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Full Length Article

Endurance exercise *per se* reduces the cardiovascular risk marker t-PA antigen in healthy, younger, overweight men

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ABSTRACT

Introduction: The cardiovascular risk marker tissue plasminogen activator antigen (t-PA:Ag) can be reduced by long-term exercise interventions, but it is unknown, whether this is due to the weight loss induced by physical activity or due to the physical activity *per se*.
Materials and methods: This was tested in 60 healthy, younger (20–40 years), overweight (BMI: 25–30 kg/m^2) men randomly assigned to 12 weeks of intervention in one of four groups: training (T); energy-reduced diet (D); training and increased diet (T-iD); sedentary lifestyle and unchanged diet (controls, C). Fasting blood samples were obtained before and after 12 weeks of intervention and analyzed for plasma t-PA:Ag.
Results: Body weight was reduced in groups T and D. We observed a decrease in t-PA:Ag from baseline to 12 weeks in all three exercise and diet intervention groups, and no change in the control group. A between-group difference in t-PA:Ag was observed at 12 weeks ($p = 0.001$), and this was due to lower values in T ($p = 0.0005$), D ($p = 0.005$) and T-iD ($p = 0.009$) compared with the control group. Total body fat mass was reduced in all three exercise groups, and we observed a positive correlation between changes in t-PA:Ag and changes in intra-abdominal and subcutaneous adipose tissue volume.
Conclusions: Our results demonstrate that t-PA:Ag was reduced in all three intervention groups. This suggests that 12 weeks of endurance training *per se*, irrespective of concomitant weight loss, beneficially affects cardiovascular risk in healthy, younger, overweight men.

1. Introduction

Obesity is increasing worldwide and is associated with increased risk of cardiovascular disease (CVD) [1]. It is therefore important to identify interventions that can reduce this risk in overweight individuals. Cardiovascular disease is often caused by atherothrombosis as a consequence of fibrin clot formation after rupture of atherosclerotic plaques indicating that atherosclerosis and haemostasis play important roles in CVD. The fibrinolytic protein tissue plasminogen activator antigen (t-PA:Ag) is identified as one of the most important CVD risk markers among the haemostatic factors [2] with high protein concentrations being associated with increased CVD risk [3–6]. The protein is primarily synthesized in the vascular endothelium and is a marker of endothelial function [7]. It was previously demonstrated that t-PA:Ag can be reduced by long-term exercise interventions [8–10]. In these studies, it was not possible to reveal, whether the beneficial effect on t-PA:Ag was due to the weight loss induced by physical training, or whether the beneficial effect was due to the physical training by itself (*per se*).

It is often very difficult for overweight people to lose weight or to maintain a weight loss for a longer period of time [11]. In this respect it is good news that a recent meta-analysis concludes that being fit and physically active is superior to keeping a normal body weight in order to reduce mortality risk [12]. Therefore, it is interesting to know, if physical training *per se* can reduce the cardiovascular risk, or whether a risk reduction can only be obtained by physical training accompanied by weight loss.

It was recently reported that 12 weeks of endurance training *per se*, irrespective of concomitant weight loss, beneficially affected body composition and cardiovascular fitness, and increased peripheral insulin sensitivity in healthy, younger, overweight men [13]. The aim of the present study was, in the same randomized controlled trial, to investigate, whether 12 weeks of energy-reduced diet or endurance exercise, with or without a concomitant weight loss, affect the CVD risk marker

Abbreviations: AAT, total abdominal adipose tissue volume; ANCOVA, analysis of covariance; ANOVA, analysis of variance; BMI, body mass index; CVD, cardiovascular disease; FFM, fat free mass; IAAT, intra-abdominal adipose tissue volume; PAI-1, plasminogen activator inhibitor type 1; SAAT, subcutaneous abdominal adipose tissue volume; t-PA:Ag, tissue plasminogen activator antigen.

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t-PA:Ag in order to evaluate the influence of endurance exercise *per se* on cardiovascular health.

2. Materials and methods

2.1. Study population and design

The study was conducted at the Department of Biomedical Sciences, University of Copenhagen. The overall design and the primary findings have been described in detail elsewhere [13–15]. All subjects gave oral and written informed consent, and the Ethics Committee of the Capital Region of Denmark (H-KF-2006-6443) approved the study according to the Helsinki Declaration. The trial was registered at http://www.clinicaltrials.gov as NCT01090869.

Briefly, 60 healthy, younger (20–40 years), overweight (BMI 25–30 kg/m^2, body fat ≥25%), sedentary (VO_2peak < 45 ml O_2/kg/min) Caucasian men were randomly assigned to 12 weeks of intervention in one of four groups: 1) endurance training corresponding to an exercise-induced energy deficit of 600 kcal/day (Group T); 2) energy-reduced diet with an energy deficit of 600 kcal/day and a sedentary lifestyle (Group D); 3) endurance training corresponding to an exercise-induced energy deficit of 600 kcal/day and energy-increased diet providing 600 kcal/day (Group T-iD); 4) sedentary lifestyle and unchanged diet in a control group (Group C). Subjects in group T, D and T-iD were in frequent contact with the research staff to ensure high intervention compliance. Adherence to the intervention protocol, evaluated by VO_2peak, body weight, energy intake and expenditure, was documented for all four study groups [13]. To avoid seasonal variation the number of participants were evenly distributed between spring and autumn.

