Differential effects of endurance training and weight loss on plasma adiponectin multimers and adipose tissue macrophages in younger, moderately overweight men

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Submitted 11 December 2012; accepted in final form 5 July 2013

Auerbach P, Nordby P, Bendtsen LQ, Mehlsen JL, Basnet SK, Vestergaard H, Ploug T, Stallknecht B. Differential effects of endurance training and weight loss on plasma adiponectin multimers and adipose tissue macrophages in younger, moderately overweight men. Am J Physiol Regul Integr Comp Physiol 305: R490–R498, 2013. First published July 10, 2013; doi:10.1152/ajpregu.00575.2012.—Obese individuals are characterized by low circulating adiponectin concentrations and an increased number of macrophages in adipose tissue, which is believed to be causally associated with chronic low-grade inflammation and insulin resistance. Regular physical exercise decreases overall morbidity in obese subjects, which may be due to modulations of inflammatory pathways. In this randomized clinical trial we investigated the separate effects of endurance training-induced weight loss, diet-induced weight loss, and endurance training per se (without weight loss) on plasma adiponectin multimer composition (Western blotting) and adipose tissue macrophage content (immunohistochemistry) in young, moderately overweight men. Weight loss and endurance training per se decreased whole body fat percentage in an additive manner. No intervention-induced changes were observed for plasma total adiponectin. Surprisingly, endurance training, irrespectively of any associated weight loss, shifted the adiponectin multimer distribution toward a lower molecular weight (21% decrease in HMW/LMW, \(P = 0.015\)), whereas diet-induced weight loss shifted the distribution toward a higher molecular weight (42% increase in HMW/MMW, \(P < 0.001\)). Furthermore, endurance training per se increased the number of anti-inflammatory CD163+ macrophages [from 12.7 ± 2.1 (means ± SE) to 16.1 ± 3.1 CD163+ cells/100 adipocytes, \(P = 0.013\)], whereas diet-induced weight loss tended to decrease CD68+ macrophages in subcutaneous abdominal adipose tissue. Thus regular physical exercise influences systemic and adipose tissue inflammatory pathways differently than diet-induced weight loss in younger, moderately overweight men. Our data suggest that some of the health benefits of a physically active lifestyle may occur through modulations of anti- rather than pro-inflammatory pathways in young, overweight men.

- aerobic exercise; obesity; low-grade inflammation; adipokines; cytokines

**ADIPONECTIN** is an adipocyte-derived secretory protein that demonstrates anti-inflammatory properties (33) and insulin-sensitizing effects (12, 42). Adiponectin circulates in different quaternary structures due to the formation of trimers [low molecular weight (LMW)], hexamers [medium molecular weight (MMW)], and larger multimers [high molecular weight (HMW)] (36). Plasma levels of adiponectin are higher in lean than in obese individuals (2). This may be related to the presence of an increased number of macrophages in adipose tissue of obese individuals (41), which is believed to be causally associated with a chronic low-grade inflammatory state characterized by persistent minor (2- to 3-fold) elevations in systemic levels of inflammatory markers such as C-reactive protein (CRP), IL-6, and TNF-\(\alpha\) (13). Depending on the tissue microenvironment, adipose tissue macrophages (ATMs) develop into specialized subtypes with different functional properties and a concept of M1/M2 polarization has been suggested, representing two extremes of a continuum of functional states (16, 29). Based on observations in mice, Lumeng et al. (26) have proposed that ATMs are polarized toward an anti-inflammatory M2 state in lean animals, but that high-fat diet and obesity promote recruitment of pro-inflammatory M1-polarized macrophages and hence the induction of low-grade inflammation.

Lifestyle interventions such as weight loss and increased physical activity level decrease overall morbidity in obese subjects (10), and it may be speculated that these health-improving effects are exerted at least partly through a modulation of inflammatory pathways. Previous studies have indeed shown that a marked reduction in body weight reduces systemic levels as well as the expression and secretion of pro-inflammatory cytokines from white adipose tissue in obese subjects (7, 43). Furthermore, we found that a combination of exercise and a hypocaloric diet lowers systemic inflammation and reduces adipose tissue expression of macrophage markers and various pro-inflammatory cytokines and increases the expression of adiponectin in severely obese subjects (6). Cross-sectional studies have consistently shown that a higher physical activity level is associated with lower concentrations of pro-inflammatory markers (3) and a higher concentration of adiponectin in plasma (38). However, data from intervention studies are conflicting and randomized controlled trials are lacking (3, 38). Furthermore, it is not clear whether the anti-inflammatory health benefits of a physically active lifestyle, if...
any, are due to endurance training per se or result from favorable changes in body composition (40).

