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Circulating Adiponectin and Expression of Adiponectin Receptors in Human Skeletal Muscle: Associations with Metabolic Parameters and Insulin Resistance and Regulation by Physical Training

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Context: Adiponectin, an adipocyte-secreted hormone, is associated with insulin resistance and the metabolic syndrome.

Objective: The physiological regulation of circulating adiponectin levels and mRNA expression of its receptors (AdipoR1 and AdipoR2) in skeletal muscle remains to be fully elucidated.

Design/Patients: We assessed circulating adiponectin and AdipoR1/R2 mRNA expression in human skeletal muscle in a cross-sectional study of 140 subjects with normal or impaired glucose tolerance or type 2 diabetes. In the context of an interventional study, the same measurements were performed in 60 of these subjects (20/glucose tolerance group) before and after 4 wk of physical training. Finally, we measured these same variables in addition to protein levels of AMP kinase (AMPK), acetyl phosphorylated AMPK, coenzyme A carboxylase, phosphorylated coenzyme A carboxylase, and phosphatidylinositol 3-kinase in muscle before and after 3 h of intensive exercise in a subgroup of five subjects.

Setting: This study was performed at an academic clinical research center.

Results: Circulating adiponectin was negatively associated, whereas AdipoR1/R2 mRNA levels were positively associated with obesity, glucose and lipid levels, and insulin resistance. Physical training for 4 wk resulted in increased circulating adiponectin levels and AdipoR1/R2 mRNA expression in muscle. Exercise for 3 h increased AdipoR1/R2 mRNA expression as well as phosphorylation of AMPK and acetyl coenzyme A carboxylase in muscle, but had no effect on circulating adiponectin.

Conclusions: Adiponectin, AdipoR1, and AdipoR2 are all associated with body composition, insulin sensitivity, and metabolic parameters. Physical training increases circulating adiponectin and mRNA expression of its receptors in muscle, which may mediate the improvement of insulin resistance and the metabolic syndrome in response to exercise. **(*J Clin Endocrinol Metab* 91: 2310–2316, 2006)**

ADIPONECTIN IS AN adipocyte-secreted hormone with insulin-sensitizing effects (1–6). Serum adiponectin concentrations are inversely associated with obesity, insulin resistance, and type 2 diabetes in rodents and humans (3, 7, 8) and are positively associated with improved insulin sensitivity (1, 2, 9, 10). Adiponectin mediates these beneficial effects through at least two cell membrane receptors, adiponectin receptors, AdipoR1 and AdipoR2 (11).

Two studies (one in diabetic and streptozotocin-treated mice and one in lean humans) have demonstrated that although mRNA expression of both AdipoR1 and R2 in skeletal muscle is associated with glucose and lipid levels *in vivo*, AdipoR1 appears to be more closely associated with insulin secretion and sensitivity (12, 13). Another study has shown that obese subjects tend to have elevated AdipoR1 expression levels compared with lean controls (14). Adiponectin and its receptors have not been studied rigorously in groups of lean, obese, and diabetic subjects, and whether their concentrations are associated with body composition, glucose and lipid metabolism, and insulin sensitivity after controlling for known cardiovascular risk factors is unknown. Additionally, it remains to be determined whether the well-established improvements of insulin sensitivity associated with increasing physical activity are mediated through regulation of circulating adiponectin and expression of its receptors. Thus, we hypothesized that in the steady state, adiponectin levels would be lower, and its receptor levels would be higher to compensate for the low adiponectin levels observed in subjects with obesity and insulin resistance. Because exercise improves insulin resistance, acting, in part, through AMP kinase (AMPK), which is also activated by adiponectin (15), we also hypothesized that exercise would increase adiponectin levels and/or expression of adiponectin receptors. Finally, we hypothesized that prolonged training could increase both adiponectin and adiponectin receptor mRNA levels directly and/or by decreasing body weight and im-

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Abbreviations: ACC, Acetyl coenzyme A carboxylase; AMPK, AMP kinase; BMI, body mass index; FFA, free fatty acid; HDL, high-density lipoprotein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; pACC, phosphorylated ACC; pAMPK, phosphorylated AMPK; PI3K, phosphatidylinositol 3-kinase; T2D, type 2 diabetes; WHR, waist/hip ratio.

