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Weight loss is superior to exercise in improving the atherogenic lipid profile in a sedentary, overweight population with stable coronary artery disease: A randomized trial

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ABSTRACT

Background: Dyslipidemia and low-grade inflammation are integral in the pathogenesis of atherosclerosis. We aim to compare the effects of a considerable weight loss and intensive exercise training on lipid atherogenicity and low-grade inflammation in a high-risk population with coronary artery disease (CAD).
Methods: Seventy non-diabetic participants with CAD, BMI 28–40 kg/m^2, age 45–75 years were randomized to 12 weeks' aerobic interval training (AIT) at 85–90% of peak heart rate three times/week or a low energy diet (LED, 800–1000 kcal/day) for 8–10 weeks followed by 2–4 weeks' weight maintenance diet. Lipid profile atherogenicity was described using lipoprotein particle size and density profiling. Low-grade inflammation was evaluated by tumor necrosis factor alpha (TNFα), C-reactive protein, interleukin 6 and soluble urokinase plasminogen activator receptor.
Results: Twenty-six (74%) AIT and 29 (83%) LED participants completed intervention per protocol. AIT and LED decreased total (AIT: −518 {−906;−129},P = 0.011, LED: −767 {−1128:−406},P < 0.001) and low-density lipoprotein (LDL, AIT: −186 {−306;−65},P = 0.004, LED: −277 {−433;−122},P < 0.001) assessed as the area under the density profile curve. LED was superior to AIT in decreasing atherogenicity reflected by increased LDL (between-group: 1.0 Å {0.4; 1.7},P = 0.003) and high-density lipoprotein (between-group: 1.2 Å {0.2; 2.4},P = 0.026) particle size and a decreased proportion of total lipoprotein constituted by the small, dense LDL_5 subfraction (between-group: −5.0% {−8.4;−1.7},P = 0.004). LED decreased TNFα (9.5% {−15.8;−2.6},P = 0.009). No changes were seen following AIT.
Conclusion: LED and AIT decreased total and LDL lipoprotein. LED was superior in decreasing atherogenicity assessed by a shift in density profile and increased particle size. Effect on low-grade inflammation was limited.

1. Introduction

Initiation and progression of atherosclerotic plaque are mediated by deposition of atherogenic lipoproteins in the intima and media of the arterial wall eliciting an inflammatory response. Unstable atherosclerotic plaque prone to rupture is characterized by perivascular inflammation and a lipid-rich core. Plaque rupture is believed to be the cause of 60–70% of myocardial infarctions [1,2]. In clinical practice amounts of low and high-density lipoprotein

Abbreviations: AIT, Aerobic interval training; BMI, Body mass index; CAD, Coronary artery disease; CRP, C-reactive protein; HDL, High density lipoprotein; FFM, Fat free mass; IL6, Interleukin 6; LDL, Low density lipoprotein; LED, Low energy diet; TNFα, Tumor necrosis factor alpha; SuPAR, Soluble urokinase plasminogen activator receptor; TRL, Triglyceride-rich lipoprotein; VO_2peak, Peak aerobic capacity.

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(LDL and HDL) cholesterol in blood are used to monitor lipid-lowering treatment and assess cardiovascular risk [3]. However, measurement of lipoprotein density and particle size could provide a more accurate risk estimation by taking into account the small, dense atherogenic lipoprotein particles with triglyceride-rich, cholesterol-depleted cores [4–8].

The pro-inflammatory cytokines tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) are believed to be involved in the pathogenesis of atherosclerosis [2]. A meta-analysis comprising 29 studies associated IL6, TNFα and the inflammatory marker C-reactive protein (CRP) to increased cardiovascular risk independent of traditional risk factors in healthy populations [9]. Increased IL6, CRP and TNFα predicts a poor prognosis in stable and unstable CAD [10–12]. Recently, the inflammatory marker soluble urokinase plasminogen activator receptor (suPAR) was linked to atherogenesis, cardiovascular risk in healthy populations and prognosis following ST-elevation myocardial infarction [13–15].

Dyslipidemia and low-grade inflammation are associated with obesity and sedentarism [2,3,16]. The current paper compares the effects of 12 weeks' aerobic interval training (AIT) to those of a rapid weight loss using a low energy diet (LED) on lipid profile atherogenicity and low-grade inflammation. We recently demonstrated that both interventions elicited similar decreases in total cholesterol, triglycerides and nonHDL-cholesterol and reduced visceral abdominal fat; albeit, LED was superior to AIT [17]. Since low-grade inflammation and lipoprotein particle size [18,19] have been associated with visceral adipose tissue we hypothesize a superior effect of LED in terms of decreasing inflammatory response and lipoprotein atherogenicity.

2. Methods

2.1. Study design

Population, study design and intervention of this randomized trial has been described in detail previously [20]. Main inclusion criteria were CAD diagnosed >6 months prior to inclusion, BMI 28–40 kg/m^2, age 45–75 years and no diabetes. Participants were randomized to either 12 weeks' supervised AIT at 85–90% of VO_2peak three times/week or a weight loss program using an LED (800–1000 kcal/day, the Cambridge Weight Plan, Northants, UK) for 8–10 weeks, followed by a 2–4 week weight maintenance diet to ensure a non-catabolic state at follow-up. The AIT group was examined >18 h after the last exercise session [20].

Per protocol adherence to intervention was a priori defined as: ≥5% weight loss in the LED group and training attendance ≥60% overall and ≥50% the last two weeks of the intervention in the AIT group. The aim of the study was a direct comparison of weight loss and exercise training thus the main results are the per protocol analyses. All participants who were examined at 12 weeks entered into the intention-to-treat analyses (Fig. 1).

Written informed consent was obtained from each participant.The study obtained approval from the Danish Dataprotection Agency and the regional ethics committee of the Capital Region of Denmark (H-4-2010-146) and adheres to the Helsinki declaration.

2.2. Body composition and peak aerobic capacity

As previously published [17] body fat mass and fat free mass (FFM) was determined by dual X-ray absorptiometry. Magnetic resonance imaging (MRI) estimated abdominal visceral and subcutaneous fat. For logistic and medical reasons only 33 participants underwent MRI at both baseline and follow-up. A bicycle ergometer and breath-by-breath gas exchange measurements determined peak aerobic capacity (VO_2peak). To account for changes in body composition VO_2peak was expressed as mL/kg FFM$^{0.67}$/min.

