Diet-Induced Weight Loss and Exercise Alone and in Combination Enhance the Expression of Adiponectin Receptors in Adipose Tissue and Skeletal Muscle, but Only Diet-Induced Weight Loss Enhanced Circulating Adiponectin

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Objective: The aim of the study was to investigate the effect of weight loss and exercise independently and in combination on circulating levels of adiponectin including low molecular weight, medium molecular weight, and high molecular weight adiponectin and expression of adiponectin and adiponectin receptors (AdipoR) in adipose tissue (AT) and skeletal muscle (SM).

Design and Methods: Seventy-nine obese males and females were randomized into the following: 1) exercise only (12 wk of exercise without diet restriction); 2) hypocaloric diet [8 wk of very low energy diet (600 kcal/d) followed by 4 wk with a weight maintenance diet]; and 3) hypocaloric diet and exercise (DEX; 8 wk very low energy diet 800 kcal/d followed by 4 wk weight maintenance diet combined with exercise throughout the 12 wk). Blood samples and biopsies from sc abdominal AT and SM were collected at baseline and after 12 wk. The molecular subforms of adiponectin in serum were determined by Western blot.

Results: The mRNA expression of AdipoR1 and -2 in SM was increased significantly in the exercise-only and DEX groups (both $P < 0.05$). The mRNA expression of adiponectin and AdipoRs in AT was increased significantly in all three groups (all $P < 0.01$), whereas serum total circulating adiponectin was significantly increased only in the DEX and hypocaloric diet groups (both $P < 0.01$). All the adiponectin subforms changed in a similar manner as total adiponectin, indicating no specific regulation of any of the subforms by the intervention.

Conclusion: Exercise alone and in combination with a diet-induced weight loss enhance the mRNA expression of adiponectin receptors in AT and in SM but only a pronounced hypocaloric-induced weight-loss increases circulating adiponectin in obese subjects. **(*J Clin Endocrinol Metab* 95: 911–919, 2010)**

Adiponectin is an adipose secreted protein, and the level of adiponectin has been associated with type 2 diabetes, the metabolic syndrome, and cardiovascular disease (1). Although adiponectin is exclusively produced in the adipose tissue (AT) the expression in AT and the circulating levels of adiponectin are reduced in obesity and up-regulated after weight loss. Adiponectin is recognized as a key player in regulation of insulin sensitivity and has antiatherosclerotic and antiinflammatory effects as well (2, 3). Adiponectin mediates its effects through two

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Abbreviations: AdipoR, Adiponectin receptor; AT, adipose tissue; BMI, body mass index; HDL, high-density lipoprotein; HMW, high molecular weight; HOMA, homeostasis model assessment; LMW, low molecular weight; MRI, magnetic resonance imaging; MWW, medium molecular weight; SM, skeletal muscle; VAT, visceral abdominal fat; VO_2max, maximal oxygen uptake.

distinct membrane receptors: adiponectin receptor (AdipoR)-1 and AdipoR2 (4). AdipoR1 and AdipoR2 are expressed in various cell types including liver cells (4), adipocytes (5), and muscle cells (6) and are suggested to mediate the effect of adiponectin through different pathways in which some of the more important are activation of the AMP-activated protein kinase system, stimulation of peroxisomal proliferator-activated receptor-α activities, and increasing fatty acid oxidation and glucose uptake (7). Circulating adiponectin is found in different isoforms including low molecular weight (LMW), medium molecular weight (MMW), and high molecular weight (HMW) with the latter suggested as being the active biological form of the protein. Recent studies suggested that HMW adiponectin, the ratio of HMW form to total adiponectin, is more likely to be associated with type 2 diabetes and the metabolic syndrome than total level of adiponectin (8, 9).

Whether diet-induced weight loss differentially affects multimeric complexes of adiponectin is, however, unknown, and contradictory results have been published (10–13). Both weight loss and exercise enhance the insulin sensitivity (14, 15) and the weight loss-induced improvement in insulin sensitivity may partly be mediated by up-regulation of adiponectin, resulting in enhanced adiponectin effects (16). Moreover, we recently found that weight loss also up-regulated the gene expression of AdipoRs in human AT (5). Thus, weight loss may increase the action of adiponectin by both up-regulating adiponectin itself and increasing the number of receptors. Whether the improvement in insulin sensitivity induced by exercise also involves the adiponectin system is still not fully elucidated. In a recent review, the main part of the studies found no effect of exercise on adiponectin in the circulation (17). In relation to the adiponectin receptors, some studies (18, 19) but not all (20, 21) reported an increased level of AdipoR expression in AT and skeletal muscle (SM) in response to regular exercise. Thus, the link between exercise and improved insulin sensitivity may involve the AdipoRs. In contrast to exercise alone, diet-induced weight losses of a given magnitude ($\geq$10%) are generally followed by increased circulating levels of adiponectin (22).