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proving body composition. Thus, we first performed a cross-sectional observational study to assess whether circulating adiponectin and mRNA expression of its receptors are associated with anthropometric and metabolic parameters, glucose and lipid levels, and insulin resistance in subjects with normal (NGT) and impaired (IGT) glucose tolerance and type 2 diabetes (T2D). We then assessed whether acute (3 h) and chronic (4 wk) physical training alters levels of adiponectin and its receptors *in vivo* and studied the acute effect of intensive exercise on molecules that have previously been demonstrated to mediate adiponectin-stimulated free fatty acid (FFA) oxidation and/or glucose uptake, *i.e.* AMPK, phosphorylated AMPK (pAMPK), acetyl coenzyme A carboxylase (ACC), phosphorylated ACC (pACC), and phosphatidylinositol 3-kinase (PI3K) in muscle (11, 16, 17).

Subjects and Methods

We studied 140 Caucasian men and women with no acute or chronic inflammatory disease, alcohol or drug abuse, or diabetic retinopathy or nephropathy. All baseline blood samples and skeletal muscle samples were collected between 0800 and 1000 h after an overnight fast. Skeletal muscle was obtained under local anesthesia from the right vastus lateralis muscle and was immediately frozen in liquid nitrogen. The study was approved by the ethics committee, and all subjects gave written informed consent.

A subgroup of 60 subjects with NGT (n = 20; nine males and 11 females), IGT (n = 20; nine males and 11 females), and T2D (n = 20; 11 males and nine females) were enrolled in 60 min of supervised physical training sessions, 3 d/wk. Each training session included 20 min of biking or running, 20 min of swimming, and 20 min of warming up/cooling down periods. All subjects completed a graded bicycle-ergometer test to volitional exhaustion and had maximal oxygen uptake measured with an automated open-circuit gas analysis system at baseline. The highest oxygen uptake per minute reached was defined as the maximal oxygen uptake, and subjects subsequently trained at their individual submaximal heart rate using heart rate monitors. At baseline and after 4 wk of training (48 h after the last training session), skeletal muscle and blood samples were obtained in the fasting state, and dual-energy x-ray absorptiometry analyses and measurements of anthropometric parameters were performed. In a subgroup of five healthy female subjects, muscle biopsies were also taken in the fed state after a standardized breakfast of 1000 kcal at 0800 h (baseline) and also after 3 h of intensive training (biking) to investigate the acute effect of exercise on AdipoR1 and -R2 mRNA expression as well as on protein levels of AMPK, pAMPK, ACC, pACC, and PI3K in skeletal muscle.

Basal and fasting blood samples were taken after an overnight fast with subjects in the supine position for 30 min to determine plasma FFA, leptin, adiponectin, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride levels. Insulin sensitivity was assessed in all subjects at baseline and in the exercise group at baseline and after 4 wk of training using the euglycemic-hyperinsulinemic clamp method, as previously described (18–20). Plasma insulin, FFA, adiponectin, leptin (21–23), total cholesterol, HDL, LDL, and triglyceride levels were assayed as previously described (9).

Immunoprecipitations and Western blot analyses were performed on homogenates from skeletal muscle of five healthy female subjects. For the analysis of AMPK, pAMPK, ACC, pACC, and PI3K, protein extracts from skeletal muscle were subjected to immunoprecipitation using specific antisera for the proteins specified (for AMPK and pAMPK, antibodies were obtained from Upstate USA, Inc., Charlottesville, VA; for ACC, pACC, and PI3K, antibodies were obtained from Santa Cruz Biotechnology, Inc., Santa Cruz, CA), followed by Western blot analysis with the same antibody.

Human AdipoR1 and -R2 mRNA expression was measured by quantitative real-time RT-PCR in a fluorescent temperature cycler (TaqMan, Applied Biosystems, Darmstadt, Germany) using previously published human-specific AdipoR1 or -R2 primers (11, 22) and was normalized to relative 36B4 and 18S values, as previously described (23).