2.3. Blood samples: collection and analyses

All blood samples were drawn from an intravenous cannula in an antecubital vein in the morning after a 10-h overnight fast. Samples were centrifuged for 10 min at 3500 rpm (Universal 320R, Hettich Centrifugen, Tuttlingen, Germany) and plasma was stored at −80 °C.

2.3.1. Density profiling

Density profiling was carried out using ultracentrifugation [21,22] with a bismuth sodium ethylenediaminetetraacetic acid (NaBiEDTA, TCI America, Portland, OR) gradient solution and lipoproteins pre-stained with the lipophilic fluorescent probe NBD C6 (ceramide (6-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoyl)sphingosine, Invitrogen, Carlsbad, CA). Each sample was centrifuged in an ultracentrifuge tube (Beckman Coulter Inc., Palo Alto, CA) at 120,000 rpm, for 6 h using an Optima TLX ultracentrifuge and a TLA 120.2 fixed-angle rotor (Beckman–Coulter, Palo Alto, CA). An image of tube was obtained and analyzed using a digital OptronicsMicrofireCamera (S99808, Goleta, CA) with a Fiber-Lite MH-100 Illuminator as a light source (MH100A, Edmund Industrial Optics, Barrington, NJ). A digital color microscope camera (S99808, Optronics, Goleta, CA) was used to record the image. The image was converted to a density profile using a commercially available software program (OriginPro 7.5, Microcal Software Inc., Northampton, MA) depicting fluorescence emission in pixels at any given point versus tube coordinate (6–33 mm length). For a more detailed description of the method and an example of the density profile curve see online appendix B.

The lipoproteins were divided into subfractions based on density. The amount of each subfraction was determined as the area under the density profile curve which is determined as the total amount of pixels per defined subfraction as indicated by the length along the tube. Lipoprotein density is inversely related to lipoprotein particle size (Table 2) [4]. Average particle size was calculated using the percentage each subfraction constituted of total LDL or HDL.

2.3.2. Inflammatory markers

TNFα was determined using an enzyme-linked immunosorbent assay (ELISA, DRG instruments Marburg, Germany). SuPAR was analyzed using suPARnostic® ELISA (ViroGates, Copenhagen, Denmark). CRP was determined using a high sensitivity assay ELISA with a lower detection limit of 0.2 mg/L. The ELISA used for IL6 had a lower detection level of 2 pg/mL (Immulite 2000, Siemens Healthcare diagnostics, LA, CA). All kits were used according to the manufacturer instructions. The intra-assay variation was 3.2%, 3.3–4.5%, 2.8–8.7% and 3.3–4.9% whereas inter-assay variation was 5.4%, 6.3–6.6%, 3.1–8.7% and 4.6–7.2% for suPAR, TNFα, CRP and IL6, respectively. Baseline and 12-week samples were analyzed using the same assay.

2.4. Statistics

Sample size calculation was published earlier and was based on the primary endpoint of the study, coronary flow reserve [20,23]. Categorical data, including IL6 dichotomized at 2 ng/mL, are presented as number (percentage) and compared using χ^2 or Fischer's exact test. McNemar's test was used for within-group comparison of IL6. Continuous data are presented as mean (SD) or median (Q1, Q3) if not normally distributed. An appropriate t-test was used for comparisons of paired (within-group) and unpaired (between-group) continuous data. Logarithmic transformation (log10) was

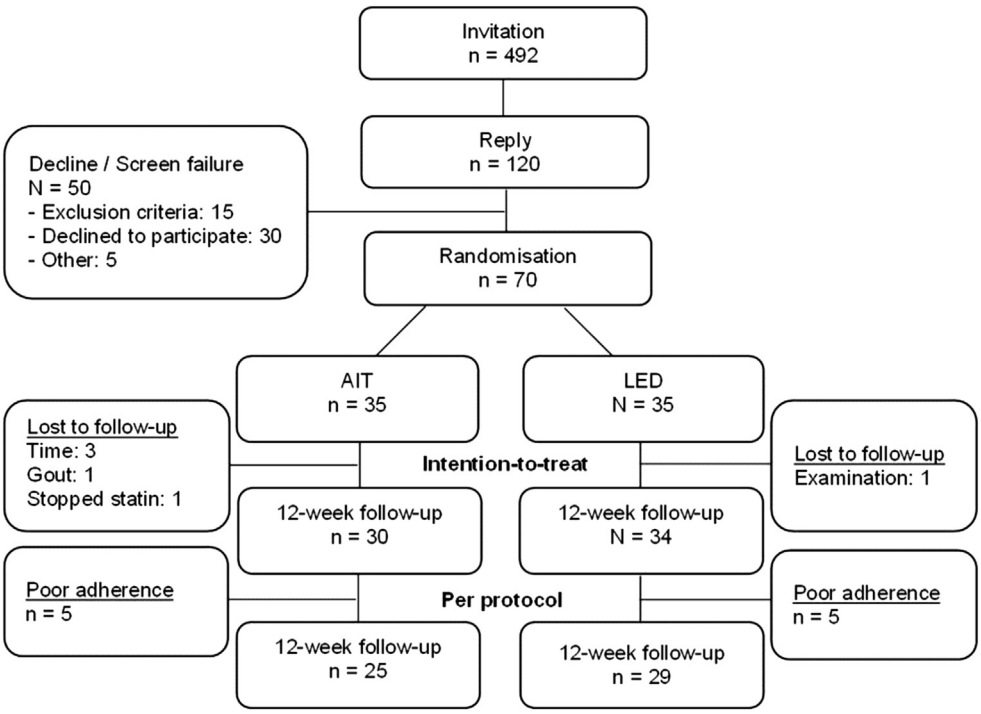

Fig. 1. Inclusion and course of the study. AIT: aerobic interval training, LED: Low energy diet

Table 1
Baseline characteristics.