Thus the aim of the present randomized clinical trial was to investigate the separate effects of endurance training-induced weight loss, diet-induced weight loss, and endurance training per se (without weight loss) on plasma adiponectin, subcutaneous ATMs and systemic and local adipose tissue pro- and anti-inflammatory markers. We investigated moderately overweight, clinically healthy young men as they possess a great therapeutic potential for prevention of lifestyle-related diseases.

MATERIALS AND METHODS

Subjects. Caucasian overweight men [body mass index (BMI) range 25–30 kg/m²] aged 20–40 years with a body fat percentage >25% were recruited. Participants were healthy according to an interview, took no medications, and had a blood pressure <140/90 mmHg (Omron M7, Omron Healthcare, Kyoto, Japan), a fasting capillary blood glucose <6.1 mM (Hemocue glucose 201+, Hemocue AB, Angelholm, Sweden), and no first-degree relatives with Type 2 diabetes. Only participants that did not engage in regularly physical activity were included as judged by an interview, a questionnaire, and a maximal oxygen uptake ($V_{\text{O}_{2}\text{max}}$) <45 ml O₂·kg⁻¹·min⁻¹.

Sixty men were randomly assigned to a 12-wk intervention of the following: endurance training-induced weight loss (T); diet-induced weight loss (D); endurance training and increased diet without weight loss (T-iD) or control (C). All participants gave fully informed oral and written consent before participation, and the study was conducted in accordance with the Helsinki II Declaration. The study was approved by the Ethical Committee of Copenhagen (H-KF 2006-6443) and registered at http://www.clinicaltrials.gov with the identifying code NCT01090869.

Eight participants withdrew from the study before intervention start because they were dissatisfied with their group assignment (n = 6) or were afraid of the invasive procedures (n = 2). Four participants from the T group did not complete the intervention due to either health-related issues (n = 1), job transfer (n = 1), or because of loss of motivation (n = 2).

The data presented in this article are part of a larger study on the effects of endurance training per se on metabolic health parameters conducted at the University of Copenhagen, Denmark (31).

Diet and exercise protocol. The intervention protocols have been described in details elsewhere (31). In brief, participants in the T and T-iD groups had endurance training (i.e., jogging, cycling, rowing, cross training) prescribed 7 days per week equivalent to an increase in daily energy expenditure of 600 kcal. Three to four days per week the training was intense (~85% of their maximal heart rate (HR) reserve) and for the remaining sessions the intensity was moderate (~65% of HR reserve). The participants wore HR monitors (S640, Polar Electro Oy, Kempele, Finland), which stored energy expenditure and HR data for each exercise session. Participants in the T group were told not to change their energy intake, whereas participants in the T-iD group continued a sedentary lifestyle and reduced daily energy intake with 600 kcal. Participants in the C group were instructed to continue a sedentary lifestyle with an unchanged diet.

Tests. At baseline and postintervention all participants had their height and weight recorded and body composition assessed by dual-energy X-ray absorptiometry (DEXA, DPX-IQ X-ray bone densitometer version 4.7e, Lunar, Madison, WI) after 12 h of fasting. $V_{\text{O}_{2}\text{max}}$ was determined using a graded bicycle ergometer (Ergomulti 800S, Ergo-line, Bitz, Germany) exercise test. An automated on-line system (Oxycon Pro system, Jaeger, Würzburg, Germany) measured pulmonary O₂ uptake and CO₂ production, and HR was monitored by telemetry (Polar WearLink 31 transmitter).

Experimental day. An experimental day was carried out at baseline and after the 12-wk intervention period. Participants were not allowed to exercise the day before the experimental day and arrived by car or public transportation at the laboratory in the morning after 12 h of fasting. The participants recorded their dietary intake the day before baseline experiments and repeated this diet the day before postintervention experiments. A dorsal hand vein was catheterized in retrograde direction and heated in a heating pad before arterialized blood samples were collected. Next, after anesthesia of skin and muscle fascia by lidocaine, large-pore microdialysis catheters (molecular mass cut-off above 950 kDa; Plasmaflo OP-05, Asahi Medical, Tokyo, Japan) were inserted, by use of an 18-gauge cannula, under sterile conditions into the subcutaneous abdominal and femoral adipose tissue as well as into femoral muscle (m. vastus lateralis). The catheters were manufactured in our laboratory as previously described (30) and were perfused with Ringer acetate containing 2 mM glucose at a rate of 1 µl/min using a high precision syringe pump (CMA400, CMA Microdialysis, Solna, Sweden). To obtain steady state after the insertion trauma, microdialysate from the initial 120 min was discarded and hence microdialysate was collected for 60 min. During the initial 120-min period subcutaneous abdominal adipose tissue biopsies were obtained by the method of Bergström (4). The skin of the periumbilical area was anesthetized with lidocaine before making a small incision. Approximately 300 µg of adipose tissue was removed under sterile conditions. Part of the samples were immediately fixed in Zamboni fixative (2% freshly depolymerized paraformaldehyde and 0.15% picric acid) and embedded in paraffin.