Statistical analyses

Comparisons of descriptive characteristics were expressed as the mean ± SE. Nonparametric Kruskal-Wallis tests were also conducted and yielded similar results (data not presented). We calculated nonparametric Spearman correlation coefficients between variables to examine cross-sectional associations of adiponectin and its receptors with anthropometric and insulin resistance-related parameters. Analyses were repeated, adjusting for gender. Simple and multivariate linear regression models produced crude and adjusted standardized coefficients between adiponectin receptors and insulin resistance and related measures. For the interventional study, linear mixed effects models of log-transformed variables accounting for within-subject variability due to repeated measures were used to assess the effects of training, glucose tolerance group, and the potential interaction between training and treatment group on measurements of body weight and composition, fasting plasma glucose and insulin, whole body glucose uptake, FFAs, adiponectin, AdipoR1, and AdipoR2. *Post hoc* comparisons of baseline and after-training measures were made using paired *t* tests within groups of glucose tolerance (NGT, IGT, and T2D), and differences in change in measurements between groups was assessed using one-way ANOVA. A search for outliers using standardized methodology revealed no outlying values that could have substantially influenced the results reported below. A level of $\alpha = 0.05$ was used to determine statistical significance. Bonferroni corrections were used to account for multiple comparisons as appropriate. Statistical analyses were performed using SPSS 8 (SPSS, Inc., Chicago, IL) and SAS 9 (SAS Institute, Cary, NC).

Results

Cross-sectional observational study

This study population, comprised of 140 Caucasian men and women, had a mean age of 46.8 ± 1.2 yr, a body mass index (BMI) of 28.2 ± 0.5 kg/m^2, a waist/hip ratio (WHR) of 1.08 ± 0.02, and a body fat mass of 32.3 ± 0.9%. Additionally, 48 (34%) of the subjects had a low physical activity level, and 34 (24%) used tobacco. Descriptive measures of the study group divided by level of glucose tolerance (NGT, IGT, and T2D) are presented in Table 1. Substantial differences were apparent across the groups, including significantly decreased plasma adiponectin concentration and significantly increased AdipoR1 and -R2 mRNA expression in the IGT and T2D groups compared with the NGT subgroup.

We confirmed, using Spearman correlational analysis, well-described positive associations of BMI with age, WHR, percent fat mass, FFA, and total cholesterol, LDL, triglyceride, and leptin levels ($P < 0.001$). Fasting plasma glucose, 2-h oral glucose tolerance test (OGTT) glucose levels, and fasting insulin levels were also positively correlated with age, BMI, WHR, percent fat mass, total cholesterol, LDL, and triglycerides ($P < 0.001$). Whole body glucose uptake and HDL were negatively associated with all markers of obesity, insulin resistance, and hyperlipidemia ($P < 0.001$) and were positively associated with each other ($P < 0.001$). We repeated the analysis using Spearman partial correlations adjusting for gender and found no substantial changes compared with reported associations (data not presented).

The plasma adiponectin concentration correlated inversely to mRNA expression of both AdipoR1 and -R2 (Table 2). Consistently, although adiponectin showed significant direct correlations with whole body glucose uptake and HDL cholesterol, expression of its receptors had inverse associations with these variables. Similarly, adiponectin was associated negatively and AdipoR1 and -R2 expression positively with BMI; WHR; fat mass percentage; fasting and 2-h plasma

TABLE 1. Cross-sectional evaluation of 140 subjects: baseline anthropometric and metabolic characteristics in subjects with NGT, IGT, and T2D