	AIT (n = 26)	LED (n = 29)	*P*
Male (%)	22 (85%)	21 (72%)	0.19
Age (years)	62.3 (5.7)	63.6 (6.8)	0.61
Body mass index (kg/m^2)	31.7 (29.6; 34.8)	31.1 (29.9; 32.7)	0.48
Visceral fat (cm^3, n = 14/14)	246 (62)	277 (99)	0.34
Subcutaneous fat (cm^3, n = 14/14)	256 (70)	244 (81)	0.80
Body fat mass (kg)	33.3 (8.3)	33.3 (7.9)	0.99
VO_2peak (mL/kg/min)	20.7 (4.9)	20.6 (5.0)	0.83
VO_2peak (mL/kg FFM$^{0.67}$/min)	124 (24)	124 (25)	1.00
Total-cholesterol (mmol/L)	4.3 (0.8)	4.1 (0.7)	0.28
HDL-cholesterol (mmol/L)	1.2 (0.3)	1.1 (0.2)	0.50
NonHDL-cholesterol (mmol/L)	3.1 (0.7)	3.0 (0.7)	0.37
Systolic blood pressure (mmHg)	127 (13)	129 (16)	0.67
Diastolic blood pressure (mmHg)	74 (9.1)	71 (8.0)	0.16
Medication			
ACE-inhibitor/ARB	16 (62%)	21 (72%)	0.39
Acetylsalicylic Acid	22 (88%)	26 (90%)	1.00
Beta blocker	13 (50%)	15 (52%)	1.00
Calcium antagonist	6 (23%)	11 (3%)	0.23
Statin	25 (96%)	28 (97%)	1.00
Other cholesterol lowering drug	5 (19%)	2 (7%)	0.24

Categorical data: number (percentage). Continuous data: mean (SD) or median (Q1,Q3). AIT: Aerobic interval training, LED: Low energy diet, HDL: High density lipoprotein, ACE: Angiotensin converting enzyme, ARB: Angiotensin receptor blocker.

performed on TNFα, CRP and suPAR ensuring a normal distribution. Linear regression including the entire population was used to describe the correlation between changes in body fat distribution and triglycerides, and lipoprotein particle size and log transformed inflammatory markers. The significance level was set to $P < 0.05$. Stata 13.1 software (StataCorp, College Station, TX, USA) was used for all analyses.

3. Results

3.1. Population and intervention

Seventy participants were included. Of them 57 (81%) were male, median age 63 (58; 71) years and median BMI 31.3 (29.7; 33.7). After 12 weeks 65 (93%) participants were re-examined; 31 (88.6%) and 34 (97.1%) in the AIT and LED group, respectively, and included in the intention-to-treat analyses whereas 26 (74.2%) and 29 (82.9%) met per protocol criteria in the AIT and LED group, respectively. In the present paper, one AIT participant was excluded from the analyses due to discontinuation of statin treatment prior to follow-up possibly affecting the lipid profile and low-grade inflammation (Fig. 1). The population was largely asymptomatic and contemporarily treated with regard to blood pressure and lipids [17]. Baseline characteristics for participants included in per protocol analyses are presented in Table 1.

As previously reported [17], the LED group obtained a 10.6% weight loss versus 1.6% after AIT. Fat mass decreased 26.6% and 5.5%, respectively. AIT improved VO_2peak 10.4% vs −3.0% following LED (all between-group $P < 0.001$). Intention-to-treat analyses were similar to per protocol analyses and presented in the online appendix A.1, 2 and 4.

3.2. Lipoprotein density profiling

Total lipoprotein decreased significantly in both groups (Table 2). The atherogenic triglyceride-rich lipoproteins (TRL, i.e. chylomicrons, very low and intermediate density lipoprotein) [4] decreased following both interventions, although only significantly after LED. Total LDL decreased significantly in both groups. LED and AIT decreased LDL_2, LDL_3 and LDL_4 amounts similarly whereas a trend for LED to have greater effect on LDL_5 was seen. This resulted in a less atherogenic lipoprotein profile following LED with a larger contribution to total LDL from the large, less dense LDL_1 and LDL_3 and a simultaneous decrease in the contribution of

Table 2
Lipoprotein density profile.

	Density (mg/dL)	Average size (Å)	AIT (n = 25)			LED (n = 29)			Between-group	
			Baseline	Change	P	Baseline	Change	P	Difference	P
TRL	<1.019		417 (239)	−90 (−190; 10)	0.077	433 (256)	−161 (−257;−65)	0.002	−71 (−207; 65)	0.299
LDL										
LDL_1	1.019−1.023	270	65 (35)	−4.0 (−19; 11)	0.592	57 (30)	−2.6 (−14; 9)	0.647	1.4 (−17; 20)	0.880
LDL_2	1.023−1.029	255	108 (44)	−21 (−34;−9)	0.002	97 (42)	−19 (−35;−2)	0.029	2.8 (−18; 23)	0.786
LDL_3	1.029−1.039	247	273 (81)	−32 (−62;−1.6)	0.040	271 (95)	−31 (−66; 4.5)	0.085	1.4 (−45; 47)	0.951
LDL_4	1.039−1.050	237	611 (251)	−72 (−131;−13)	0.020	598 (219)	−96 (−174;−17)	0.018	−24 (−122; 75)	0.630
LDL_5	1.050−1.063	226	436 (180)	−56 (−105;−7.8)	0.025	401 (169)	−130 (−187;−73)	<0.001	−73 (−148; 1.4)	0.054
Total LDL			1493 (440)	−186 (−306;−65)	0.004	1425 (362)	−277 (−433;−122)	0.001	−91 (−288; 105)	0.355
HDL										
HDL_{2b}	1.063−1.091	111	598 (283)	−30 (−157; 98)	0.635	489 (184)	−12 (−74; 49)	0.682	−20 (−114; 149)	0.794
HDL_{2a}	1.091−1.110	93	559 (249)	−90 (−179;−0.36)	0.049	496 (150)	−57 (−102;−12)	0.014	33 (−61; 126)	0.487
HDL_{3a}	1.110−1.133	85	717 (228)	−106 (−200;−12)	0.028	687 (145)	−125 (−175;−75)	<0.001	−19 (−119; 81)	0.702
HDL_{3b}	1.133−1.156	80	464 (148)	−16 (−92:60)	0.659	466 (128)	−99 (−146;−52)	<0.002	−83 (−167; 1.95)	0.055
HDL_{3c}	1.156−1.179	75	179 (−27; 27)	0.17 (−27; 27)	0.990	181 (45)	−35 (−53;−17)	<0.001	−35 (−66;−4.2)	0.026
Total HDL			2517 (774)	−242 (−511; 27)	0.076	2319 (512)	−329 (−497; 161)	<0.001	−87 (−388; 214)	0.564
Total			4427 (942)	−518 (−906;−129)	0.011	4176 (907)	−767 (−1128;−406)	<0.001	−249 (−767; 268)	0.338