Biochemical analysis of plasma and microdialysate. Blood samples were drawn into cooled glass tubes containing either EDTA (for the determination of adipokines and cytokotyes) or Trasylol/EDTA (for the determination of insulin), and plasma was immediately isolated by centrifugation at 4°C and stored at −80°C together with microdialysate until analyzed. Commercially available ELISA kits were used to determine plasma insulin (Dako, Glostrup, Denmark), YKL-40 (Quidel, San Diego, CA), IL-1 receptor antagonist (IL-1ra), and total adiponectin (Quantikine, R&D Systems, Minneapolis, MN). Plasma high sensitivity CRP was determined by nephelometry (Dade Behring Diagnostics, Marburg, Germany). Concentrations of IL-6, TNF-α, IL-1β, IL-10, monocyte chemotactic protein-1 (MCP-1), and leptin in plasma and microdialysate were determined by multiplexed flow cytometric assays on a Luminex 100 system (Luminex, Austin, TX). For the measurement of IL-6, TNF-α, IL-1β, and IL-10 in plasma, a high sensitivity human cytokine kit (HSCYTO-60SK, Linco Research, St. Charles, MO) was used. For the measurement of IL-6 and TNF-α in microdialysate and MCP-1 and leptin in both plasma and microdialysate a human adipokine kit (HADK2-61-K-B, Linco Research) was used.

The relative amounts of plasma total adiponectin and adiponectin multimers were determined in plasma by reducing SDS-PAGE and nonreducing native PAGE, respectively, and Western blotting. For determination of total adiponectin concentration one volume of plasma was mixed with one volume of reducing sample buffer [125 mM Tris (pH 6.8), 4% SDS, 2.5 mM EDTA, 0.0005% bromophenol blue, 20% glycerol, and 100 mM DTT], heated to 100°C for 8 min and separated on a 4–15% Criterion gel (Bio-Rad, Copenhagen, Denmark) under denaturing conditions (192 mM glycine, 0.1% SDS, 24 mM Tris, pH 8.3). A volume corresponding to 0.5 µl plasma was loaded in each well. For adiponectin multimer assessment one volume of plasma was mixed with one volume of native sample buffer [200 mM Tris-HCl (pH 8.6), 20% glycerol, 0.0005% bromophenol blue] at room temperature and separated on a 4–15% Criterion gel (Bio-Rad) under native conditions (192 mM glycine, 24 mM Tris, pH 8.3). A volume corresponding to 1.5 µl plasma was loaded in each well. NativeMARK Unstained Protein Standard (LC0725, Life Technologies, Carlsbad, CA) was used as a molecular weight marker for the...
native PAGE. In both cases proteins were electrophoretically transferred to PVDF membranes (Hybond-P, GE Healthcare, Chalfont St. Giles, UK) for 1 h at 100 V in a tank buffer system (25 mM Tris, 192 mM glycine, 0.008% SDS, and 20% methanol). Membranes were blocked in 5% defatted milk powder in TS buffer [10 mM Tris (pH 7.4), 150 mM NaCl] and incubated for 90 and 60 min with primary and horseradish peroxidase-labeled secondary antibodies, respectively, diluted in blocking solution. Antigen-antibody complexes were visualized by ECL and a CCD system (LAS-3000, Fujifilm, Japan) and quantitated by the analysis software “Multi Gauge” (ver. 3.0, Fujifilm). Aliquots of the same human plasma sample were included in quadruplicates on all gels, and the mean value of their specific signals was used as an “internal standard” for normalization between gels. As primary antibody a rabbit polyclonal antibody against recombinant human adiponectin (RD181023220, BioVendor, Brno, Czech Republic) was used.

