were randomized into one of four intervention groups: no intervention (control) (n=43), dietary changes alone (n=55), exercise alone (n=54) or the combination of the two interventions (n=67). A written declaration of willingness was signed before the randomization took place. The study was approved by the regional ethical committee.

The intervention program

Dietary counseling was given to participant and spouse together at the start, and then to the participants alone after three and nine months. The advice was individualized and adapted according to each person's dietary history and risk profile (estimated from total cholesterol, HDL-c, triglycerides, blood pressure and body weight). The intervention focused primarily on energy restriction in those who were overweight. Consumption of fish and fish products, and reduced intake of saturated fat and cholesterol, was recommended to all participants but especially to those whose elevated total cholesterol was the most important component of the risk profile. In order to assess dietary compliance, each participant responded to a 180-item food frequency questionnaire (19); also plasma free fatty acids were measured (20). Smoking habits were recorded by a questionnaire as well as estimated through serum thiocyanate concentration.

The exercise program focused on endurance type of exer-

TABLE 1
Blood components measured and methods used in ODES.

Component	Type of plasma	Method
ECLT	Fresh plasma	Clot lysis time
PAI-1	Frozen plasma	Chromogenic
t-PA	Frozen plasma	Chromogenic
Fibrinogen	Frozen plasma	Thrombin clotting time
Coagulation factor VII	Frozen plasma	Chromogenic
Platelet volumes	Fresh blood	Cell counter
Platelet count	Fresh blood	Cell counter
β-Thromboglobulin	Frozen plasma	Radioimmunoassay
Cholesterol	Frozen serum	Enzymatic
Triglycerides	Frozen serum	Enzymatic
HDL cholesterol	Frozen serum	Heparin-Mn precip, enzymatic
Total phospholipid fatty acids	Frozen plasma	Gas liquid chromatography
Serum thiocyanate	Frozen serum	Colorimetric

ECLT, euglobin clot lysis time; PAI-1, plasminogen activator inhibitor; t-PA, tissue plasminogen activator; HDL, high density lipoprotein

cise such as aerobics, circuit training and fast walking/jogging. The "exercise only" and the "exercise and diet" groups were not separated. Workouts were done three times a week in supervised groups consisting of 14-20 persons. The intensity of the training was 60-80% of the participants' individual peak heart rate as measured by treadmill test at baseline. Polar Sportstester heart rate recorder was used to measure training-intensity. Initially, there were eight weeks of progression in intensity and duration of the program, after which the intensity was maintained at the level described above. The duration of each workout was one hour. Approximately 60% of the scheduled workout were aerobics, 25% circuit training and 15% jogging/fast walking programs. The attendance at each training session was recorded. Exercise adherence was defined as presence at the scheduled workouts and it also included the workouts that some participants did otherwise according to their training log book. The protocol required an average of three workouts per week throughout the intervention year. If a participant exercised more, the adherence per cent would thus exceed 100.

Participants in the control group were told not to change their life-style during the trial but, as all the other participants, they were advised against smoking. At randomization, the control group participants were told that after the 1-year trial period they would be offered dietary advice and supervised physical training.

Laboratory procedures

Each participant was examined for risk factors before and after the one-year of intervention. Blood pressure was measured in the supine position after 10 minutes rest in a quiet room with a room temperature of 22 °C. Three recordings were made at one minute intervals with automatic equipment (Vita-Stat- oscillometric method) and the mean of the last two measurements was used for statistical analysis.

Maximal oxygen uptake ($Vo_{2\ max}$) was measured according to a modified Balke protocol (21) after 15 minutes warm up. The test was ended when the participants were close to exhaustion according to the Borg scale (22) (above 18) and/or when the increase in oxygen uptake with increasing workload was leveling off with a respiratory exchange ratio above 1.05. Expired air was analyzed using a SENSOR MEDICS MMS HORIZON ™ System.

Participants were nude when body weight and various circumference measurements were performed.

Four to seven days after completing the exercise test, the participants were referred to the Haematological Research Laboratory for collection of blood samples. In this period they were requested not to perform major physical exercise. Those who for some reason had temporarily used acetyl salicylic acids were referred to the Laboratory two weeks after the last intake, and females were requested to attend within the first two weeks after the first day of menstruation.