Baseline values: mean (SD) of the area under the curve for each subfraction (defined in section 2.3.1). The density profiling curves are presented in Appendix B.
Within-group: change (95% C.I) and P-value. Between-group: difference (95% C.I) and P-value. Density and particle size corresponding to each subfraction are listed.
AIT: Aerobic interval training, LED: Low energy diet, TRL: triglyceride-rich lipids, LDL: Low density lipoprotein, HDL: High density lipoprotein.

the small, dense LDL_5. No such decrease in subfraction distribution was seen following the AIT (Fig. 2A, appendix A.3). Total HDL decreased similarly in both groups (Table 2). With the exception of HDL_{2b}, all HDL subgroups decreased significantly following LED whereas this was only the case for HDL_{3a} after AIT. For HDL_{3c} the between-group difference reached statistical significance. Again, a shift towards larger, less atherogenic HDL subfractions was seen after LED but not AIT (Fig. 2B, appendix A.3). The contribution of HDL to total lipoprotein at baseline was 56.2% (SD 9.5%) and 55.7% (SD 5.5%) in the AIT and LED group, respectively. This contribution increased by 2.2% (2.1%; 6.6%, P = 0.300) and 2.8% (0.7%; 4.8, P = 0.010) following AIT and LED, respectively, reflecting an increase in the proportion of total lipoprotein constituted by HDL despite the absolute decrease in total HDL.

The reduced atherogenicity following LED is also reflected by increased average particle size of LDL (baseline: 238 Å {SD 1.7}, within-group difference: 1.0 Å {95% CI 0.5; 1.4}, P = 0.004) and HDL (baseline 90 Å {SD 1.6 Å}, within-group difference: 0.9 Å {95% CI 0.3; 1.5}, P = 0.004). No change was seen following AIT for neither LDL (baseline: 238 Å {SD 2.4}, within-group difference: −0.1 Å {95% CI −0.6; 0.05}, P = 0.831) nor HDL (baseline 91 Å {SD 2.5} within-group difference −0.4 Å {95% CI −1.4; 0.6}, P = 0.448). LED was superior to AIT in increasing particle size (LDL: between-group difference: 1.0 Å {0.4; 1.7}, P = 0.003, HDL: between-group difference: 1.2 Å {0.2; 2.4}, P = 0.026). Nonetheless, all participants had the atherogenic LDL pattern B (LDL particle size ≤255 Å) characterized by a preponderance of small, dense LDL particles [24] both before and after intervention.

3.3. Inflammatory markers

No significant changes were seen in suPAR and CRP following the interventions. A statistically significant 9.5% decrease was seen in TNFα after LED while it remained unchanged following AIT. No between-group difference was seen on any of the inflammatory markers (Table 3). Intention-to-treat analyses comprising more participants revealed a statistically significant decrease in suPAR following LED but still no between-group difference (appendix A.1).

IL6 was dichotomized at the lower detection level of 2 ng/mL. Twenty-five (46%) and 29 (54%) participants had an IL6 <2 pg/mL before and after the intervention, respectively. No between-group (P = 0.816) or within-group (LED: P = 0.688, AIT: P = 0.727) differences were seen.

3.4. Adipose tissue, inflammatory markers and particle size

Linear regression including the entire population showed an association between increased HDL particle size and decreased visceral fat (β = −122, R^2 = 0.37, P < 0.001) and total fat mass (β = −7.5, R^2 = 0.13, P = 0.004). The association with subcutaneous fat was borderline significant (β = −58, R^2 = 0.10, P = 0.066). When adding the intervention to the model an independent effect was seen. Univariate regression divided by study group revealed a significant effect of LED but not AIT. The latter is possibly due to a smaller range in the difference of particle size and visceral fat. No associations were seen between LDL particle size and visceral fat (β = −42, R^2 = 0.02, P = 0.467) or subcutaneous fat (β = −84, R^2 = 0.09, P = 0.095) nor when regression was performed by group. Change in total fat mass and LDL particle size were associated (β = −7.8, R^2 = 0.06, P = 0.045). Linear regression showed no association between reduced triglyceride-levels and increased HDL (β = −0.02, R^2 = 0.01, P = 0.505) or LDL (β = 0.01, R^2 = 0.003, P = 0.688) particle size.

Reduced visceral fat was associated with decreased logCRP (β = 0.004, R^2 = 0.21, P = 0.008) whereas no association was seen between decreased logCRP and total body fat (β = 0.02, R^2 = 0.07, P = 0.065) or subcutaneous fat (β = 0.002, R^2 = 0.05, P = 0.215); suggesting a selective effect of visceral fat on logCRP. No association was seen between reduced adipose tissue and decreased logTNFα (visceral fat: β = −2.4, R^2 < 0.001, P = 0.976, subcutaneous fat: β = 13, R^2 = 0.001, P = 0.850, total fat mass: β = 3.4, R^2 = 0.006, P = 0.543) or logsuPAR (visceral fat: β = −39, R^2 = 0.005, P = 0.693, subcutaneous fat: β = −57, R^2 = 0.01, P = 0.514, total fat mass: β = 2.8, R^2 = 0.003, P = 0.673).