The aim of the present study was to investigate the effect of weight loss and exercise independently and in combination on the adiponectin system: serum adiponectin (total adiponectin and the three molecular forms of adiponectin, HMW, MMW, and LMW) and expression of adiponectin and AdipoR expression in AT and SM to determine the possible effect of the adiponectin system on the biological effects of weight loss and exercise on metabolic risk factors. This was investigated in a randomized study in which overweight subjects were treated with diet-induced weight loss, exercise, or a combination of both for 12 wk.

Subjects and Methods

Subjects

Seventy-nine obese but otherwise healthy Caucasian males and females were recruited via advertisements in local newspapers. The subjects were eligible for inclusion if they were aged 18–45 yr, obese [30 kg/m^2 $<$ body mass index (BMI) $<$ 40 kg/m^2], physically inactive ($<$30 min/d), and weight stabile for at least 3 months ($\pm$2 kg of current body weight). Exclusion criteria were cardiovascular disease, type 2 diabetes, pregnancy, or orthopedic difficulties causing inability to undertake an exercise program. No subjects received medication that could affect the investigated metabolic markers. The study was approved by the local ethic committee in the county of Aarhus.

Study design

Seventy-nine subjects were randomized into the 12-wk intervention study consisting of the following: 1) exercise only (EXO; n = 25), 2) hypocaloric diet (DIO; n = 29), or 3) hypocaloric diet and exercise (DEX; n = 25). Twenty subjects did not complete the study (eight women and 12 men; BMI 35.7 $\pm$ 4 kg/m^2; P = 0.2 compared with subjects who completed the study).

Diet regimen

Subjects in the DIO and DEX groups were prescribed a liquid VLED (Nupo, Copenhagen, Denmark) of, respectively, 600 and 800 kcal/d (proteins 41 g, carbohydrates 29 g, fat 5.6 g per 100 g) for 8 wk followed by a weight maintenance diet for 4 wk. The subjects in the DEX group were allowed to consume 150–200 kcal more per day compared with the DIO group, reflecting the estimated extra energy expenditure of 1500 kcal/wk during exercise activity to obtain similar weight losses in the two groups. In the weight-maintenance phase, the subjects consumed a diet with the following energy contents: 55% from carbohydrates, 15% from protein, and less than 30% from fat. The subjects in the EXO group were advised to maintain an isocaloric diet for the duration of the intervention. The daily energy intake was comparable in the EXO group before and at the end of the study (2610 $\pm$ 582 *vs.*2467 $\pm$ 410 kcal; P = 0.6), and the energy distribution was similar with 55% carbohydrates, 15% protein, and 30% fat. All subjects in the three groups were asked to keep dietary intake records over a 2-wk period.

Exercise regimen

The exercise intervention for subjects in the EXO and DEX groups consisted of supervised aerobic exercise three times per week with a duration of 60–75 min per training session, with an estimated energy expenditure of 500–600 kcal per session (23). The subjects were required to keep records of training sessions during the whole intervention.

Maximal rate of oxygen uptake

At baseline and after 12 wk, each subject completed a progressive maximal exercise test using a stationary cycle ergometer (Monark 828; Monark Exercise AB, Vansbro, Sweden) and stan-

dard open-circuit spirometry techniques (AMIS 2001; Innovision, Odense, Denmark).

Anthropometry, body fat distribution, and metabolic risk factors

Body weight and waist and hip circumference were measured at baseline, at wk eight, and after 12 wk. Blood pressure was measured on the left arm with the use of an automated blood pressure monitor after the subjects had 5 min at rest. To determine changes in fat mass in various abdominal fat depots, a multislice magnetic resonance imaging (MRI) scanning was performed before and at the end of the interventions as previously described (23). Blood samples were collected after an overnight fast and at least 24 h after the subjects had finished the last exercise session.

AT and SM biopsies

At baseline and after 12 wk, AT biopsies were obtained from the abdominal sc AT depot. The skin was anesthetized with lidocaine (10 mg/ml) before a small incision was made and about 200 mg of AT were removed under sterile conditions using a liposuction needle. Immediately after removal, the AT sample was washed in isotonic NaCl, snap frozen in liquid nitrogen, and kept at −80 C until RNA extraction. The SM biopsies were obtained from the vastus lateralis muscle at baseline and after 12 wk. Skin and muscle fascia were anesthetized with lidocaine (10 mg/ml), and under sterile conditions a 1-cm incision was made, in which after about 100 mg of muscle tissue were removed using the conchotome biopsy technique. The SM biopsies were dissected free of visible fat, snap frozen in liquid nitrogen, and kept at 80 C until mRNA extraction. To minimize a carryover effect of the last exercise bout, the biopsies in AT and SM were taken 24–48 h after the last exercise bout.