Blood samples were collected between 8 and 10 a.m. after an overnight fast and abstinence from smoking. The participants were supine and had rested for at least 10 minutes before sampling. Blood samples were drawn before venous occlusion (VO), and immediately before deflating the cuff following VO. Samples were drawn into Vacutainer® tubes. The VO test was carried out according to Robertson et al. (23) (20 min duration, cuff pressure midway between systolic and diastolic pressure). Following centrifugation (3000 g, +4 °C, 15 min), serum and plasma samples were stored in aliquots at -70 °C.

ECLT, mean platelet volume and count were measured in fresh blood, otherwise the components were measured batchwise from frozen samples at the end of the trial (Table 1). PAI-1 and t-PA activity were determined with chromogenic assays (COATEST PAI and COA-SET t-PA, Kabi Diagnostica, Sweden). ECLT measuring procedure

TABLE 2
Baseline values (mean SE) of the primary and secondary variables in each intervention group.

Variables n	Control 43	Diet 52	Exercise 49	Diet+Exercise 65
Primary hemostatic				
ECLT (min)	118 (17)	134 (17)	107 (14)	116 (14)
Fibrinogen (g/l)	2.9 (0.1)	2.9 (0.1)	3.0 (0.1)	2.8 (0.1)
Factor VII (%)	133.5 (5.4)	133.5 (6.8)	134.0 (5.6)	126.8 (4.6)
Platelet volume (x 10^{-15}l)	6.9 (0.1)	6.9 (0.1)	6.8 (0.1)	6.9 (0.1)
Other hemostatic				
PAI-1 (u/ml)	18.6 (13.6)	22.6 (17.3)	18.6 (9.9)	21.3 (16.3)
t-PA (iu/ml)	14.9 (1.4)	16.4 (2.0)	18.0 (2.1)	17.2 (11.5)
BTG (ng/ml)	39.3 (5.1)	48.0 (7.1)	38.5 (5.5)	42.0 (3.9)
Platelet count (x 10^9/l)	225.4 (7.7)	227.4 (6.0)	221.9 (7.6)	210.7 (6.2)
Lipids (mmol/l)				
Triglycerides	2.18 (0.13)	2.38 (0.18)	2.23 (0.13)	2.31 (0.16)
Total cholesterol	6.58 (0.13)	6.37 (0.13)	6.24 (0.10)*	6.20 (0.10)*
HDL cholesterol	1.04 (0.03)	1.01 (0.03)	1.01 (0.02)	1.01 (0.02)
LDL cholesterol	4.57 (0.13)	4.27 (0.12)	4.22 (0.10)*	4.22 (0.10)*
Blood pressure (mmHg)				
Systolic	128.7 (1.5)	132.8 (2.1)	132.1 (1.6)	131.8 (1.5)
Diastolic	87.0 (1.1)	87.5 (1.2)	89.2 (1.1)	88.0 (1.0)
Anthropometry				
Bodyweight (kg)	89.3 (2.1)	93.4 (1.8)	89.7 (1.7)	90.2 (1.6)
Waist circumference (cm)	102.3 (1.4)	105.0 (1.3)	102.6 (1.4)	102.7 (1.2)
Waist/hip	0.988 (0.007)	0.997 (0.006)	0.988 (0.007)	0.990 (0.006)
BMI (kg/m^2)	28.30 (0.48)	29.54 (0.54)	28.56 (0.46)	28.57 (0.43)
Aerobic capacity				
$\dot{V}O_{2max}$(ml•kg^{-1}•min^{-1})	34.3 (0.8)	34.4 (0.7)	36.9 (0.8)*	35.6 (0.7)

* $p<0.05$ as compared with control by two sample t test.

has been described by Nordby et al. (24). Cut-off point was 300 min. Fibrinogen was quantified by the method of Clauss (25) by means of semi-automated coagulometer (Thrombotrack 1, NYCOMED AS, Norway). Factor VII activity was measured with a chromogenic assay (COASET FVII, Kabi Diagnostica, Sweden). Platelet count and volume distribution were determined in whole blood by the 147 Compact Thrombocyte Analyser (Analys Instrument AB, Stockholm, Sweden). Plasma β-thromboglobulin was measured with commercial radioimmunoassay kit (Kodak Clinical Diagnostics Ltd, Amersham, UK). Hemostatic and fibrinolytic variables, except ECLT, were analysed in duplicate. The analytical day-to-day coefficient of variation was 7% for factor VII, 5% for fibrinogen, 12% for PAI-1, 16% for t-PA, 5% for platelet count, 3% for platelet volume and 6% for β-thromboglobulin.

