Expression of Adiponectin Receptor mRNA in Human Skeletal Muscle Cells Is Related to In Vivo Parameters of Glucose and Lipid Metabolism

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The adiponectin receptors, AdipoR1 and AdipoR2, are thought to transmit the insulin-sensitizing, anti-inflammatory, and atheroprotective effects of adiponectin. In this study, we examined whether AdipoR mRNA expression in human myotubes correlates with in vivo measures of insulin sensitivity. Myotubes from 40 metabolically characterized donors expressed 1.8-fold more AdipoR1 than AdipoR2 mRNA (588 ± 35 vs. 321 ± 39 fg/μg total RNA). Moreover, the expression levels of both receptors correlated with each other ($r = 0.45$, $P < 0.01$). AdipoR1 mRNA expression was positively correlated with in vivo insulin and C-peptide concentrations, first-phase insulin secretion, and plasma triglyceride and cholesterol concentrations before and after adjustment for sex, age, waist-to-hip ratio, and body fat. Expression of AdipoR2 mRNA clearly associated only with plasma triglyceride concentrations. In multivariate linear regression models, mRNA expression of AdipoR1, but not AdipoR2, was a determinant of first-phase insulin secretion independent of insulin sensitivity and body fat. Finally, insulin did not directly modify myotube AdipoR1 mRNA expression in vitro. In conclusion, we provide evidence that myotube mRNA levels of both receptors are associated with distinct metabolic functions but not with insulin sensitivity. AdipoR1, but not AdipoR2, expression correlated with insulin secretion. The molecular nature of this link between muscle and β-cells needs to be further clarified. *Diabetes* 53: 2195–2201, 2004

Adipose tissue communicates with other peripheral tissues via the release of numerous humoral mediators (for review, see Frühbeck et al. [1] and Arner [2]). Among these, free fatty acids and adipokines (leptin, adiponectin, tumor necrosis factor-α, and interleukin-6) have attracted much interest due to their dysregulated secretion and potential role in obesity-linked disorders, such as hypertension, atherosclerosis, insulin resistance, and type 2 diabetes. In contrast to the other adipokines, the plasma concentrations of adiponectin are not elevated but significantly reduced in obesity, insulin resistance, and coronary heart disease (3–6). Because of adiponectin's beneficial effects on muscle and liver insulin sensitivity as well as vascular inflammation, its downregulation is suggested to contribute to the pathogenesis of these disorders.

In animal models of obesity and type 2 diabetes, administration of adiponectin promotes weight loss as well as an improvement of insulin sensitivity and glucose tolerance (7,8). Molecularly, adiponectin was shown to increase insulin sensitivity by enhancing insulin's suppressive effect on gluconeogenesis (9,10) and by reducing hepatic triglyceride content resulting from an increase in fatty acid oxidation (8). The latter effect was demonstrated in skeletal muscle as well and was shown (7,11) to depend on the activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator–activated receptor-α. Adiponectin's anti-inflammatory and antiatherogenic effects are thought to be caused by diminished monocyte adhesion to the endothelium (12), decreased smooth muscle cell proliferation (13–15), and reduced foam cell formation (16).

All of these effects are postulated to be mediated by the recently cloned adiponectin receptors, AdipoR1 and AdipoR2 (17). Both structurally highly related proteins derive from different genes located on chromosomes 1q32 and 12p13, respectively, and constitute a novel class of seven transmembrane domain receptors that are not coupled with known G-proteins. As was shown by Northern blot analysis of multiple murine and human tissues (17), AdipoR1 is ubiquitously expressed, whereas AdipoR2 expression is more restricted to skeletal muscle and liver. Overexpression and knockdown experiments furthermore demonstrated (17) the receptors' ability to ligand-dependently activate AMPK, p38 mitogen-activated protein kinase, and peroxisome proliferator–activated receptor-α and to stimulate fatty acid oxidation and glucose uptake in murine hepatocytes and C2C12 myocytes.

Because of their putative role in insulin sensitization, we tested in this study whether the mRNA expression levels of AdipoR1 and AdipoR2 in primary cultured human skeletal muscle cells from 40 metabolically characterized nondiabetic subjects correlate with in vivo measures of insulin sensitivity.

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Received for publication 20 November 2003 and accepted in revised form 26 May 2004.

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AdipoR, adiponectin receptor; AMPK, AMP-activated protein kinase; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test.