Determination of adiponectin, plasma lipids, glucose, and insulin

Adiponectin was measured using a human specific high sensitive ELISA method (B-Bridge International, San Jose, CA) with an intraassay coefficient of 5% (n = 12).

Cholesterol, triglycerides, and glucose were analyzed at the local university department of clinical biochemistry. Insulin was analyzed with an ELISA (Dako, Cambridgeshire, UK). The homeostasis model assessment (HOMA) insulin resistance index was calculated using the formula: fasting insulin (microunits per milliliter) fasting glucose (millimoles per liter)/22.5 (24).

Quantitation of adiponectin oligomers by Western blot

One volume of serum (1.5 μl) was mixed with one volume of 2× native sample buffer [200 mM Tris-HCl (pH 8.6), 20% glycerol, 0.005% Bromophenol Blue] at room temperature, separated on a 4–15% Criterion gel (Bio-Rad Laboratories, Herlev, Denmark) under native conditions [192 mM glycine, 24 mM Tris (pH 8.3)] and electrophoretically transferred to polyvinylidene difluoride membranes for 1 h at 100 V in a tank buffer system (25 mM Tris, 192 mM glycine, 0.008% sodium dodecyl sulfate, 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 enhanced chemiluminescence and a charge-coupled device system (LAS-3000; Luminescent image analyzer; Fujifilm, Tokyo, Japan) and quantitated by the analysis software Multi Gauge (version 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. The average relative abundance of the HMW adiponectin was 43%, MMW adiponectin 25%, and LMW adiponectin 18%.

RNA isolation and RT-PCR analysis

RNA was isolated as previously described (20). The mRNA levels of the target genes (adiponectin, AdipoR1, AdipoR2) were expressed relative to the housekeeping gene (β-actin). Quantification was as previously described, performed with a SYBR Green real-time PCR assay using an iCycler PCR machine (Bio-Rad Laboratories) (20). All samples were determined in duplicate. The threshold cycle was calculated, and the relative gene expression was calculated essentially as described in the user bulletin 2 (1997; PerkinElmer Cetus, Norwalk, CT). The oligonucleotide primer pairs used were: adiponectin, sense primer, 5′-CATGACCAGGAAACCACGACT-3′, antisense primer, 5′-TGAATGCTGAGCGGTAT-3′; AdipoR1 sense primer, 5′-CCGGTTTGCCACTCCTAAGC-3′, antisense primer, 5′-TGACAAAGCCCTCAGCGATAG-3′; AdipoR2, sense primer, 5′-AGGCCGCCACCATAGGG-3′, antisense primer, 5′-CGCCGATCAAACGAAACT-3′; and β-actin primer: ACGGGGTCACCCACACTGTGC and CTAGAAGCATTTGCGGTGGACGATG.

Statistical analysis

The statistical software packet SPSS (SPSS, Chicago, IL) was used for all statistical evaluation. Descriptive statistics are presented as means ± SD or as means with confidence interval of 95%. Group differences at baseline were examined using multivariate analysis of variance with adjustment for multiple comparisons. The absolute changes and percent changes from baseline to wk 12 were calculated for selected variables. Multivariate analysis of variance was used to test the interaction of treatment (EXO, DEX, DIO) and gender (male and female). A linear regression model was used to test the association between selected variables. A nonparametric test was used for variables with a nonnormal distribution. The chosen significance level was a two tailed $P < 0.05$.

Results

Body weight, metabolic markers, and maximal oxygen uptake (VO_2max)

Changes in body weight, fat distribution, and VO_2max after the 12-week intervention are displayed in Fig. 1 and Table 1. Subjects in the EXO group improved their VO_2max by 18% ($P < 0.01$). Body weight was reduced by 3.5% (−3.5 ± 3 kg; $P < 0.01$) together with a significant

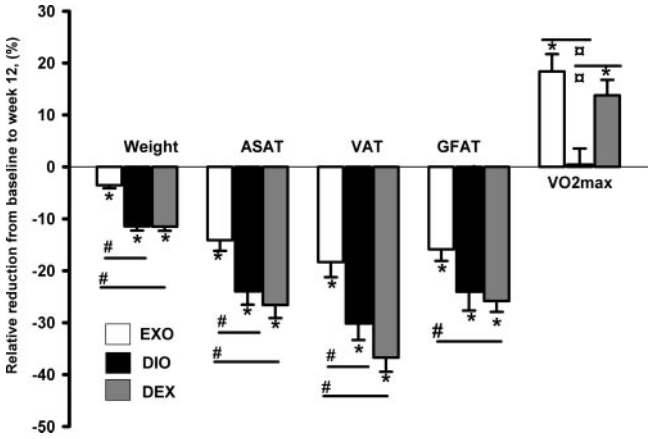

FIG. 1. Changes in body weight, fat mass, and VO_2max during the intervention. Relative changes in body weight, sc abdominal fat (ASAT), VAT, and gluteal femoral adipose tissue (GFAT) determined by MRI and VO_2max in the three groups: EXO, DIO, and DEX. *, $P < 0.01$ compared with baseline; #, $P < 0.01$ compared with changes in the EXO group; ¤, $P < 0.01$ compared with changes in the DIO group.