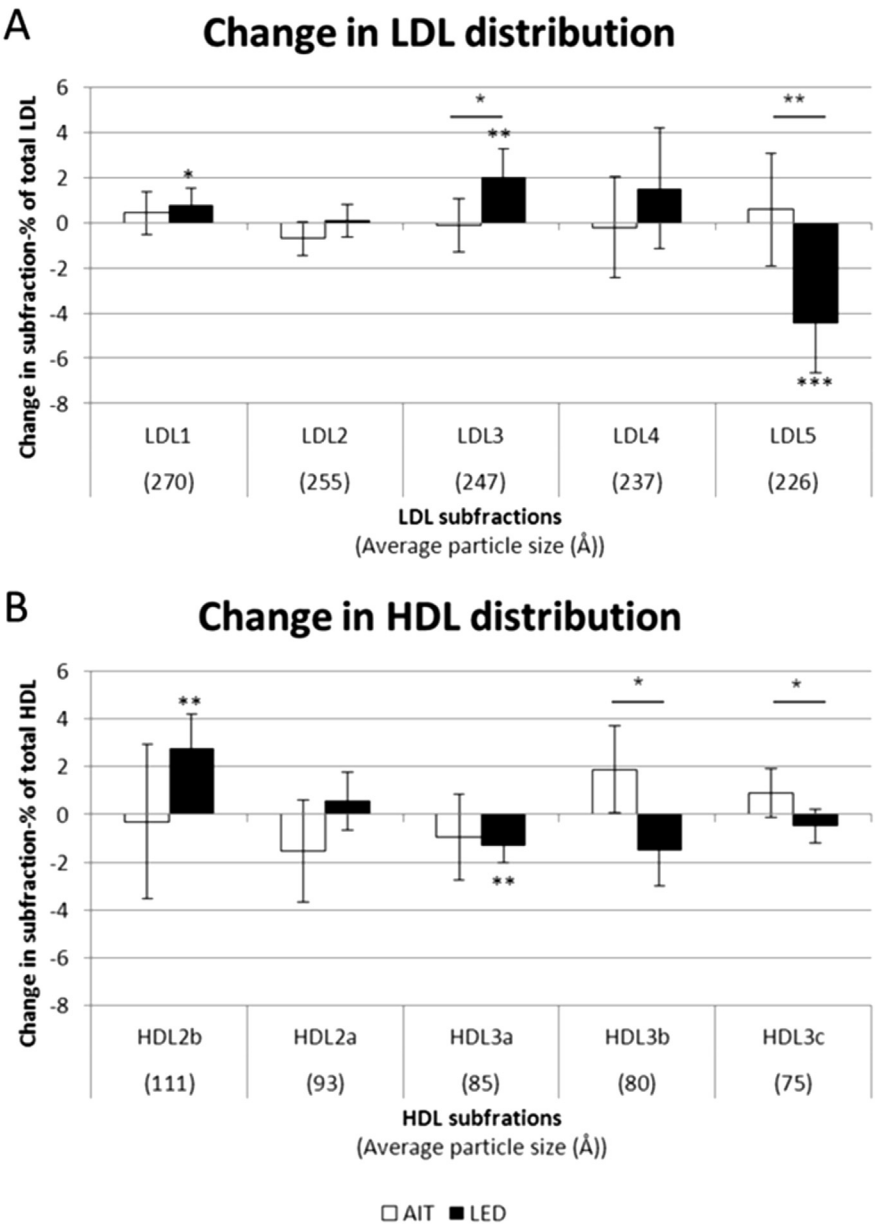

Fig. 2. A: Changes in the distribution of LDL-subfractions by the change in the proportion that each subfraction constitutes of total LDL. B: Changes in the distribution of HDL-subfractions by the change in the proportion that each subfraction constitutes of total HDL. Error-bars: 95% C.I. Stars above error-bars: within-group difference. Stars above horizontal lines: between-group difference. *P < 0.05,**P < 0.01, ***P < 0.001. AIT: Aerobic interval training, LED: Low energy diet, LDL: Low density lipoprotein, HDL: High density lipoprotein.

Table 3
Inflammatory markers.

	AIT (n = 25)			LED (n = 29)			Between group
	Baseline	Change (%)	*P*	Baseline	Change (%)	*P*	*P*
CRP (mg/L)	1.86 (2.72)	−8.9 (−35.9; 29.5)	0.590	1.74 (3.07)	−26.3 (−50.4; 9.4)	0.125	0.415
TNFα (pg/mL)	5.98 (1.34)	−3.7 (−13.6; 7.38)	0.486	5.92 (1.21)	−9.5 (−15.8;−2.6)	0.009	0.326
suPAR (ng/mL)	4.49 (1.23)	−1.62 (−9.38; 6.81)	0.686	5.20 (1.32)	−3.13 (−8.84; 2.95)	0.294	0.753

Baseline values: Mean (SD). Within-group change: Percentage change (95% C.I.). P-value: Within- and between-group differences. AIT: Aerobic interval training, LED: Low energy diet, CRP: C-reactive protein, TNFα: Tumor necrosis factor α; suPAR: Soluble urokinase plasminogen activator receptor.

4. Discussion

The current paper demonstrates that both a rapid weight loss using an LED and 12-weeks' of AIT decrease total lipoprotein, TRL and LDL whereas LED was superior to AIT in improving lipoprotein atherogenicity reflected by increased LDL and HDL particle size and

a decrease in the proportion of LDL constituted by the small, dense LDL_5-subfraction. Moreover, TNFα was decreased following LED. It is the first study to compare the effects of weight loss obtained by an LED to those of an AIT program on lipoprotein atherogenicity and central markers of low-grade inflammation in a high-risk population with CAD and dyslipidemia. AIT was superior to LED in increasing VO_2peak while both interventions facilitated significant changes in body weight and composition; albeit, largest after LED as previously published [17].

4.1. Density profile

We have demonstrated a decrease in total cholesterol, nonHDL-cholesterol, total cholesterol/HDL-cholesterol-ratio and triglycerides following both interventions with no between-group difference [17]. These are all easily accessible measures of dyslipidemia and predictors of cardiovascular risk used in clinical practice [25,26]. Nevertheless, these LDL and HDL cholesterol assessments may have very different distributions of the small dense atherogenic lipoproteins with triglyceride-rich, cholesterol-depleted cores [1,4,5]. LDL cholesterol used in clinical practice is often calculated using Friedewald's formula [3,4] and the accuracy of this estimate in subjects with the metabolic syndrome is still debated [27,28]. Thus, in clinical research lipoprotein density and size provide important additional information.

Most previous studies have used particle size to evaluate the effect of various interventions on lipoprotein atherogenicity. In the present study the LED group exhibited a shift toward a less atherogenic lipoprotein profile based on both particle size and density profile; the latter reflected by a relative improvement following LED compared to AIT in the atherogenic LDL_5, HDL_{3b} and HDL_{3c}-subfractions. A randomized trial comparing 5% weight loss by moderate intensity exercise or caloric restriction showed that weight loss by caloric restriction caused a shift toward larger LDL-particles while LDL particle size remained unchanged after exercise-induced weight loss [29]. Thus, the effect of weight loss may depend on the weight loss mechanism. In overweight men a 10 kg weight loss increased LDL particle size without increasing HDL cholesterol in participants with LDL pattern B but not with LDL pattern A (average LDL size >255 Å) [24] suggesting that the effects of weight loss depends on a low baseline LDL particle size. With respect to HDL, a study comparing weight loss induced by diet or exercise found increased total HDL, HDL_2 and HDL_3 in both groups but no change in LDL cholesterol concentration in either group; however, LDL subfractions were not measured [30].