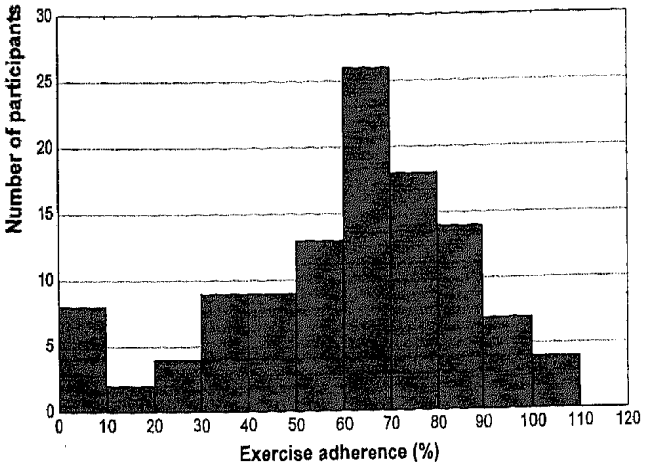
The calculation of expected CVD risk reduction as a result of 1-year changes in important risk factors is based on data from Nabulsi et al. (26) and Collins et al. (27). The variables used in the calculations were total cholesterol, factor VII, fibrinogen and diastolic blood pressure.

The calculations were done by using the regression coefficients from logit models, and then multiplying the estimated effect per variable based on the observed changes in ODES.

Assumptions lying behind usage of the predictive function are independent and causally fully reversible risk as a result of risk factor modification, maintained for many years.

Statistical analyses

FIGURE 1
Number of participants by percent exercise adherence (technically some could exceed 100%).



The response to intervention was measured as the difference between start and final value for all continuous variables (year 1- year 0). A 4-dimensional variable consisting of 1-year responses for the primary variables ECLT, fibrinogen, factor VII and platelet volume, was calculated for each participant and subjected to multivariate analyses of variance (MANOVA). By the MANOVA model we first tested an interaction effect between diet (dichotomous) and exercise (dichotomous) interventions by adding a multiplicative interaction term of the two. In case of no interaction, main effects of diet and exercise intervention were tested without the interaction term in the model. The trial was said to have a statistically significant outcome if at least one of the two intervention F-tests gave *p*-values less than 5% or if an interaction term was found with *p*-value less than 5%. A similar unidimentional analysis of variance procedure was done for each variable. A priori, four contrasts were specified to be of interest: diet+exercise vs. diet; diet+exercise vs. exerxise; diet vs. control group; and exercise vs. control group. These contrasts were tested by simple *t* tests for each of the four variables using Bonferroni's adjustment of *p*-values with 5% overall significance level. Testing of, and confidence intervals for between intervention group contrasts for other variables were done by the Student *t*-method without adjustments for multiple comparisons. The analyses were done using the statistical package STATISTICA for Windows (28) and JMP statistics for the Apple Macintosh (29).

Results

Results before and after the one-year intervention were obtained in 209 of the 219 participants and were analyzed according to the intention-to-treat principle. Five participants were excluded during the one year of intervention (1 due to malignant immunodeficient disease, 1 due to cancer, 1 due to cardiac event, 1 due to prolapse in the lumbar region not related to exercise and 1 due to a leg injury related to the exercise program), and five were unwilling to return for the 1-year test. Five of the ten were from the exercise group, three from the diet group and two from the combined group. The ten dropouts did not differ significantly from the remaining participants with respect to the mean values of the inclusion variables.