reduction in waist circumference (−5.3 cm; $P < 0.01$, Table 1). The relative reduction in all of the anthropometrical parameters was, however, significantly smaller in the EXO group compared with the reductions found in the DIO and DEX groups (Table 1.) Weight losses in the DEX and DIO groups were about 11% after 12 wk. Reduction of all the other anthropometric parameters in the DEX and DIO groups were also comparable ($P > 0.05$, Table 1). Subjects in the DEX group increased their VO_2max with 14% ($P < 0.01$), whereas no changes in VO_2max were observed in the DIO group. In the DEX group, high-density lipoprotein (HDL) cholesterol was increased significantly after 12 wk ($P < 0.05$). HOMA was reduced significantly after 12 wk in the DIO and DEX groups (both $P < 0.01$ compared with baseline), but in EXO only a nonsignificant trend reduction in HOMA was observed ($P = 0.09$, Table 1.)

Circulating adiponectin

In the DIO group, total adiponectin after 8 wk was nonsignificantly increased by 9% ($P = 0.1$), but after 12 wk total adiponectin was significantly increased by 19% ($P < 0.01$, Table 1 and Fig. 2). In the DEX group, total adiponectin was significantly increased after 12 wk by 20% ($P < 0.01$, Table 1 and Fig. 2), similar to the increase in the DIO group. In the EXO group, total adiponectin was not increased by the exercise intervention; actually a nonsignificant reduction by 6%; ($P = 0.2$) was observed (Fig. 2). So the changes in total adiponectin in the DIO and DEX groups were significantly higher than in the EXO group ($P < 0.01$, Table 1 and Fig. 2).

To access whether the adiponectin multimeric complexes were differently regulated during the intervention, we compared the relative changes after 12 wk for each of

TABLE 1. Baseline values and changes at wk 8 and 12

	EXO			DIO			DEX		
	Baseline	Change wk 8	Change wk 12	Baseline	Change wk 8	Change wk 12	Baseline	Change wk 8	Change wk 12
n	19 (10♀ and 9♂)			19 (9♀ and 10♂)			21 (11♀ and 10♂)		
Age (yr)	37.2 ± 7			35.6 ± 7			37.5 ± 8		
Anthropometry									
Weight (kg)	100.9 ± 10	−2.9 (−4.0, −1.9)[a]	−3.5 (−4.7, −2.1)[a]	107.8 ± 12	11.2 (−12.5, −9.2)[a,b]	−12.3 (−14.4, −10.3)[a,b]	105.8 ± 15	12.1 (−14.2, −9.9)[a,b]	−12.3 (−14.6, −10.0)[a,b]
BMI (kg/m^2)	33.4 ± 4	−0.9 (−1.5, −0.7)[a]	−1.1 (−1.6, −0.7)[a]	35.3 ± 4	−3.7 (−4.1, −3.2)[a,b]	−4.0 (−4.7, −3.4)[a,b]	34.2 ± 3	−3.9 (−4.5, −3.3)[a,b]	−4.0 (−4.6, −3.3)[a,b]
Waist (cm)	104.5 ± 6	−4.8 (−6.9, −3.7)[a]	−5.3 (−6.8, −3.7)[a]	110.8 ± 9	−10.0 (−13.4, −9.2)[a,b]	−11.5 (−13.5, −9.4)[a,b]	109.5 ± 10	−12.3 (−13.4, −9.2)[a,b]	−12.3 (−14.4, −10.1)[a,b]
Metabolic									
HDL cholesterol (mmol/liter)	1.3 ± 0.4		−0.04 (−0.18, 0.09)	1.2 ± 0.3		−0.02 (−0.11, 0.00)	1.2 ± 0.3		1.4 (0.4, 2.3)[a,b]
HOMA	2.2 ± 1		−.4. (−.9, .1)	3.1 ± 2		−1.0 (−1.5, −0.5)[a]	3.2 ± 2		−1.2 (−2.0, −0.3)[a]
VO_2max (liters/$O_2 \cdot m^{-1}$)	2.8 ± 0.7		0.49 (0.34, 0.64)[a,c]	2.8 ± 0.7		0.00 (−0.17, 0.17)	3.0 ± 0.6		0.40 (0.19, 0.62)[a,c]
Adiponectin									
Total adiponectin (ng/nl)	9.2 ± 3		−0.54 (−0.4, 1.5)	7.4 ± 3		1.5 (0.6, 2.5)[a,b]	7.8 ± 2		1.4 (0.6, 2.1)[a,b]
Total adiponectin (%)			−5.7 (4, −15)			18.6 (6.2, 31.1)[a,b]			19.7 (9.4, 29.6)[a,b]
Complexes (%)									
LMW adiponectin			−15 (−30, 11)			11 (42, −14)			6 (23, −11)
MMW adiponectin			−10 (−20, 18)			23 (7, 36)			4 (20, −11)
HMW adiponectin			−8 (−30, 11)			4 (−3, 56)			2 (10, −19)

Baseline data are presented in mean ± SD. Changes (δ) in anthropometric and metabolic variables from baseline to wk 8 and 12 are presented with mean and 95% confidence interval. Changes concerning adiponectin complexes are shown with mean relative values and 95% confidence interval.