	NGT (n = 45)	IGT (n = 69)	T2D (n = 26)
Male (no.)/female (no.)	21/24	28/41	13/13
Age (yr)	32.8 ± 1.7	53.6 ± 1.4[a]	53.0 ± 1.6[a]
Body weight (kg)	69.2 ± 2.0	86.2 ± 3.0[a]	99.1 ± 4.0[a,b]
BMI (kg/m^2)	24.2 ± 0.2	29.3 ± 0.7[a]	32.5 ± 0.8[a,f]
WHR	0.84 ± 0.01	1.16 ± 0.03[a]	1.31 ± 0.03[a,f]
Fat mass (%)	24.2 ± 0.5	34.3 ± 1.4[a]	41.2 ± 1.9[a,f]
Tobacco use (no.)	8 (18)	18 (26)	8 (30)
Physical activity level (no.)			
Low	5 (11)	22 (32)[c]	21 (81)[a,d]
Medium	21 (47)	35 (51)	4 (15)[a,d]
High	19 (42)	12 (17)[c]	1 (4)[a,d]
FPG (mmol/liter)	5.3 ± 0.1	5.6 ± 0.1[a]	6.3 ± 0.1[a,d]
2-h OGTT glucose (mmol/liter)	5.9 ± 0.1	9.7 ± 0.1[a]	12.9 ± 0.3[a,d]
FPI (pmol/liter)	64 ± 6	433 ± 47[a]	379 ± 67[a]
2-h OGTT insulin (pmol/liter)	183 ± 14	961 ± 57[a]	1270 ± 101[a,f]
WBGU (μmol/kg·min)	1.86 ± 0.01	1.25 ± 0.03[a]	1.32 ± 0.03[a]
FFA (mmol/liter)	0.41 ± 0.03	0.51 ± 0.03[e]	0.54 ± 0.05[e]
Fasting leptin (pmol/liter)			
Males	2.3 ± 0.4	17.0 ± 3.2[a]	5.8 ± 1.6[f]
Females	7.7 ± 0.9	32.8 ± 4.0[a]	11.8 ± 1.6[f]
Total cholesterol (mmol/liter)	4.57 ± 0.07	5.22 ± 0.08[a]	5.52 ± 0.14[a,b]
Total HDL (mmol/liter)	1.60 ± 0.05	1.22 ± 0.03[a]	1.30 ± 0.05[a]
Total LDL (mmol/liter)	2.48 ± 0.06	3.26 ± 0.06[a]	3.49 ± 0.15[a]
TG (mmol/liter)	1.35 ± 0.04	2.54 ± 0.07[a]	3.02 ± 0.12[a,d]
Adiponectin (μg/ml)	8.88 ± 0.39	4.34 ± 0.28[a]	3.42 ± 0.38[a]
AdipoR1 (AU)	334 ± 17	903 ± 60[a]	898 ± 99[a]
AdipoR2 (AU)	224 ± 20	369 ± 21[a]	508 ± 43[a,f]

FPG, Fasting plasma glucose; FPI, fasting plasma insulin; 2-h OGTT glucose, 2-h OGTT plasma glucose; TG, triglycerides; WBGU, whole body glucose uptake. Data are expressed as the mean ± SE or as the number of subjects (percentage) and were compared using ANOVA with Bonferroni corrections for *post hoc* tests or χ^2 tests.

[a] $P < 0.001$ *vs.* NGT group.
[b] $P < 0.05$ *vs.* IGT group.
[c] $P < 0.01$ *vs.* NGT group.
[d] $P < 0.001$ *vs.* IGT group.
[e] $P < 0.05$ *vs.* NGT group.
[f] $P < 0.01$ *vs.* IGT group.

glucose; insulin, total, and LDL cholesterol; triglycerides; and leptin (Table 2). Adjusting for gender did not alter these associations.