Baseline values

Table 2 gives the baseline mean values for the primary and secondary variables in each of the four intervention groups. Compared to the control group, total cholesterol and LDL-c were significantly lower in both the exercise group and the combined group, and $\dot{V}o_{2max}$ was significantly higher in the exercise group. Otherwise, no significant difference was found.

Adherence to the intervention programs

Average exercise adherence was 57% in the exercise and 63% in the combined group (Fig. 1). This corresponds to an average of 1.8 hours per week throughout the year. The diet and the control group did not change their physical activity habits. The average exercise intensity estimated from the heart rate-recordings was approximately 75% of peak heart rate (data not shown). Peak oxygen consumption increased significantly in the exercise groups compared with the control group (Table 3).

The energy intake based on the food frequency questionnaire was substantially decreased in the diet group and in the diet+exercise group compared to the control group and the exercise group (Table 3). Also the composition of the diet changed in those who received dietary counseling, by decreased total fat intake combined with increased polyunsatured/saturated fatty acids ratio. Plasma level of docosahexaenoic free fatty acids increased significantly in both the diet group and the combined group compared to the control and the exercise groups. No change in any of the measured dietary variables took place in the control or the exercise group. There were no statistically significant differences in smoking habits in either of the groups assessed by ques-

Variables	Baseline all groups	Control	Diet	Exercise	Diet+ exercise
n	209	43	52	49	65
Aerobic capacity					
$\dot{V}O_{2max}$(ml•kg^{-1}•min^{-1}) 4.7 (0.5)*#	35.4 (0.4)	-2.0 (0.5)		-0.3 (0.5)*	2.0 (0.7)*#
Length on treadmill (m)	783.2 (12.9)	8.2 (14.0)	56.6 (16.9)*	156.1 (23.4)*#	236.0(15.1)*#
Energy intake					
Total energy intake (kJ/day)	10500 (208)	-589 (450)	-2268 (356)*$	-497 (400)	-2003 (357)*$
Energy from fat (%)	33.7 (0.4)	-0.6 (0.7)	-5.5 (0.8)*$	-1.5 (0.7)	-5.7 (0.7)*$
Fatty acids					
Saturated (g/day)	35.8 (0.9)	-1.9 (2.0)	-14.0 (1.8)*$	-3.0 (1.8)	-13.7 (1.4)*$
p/s ratio	0.48 (0.01)	-0.001 (0.02)	0.07 (0.02)*$	-0.01 (0.02)	0.11 (0.02)*$
Plasma EPA (%)	2.23 (0.10)	0.18 (0.18)	0.60 (0.20)	0.15 (0.20)	0.85 (0.29)
Plasma DHA (%)	6.91 (0.12)	0.37 (0.20)	1.26 (0.23)*	0.72 (0.25)	1.24 (0.26)*
Smoking marker					
Thiocyanate(μmol/l)	77.7 (3.7)	-0.9 (4.1)	-5.6 (3.9)	-3.9 (5.0)	-10.7 (3.7)

* $p<0.05$ compared to control; # $p<0.05$ compared to diet; $ $p<0.05$ compared to exercise group; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

TABLE 3
Baseline and changes in exercise and dietary specific variables and serum thiocyanate after one year [year 1 - year 0, mean (SE)].

tionnaire or as estimated from serum thiocyanate. However, the thiocyanate results show that the combined group tended to reduce their smoking habits more than the other groups.

One-year intervention effects

Primary hemostatic variables

Table 4 shows that the decrease in ECLT was 22 minutes, 20 minutes and 17 minutes in the combined group, diet group and the exercise group, respectively–all statistically non significant. The net difference change between the combined group and the control group was nearly significant, p= 0.056. Since there was no interaction effect between diet and exercise with respect to change in ECLT, the factorial design made it possible to test the effect of diet and exercise separately, using all the participants in this comparison. However, neither of the two interventions showed any statistically significant effect on the change in ECLT even with this method of analysis (data not shown). ECLT had a truncated distribution at 300 minutes in this study. When analyses were repeated on the subset of subjects, where all recorded times were less than 300 minutes (n=169), the combined group had a significantly greater mean ECLT change compared to controls ($p<0.05$). A separate exploratory statistical regression modelling was done where the logarithm of the last ECLT value was defined as the dependent response variable and treatment groups (diet/ no diet) and (exercise/no exercise) as exposure variables. In addition, the