[a] $P < 0.05$ as compared with baseline; [b] $P < 0.05$ as compared with EXO; [c] $P < 0.05$ as compared with DIO.

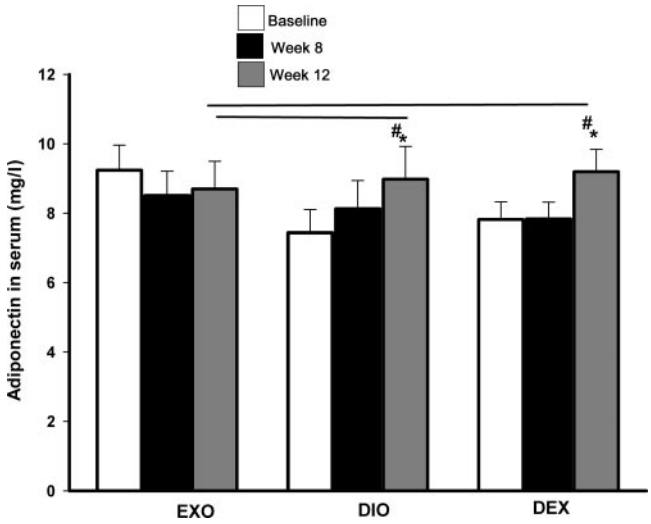

FIG. 2. Changes in circulating adiponectin. Serum levels of total adiponectin at baseline, wk 8m and after 12 wk in the three groups: EXO, DIO, and DEX. *, $P < 0.01$ compared with baseline; #, $P < 0.01$ changes in the different isoforms of adiponectin from baseline to wk 8 or wk 12 in the DIO and DEX groups compared with changes in the EXO group.

the three adiponectin forms (HMW, MMW, and LMW) with a Western blot method as described in *Patients and Methods*. The changes in the three molecular subforms in the three intervention groups followed generally the same changes as observed for total adiponectin (small decrements in the EXO group and increments in the DIO and DEX groups for all of the three adiponectin subforms), but the changes did not reach significance between any of the groups (Table 1 and Fig. 3).

Expression of adiponectin and AdipoRs in AT

As shown in Fig. 4, the expression of adiponectin in AT was significantly increased after the 12-wk intervention in all three groups (100–120% increase as compared with baseline; $P < 0.01$ for all three groups), with no significant differences between the three groups. Almost similar increments were found for the AdipoR1 and AdipoR2 in AT after the intervention with an increase of 70–100% compared with baseline ($P < 0.01$, Fig. 4) with no differences between the three groups.

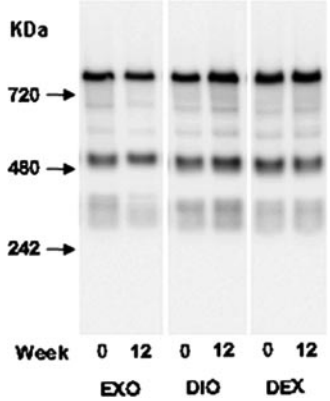

FIG. 3. Western blot of adiponectin multimeric complexes. A representative Western blot of serum levels of adiponectin multimeric complexes separated by NATIVE-PAGE (non-heated, non-denatured) at baseline and after 12 wk of intervention in the three groups: EXO, DIO, and DEX.

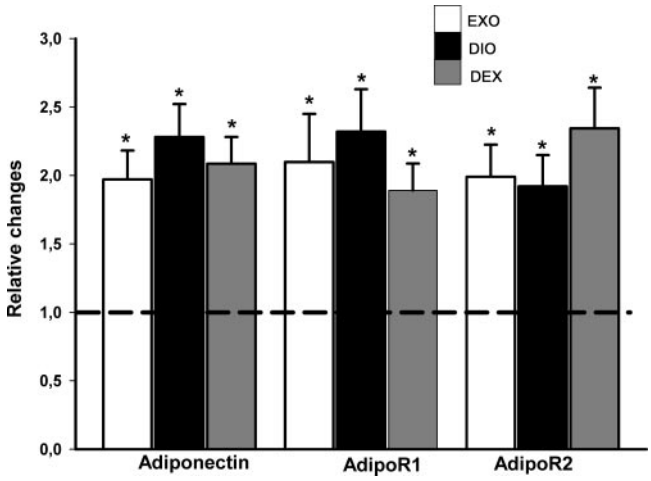

FIG. 4. Changes in adiponectin expression in AT. Relative changes (calculated as the ratio of wk 12 and baseline relative to index 1) in adiponectin and AdipoR1 and AdipoR2 relative to β-actin in sc abdominal adipose tissue after 12 wk in the three groups: EXO, DIO, and DEX. *, $P < 0.01$ compared with baseline.