Immunohistochemical analysis of subcutaneous abdominal adipose tissue. The paraffin-embedded adipose tissue samples were cut in 5-μm thick sections, deparaffinized, and subjected to heat-induced epitope retrieval at 95°C for 15 min in target retrieval solution (S2367, Dako). Endogenous peroxidase was blocked (S2023, Dako) and sections were incubated with a primary antibody against either CD68 (Ab955: Abcam, Cambridge, UK), CD163 (NCL-163: Dako) and sections were incubated with a primary antibody against either CD68 (Ab955: Abcam, Cambridge, UK), CD163 (NCL-163: Dako), or IL-10 (AH9102: Invitrogen, San Diego, CA). All primary antibodies were detected using Dako’s REAL Envision Detection system (K5007, Dako) and 3’,3’-diaminobenzidine as a chromogen. Because of detection sensitivity concerns only sections incubated with primary antibodies against CD68 and CD163 were counterstained with Mayer’s hematoxylin (S3301, Dako). Negative control sections were incubated without either primary or secondary antibody. To decrease the influence of batch-to-batch variation, the same subject’s pre- and postsamples were run in the same batch, and samples from all four groups were included in each batch. Stained sections were examined in an observer blinded manner with a light microscope (Leica DMRXe microscope, Leica Microsystems, Wetzlar, Germany) and images acquired with a Leica DFC 420C CCD camera and software (Leica Application Suite, version 2.5.0 RI). The same settings of the camera were used for all slides. For each slide, images of eight randomly chosen areas labeled with CD68, CD163, IL-10, or TNF-α were analyzed, representing an area of 200–300 adipocytes.

ATMs were quantified using a similar approach as described by Cancelli et al. (9). Both CD68 and CD163 are highly expressed by human tissue macrophages (18, 25, 34). However, CD163 exists in a soluble form in addition to a membrane bound form in monocytes/macrophages (23), and therefore CD68 and CD163 signals were only counted if an identifiable nucleus was present along with the staining. Since we wanted to determine the number of resident, parenchymal ATMs, monocytes/macrophages located in the stroma-vascular tissue were not counted. The number of macrophages in each slide was expressed as a percentage of the total number of adipocytes observed.

Staining of IL-10 and TNF-α were quantified in ImageJ software (freeware from WS Rasband, ImageJ, National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/, 1997–2008). The images where inverted to eight-bit grayscale and to exclude background intensity an identical threshold was applied to all images. The mean of the intensity of all the pixels (integrated density) above the applied threshold was calculated, representing the total immunostaining of either TNF-α or IL-10. The mean of the integrated density of the eight analyzed images from each slide represented the amount of that particular cytokine in arbitrary units.

Statistics. Data analyses were based on the 48 participants who completed the intervention. SigmaPlot 11.0 (Systat Software, Point Richmond, CA) was used for statistical analysis. Baseline data for all 48 participants are presented as means ± SD and within group changes after the intervention are presented as means ± SE. If data were not normally distributed, they were log transformed before analysis. A one-way ANOVA was used to test for differences between groups at baseline due to potential stratification bias. Also a one-way ANOVA was used to test for baseline differences in microdialysate concentrations between different tissue compartments and an unpaired t-test was used to evaluate differences in adipose tissue CD68 and CD163 content. To evaluate main effects and interactions between groups and time a two-way repeated-measurement ANOVA was performed and when appropriate followed by a post hoc test to locate changes with time within each group. A one-way ANOVA (parametric or nonparametric with appropriate post hoc test) was performed for comparison of delta changes between groups as a result of the intervention. Overall effects of endurance training (T and T-iD), weight loss (D and T), and the interaction between endurance training and weight loss were evaluated by ANCOVA with postintervention data as the dependent variable, training and weight loss as qualitative covariates, and baseline data as quantitative covariate. Pearson Product Moment Correlation (when data were normally distributed) or Spearman Rank order (when data were not normally distributed) were used to test for correlations between different variables. Threshold for significance was set at P < 0.05 giving an 80% power to detect a 35% difference between groups on a parameter with an SD of 25% when performing a one-way ANOVA.

RESULTS

Anthropometric and metabolic variables. Compliance to both training and diet protocols was excellent and anthropometric and metabolic variables improved in all three intervention groups as previously described (31) and summarized in Table 1. Weight loss in the T and D groups averaged ~6% during the 12-wk intervention, whereas T-iD and control groups remained weight stable (Table 1). Whole body fat percentage decreased from pre- to postintervention in all three intervention groups (T and D: P < 0.001; T-iD: P = 0.01), and the change in the T and D groups differed from the change in C (Table 1). VO2max increased in the T and T-iD groups (P < 0.001 for both) and the changes differed from changes in the D and C groups (Table 1).