Variables	Diet	Exercise	Diet+Exercise
ECLT (min)	-40 (-93, 13)	-38 (-88, 12)	-43 (-88, 2)
Fibrinogen (g/l)	0.15 (-0.05, 0.35)	0.04 (-0.16, 0.24)	0.16 (-0.08, 0.40)
Factor VII (%)	-1.1 (-14.0, 11.8)	-1.0 (-11.8, 9.8)	-8.8 (-18.4, 0.8)
Platelet volume (x 10^{-15}1)	0.03 (-0.17, 0.23)	0.10 (-0.15, 0.35)	0.04 (-0.16, 0.24)

TABLE 4
Mean one year net-changes (±95% confidence limits) in the four primary hemostatic variables (n=209).

TABLE 5
One year differences in secondary variables [year 1 - year 0, mean (SE)].

Variables n	Control 43	Diet 52	Exercise 49	Diet+Exercise 65
Hemostatic				
PAI-1 (u/ml)	2.4 (21.0)	0.7 (27.5)	5.2 (27.3)	2.7 (26.0)
t-PA (iu/ml)	6.4 (18.8)	6.2 (18.4)	6.7 (26.8)	3.8 (21.9)
BTG (ng/ml)	-5.2 (5.3)	-7.3 (7.8)	10.5 (7.3)	-3.8 (3.9)
Platelet count (x 10^9/1)	-1.3 (5.2)	0.1 (5.7)	-3.8 (7.1)	-3.9 (4.9)
Lipids (mmol/l)				
Triglycerides	0.17 (0.14)	-0.23 (0.14)*	-0.24 (0.10)*	-0.58 (0.12)*
Total cholesterol	-0.16 (0.09)	-0.23 (0.09)	-0.20 (0.08)	-0.48 (0.11)*
HDL cholesterol	0.015 (0.015)	0.050 (0.016)	0.040 (0.02)	0.132 (0.018)*
LDL cholesterol	-0.22 (0.09)	-0.18 (0.10)	-0.13 (0.07)	-0.39 (0.10)
Blood pressure (mmHg)				
Systolic	-0.5 (1.7)	-6.4 (1.4)*	-2.2 (1.1)	-5.9 (1.1)*
Diastolic	-0.7 (1.3)	-3.4 (1.0)	-2.7 (1.0)	-5.2 (0.9)*
Anthropometry				
Body weight (kg)	1.1 (0.4)	-4.0 (0.7)*	-0.9 (0.6)*	-5.6 (0.6)*
Waist-circumference (cm)	0.9 (0.4)	-3.7 (0.6)*	-1.9 (0.6)*	-5.7 (0.6)*
Waist/hip	-0.003 (0.003)	-0.021 (0.004)*	-0.023 (0.005)*	-0.036 (0.004)*

*$p<0.05$ as compared to control.

baseline log (ECLT) values was adjusted for. A statistically significant association between final ECLT and exercise was thus found (p= 0.016). This could not be demonstrated with respect to diet (NS).

Factor VII activity decreased slightly in the intervention groups (Table 4). Compared to the control group, none were significant. Again, utilizing the factorial design on the two interventions did not show any significance. However, within the combined group, a significant decrease took place ($p<0.05$).

Unexpectedly, fibrinogen increased within the diet groups, but not significantly as compared to controls. The increase was 7.9% and 6.6% within the combined and diet group respectively, and there was almost no change within the exercise and the control group (Table 4). When applying the factorial design, the increase in fibrinogen in the diet groups versus no diet groups did not reach statistical significance.

All four groups decreased significantly in platelet volume compared to baseline. The decrease in platelet volume in the control group may suggest a drift in the analyses. There were no statistically significant differences between groups, analysed either by single groups versus control or by two-way factorial ANOVA.