Expressions of AdipoRs in SM

In adipose tissue the expression of AdipoR1 compared with AdipoR2 relative to β-actin was 3- to 4-fold higher ($P < 0.01$; data not shown), and in skeletal muscle the expression of AdipoR1 compared with AdipoR2 was 3- to 4-fold higher ($P < 0.01$; Fig. 5). In the two exercise groups (EXO and DEX), a significant increment was found in the expression of AdipoR1 and AdipoR2 after the 12-wk intervention compared with baseline (Fig. 5). In the DIO

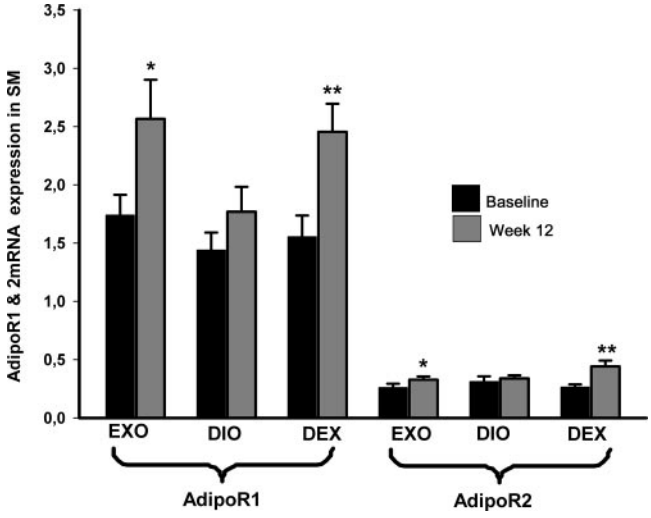

FIG. 5. Changes in AdipoR1 and AdipoR2 expression in SM. mRNA expression of AdipoR1 and AdipoR2 in SM biopsies from vastus lateralis at baseline and after 12 wk in the three groups: EXO, DIO, and DEX (expressed relative to β-actin). *, $P < 0.05$; **, $P < 0.01$ compared with baseline.

group, a nonsignificant increment in AdipoR2 expression was observed ($P = 0.12$). Comparison between the three groups revealed no significant differences in the expression levels after the intervention (Fig. 4). However, a nonsignificant higher expression of AdipoR1and AdipoR2 was found in the DEX group compared with the DIO group ($P = 0.09–0.1$; Fig. 5).

Influence of gender

The circulating levels of total adiponectin at baseline were, as expected, significantly higher in females as compared with males (9.3 *vs.* 6.7 mg/liter; $P < 0.01$). Moreover, AT expression of adiponectin tended to be higher in females (9.3 *vs.* 7.0; $P = 0.3$). Expression of AdipoR1 (1.0 *vs.* 0.6; $P < 0.05$) and AdipoR2 (0.5 *vs.* 0.3; $P < 0.05$) in AT were also higher in females, whereas in SM the expression of AdipoR1 (1.57 *vs.* 1.59; p0.9) and AdipoR2 (0.28 *vs.*0.27) were comparable between sexes (data not shown). In the DIO and EXO groups, the absolute and relative increment in total adiponectin was comparable between gender (data not shown). No other gender differences within the three groups regarding relative changes or absolute changes in expression of adiponectin or AdipoRs in AT or SM were observed (data not shown).

Correlations

Across the three groups, the changes in total adiponectin were inversely associated with relative changes in body weight ($r = -0.46$; $P < 0.01$, data not shown) and relative changes in fat mass (calculated as visceral AT + abdominal sc AT + gluteal femoral AT) (Table 2). Moreover, across the groups absolute changes in total adiponectin were associated with changes in HDL cholesterol ($r = 0.47–0.53$; $P < 0.01$; Table 2).

At baseline total adiponectin was positively associated with the gluteal-femoral fat mass ($r = 0.43$; $P < 0.01$, data not shown) and HDL cholesterol ($r = 0.62$; $P < 0.01$, Table 2) and inversely associated with the ratio of visceral abdominal fat (VAT) to gluteal fat and HOMA ($r = -0.38$; $P < 0.01$, data not shown). Moreover, baseline values of total adiponectin was correlated with mRNA levels of adiponectin in AT ($r = 0.42$; $P < 0.01$).