While two of the studies cited above compared weight loss obtained by diet or exercise, few studies have examined the effect of exercise without weight loss. In agreement with our observations, exercise inducing a small weight loss in a healthy overweight population caused no significant change in LDL or HDL particle size [31]. The STRRIDE study demonstrated that a high amount of high-intensity exercise is required to change the lipoprotein density and particle size [32]. The importance of training intensity could explain the unaltered particle size following the 5% exercise-induced weight loss in the trial cited above [29]. The participants in the STRRIDE study underwent 8 months' exercise at an intensity comparable to ours but with a higher weekly frequency of exercise sessions accentuating training duration. In our study 12 weeks' AIT three times/week may be sufficient to decrease the amount of lipoprotein but insufficient to affect atherogenicity.

In the STRRIDE study the group undergoing high-amount, high-intensity exercise obtained the greatest decrease in body weight, fat mass and abdominal obesity [33]. In the present study, LED was superior to AIT in decreasing body weight, body fat mass, subcutaneous and visceral abdominal fat [17]. Lipoprotein particle size has been linked to abundance of adipose tissue and, in particular, visceral fat [2,19] and changes in atherogenicity could be related to changes in body composition. The association between increased HDL particle size and decreased total and visceral fat mass lends support to this pathogenic mechanism. The results were less clear regarding LDL particle size, which was only associated with reduced total fat mass. This association did not persist when separating the groups.

Since increased triglyceride-levels promote the formation of small lipoprotein particles [5] the decrease in triglycerides observed previously [17] corresponds well to the shift toward larger, less dense lipoproteins. Triglycerides have been shown to predict LDL particle size in physically active and sedentary men [34]. Thus, it was unexpected that no link was seen between increased LDL and HDL particle size and decreased triglyceride-levels. Alternatively, a decrease in the enzyme hepatic lipase, which is not measured in the present study, has been connected to decreased visceral fat [19]. This enzyme catalyzes the hydrolysis of core triglycerides resulting in smaller, denser lipoproteins [35].

4.2. Inflammatory markers

Existing evidence concerning the effects of exercise on low-grade inflammation is equivocal. In the current study AIT did not lead to statistically significant changes in IL6, TNFα or CRP. A study comprising 12 participants with heart failure due to CAD undergoing 4 months' combined endurance and resistance training obtained no change in IL6 and TNFα; nonetheless, a significant decrease in soluble TNF1 and TNF2-receptors suggested an attenuated inflammatory response [36]. CAD patients enrolled in a cardiac rehabilitation program including 12 weeks' aerobic exercise achieved a significant decrease in CRP [37,38] and IL6 [38] independently of weight change and statin treatment. However, in both studies baseline CRP-levels were considerably higher than in the present population. In 96 CAD patients with CRP-levels more similar to ours an eight-week exercise-based cardiac rehabilitation program did not affect CRP, IL6 and TNFα levels despite improved VO_2peak [39]. To further support our findings, six months' aerobic exercise training in an at-risk population did not decrease CRP despite improved fitness and body composition [40]. The acute increase in inflammatory markers subsequent to exercise [41,42] could mask a more long-term decrease in inflammatory markers. However, CRP and IL6 are both normalized 20 h after exercise [41,42]. In the current study blood samples were taken >18 h after the last exercise session.

A review comprising 33 interventional studies conclude that CRP decreases following weight loss [43]. Our LED group achieved a significant decrease in TNFα suggesting decreased low-grade inflammation. This was accompanied by a 26.3% decrease in CRP but statistical significance was not reached. Intention-to-treat analyses including more participants showed a borderline significant 27.5% decrease in CRP ($P = 0.059$, appendix A.1) indicating that the small sample size could explain the lack of statistical significance in per protocol analyses. High intra-assay variability of the high-sensitivity CRP assay and high within-individual variability of CRP [44] presuppose a large sample size to detect an effect of the intervention.; in particular, when baseline CRP concentrations are modest as in the present study. Mean CRP was only slightly higher than in a healthy population [45] and considerably lower than in overweight CAD patients participating in cardiac rehabilitation [37,38]. This was also reflected by the IL6-measurement with IL6 below the lower detection-limit in 46% of the participants. Thus, the limited effect on low-grade inflammation could relate to the low baseline values.

As opposed to the other inflammatory markers presented there

is a paucity of evidence regarding the effect of weight loss and exercise on suPAR. A cross-sectional study has shown that suPAR is positively associated with self-reported physical activity while there is a negative association with BMI and waist circumference; however, this association was only seen in smokers [46]. In 12 professional football players no acute effect on suPAR levels was seen after a match [47]. This is the first study to examine the effects of an AIT and weight loss program on suPAR levels revealing a modest decrease following LED, though only statistically significant in the intention-to-treat analyses.

Low-grade inflammation is linked to visceral adipose tissue [2,18] and changes in low-grade inflammation could be related to changes in body composition. TNFα was reduced after LED and it was somewhat unexpected that no association was seen between decreased TNFα and decreased measures of body fat while decreased CRP was correlated to decreased visceral fat. However, the same applies in a cross-sectional analyses based on the Framingham study demonstrating that visceral fat is related to the level of CRP but not TNFα [18].

4.3. Limitations

When transferring the results to the general population of CAD patients generalizability must be considered. Our participants enrolled voluntarily and could be more inclined to change their lifestyle than CAD patients in general. Engaging in intensive lifestyle changes requires a certain mental and physical discipline from the participants and support from family and employers. This is supported by the observation reported previously [17] that a large proportion (87%) of the participants had >10 years education and was cohabitant (71%). A larger proportion of those who did not complete the intervention were employed compared to those who did. Based on the exclusion criteria a direct translation of the results to CAD patient with co-morbidities such as diabetes, severe heart failure or COPD should be done with caution.

Regarding study design the sample size calculation was based mainly on the trial's primary end point: coronary flow reserve [23]. The relatively small sample size could partly explain the limited effect on low-grade inflammation as discussed above. Since the aim of the study was to compare two intervention modalities the design did not include a control group. A control group, although not blind to the intervention, could elucidate any placebo effect. However, participants were all motivated for lifestyle changes and requiring them to refrain from changing their lifestyle for the entire duration of the trial, 12 months, was thought to be problematic since it is not in line with current guidelines [3].