For various reasons, 12 participants dropped out during the intervention period leaving 12 participants in each of the four study groups [13]. The subjects' characteristics before and after 12 weeks of intervention are described in Table 1 and show that body weight decreases in groups T and D, but not in T-iD and C. These results are published [13].

2.2. Anthropometric measures

Participants underwent a panel of tests before and after the intervention [13]. In short, fat mass and fat free mass were assessed by dual-energy X-ray absorptiometry (Prodigy Bone Densitometer System; GE Lunar, Madison, WI), and volumes of abdominal adipose tissue (total, subcutaneous, intra-abdominal) were determined by magnetic resonance imaging (1.5 T General Electric Signa Horizon scanner).

2.3. Blood sampling

Blood samples were drawn from a hand vein catheter in the morning (between 08:00 and 09:00) after 12 h of fasting and 36 h of abstinence from any kind of exercise. For the present study, 3 ml blood was collected in EDTA-tubes (K_2-EDTA: 5.4 mg) and used for the analysis of t-PA:Ag. The tubes were centrifuged at 4 °C for 10 min at 2100*g*. Plasma was pipetted into Eppendorf tubes and stored at −80 °C until analysis.

2.4. Blood analyses

Plasma samples were rapidly thawed in a water bath at 37 °C and analyzed in duplicate and in one series for each individual. The concentration of t-PA:Ag (ng/ml) was determined by a TriniLIZE t-PA Antigen kit (Trinity Biotech, Bray, Co. Wicklow, Ireland). Due to the lack of plasma in 3 samples ($n = 2$ in group D; $n = 1$ in group C) and very high t-PA concentrations (>26 ng/ml) in one sample (group C) results for t-PA:Ag are presented for 44 study participants.

2.5. Statistics

Within-group differences between baseline and 12 weeks (in T, D, T-iD and C groups, respectively) were analyzed with paired *t*-tests. Baseline values were compared between the four groups using an analysis of variance (ANOVA). Between-group differences at 12 weeks were determined with an analysis of covariance (ANCOVA) with baseline t-PA:Ag values as covariate. When significant between-group effects were observed at 12 weeks, pair wise *post hoc* analyses were performed with the method of Least Significant Differences. The Pearson correlation coefficient (r) was calculated to describe associations between changes in t-PA:Ag and changes in body composition with all study groups combined, irrespective of exercise modality.

Results are presented as mean (SD). A *p*-value of <0.05 was considered statistically significant. The SPSS program (version 21; IBM SPSS Inc., Chicago, IL, USA) was used for all the statistical analyses.

Table 1
Subjects' characteristics at baseline and after 12 weeks of intervention.

Variable		T ($n = 12$)	D ($n = 12$)	T-iD ($n = 12$)	C ($n = 12$)	*p*-Value
Age, years	Baseline	28 (5)	32 (7)	32 (6)	31 (7)	NS
	12 weeks	–	–	–	–	–
Weight, kg	Baseline	94.5 (8.0)	91.2 (6.2)	96.0 (8.3)	92.2 (9.3)	NS
	12 weeks	88.6 (7.8)***	85.9 (7.6)***	95.0 (8.3)	92.1 (8.8)	<0.001
BMI, kg/m^2	Baseline	28.3 (1.1)	28.0 (1.3)	28.0 (1.4)	28.0 (1.5)	NS
	12 weeks	26.5 (1.3)***	26.4 (1.7)***	27.7 (1.3)	28.0 (1.5)	<0.001
Fat mass, kg	Baseline	28.5 (4.9)	29.5 (4.8)	27.4 (4.6)	27.8 (5.8)	NS
	12 weeks	20.8 (5.9)***	25.1 (5.9)***	25.5 (4.8)***	27.9 (4.7)	<0.001
AAT, l	Baseline	4.58 (0.99)	5.25 (1.24)	4.71 (1.12)	5.09 (0.98)	NS
	12 weeks	3.29 (1.08)***	4.39 (1.28)***	4.30 (1.04)*	5.09 (0.82)	<0.001
SAAT, l	Baseline	2.97 (0.84)	3.41 (0.77)	2.78 (0.61)	2.96 (0.72)	NS
	12 weeks	2.23 (0.83)***	2.79 (0.83)***	2.62 (0.79)	2.95 (0.65)	<0.001
IAAT, l	Baseline	1.60 (0.41)	1.83 (0.63)	1.83 (0.59)	2.12 (0.74)	NS
	12 weeks	1.06 (0.43)***	1.58 (0.60)**	1.67 (0.63)	2.13 (0.70)	<0.001
FFM, kg	Baseline	66.0 (6.9)	62.0 (4.5)	68.6 (5.4)	64.5 (6.0)	NS
	12 weeks	67.8 (7.4)**	60.8 (4.1)**	69.5 (5.0)**	64.3 (5.8)	<0.001

Mean (SD) values at baseline and 12 weeks were compared with a paired *t*-test within groups. Between-group comparisons at baseline were performed with an ANOVA. Between-group comparisons at 12 weeks were performed with an ANCOVA adjusted for baseline values. T, training; D, energy-reduced diet; T-iD, training and increased diet; C, control group; BMI, body mass index; AAT, total abdominal adipose tissue volume; SAAT, subcutaneous abdominal adipose tissue volume; IAAT, intra-abdominal adipose tissue volume; FFM, fat free mass; NS, non-significant. Subjects' characteristics were previously reported in Nordby et al. [13].

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$