Discussion

Adiponectin is associated with insulin sensitivity, type 2 diabetes, and cardiovascular disease and is suggested to mediate its effect through two distinct adiponectin membrane receptors: AdipoR1and AdipoR2. Because both exercise and weight loss have been shown to increase insulin sensitivity and prevent type 2 diabetes and cardiovascular diseases, we investigated in the present study how the adiponectin system (adiponectin and its receptors) may be affected by exercise and weight loss, respectively. We found that circulating adiponectin increased only in association with diet-induced weight loss and not after 12 wk of exercise training. These findings were independent of whether exercise was performed alone or in combination with the diet-induced weight loss and independent of the fact that exercise alone was also associated with a weight loss of 3.5 kg. In contrast, it was shown that both diet-induced weight loss and exercise enhance the expression of adiponectin and adiponectin receptors in AT, whereas only exercise

TABLE 2. Correlation matrix of baseline values and changes during the intervention

Values	Total adiponectin	Adiponectin (AT)	AdipoR1 (AT)	AdipoR2 (AT)	AdipoR1 (SM)	AdipoR2 (SM)
Baseline values						
Fat mass (cm^3)[a]	0.17	−0.05	0.02	0.04	0.07	0.06
VAT (cm^3)	−0.54**	−0.23	−0.28*	−0.26	0.02	0.04
HDL cholesterol	0.62**	0.32*	0.31*	0.23	0.04	−0.02
HOMA	−0.36**	0.05	0.10	0.14	−0.07	0.10
Insulin	−0.37**	−0.07	0.13	0.18	−0.04	0.13
Total adiponectin		0.42**	0.35**	0.26	−0.04	−0.20
	Δ Total-adiponectin	**Δ Adiponectin (AT)**	**Δ AdipoR1 (AT)**	**Δ AdipoR2 (AT)**	**Δ AdipoR1 (SM)**	**Δ AdipoR2 (SM)**
δ Values during the intervention						
Δ Fat mass (cm^3)[b]	−0.52**	−0.08	−0.06	0.01	−0.08	−0.16
Δ VAT (cm^3)[b]	−0.21	−0.16	−0.2	−0.12	0.04	−0.12
Δ HDL cholesterol	0.53**	0.06	−0.08	0.11	0.22	0.12
Δ HOMA	−0.14	0.14	−0.05	0.12	0.03	−0.08
Δ Insulin	−0.11	0.15	−0.04	0.13	0.03	−0.02
Δ Total adiponectin		0.17	0.03	0.21	0.22	0.09

[a] Fat mass calculated as visceral fat + sc abdominal fat + gluteal femoral fat quantified with MRI technology; [b] relative changes; *, $P < 0.05$; **, $P < 0.01$.

alone and in combination with a diet-induced weight loss enhanced the expression of adiponectin receptors in SM.

The effect of regular aerobic exercise without diet restriction in most studies (25, 26) but not all (18) are found to have no or little effect on the circulating level of adiponectin, which is in accordance with our present study. Circulating adiponectin is found in different isoforms, LMW, MMW, and HMW adiponectin, in which the latter have been suggested to be the biologically most active form (8, 9). Changes in the adiponectin multimeric complexes in response to diet-induced weight loss yielded contradictory results with an increase in all multimeric complexes after a diet-induced weight loss in one study (12), but in others only an increase in MMW (10) or HMW (13) were observed. The reason for this diversity is not known but could be due to differences in the obtained weight loss, different weight loss periods, problems in measuring the adiponectin subforms, or too small sample sizes. In the present study the changes in the three adiponectin forms were comparable in all three groups (Fig. 3). Thus, in the present group of obese subjects, we found no differential regulation of the adiponectin isoforms induced by neither exercise nor diet-induced weight loss.

We and others have proposed that to increase circulating levels of adiponectin, a weight loss of at least 10% of the initial body weight is needed (22). Our data confirm to some extent this hypothesis as subjects in the DIO and DEX group at the end of the study had reduced their body weight with 11% accompanied with a 20% increase in total circulating adiponectin. In addition to the magnitude of the weight loss, the duration of the weight loss period could possibly also play a role for establishing significant changes in the circulating adiponectin concentration. Recently it was shown that circulating levels of adiponectin were unchanged in obese subjects after 8 wk of VLED despite a 11% weight loss (27). Interestingly, we also found unchanged level of adiponectin after 8 wk of VLED with an 11–12% weight loss, whereas during the following 4 wk of weight maintenance, adiponectin was increased with 20% (Fig. 2). Thus, as our data suggest, the concentration of adiponectin may be influenced by both the magnitude of the weight loss and the duration of the weight loss period and possibly also of the actual energy balance (*e.g.* negative *vs.* neutral energy state), which perhaps partly can explain the discrepancies in the literature regarding the effect of weight loss on circulating adiponectin.