Consistent with overall measures, adiponectin was negatively associated with BMI and percent fat mass in the NGT subgroup. Additionally, the data showed a positive trend for adiponectin with whole body glucose uptake, concurrent, although attenuated, from the results seen in all subjects. There was also a trend toward a negative association between adiponectin and FFA level that was, in fact, stronger than the correlation seen in all subjects, but not statistically significant due to decreased power from the smaller sample size. AdipoR1 expression correlated directly to LDL cholesterol in NGT subjects, similar to all subjects, and like adiponectin, AdipoR2 expression was more strongly positively related to FFAs in the NGT subgroup than in the total sample. Expression of AdipoR2 also showed a positive trend with percent fat mass in NGT subjects similar to all, but exhibited a negative trend with 2-h OGTT results, where the association was positive in all subjects (Table 2). To determine whether the observed significant bivariate correlations of AdipoR1 and -R2 with measures of insulin resistance and lipid levels could be driven by confounding factors, multivariate linear regression models controlling for multiple factors were constructed. Adjusting for adiponectin concentration alone did not affect reported associations of AdipoR1 and -R2 materially. After controlling for age, BMI, WHR, physical activity, and tobacco use in addition to adiponectin concentration, a significant positive association remained between AdipoR1 with 2-h OGTT ($P < 0.05$) and total cholesterol ($P < 0.05$) as well as a negative association with whole body glucose uptake ($P < 0.05$). There was no significant relationship between AdipoR1 and triglycerides or HDL or LDL cholesterol after multivariate adjustment. Like AdipoR1, AdipoR2 was significantly positively associated with 2-h OGTT ($P < 0.01$), but showed no association with any of whole body glucose uptake, triglycerides, or total, HDL, or LDL cholesterol after controlling for confounders (data not shown).

Interventional study

Overall, 4 wk of physical training resulted in significant changes in body weight, body fat percentage, fasting plasma insulin, whole body glucose uptake, adiponectin, AdipoR1, AdipoR2, as well as fasting plasma glucose, but not FFAs. The significant interaction of training and glucose tolerance group on body weight, body fat percentage ($P < 0.01$), fasting plasma insulin ($P < 0.001$), whole body glucose uptake ($P < 0.0001$), and adiponectin ($P = 0.0001$) indicates that the effect

TABLE 2. Spearman correlation matrix of adiponectin, AdipoR1, and AdipoR2 with study variables

	All subjects (n = 140)			NGT (n = 45)		
	Adiponectin	AdipoR1	AdipoR2	Adiponectin	AdipoR1	AdipoR2
Age (yr)	−0.30[a]	0.36[a]	0.31[a]	0.12	0.12	−0.02
BMI (kg/m^2)	−0.39[a]	0.34[a]	0.39[a]	−0.40[b]	−0.17	0.23
WHR	−0.45[a]	0.40[a]	0.38[a]	−0.12	0.13	0.04
FM (%)	−0.43[a]	0.35[a]	0.41[a]	−0.35[b]	−0.10	0.29
FPG (mmol/liter)	−0.31[a]	0.25[b]	0.30[a]	0.16	0.24	0.01
2-h glucose (mmol/liter)	−0.61[a]	0.47[a]	0.47[a]	−0.14	0.07	−0.28
Insulin (pmol/liter)	−0.45[a]	0.47[a]	0.37[a]	0.13	0.16	−0.06
WBGU (μmol/kg·min)	0.53[a]	−0.48[a]	−0.36[a]	0.26	0.06	0.06
FFA (mmol/liter)	−0.15	0.20[b]	0.26[b]	−0.29	−0.01	0.40[b]
Total cholesterol (mmol/liter)	−0.41[a]	0.37[a]	0.21[b]	0.21	−0.04	−0.01
HDL (mmol/liter)	0.33[a]	−0.33[a]	−0.14	0.11	0.12	0.18
LDL (mmol/liter)	−0.45[a]	0.25[b]	0.18[b]	−0.24	−0.32[b]	−0.16
TG (mmol/liter)	−0.56[a]	0.39[a]	0.34[a]	0.16	−0.04	−0.25
Leptin (pmol/liter)	−0.22[a]	0.28[a]	0.11	0.21	0.00	−0.01
Adiponectin (μg/ml)		−0.41[a]	−0.27[a]		0.17	−0.02
AdipoR1 (AU)			0.28[a]			0.22

AdipoR1, AdipoR1 mRNA expression; AdipoR2, AdipoR2 mRNA expression; FM, fat mass; FPG, fasting plasma glucose; 2-h glucose, 2-h OGTT glucose; TG, triglycerides; WBGU, whole body glucose uptake. Adjustment for multiple testing (48 comparisons) with Bonferroni correction would necessitate a value of $P < 0.001$ for statistical significance, and P values between 0.001 and 0.05 would be regarded as trends toward significance.

[a] $P < 0.001$.