Test of primary hypothesis

The one year changes for all four primary research variables (ECLT, fibrinogen, factor VII, and platelet volume) were put into a four-dimensional variable per participant and tested by the MANOVA procedure. A test of interaction between diet and exercise on the vector was not found to be significant ($F_{4,181}$=0.37; NS). Removing the interaction term from the multivariate model, the MANOVA test for main effect of diet gave $F_{4,182}$=1.64; p=0.165. The main effect of exercise gave $F_{4,182}$=1.01; p=0.403, i.e. no statistically significant outcome of the primary variables. If the 21 females were excluded, the test for males gave F=2.24; p=0.078 for diet and F=1.11; p=0.35 for exercise. The four contrasts that were set prior to the study, concerning the primary variables, did not show any statistical difference between groups by any statistical analysis (Table 4).

Secondary hemostatic variables

The results obtained with PAI-1, t-PA, β-thromboglobulin and platelet count are shown in Table 5. None of the variables changed significantly when compared within groups, with controls or when using the factorial design. Unexpectedly, there was a slight increase in β-thromboglobulin (p=0.09) in the exercise alone group.

Variables	Exercise vs no exercise		Diet vs no diet	
	all	males only	all	males only
Triglycerides	F=8.77, p=0.0034	F=10.32, p=0.0016	F=8.31, p=0.004	F=9.12, p=0.0029
Total cholesterol	F=2.52, p=0.11	F=4.12, p=0.044	F=3.58, p=0.059	F=8.27, p=0.0045
HDL-c	F=9.74, p=0.002	F=7.99, p=0.005	F=13.19, p=0.0004	F=15.34, p=0.0001
LDL-c	F=0.63, p=0.42	F=1.03, p=0.31	F=1.79, p=0.18	F=5.58, p=0.019
Systolic BP	F=0.12, p=0.73	F=0.46, p=0.50	F=13.35, p=0.0003	F=14.65, p=0.0002
Diastolic BP	F=3.50, p=0.063	F=5.32, p=0.022	F=6.26, p=0.013	F=6.68, p=0.011

TABLE 6
Statistical F-tests with *p*-values for 2x2 factorial design without interaction. Comparison diet vs. no diet and exercise vs. no exercise for some of the secondary variables with and without inclusion of females.

Lipids and lipoproteins

Single and combined intervention had significant effects on triglycerides in all groups compared to the control group (Table 5). The combined group had a reduction of 32% as compared to the control group, with about equal effect (17%) of diet and exercise intervention. Only the combined intervention had significant effect on total cholesterol compared to the control group. HDL-c increased in all intervention groups but significantly only in the combined group when comparing with the control. There were no significant effects on LDL-c in either of the groups compared to the control. Since no interaction between interventions were found on any variable, the factorial design was used to compare the effect of diet and exercise separately for all participants and for males separately (Table 6). In the comparison diet vs. no diet, and exercise vs. no exercise, triglycerides and HDL-c were statistically highly significant. Excluding the females from these analyses gave, in general, more significant findings in both comparisons. Also total cholesterol change was significant for both interventions in these comparisons when females were excluded. In addition, LDL-c change was significantly different in the comparison diet vs. no diet.

Blood pressure

The combined intervention had significant effects on diastolic and systolic blood pressure as compared to control (Table 5). In the diet alone group the systolic blood pressure decreased by 6.4 mmHg ($p<0.05$) and diastolic blood pressure by 3.4 mmHg (not significant) as compared to the control group. Exercise alone did not influence blood pressure significantly. Using the factorial design, diet intervention reduced both systolic (-6.1 mmHg, $p<0.001$) and diastolic blood pressure (-4.4 mmHg, $p<0.01$). Also exercise intervention reduced diastolic blood pressure (-4.5 mmHg, $p=0.02$) in males (Table 6).

Anthropometry

Compared with the control group, all intervention groups had a significant change in body weight, waist circumference and the waist/hip ratio (Table 5). Again, an additive model of the two interventions was indicated (since interaction terms in models were not significant).

Calculated risk reduction of CVD

Estimation of the ODES' 1-year changes potential effect on the risk of CVD if maintained over years, gave the following net effect compared to the control group: -4.6% in the diet group, -7.1% in the exercise group and -29.5% in the combined group (Fig. 2).

Discussion

The present study is the first one to examine the extent to which the hemostatic system is influenced by long-term exercise and diet in middle-aged subjects who still were healthy, but had a high level of cardiovascular risk factors.