5. Conclusion

The present study demonstrates that both LED and AIT decrease total and LDL lipoprotein whereas LED is superior to AIT in decreasing lipoprotein atherogenicity. This may be explained by a larger decrease in total fat mass and visceral abdominal fat following LED. Neither intervention had marked effects on inflammatory markers although a significant decrease in TNFα was seen following LED. The present study concerns the short-term effect of weight loss and exercise; however, in secondary prevention sustainability is pivotal. The study design comprises a 40-week maintenance period and a one-year follow-up. Future analyses will reveal whether the effects on inflammation and atherogenic markers are sustainable.

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Disclosures

JEO is a founder, shareholder and board member of ViroGates A/S, Denmark, the company that produces the suPARnostic® assay. JEO and SBH are inventors on a patent on suPAR and risk. Hvidovre Hospital, University of Copenhagen, Denmark, owns the patent, which is licensed to ViroGates A/S.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2016.01.001.

References

[1] E. Falk, Pathogenesis of atherosclerosis, J. Am. Coll. Cardiol. 47 (2006) C7–C12.
[2] G. Hansson, I. Kriszbacher, M. Koppán, J. Bódis, Inflammation, atherosclerosis, and coronary artery disease, N. Engl. J. Med. 352 (2005) 1685–1695.
[3] J. Perk, G. De Backer, H. Gohlke, I. Graham, Z. Reiner, M. Verschuren, et al., European guidelines on cardiovascular disease prevention in clinical practice (version 2012), Eur. J. Prev. Cardiol. 19 (2012) 585–667.
[4] A. Ellington, I. Kullo, Atherogenic lipoprotein subprofiling, Adv. Clin. Chem. 46 (2008) 295–317.
[5] J.D. Otvos, E.J. Jeyarajah, W.C. Cromwell, Measurement issues related to lipoprotein heterogeneity, Am. J. Cardiol. 90 (2002) 22–29.
[6] A. Pascot, I. Lemieux, D. Prud'homme, A. Tremblay, A. Nadeau, C. Couillard, et al., Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity, J. Lipid Res. 42 (2001) 2007–2014.
[7] B. Lamarche, S. Moorjani, B. Cantin, G.R. Dagenais, P.J. Lupien, J.-P. Despres, Associations of HDL2 and HDL3 subfractions with Ischemic heart disease in men prospective results from the Québec cardiovascular study, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 1098–1105.
[8] B. Lamarche, S. Moorjani, B. Cantin, Small, dense low-density lipoprotein particles as a predictor of the risk of Ischemic heart disease in men prospective results from the Québec cardiovascular, Circulation 95 (1997) 69–75.
[9] S. Kaptoge, S.R.K. Seshasai, P. Gao, D.F. Freitag, A.S. Butterworth, A. Borglykke, et al., Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis, Eur. Heart J. 35 (2014) 578–589.
[10] P.M. Ridker, N. Rifai, M. Pfeffer, F. Sacks, S. Lepage, E. Braunwald, Elevation of tumor necrosis factor-α and increased risk of recurrent coronary events after myocardial infarction, Circulation 101 (2000) 2149–2153.
[11] M.S. Sabatine, D. Morrow, K. Jablonski, M.M. Rice, J.W. Warnica, M.J. Domanski, et al., Prognostic significance of the centers for disease control/American Heart Association high-sensitivity C-reactive protein cut points for cardiovascular and other outcomes in patients with stable coronary artery disease, Circulation 115 (2007) 1528–1536.
[12] E. Lindmark, E. Diderholm, L. Wallentin, A. Siegbahn, Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy, Jama 286 (2001) 2107–2113.
[13] J. Eugen-Olsen, O. Andersen, A. Linneberg, S. Ladelund, T.W. Hansen, A. Langkilde, et al., Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population, J. Intern. Med. 268 (2010) 296–308.
[14] S. Lyngbæk, J.L. Marott, D.V. Møller, M. Christiansen, K.K. Iversen, P.M. Clemmensen, et al., Usefulness of soluble urokinase plasminogen activator receptor to predict repeat myocardial infarction and mortality in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous intervention, Am. J. Cardiol. 110 (2012) 1756–1763.