[b] $P < 0.05$.

of training on these variables, but not on AdipoR1 or -R2, differed among groups of glucose tolerance. Presented in Table 3 are pre- and posttraining anthropometric, insulin resistance, and hormonal measures for the NGT, IGT, and T2D groups. Prolonged physical training resulted in significant decreases in body weight and percent body fat in each of the glucose tolerance level groups. Moreover, after 4 wk of training, whole body glucose uptake significantly improved, and fasting insulin concentrations decreased regardless of the level of insulin resistance. In patients with T2D, there was an additional significant decrease in fasting plasma glucose after 4 wk of physical training, whereas no such difference was detected in the NGT and IGT groups (Table 3).

Plasma adiponectin increased by 13% in the NGT group after 4 wk of physical training and was elevated significantly more in the IGT and T2D groups, with increases of 97 and 86%, respectively (Table 3). The greater improvements in adiponectin levels among individuals with any form of IGT compared with those with no insulin resistance could not be fully explained by changes in body weight, body fat, or fasting plasma insulin, because the interaction of training and glucose tolerance group weakened, but remained significant after adjusting for each of these factors ($P = 0.01$ for all). AdipoR1 and -R2 mRNA expression was significantly increased after 4 wk of physical training in all groups. There was a strong positive correlation between changes in adiponectin levels and changes in whole body glucose uptake ($r = 0.44$; $P < 0.001$) and a negative correlation between changes in adiponectin levels and changes in FFA levels ($r = -0.46$; $P < 0.001$). In contrast to adiponectin, there was only a trend toward associations between changes in levels of AdipoR1 and -R2 expression with changes in whole body glucose uptake and no associations with FFA levels (data not shown).

There were no significant differences in plasma adiponec-

TABLE 3. Interventional study: anthropometric, metabolic, and hormonal parameters at baseline and after 4 wk of intensive physical training in subjects with NGT, IGT, and T2D

	NGT (n = 20)			IGT (n = 20)			T2D (n = 20)		
	Baseline	Postintervention	% Change	Baseline	Postintervention	% Change	Baseline	Postintervention	% Change
Body weight (kg)	69.6 ± 3.2	68.2 ± 3.1[b]	−2.0	87.6 ± 3.7	84.4 ± 3.6[c]	−3.7	94.6 ± 4.4	93.0 ± 4.0[a]	−1.7
Fat mass (%)	24.5 ± 0.7	23.2 ± 0.6[c]	−5.3	34.9 ± 1.9	31.5 ± 1.7[c]	−9.7[d]	38.1 ± 1.8	35.2 ± 1.8[c]	−7.6[d]
FPG (mmol/liter)	5.1 ± 0.1	5.0 ± 0.1	−2.0	5.6 ± 0.1	5.4 ± 0.1	−3.6	6.2 ± 0.13	5.8 ± 0.1[b]	−6.5
FPI (pmol/liter)	66 ± 8	58 ± 6[a]	−12.1	695 ± 110	379 ± 70[c]	−45.5[e]	319 ± 50	234 ± 28[b]	−26.6
WBGU (μmol/kg·min)	76 ± 4	85 ± 3[c]	11.8	19 ± 2	36 ± 4[c]	89.5	21 ± 2	32 ± 3[c]	52.4
FFA (mmol/liter)	0.41 ± 0.04	0.39 ± 0.04	−4.9	0.53 ± 0.06	0.51 ± 0.05	−3.8	0.56 ± 0.06	0.47 ± 0.05	−16.1
Adiponectin (μg/ml)	8.7 ± 0.6	9.8 ± 0.6[b]	12.6	3.4 ± 0.26	6.7 ± 0.7[c]	97.1[d]	3.5 ± 0.4	6.5 ± 0.6[c]	85.7[d]
AdipoR1 (AU)	338 ± 29	670 ± 48[c]	98.2	825 ± 112	1306 ± 107[c]	58.3	922 ± 130	1420 ± 154[b]	54.0
AdipoR2 (AU)	227 ± 31	537 ± 65[c]	136.6	341 ± 34	734 ± 93[c]	115.2	524 ± 54	1018 ± 124[b]	94.3

Data are expressed as the mean ± SE, with baseline and postintervention measures compared using paired t tests and differences in change in measurements assessed using ANOVA with Bonferroni correction for *post hoc* tests. AU, Arbitrary units; FPG, fasting plasma glucose; FPI, fasting plasma insulin; WBGU, whole body glucose uptake.