Compared to the control group, participants in the exercise groups increased their peak oxygen uptake significantly during the one year of intervention. The increase was of a magnitude to be expected with this type of exercise program (30). Also, the 5-7 kg body weight reduction found in the diet groups compared to the control group is consistent with findings from other trials with randomized design (31, 32). Changes in the fatty acids pattern indicated a successful adherence with dietary advice.

Despite a good adherence to the intervention program and comparatively large study groups, this trial could not prove any statistically significant effect on the four primary hemostatic variables, ECLT, fibrinogen, factor VII and mean platelet volume analyzed by multi or single variate analyses of variance. Nor could it demonstrate effects on the other hemostatic and fibrinolytic variables. However, the direction of change in the fibrinolytic variables and fac-

tor VII was favorable according to current knowledge (4, 5). For instance, ECLT decreased significantly in the combined group compared to the control group when the truncated values were excluded in the analyses. This was also found when the last value of ECLT was regressed against the intervention group and adjusted for baseline ECLT. The effect was mainly due to the exercise component since special regression analyses showed that the exercise was significantly associated to the last value of ECLT. Furthermore, factor VII decreased significantly within the combined group. An increase in fibrinogen was indicated in the dietary groups. Subgroup analyses, however, including only fibrinogen values above the median at baseline (fibrinogen >2.80 g/l, n=104) showed that no changes occurred in that group as compared to the control. Hence, there seems to be no negative effect of this type of intervention in subjects with high fibrinogen levels.

The overall direction of change and the subgroup analyses are in agreement with those of Folsom et al. (14) who recently showed a decrease in factor VII, PAI-1 and t-PA antigen, but no change in fibrinogen after six months of dietary intervention. Also the results of Stratton et al. (16), relating to the younger participants (24-30 years) in that study, are comparable to those of ODES. Stratton et al. found that exercise enhanced fibrinolytic activity, but in contrast to our results, they found a decrease in fibrinogen level. However, this exercise study was non-randomized and lacked a control group. Furthermore, the significant changes found in that study were observed in the older group of participants (60-80 years) in which the intervention potential was high.

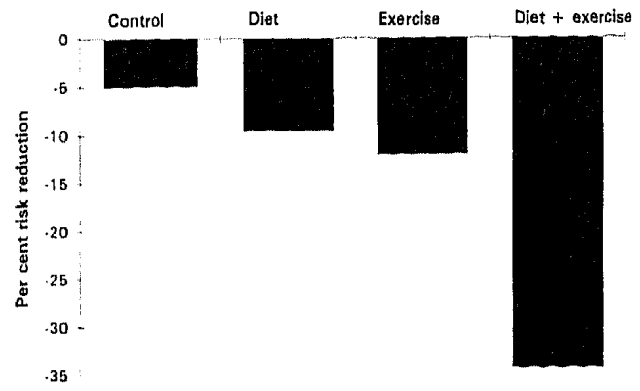
There are different possible explanations for our results concerning fibrinolytic and hemostatic variables. Fibrinolytic activity shows a substantial biological variation both within the day and between days (33). Seasonal and within-week variation were eliminated due to one-year trial and corresponding day-matched blood sampling. Different non-seasonal factors such as smoking, physical activity (34, 35) and dietary fat (36) may also influence the hemostatic and fibrinolytic system acutely. However, despite good standardization, participants still had a considerable biological variation as assessed from the coefficient of variation (CV) of 1-year change in the control group data (%CV was 15 for fibrinogen, 103 for ECLT, 7 for mean platelet volume and 20 for factor VII). Rather high biological variation in fibrinolytic and hemostatic factors has, however, also been observed by other investigators during shorter time observations (37, 38). Analysis of repeated samples, drawn both at baseline and after the intervention could have reduced this variability, however at the expense of increasing costs.

The inclusion criteria did not include measurements of hemostatic variables. The intervention potential of these variables, however, is important to assess, since it is usually more difficult to achieve changes following intervention on variables within reference range. The baseline ECLT median was 70 min, whereas the upper reference limit is 60 min. Mean t-PA value at baseline was within normal range but PAI-1 was in the upper part of the reference range, reflecting the elevated ECLT. However, both t-PA and PAI-1 have a rather large assay variability associated with them, which may affect comparisons of the reference ranges. Platelet variables, fibrinogen and factor VII were all within reference range at baseline. Thus, there was a clear intervention potential on ECLT, but less so with the coagulation factors.