Table 1. Baseline anthropometric and metabolic variables and changes from baseline after 12 wk of intervention

<table>
<thead>
<tr>
<th></th>
<th>Means ± SD, Baseline</th>
<th>Changes From Baseline to Postintervention, means ± SE</th>
<th>P Value Group Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 48)</td>
<td>T (n = 12)</td>
<td>D (n = 12)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.1 ± 1.3</td>
<td>-1.8 ± 0.2*†§</td>
<td>-1.6 ± 0.2*§</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>93.5 ± 8.0</td>
<td>-5.9 ± 0.7*§</td>
<td>-5.3 ± 0.7*§</td>
</tr>
<tr>
<td>Whole body fat percentage, %</td>
<td>31.3 ± 4.1</td>
<td>-6.8 ± 0.8*†§</td>
<td>-3.2 ± 0.7*†</td>
</tr>
<tr>
<td>VO2max, ml·kg⁻¹·min⁻¹</td>
<td>37.0 ± 5.5</td>
<td>9.0 ± 1.3*†‡</td>
<td>1.2 ± 0.9</td>
</tr>
</tbody>
</table>

T, training; D, diet; T+iD, training and increased diet; C, control; BMI, body mass index; VO2max, maximal oxygen uptake. No statistical differences between the four groups were present at baseline (P > 0.05), and baseline data are therefore presented as means ± SD of all 48 subjects. *Significant change from baseline to postintervention within group; †significantly different from C; ‡significantly different from D; §significantly different from T-iD.
Table 2. Baseline plasma concentrations of adipokines and inflammatory markers and changes from baseline after 12 wk of intervention

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Means ± SD, Baseline (n = 48)</th>
<th>Changes From Baseline to Postintervention: Means (SE)</th>
<th>P Value Group Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T (n = 12)</td>
<td>D (n = 12)</td>
<td>T-iD (n = 12)</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>11 ± 8</td>
<td>−4.6 ± 1.2†</td>
<td>−7.0 ± 2.3†</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>4.3 ± 2.3</td>
<td>−0.4 ± 0.3</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>Adiponectin, AUa</td>
<td>87 ± 59</td>
<td>−3.3 ± 7.2</td>
<td>5.7 ± 5.4</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>1.4 ± 0.5b</td>
<td>0.0 ± 0.1</td>
<td>−0.3 ± 0.2</td>
</tr>
<tr>
<td>YKL-40, ng/ml</td>
<td>33 ± 15</td>
<td>−2.3 ± 7.8</td>
<td>4.4 ± 4.9</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>7.1 ± 2.4</td>
<td>0.0 ± 0.4</td>
<td>−0.3 ± 0.7</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>15 ± 27</td>
<td>0.2 ± 0.8</td>
<td>−2.2 ± 1.3*</td>
</tr>
<tr>
<td>IL-10, pg/ml</td>
<td>11 ± 11</td>
<td>−0.8 ± 1.9</td>
<td>−1.2 ± 0.8</td>
</tr>
<tr>
<td>IL-1β, pg/ml</td>
<td>0.9 ± 1.0</td>
<td>−0.2 ± 0.2</td>
<td>−0.2 ± 0.1</td>
</tr>
<tr>
<td>IL-1ra, pg/ml</td>
<td>251 ± 120</td>
<td>−24 ± 11</td>
<td>−49 ± 42</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>102 ± 36</td>
<td>−0.7 ± 6.3</td>
<td>−8.8 ± 4.6</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; IL-1ra, IL-1 receptor antagonist; MCP-1, monocyte chemoattractant protein-1; AU, arbitrary units. No differences between the four groups were present at baseline (P > 0.05), and baseline data are therefore presented as means ± SD of all 48 subjects. *Significant change from baseline to postintervention within group; †significantly different from T-iD; #endurance training (T, D, T-iD) independently predicts changes in HMW (band 1) and LMW (band 5) as evaluated by ANCOVA with postintervention data as the dependent variable, training and weight loss as qualitative covariates, and baseline data as quantitative covariate.