The expression of adiponectin was enhanced in AT after both exercise and diet-induced weight loss. It is well known that weight loss is associated with increased adiponectin expression (5, 20, 28), but the effect of exercise on AT adiponectin is less investigated. To what extent the observed increase in adiponectin expression in AT in the EXO group is mediated by exercise *per se* is unknown, but because exercise in the EXO group was also associated with a 3.5% weight loss, it is suggested that the increase in adiponectin expression in AT after exercise may be mediated by the exercise-induced weight loss. Why weight loss may up-regulate adiponectin expression in AT is not fully elucidated, but the weight loss-induced reduction of macrophage infiltration in AT and reduced AT inflammation (29) may play a role because we previously have shown that proinflammatory cytokines decrease adiponectin mRNA in AT (30). Why enhanced adiponectin expression in AT after diet-induced weight loss is followed by increased levels of circulating adiponectin and similar enhanced expression of adiponectin after exercise was not is unknown. Although at baseline we found a significant correlation between total adiponectin and expression of adiponectin in AT, the association between gene expression and protein levels may be dubious and possibly influenced by several factors. The release of adiponectin from AT into the circulation involves a complex and not fully understood regulation in adipocytes (31). The blood concentration of adiponectin is the balance between secretion and degradation and the effect of exercise on adiponectin degradation is completely unknown but might be enhanced. Finally, the exercise-induced weight loss in the EXO group may not be large enough to affect the circulating level of adiponectin (22).

Both exercise and diet-induced weight loss increased the expression of AdipoR1 and AdipoR2 in AT and SM. However, only in the exercise groups (EXO and DEX), the expression of AdipoRs in SM was increased significantly. In animal models, reduced plasma levels of insulin have been found to be associated with an increase in the AdipoR expression, whereas insulin treatment decreased the AdipoR expression (32). Thus, the regulation of the AdipoR expression may be inversely regulated by plasma levels of insulin, although we were unable to detect correlations between levels of insulin and AdipoR expression in AT or SM in the present study. It has previously been shown that 4 wk of exercise training was associated with an increase in the AdipoR expressions in SM in diabetics subjects and subjects with impaired glucose tolerance and that the exercise-induced improvements in insulin resistance were associated with this increase in the SM AdipoR expression (19). Thus, in accordance with this study, our data are in agreement with the suggestion that the AdipoR may play a role for the insulin-sensitizing effect of physical activity (19). It has been shown in some studies that the AdipoR1 and AdipoR2 expression in AT, in agreement with our results, were enhanced after diet-induced weight loss in obese subjects (5, 20), whereas other studies have found no effect of neither exercise (21) nor diet restriction (20) on the

AdipoRs in AT. Thus, changes in AdipoR1 and AdipoR2 in AT and SM in response to weight loss and exercise training have so far yielded conflicting results (5, 19, 20, 33, 34), which highlights the need for further studies to elucidate the regulation of the AdipoR.

Concerning gender differences, we found as expected that circulating levels of adiponectin were increased in females compared with males. In addition, we observed higher expression of adiponectin and AdipoR1 in AT in females compared with males, whereas no gender differences were observed in AdipoR2 in AT and AdipoR1 and 2 in SM. In contrast to these sex dimorphisms at baseline, we found no gender differences in the effect of exercise and weight loss on the adiponectin system.

A major strength in this study is the randomized control combined with a careful monitored diet regimen throughout the 12 wk and the supervised exercise sessions in the two exercise groups. There are some limitations to the study. It is not known whether the enhanced gene expression of AdipoR1 and AdipoR 2 in AT and SM reflects increased receptor protein. It has previously been found that even though the expression of AdipoR1 and AdipoR2 in SM was increased in response to exercise, the protein level was unchanged (35). Moreover, some recent studies questioned the importance of the adiponectin receptors in mediating the effect of adiponectin (36). In this context, T-cadherin, an adhesion molecule expressed on the vascular endothelial cells, has recently been suggested to act as a third AdipoR (37). Because T-cadherin is found abundantly expressed in injured endothelial cells in atherosclerotic regions, the antiatherogenic effect of adiponectin may in part be mediated through this receptor. Another limitation of the study is the differences in the obtained weight loss. Thus, due to the higher reduction in body weight and body fat distribution in the DIO and DEX group as compared with the EXO group, a direct comparison between the results achieved in the DIO and DEX group with the EXO group could underestimate the effect of regular exercise.

In summary, we have shown that only diet-induced weight loss increases circulating adiponectin in obese subjects. Moreover, we have shown that exercise alone and in combination with a diet-induced weight loss enhance the mRNA expression of AdipoRs in AT and SM, but both diet-induced weight loss and exercise enhance the expression of adiponectin and AdipoRs in AT and SM. Whether changes in the expression of AdipoRs are reflected to changes in the receptor protein and the biological effects remains to be elucidated and should be investigated in future studies. The study has, however, demonstrated that the health-promoting effect of weight loss and exercise may involve the adiponectin system.

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