A large number of laboratory tests are available for the study of homeostasis. Thus, the selection of tests can always be debated. We selected the four outcome variables (mean platelet volume, coagulation factor VII, fibrinogen and ECLT) because each assay had some documentation suggesting that they were relevant for the present study, and they were established in our laboratories. Also, including a larger number of tests would increase the chances of type II error. However, other tests within hemostasis might have been more appropriate.

FIGURE 2

Estimated risk reduction for CVD in the four intervention groups due to observed ODES changes in total cholesterol, coagulation factor VII, fibrinogen and diastolic blood pressure.



Triglycerides may be predictors for ischemic heart disease (39). The reduction in triglycerides was 32%, 17% and 18% in the combined, diet and exercise group respectively. These reductions were more pronounced than in most other trials (32, 40, 41). This might be due to higher baseline levels and thereby larger intervention potential in our participants.

HDL-c is a powerful independent predictor of CVD (42). Dietary changes such as reduction of total energy intake combined with reduced intake of saturated fat and dietary cholesterol might reduce HDL-c concentration (43). However, others have shown the opposite effect of diet intervention (44). There are also disagreements in reports concerning the effect of exercise upon HDL-c (45). However, in this trial HDL-c increased by 12% compared to the control group in the combined group which is similar to the effect in the combined group in the study of Wood et al. (32). Utilizing the factorial design with test procedures, both exercise vs. no exercise and diet vs. no diet gave significant results concerning HDL-c, indicating that both interventions were effective and that there was an additive effect of exercise and diet intervention. The lack of statistically significant effect on LDL-c compared to the control group might be due to a marked drop within the latter. However, this result is similar to the result of Wood et al. (32).

The participants had mean baseline systolic and diastolic blood pressure of 131/88 which is in the upper reference range. In spite of these rather near normal levels, both systolic and diastolic blood pressure fell significantly as a result of both interventions, except systolic BP in the exercise group. The magnitude of the changes is consistent with those found by other investigators (32, 46, 47).

Central obesity as measured by waist/hip ratio is reported to be more strongly correlated with CVD than BMI, body fat and other body composition measurements (48, 49). In this trial, all intervention groups compared with control had a significant effect on waist/hip ratio and waist circumference. For instance, the net difference in waist circumference between the control group and the combined group was 6.7 cm. The combined effect was superior to both exercise alone and diet alone, and again it seemed that the effects were additive on the arithmetic scale.

The finding that diet and exercise intervention has an additive effect on lipoprotein, blood pressure and anthropometric variables, and therefore is superior to diet and exercise intervention alone, is consistent with Wood et al. (32) and the DAMET study (40), but inconsistent with the findings of Hellenius et al. (41). The latter study concluded that diet and exercise were equally effective in reducing coronary risk factors of CVD, and the combined effect did not further decrease the risk. Moreover, combined intervention will have the potential of influencing a different sets of risk factors and thus is more effective than each isolated intervention.

When estimating the potential impact on the risk of CVD by risk factor reduction, the combined effect, if causal (i.e. a reduction will result in a correspondingly improved prognosis), gives an almost 30% reduction in CVD risk, whereas exercise alone and diet alone gives 7% and 5% reduction respectively compared to the control group. If true, this might have important implications for preventive cardiology: exercise and diet intervention combined may be more effective in reducing the risk of CVD than diet changes and exercise changes alone.

Conclusions

The present study demonstrates that sedentary subjects with a high cardiovascular risk can be influenced to improve their diet and exercise regularly, with major benefits to their cardiovascular risk profile. These interventions, however, did not alter hemostatic or fibrinolytic variables significantly. The combined group was superior to diet alone and exercise alone for most of the reported variables. Future trials to study intervention effects on hemostatic and fibrinolytic function must take biological variation into consideration in addition to methodological difficulties and variations. The level of abnormality in such variables should also be carefully considered when including trial participants.

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