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ *vs.* baseline measurements within each group.

[d] $P < 0.05$ *vs.* NGT group.

[e] $P < 0.01$ *vs.* NGT group.

tin levels after 3 h of intensive exercise (data not shown) in five NGT women. However, AdipoR1 mRNA expression increased 3-fold, and AdipoR2 expression increased 5-fold in skeletal muscle after 3 h of acute training ($P < 0.0001$); phosphorylation of AMPK and ACC, but not PI3K, was significantly increased after 3 h of intensive exercise ($P < 0.0001$).

Discussion

Adiponectin plays a significant role in insulin resistance and IGT (1–8, 10, 20, 24), acting through at least two cell membrane receptors, AdipoR1 and AdipoR2 (11). Little is known about the physiological regulation of adiponectin and its receptors in humans.

We show in this study that baseline plasma adiponectin levels are significantly decreased (3, 7, 8) and that AdipoR1 and -R2 mRNA expression in muscle is significantly increased in subjects with IGT and T2D compared with subjects with NGT. Plasma adiponectin is negatively associated, whereas levels of AdipoR1 and -R2 expression are positively associated, with markers of obesity, IGT, and insulin resistance. Strong positive associations of plasma adiponectin and negative associations of AdipoR1 and -R2 expression were observed with whole body glucose uptake. Adjustment for several potential confounders revealed that the significantly increased expression of AdipoR1 and -R2 in the IGT and T2D groups is mediated primarily by differences in plasma adiponectin, age, and BMI, consistent with the hypothesis that up-regulation of AdipoR1 and -R2 in the insulin-resistant state may be largely compensatory for the low adiponectin levels observed in this state. In addition, associations of AdipoR1 with measures of insulin sensitivity and total cholesterol remain significant, whereas only the association between AdipoR2 and 2-h OGTT glucose levels remains significant after adjusting for adiponectin or other potential confounders. Associations with whole body glucose uptake and triglycerides are of similar magnitude for AdipoR1 and -R2. These findings are consistent with and significantly extend the results of a recent study of humans demonstrating that AdipoR1, but not AdipoR2, expression in human skeletal muscle is positively correlated with *in vivo* parameters of glucose metabolism and first-phase insulin secretion (12) and the observation that obese subjects tend to have elevated AdipoR1 expression levels compared with lean controls (14). Another small study in 18 nondiabetic Mexican-Americans (25) demonstrated that AdipoR1 and -R2 mRNA expression was positively associated with whole body glucose uptake; however, this trend was only apparent in subjects with no family history of diabetes (n = 10). The association appeared null or negative in subjects with a family history of diabetes (n = 8). We found no significant association of AdipoR1 or -R2 with whole body glucose uptake in subjects with NGT, but our sample most likely included both subjects with and without a family history of diabetes in unknown proportions. Our study goes on to demonstrate in a population with diverse glucose tolerance (normal, impaired, or diabetic) that the expression of AdipoR1 and possibly AdipoR2 is negatively associated with whole body glucose uptake, similar to NGT subjects with family history of diabetes studied previously (25). More research is needed to fully elucidate the roles of familial and genetic factors in the expression of adiponectin receptors.