Adipokines and inflammatory markers in plasma and microdialysate. No intervention-induced changes were observed for total plasma adiponectin (Table 2). Adiponectin multimers were separated into five distinct bands by nonreducing native PAGE (Fig. 1A) where band 1, 4, and 5 represent HMW, MMW, and LMW, respectively, as also suggested earlier by Ebinuma et al. (14). Band 2 and 3 are likely to represent smaller fractions of HMW adiponectin consisting of...
Plasma IL-6 decreased from pre- to postintervention in the D group ($P < 0.001$), but the change was not different from the other three groups (Table 2). Neither plasma CRP, YKL-40, TNF-α, IL-10, IL-1β, IL-1ra, MCP-1 (Table 2) nor microdialysate TNF-α, IL-6, and MCP-1 (Table 3) and adipose tissue TNF-α and IL-10 (Fig. 2, C and D) changed as a result of the intervention, compared with C. However, all of these variables showed large interindividual variation reflected by large SDs ($>25\%$), and the power of the statistical tests were therefore below the desired 80%. For the clinically most relevant inflammatory marker, plasma CRP, the intervention-induced changes compared with C were as follows: T vs. C: $-0.03$ ($-0.26$, $0.20$) [mean (95% confidence interval)]; D vs. C: $-0.35$ ($-0.58$; $-0.12$); and T-iD vs. C: $0.18$ ($-0.05$; $0.41$) mg/l, $P > 0.05$. At baseline ($n = 48$), microdialysate concentrations of TNF-α, IL-6, and MCP-1 were higher in femoral muscle than in adipose tissue, and microdialysate concentration of IL-6 was higher in abdominal than in femoral subcutaneous adipose tissue (Table 3).

### Inflammatory markers in subcutaneous abdominal adipose tissue

CD68 colocalized with CD163 in the adipose tissue as judged by the same localization in two consecutive serial sections, and a positive correlation was found between the number of CD68$^+$ and CD163$^+$ cells at baseline ($r = 0.35$, $P = 0.02$, $n = 48$). However, the actual number of CD163$^+$ cells present in the tissue was higher than the number of CD68$^+$ cells (number of positive cells/100 adipocytes at baseline: CD163: $12.7 \pm 0.9$ vs. CD68: $5.2 \pm 0.7$, $P < 0.001$).

Intervention-induced changes in the number of CD68$^+$ cells in the adipose tissue differed between the D and the T-iD groups displaying a decrease in the D group and an increase in the T-iD group (Fig. 2A). The number of CD163$^+$ cells in adipose tissue increased from pre- to postintervention in the T-iD group ($P = 0.013$) and compared with the C group (Fig. 2B). ANCOVA revealed that neither weight loss nor endurance training independently predicted changes in the number of

### Table 3. Baseline microdialysate concentrations of inflammatory markers and changes from baseline after 12 wk of intervention

<table>
<thead>
<tr>
<th>Microdialysate</th>
<th>Means ± SD, Baseline ($n = 48$)</th>
<th>T ($n = 12$)</th>
<th>D ($n = 12$)</th>
<th>T-iD ($n = 12$)</th>
<th>C ($n = 12$)</th>
<th>$P$ Value Group Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abd. AT</td>
<td>$1.2 \pm 0.7$</td>
<td>$-0.3 \pm 0.2$</td>
<td>$-0.2 \pm 0.3$</td>
<td>$0.1 \pm 0.3$</td>
<td>$0.2 \pm 0.2$</td>
<td>NS</td>
</tr>
<tr>
<td>Fem. AT</td>
<td>$1.5 \pm 1.0^{a,c}$</td>
<td>$0.3 \pm 0.3$</td>
<td>$0.2 \pm 0.4$</td>
<td>$0.0 \pm 0.3$</td>
<td>$1.0 \pm 0.5^{d}$</td>
<td>NS</td>
</tr>
<tr>
<td>Fem. MT</td>
<td>$0.6 \pm 0.4^{b,c}$</td>
<td>$-0.2 \pm 0.1^{+a}$</td>
<td>$-0.4 \pm 0.1^{+a}$</td>
<td>$-0.1 \pm 0.1$</td>
<td>$0.2 \pm 0.1$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abd. AT</td>
<td>$4.5 \pm 6.0$</td>
<td>$1.8 \pm 1.3$</td>
<td>$2.1 \pm 1.9$</td>
<td>$-3.9 \pm 2.2$</td>
<td>$0.1 \pm 1.0$</td>
<td>NS</td>
</tr>
<tr>
<td>Fem. AT</td>
<td>$5.5 \pm 5.8^{c}$</td>
<td>$0.4 \pm 1.1$</td>
<td>$3.6 \pm 2.2$</td>
<td>$-4.9 \pm 2.0^{\dagger}$</td>
<td>$-0.1 \pm 2.0^{d}$</td>
<td>0.023</td>
</tr>
<tr>
<td>Fem. MT</td>
<td>$6.2 \pm 4.3^{c,d}$</td>
<td>$-0.4 \pm 1.1^{d}$</td>
<td>$1.7 \pm 1.5$</td>
<td>$3.8 \pm 2.6$</td>
<td>$-2.5 \pm 1.9$</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abd. AT</td>
<td>$1.1 \pm 0.6$</td>
<td>$0.2 \pm 0.2$</td>
<td>$0.5 \pm 0.4$</td>
<td>$-0.4 \pm 0.3$</td>
<td>$0.3 \pm 0.4$</td>
<td>NS</td>
</tr>
<tr>
<td>Fem. AT</td>
<td>$0.7 \pm 0.8^{c}$</td>
<td>$-0.3 \pm 0.4$</td>
<td>$0.3 \pm 0.1$</td>
<td>$-0.3 \pm 0.2$</td>
<td>$0.1 \pm 0.4^{d}$</td>
<td>NS</td>
</tr>
<tr>
<td>Fem. MT</td>
<td>$2.3 \pm 1.1^{c,b,c}$</td>
<td>$0.3 \pm 0.4^{d}$</td>
<td>$1.2 \pm 0.1$</td>
<td>$0.9 \pm 0.7$</td>
<td>$-0.5 \pm 0.4$</td>
<td>NS</td>
</tr>
<tr>
<td>MCP-1, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abd. AT</td>
<td>$1.1 \pm 0.7$</td>
<td>$0.2 \pm 0.2$</td>
<td>$0.3 \pm 0.3$</td>
<td>$-0.1 \pm 0.4$</td>
<td>$0.2 \pm 0.3$</td>
<td>NS</td>
</tr>
<tr>
<td>Fem. AT</td>
<td>$1.0 \pm 1.3^{c}$</td>
<td>$-0.5 \pm 0.6$</td>
<td>$0.3 \pm 0.4$</td>
<td>$-0.2 \pm 0.2$</td>
<td>$0.0 \pm 0.2^{d}$</td>
<td>NS</td>
</tr>
<tr>
<td>Fem. MT</td>
<td>$4.2 \pm 2.3^{b,c}$</td>
<td>$-0.5 \pm 0.9^{d}$</td>
<td>$0.2 \pm 0.6$</td>
<td>$-0.2 \pm 0.8$</td>
<td>$0.5 \pm 0.9$</td>
<td>NS</td>
</tr>
</tbody>
</table>