[15] B. Fuhrman, The urokinase system in the pathogenesis of atherosclerosis, Atherosclerosis 222 (2012) 8–14.
[16] T. Pischon, S. Hankinson, G.S. Hotamisligil, N. Rifai, E.B. Rimm, Leisure-time physical activity and reduced plasma levels of obesity-related inflammatory markers, Obes. Res. 11 (2003) 1055–1063.
[17] L.R. Pedersen, R.H. Olsen, A. Jürs, A. Astrup, E. Chabanova, L. Simonsen, et al., A randomised trial comparing weight loss with aerobic exercise in overweight individuals with coronary artery disease: the CUT-IT trial, Eur. J. Prev. Cardiol. 22 (8) (2015) 1009–1017.
[18] K.M. Pou, J.M. Massaro, U. Hoffmann, R.S. Vasan, P. Maurovich-Horvat, M.G. Larson, et al., Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study, Circulation 116 (2007) 1234–1241.
[19] J.Q. Purnell, S.E. Kahn, J.J. Albers, D.N. Nevin, J.D. Brunzell, R.S. Schwartz, Effect of weight loss with reduction of intra-abdominal fat on lipid metabolism in older men, J. Clin. Endocrinol. Metab. 85 (2000) 977–982.
[20] L.R. Pedersen, R.H. Olsen, M. Frederiksen, A. Astrup, E. Chabanova, P. Hasbak, et al., Copenhagen study of overweight patients with coronary artery disease undergoing low energy diet or interval training: the randomized CUT-IT trial protocol, BMC Cardiovasc Disord. 13 (2013) 106.
[21] C.D. Larner, R.R. Henriquez, J.D. Johnson, R.D. Macfarlane, Developing high performance lipoprotein density profiling for use in clinical studies relating to cardiovascular disease, Anal. Chem. 83 (2011) 8524–8530.
[22] J.D. Johnson, N.J. Bell, E.L. Donahoe, R.D. Macfarlane, Metal ion complexes of EDTA as solutes for density gradient ultracentrifugation: influence of metal ions, Anal. Chem. 77 (2005) 7054–7061.
[23] R.H. Olsen, L.R. Pedersen, A. Jürs, M. Snoer, S.B. Haugaard, E. Prescott, A randomised trial comparing the effect of exercise training and weight loss on microvascular function in coronary artery disease, Int. J. Cardiol. 185 (2015) 229–235.
[24] L.I. Katzel, P.J. Coon, E. Rogus, R.M. Kraus, A.P. Goldberg, Persistence of low HDL-c levels after weight reduction in older men with small LDL particles, Arterioscler. Thromb. Vasc. Biol. (1995) 299–305.
[25] A.P. Van de Woestijne, A.M.J. Wassink, H. Monajemi, A.-H. Liem, H.M. Nathoe, Y. van der Graaf, et al., Plasma triglyceride levels increase the risk for recurrent vascular events independent of LDL-cholesterol or nonHDL-cholesterol, Int. J. Cardiol. 167 (2013) 403–408.
[26] B.J. Arsenault, J.S. Rana, E.S.G. Stroes, J.-P. Després, P.K. Shah, J.J.P. Kastelein, et al., Beyond low-density lipoprotein cholesterol: respective contributions of non-high-density lipoprotein cholesterol levels, triglycerides, and the total cholesterol/high-density lipoprotein cholesterol ratio to coronary heart disease risk in apparently healt, J. Am. Coll. Cardiol. 55 (2009) 35–41.
[27] J. Knopfholz, C.C.D. Disserol, A.J. Pierin, F.L. Schirr, L. Streisky, L.L. Takito, et al., Validation of the friedewald formula in patients with metabolic syndrome, Cholesterol 2014 (2014) 261878.
[28] I. Gazi, V. Tsimihodimos, T.D. Filippatos, V.G. Saougos, E.T. Bairaktari, A.D. Tselepis, et al., LDL cholesterol estimation in patients with the metabolic syndrome, Lipids Health Dis. 5 (2006) 8.
[29] K. Varady, S. Bhutani, M.C. Klempel, C.M. Kroeger, Comparison of effects of diet versus exercise weight loss regimens on LDL and HDL particle size in obese adults, Lipids Health Dis. 10 (2011) 119.
[30] P. Wood, M. Stefanick, D. Dreon, B. Frey-Hewitt, S. Garay, P.T. Williams, et al., Changes in plasma lipids and lipoproteins in overweight men during weight loss through dieting as compared with exercise, N. Engl. J. Med. 319 (1988) 1173–1179.
[31] M.T. Durheim, C.A. Slentz, L.A. Bateman, S.K. Mabe, W.E. Kraus, Relationships between exercise-induced reductions in thigh intermuscular adipose tissue, changes in lipoprotein particle size, and visceral adiposity, Am. J. Physiol. Endocrinol. Metab. 295 (2008) 407–412.
[32] W.E. Kraus, J. Houmard, B.D. Duscha, K.J. Knetzger, M.B. Wharton, J.S. McCartney, et al., Effects of the amount and intensity of exercise on plasma lipoproteins, N. Engl. J. Med. 347 (2002) 1483–1492.
[33] C.A. Slentz, B.D. Duscha, J.L. Johnson, K. Ketchum, L.B. Aiken, G.P. Samsa, et al., Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE–a randomized controlled study, Arch. Intern. Med. 164 (2004) 31–39.
[34] G. O'donovan, J. McEneny, E.M. Kearney, a Owen, a M. Nevill, K. Woolf-May, et al., LDL particle size in habitual exercisers, lean sedentary men and abdominally obese sedentary men, Int. J. Sports Med. 28 (2007) 644–649.
[35] W.C. Cromwell, J.D. Otvos, Low-density lipoprotein particle number and risk for cardiovascular disease, Curr. Atheroscler. Rep. 6 (2004) 381–387.
[36] V. Conraads, P. Beckers, J. Bosmans, L. De Clerck, W. Stevens, C. Vrints, et al., Combined endurance/resistance training reduces plasma TNF-α receptor levels in patients with chronic heart failure and coronary artery disease, Eur. Heart J. 23 (2002) 1854–1860.
[37] R.V. Milani, C.J. Lavie, M.R. Mehra, Reduction in C-reactive protein through cardiac rehabilitation and exercise training, J. Am. Coll. Cardiol. 43 (2004) 1056–1061.
[38] E. Goldhammer, A. Tanchilevitch, I. Maor, Y. Beniamini, U. Rosenschein, M. Sagiv, Exercise training modulates cytokines activity in coronary heart disease patients, Int. J. Cardiol. 100 (2005) 93–99.
[39] N.L. Oliveira, F. Ribeiro, G. Silva, A.J. Alves, N. Silva, J.T. Guimarães, et al., Effect of exercise-based cardiac rehabilitation on arterial stiffness and inflammatory and endothelial dysfunction biomarkers: a randomized controlled trial of myocardial infarction patients, Atherosclerosis 239 (2015) 150–157.
[40] K. Huffman, G. Samsa, C. Slentz, B.D. Duscha, J. Johnson, C.W. Bales, et al., Response of high-sensitivity C-reactive protein to exercise training in an at-risk population, Am. Heart J. 152 (2006) 793–800.
[41] M.R. Mouridsen, O.W. Nielsen, C.M. Carlsen, N. Mattsson, M.H. Ruwald, Z. Binici, et al., High-sensitivity C-reactive protein and exercise-induced changes in subjects suspected of coronary artery disease, J. Inflamm. Res. 7 (2014) 45–55.
[42] D.L. MacIntyre, S. Sorichter, J. Mair, A. Berg, D.C. McKenzie, Markers of inflammation and myofibrillar proteins following eccentric exercise in humans, Eur. J. Appl. Physiol. 84 (2001) 180–186.
[43] E. Selvin, N. Paynter, T. Erlinger, The effect of weight loss on c-reactive protein, Arch. Intern. Med. 167 (2007) 31–39.
[44] L. Browning, J. Krebs, S. Jebb, Discrimination ratio analysis of inflammatory markers: implications for the study of inflammation in chronic disease, Metabolism 53 (2004) 899–903.
[45] N. Rifai, P.M. Ridker, Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation, Clin. Chem. 49 (2003) 666–669.
[46] S. Lyngbæk, T. Sehestedt, J.L. Marott, T.W. Hansen, M.H. Olsen, O. Andersen, et al., CRP and suPAR are differently related to anthropometry and subclinical organ damage, Int. J. Cardiol. 167 (2013) 781–785.
[47] F. Sanchis-Gomar, C. Bonaguri, H. Parejo-Galeano, M. Gomez-Cabrera, J. Candel, J. Vina, et al., Effects of acute exercise and allopurinol administration on soluble urokinase plasminogen activator receptor (suPAR), Clin. Lab. 59 (2013) 207–210.