We demonstrate that 4 wk of supervised physical activity increases plasma adiponectin concentration and AdipoR1 and -R2 mRNA expression. We also show that physical training-induced increases in circulating adiponectin are strongly and positively associated with increases in whole body glucose uptake and are negatively associated with decreases in FFA levels. These data are consistent with previous studies of adiponectin levels in diabetic subjects (26) and sedentary elderly men (27) and extend these findings by demonstrating that increased physical activity for 4 wk is sufficient for changes in both circulating adiponectin and expression of adiponectin receptors in muscle and that the magnitude of these changes parallels changes in insulin sensitivity. The effect of exercise on AdipoR1 and -R2 does not differ with the level of glucose tolerance, whereas adiponectin was significantly more increased in IGT and T2D subjects compared with the NGT group, even after accounting for changes in body weight and/or composition and fasting plasma insulin. Another small (n = 25) 6-month study found adiponectin levels improved in patients who lost weight and exercised, but not in overweight subjects who did not have surgery or lose weight, but did complete the exercise intervention (28). Although we cannot rule out the possibility that weight loss mediated some of the observed changes in adiponectin levels, a more recent study of 19 overweight males (29) found significant increases in adiponectin with two or three exercise training sessions in more than 1 wk that were sustained after 10 wk of training with no significant weight loss.

Finally, we found that 3 h of intensive training increases AdipoR1 and -R2 expression as well as phosphorylation of AMPK and ACC in human skeletal muscle from five healthy women, but has no effect on circulating adiponectin levels. This result contrasts with the findings of a recent study of 10 subjects involving less intensive training sessions (2 h at 50% maximal oxygen uptake) than in our study (30), indicating that more rigorous exercise may be necessary to increase adiponectin receptor expression. Consistent with our data, previous studies have demonstrated that globular and full-length adiponectin stimulate the phosphorylation of AMPK and ACC in both C2C12 myocytes and hepatocytes transfected with AdipoR1 *in vitro* (11, 17), and that globular and/or full-length adiponectin increases AMPK and ACC phosphorylation in muscle of lean C57BL/6J mice and in liver of DIO-C57BL/6J mice *in vivo* (16, 17). Taken together, our interventional studies suggest that the insulin-sensitizing effect of physical activity may be mediated through increased adiponectin receptor expression in muscle in the short term and increased levels of both circulating adiponectin and its receptor mRNA expression in muscle after training over a prolonged period of time. Alterations in circulating adiponectin and AdipoR1 and -R2 expression in muscle may mediate the insulin-sensitizing effects of increased physical activity independently, acting in part through activation of AMPK. Future experiments investigating the effects of specific interventions (including exercise, weight loss, or administration of insulin sensitizers) for several time periods in larger groups of subjects are needed.

A strength of this study is the use of a reasonably large

dataset, providing adequate statistical power for documenting important associations of levels of adiponectin and expression of its receptors with markers of obesity and insulin resistance. This, in association with the consistency of results for several metabolic variables examined in this study, supports the validity of the results and provides confidence in our findings. Our study is limited by the lack of information on family history of diabetes, menopausal status, and possibly other variables that could potentially play important roles in adiponectin and adiponectin receptor regulation, raising the possibility of residual confounding, as is the case with each and every observational study. A limited degree of imprecision in laboratory measurements and possible inaccurate reporting of anthropometric or demographic variables are also theoretical possibilities that could have resulted in random misclassification. This could have only depressed reported effect estimates and corresponding *P* values. Because observational studies cannot prove causality, the mechanisms underlying the up-regulation of AdipoR1 and -R2 in insulin-resistant states remain to be fully elucidated by interventional studies. In our acute training intervention study, we had a small number of subjects ($n = 5$), but a sample of this size provides 80% power at the $\alpha = 0.05$ level to detect differences in mean values greater than or equal to 1.65 times the respective SD. Because we examined the effect of acute training in healthy females only, additional research is needed on males and individuals with IGT and possibly other conditions.

In summary, the studies presented in this report demonstrate that 1) baseline circulating adiponectin levels are decreased, whereas AdipoR1 and -R2 expression in skeletal muscle is increased, in subjects with IGT or T2D compared with subjects with NGT; 2) circulating adiponectin is strongly negatively associated, whereas AdipoR1 and -R2 expression in muscle is positively associated with markers of obesity, impaired glucose metabolism, and insulin resistance; 3) improvement of insulin resistance in response to chronic (4 wk) physical training is associated with increases not only in circulating adiponectin, but also in AdipoR1 and -R2 expression in muscle; and 4) acute (3 h) intensive exercise has no effect on circulating adiponectin, but increases AdipoR1 and -R2 expression as well as phosphorylation of AMPK and ACC in muscle.

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