MCP-1, monocyte chemoattractant protein-1; Abd., abdominal; Fem., femoral; AT, adipose tissue; MT, muscle tissue. No differences between the four groups were present at baseline ($P > 0.05$), and baseline data are therefore presented as means ± SD of all 48 subjects. *Significant change from baseline to postintervention within group; †significantly different from C; ‡significantly different from D. *Significantly different from Abd. AT ($P < 0.001$); ‡significantly different from Fem. AT ($P < 0.001$). $\alpha = 47$; $\beta = 11$. 

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CD68\(^+\) cells. However, training independently predicted the increase in the number of CD163\(^+\) cells \((P = 0.006)\) in subcutaneous abdominal adipose tissue.

**DISCUSSION**

In this randomized clinical trial we investigated the separate effects of endurance training and diet-induced weight loss on systemic and adipose tissue inflammatory pathways. The main findings were that plasma adiponectin multimer composition was shifted toward a lower molecular weight by endurance training and toward a higher molecular weight by diet-induced weight loss. Also, endurance training per se increased the number of CD163\(^+\) macrophages in subcutaneous abdominal adipose tissue.

Neither endurance training nor diet-induced weight loss affected the systemic levels of total adiponectin. This may be due to the short intervention period and relatively small weight loss (−6%), since it has been suggested that a weight loss of at least 10% is needed to increase systemic levels of total adiponectin (27). We found an increase in HMW adiponectin after diet-induced weight loss, as shown previously (5, 35). However, surprisingly, endurance training both with and without weight loss opposed the effect of diet-induced weight loss by shifting adiponectin multimers toward a lower molecular weight as shown by a decrease in the ratio of HMW to LMW adiponectin. In a randomized clinical trial Ando et al. (1) reported no changes in total plasma adiponectin or adiponectin multimer distribution after 12 wk of combined aerobic and resistance exercise \((2 \times 70 \text{ min/wk})\) in middle-aged, lean males and females. Similar negative results were observed in a noncontrolled study by Christiansen et al. (11) who included obese males and females in a 12-wk aerobic exercise interven-
tion (3 × 60–75 min/wk). However, in a noncontrolled study by O’Leary et al. (32) it was found that 12 wk of endurance training (5 × 60 min/wk) decreased MMW adiponectin without any change in total adiponectin in obese males and females. It thus seems that training-induced changes in adiponectin multimer distribution require rather large and frequent amounts of exercise (≥5 sessions/wk). It is intriguing that endurance training decreases HMW-to-LMW adiponectin ratio, since HMW is thought to be positively associated with insulin sensitivity. We speculate that endurance training may increase the turnover rate of HMW adiponectin. A recent study by Halberg et al. (17) investigated the fate of systemic adiponectin in mice. They found that the clearance rate of adiponectin was lower in high-fat diet and ob/ob models suggesting that delayed clearance is an indicator of metabolic dysfunction. If regular physical exercise increases the turnover rate of HMW adiponectin, our results may reflect an increased usage and subsequent degradation of HMW adiponectin after endurance training. This may explain the lack of an increase in systemic concentrations of total adiponectin in the face of decreased fat percentage in several training intervention studies (11, 32).

Plasma leptin decreased in all three intervention groups with similar relative decreases in the two weight loss groups. Also, we found a strong correlation between changes in fat percentage and plasma leptin. In a recent review, Klimcakova et al. (20) concluded that as little as a 1% weight reduction is enough to significantly decrease plasma leptin. It therefore seems likely that the decrease in plasma leptin in the T-iD group is a consequence of the decreased fat percentage. Plasma leptin correlated with changes in microdialyse leptin obtained from all three tissue compartments suggesting that they all contribute to the systemic decrease.

No changes were observed in systemic and local AT pro- or anti-inflammatory cytokines in the three intervention groups compared with the control group. Selvin et al. (37) showed that plasma CRP decreases with 0.13 mg/l per 1 kg of weight lost. Therefore, we would have expected a reduction in plasma CRP of ~0.7 mg/l in the two weight loss groups since they lost an average of 5.6 kg of body weight. Indeed the 95% confidence limits for the group-wise comparison of D versus C suggest a decrease of up to 0.58 mg/l in the D group if more subjects had been included. Contrary to this, we do not think a potential decrease in the T-D of 0.05 mg/l is of any clinical relevance, although this was not significantly different from the control group. However, our main significant findings were supported by ANCOVA tests revealing that endurance training, independent of weight loss, shifted plasma adiponectin multimer distribution and increased CD163+ ATMs. Moreover, as already addressed, since many of the pro-inflammatory markers showed very large individual variations, negative results should be interpreted with care. Furthermore, a 12-wk intervention is relatively short, therefore our data only reflect early intervention-induced modulations of inflammatory pathways. Improvements in local adipose tissue and systemic cytokine levels may follow after a longer intervention period. Finally, it must be emphasized that quantifying macrophage content by IHC and cell counting is subject to some variation. Nevertheless, no better method exists at present and IHC is the most commonly applied method for cell identification in solid tissues.

In conclusion, endurance training and diet-induced weight loss shift the systemic composition of adiponectin multimers in opposite directions. Also, we show for the first time that endurance training per se increases the content of CD163+ ATMs, which are thought to have anti-inflammatory properties. Contrary to this, diet-induced weight loss tends to decrease adipose tissue macrophage content. Hence, regular physical exercise influences systemic and adipose tissue inflammatory pathways differently than diet-induced weight loss.

**Perspectives and Significance**

During the past decade obesity has been identified as a low-grade inflammatory state that is thought to participate in the development of Type 2 diabetes and cardiovascular disease among others. Our data suggest that some of the health benefits of a physically active lifestyle may occur through modulations of anti- rather than pro-inflammatory pathways in young, overweight men. In the long term this could possibly oppose the effects of obesity-associated low-grade inflammation. Future studies should address whether this also applies to other study populations especially whether any gender differences exist. Also future work should provide more mechanistic
insights of the anti-inflammatory effects of exercise at the cellular level.

ACKNOWLEDGMENTS

We thank Gerda Hau, Jeppe Bach, Regitze Kraunsøe, Thomas Beck, and Ulla Kjærulf-Hansen for expert technical assistance. We also thank our devoted subjects.

GRANTS

Financial support for the study was obtained from the Danish National Research Council; the Ministry of Culture, Committee on Sports Research; the Academy of Muscle Biology, Exercise and Health Research (AMBEHR); the Novo Nordic Foundation; the Danish Diabetes Association; Aase og Einar Skabens Fremme; Else og Mogens Wedell-Wedellsborgs Fond; Fonden af Novo Nordic Foundation; the Danish Diabetes Association; Aase og Ejnar Ulla Kjærulff-Hansen for expert technical assistance. We also thank our

ACKNOWLEDGMENTS

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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