TABLE 1
Clinical characteristics of myotube donors

	Women	Men	*P*
n	19	21	—
Age (years)	27.5 ± 1.2	25.3 ± 0.9	0.20
BMI (kg/m^2)	24.3 ± 0.9	22.8 ± 0.6	0.14
Waist-to-hip ratio	0.78 ± 0.02	0.84 ± 0.01	<0.01
Body fat (%)	27.6 ± 1.8	17.5 ± 1.3	<0.0001
Fasting plasma glucose (mmol/l)	4.74 ± 0.09	4.76 ± 0.10	0.9
Fasting serum insulin (pmol/l)	44.2 ± 6.0	35.0 ± 3.4	0.3
Fasting plasma triglycerides (mg/dl)	86.5 ± 8.5	89.3 ± 8.7	0.8
Fasting plasma cholesterol (mg/dl)	193.1 ± 9.4	173.6 ± 7.1	0.10
Fasting serum free fatty acids (μmol/l)	406.1 ± 37.9	406.5 ± 38.0	1.0
Fasting serum leptin (ng/ml)	15.6 ± 2.1	3.4 ± 0.6	<0.0001
Fasting serum adiponectin (μg/ml)	15.1 ± 1.4	8.3 ± 0.7	0.0001

Data are means ± SE. *P* values were determined by Student's *t* test on log-transformed data.

RESEARCH DESIGN AND METHODS

The 40 muscle cell donors were healthy normal glucose tolerant (according to World Health Organization criteria [18]) Caucasians recruited from the Tübingen Family Study for type 2 diabetes. The subjects did not take any medication known to affect glucose tolerance, insulin sensitivity, or insulin secretion. All individuals were metabolically characterized by an oral glucose tolerance test (OGTT) and hyperinsulinemic-euglycemic clamp. Subject characteristics are given in Table 1. All subjects gave informed written consent before the biopsy. The study was approved by the local ethics committee.

Determination of blood parameters. Fasting blood parameters were determined after a 10-h overnight fast. Plasma glucose was determined using a bedside glucose analyzer (glucose oxidase method; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin and C-peptide levels were measured by microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan) and radioimmunoassay (Byk-Sangtec, Dietzenbach, Germany), respectively. Plasma free fatty acid concentrations were determined by an enzymatic method (NEFAC kit; Wako Chemicals, Neuss, Germany). Plasma triglycerides, cholesterol, and lipoproteins were measured with standard colorimetric methods using the Roche/Hitachi analyzer (Roche Diagnostics, Mannheim, Germany). Serum concentrations of leptin and adiponectin were determined by radioimmunoassay (Linco Research, St. Charles, MO).

OGTT and hyperinsulinemic-euglycemic clamp. The OGTT and hyperinsulinemic-euglycemic clamp were performed after a 10-h overnight fast as previously described (18,19). The mean infusion rate of exogenous glucose (glucose infusion rate, in micromoles per kilogram per minute) necessary to maintain euglycemia during the last 60 min (steady state) of the clamp represents a measure for insulin sensitivity of glucose disposal. The clamp-derived insulin sensitivity index (ISI, in micromole multiplied by liter per kilogram per minute per picomole) for systemic glucose uptake was calculated as glucose infusion rate divided by the steady-state insulin concentration. First-phase insulin secretion (in picomoles per liter) was estimated from plasma insulin and glucose concentrations during the OGTT using validated equations as described previously (20).

Cell culture. Primary human skeletal muscle cells were grown from satellite cells obtained from percutaneous needle biopsies performed on the lateral portion of the quadriceps femoris (vastus lateralis) muscle as recently described in detail (21). The experiments were performed on subcultured (first-pass) cells after growth to 80–90% confluence and subsequent differentiation to myotubes as described (21). On day 5 of differentiation, the cells were harvested for RNA isolation and mRNA quantification. In one experimental setting, the differentiated myotubes were chronically treated (48 h) with different concentrations of insulin (Huminsulin Normal 40; Lilly, Giessen, Germany) before RNA isolation. Murine C2C12 myoblasts were grown in Dulbecco's modified Eagle's medium (4.5 g/l glucose) supplemented with 2 mmol/l glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 10% (vol/vol) FCS. At 80–90% confluence, cells were shifted to medium containing only 0.5% (vol/vol) FCS and differentiated to myotubes for 9 days. Cells were refed with fresh medium three times a week. C2C12 myotubes were treated for different time intervals with different concentrations of insulin before RNA isolation.

RT-PCR. RNA was isolated with peqGOLD TriFast according to the manufacturer's instructions (Peqlab, Erlangen, Germany). Total RNA treated with RNase-free DNase I was transcribed into cDNA using AMV reverse transcriptase and the first-strand cDNA kit from Roche Diagnostics (Mannheim, Germany). Quantitative PCR was performed with SYBR Green I dye on a high-speed thermal cycler with an integrated microvolume fluorometer according to the instructions of the manufacturer (Roche Diagnostics, Mannheim, Germany). The following primers were obtained from Invitrogen (Karlsruhe, Germany): human AdipoR1 (forward) 5′-ATTGAGGTACCAGCCAGATG-3′, human AdipoR1 (reverse) 5′-GAGGTCTATGACCATGTAGC-3′, human AdipoR2 (forward) 5′-GATTGTCATCTGTGTGCTGG-3′, human AdipoR2 (reverse) 5′-CTGGAGACTGGTAGGTATCA-3′, murine AdipoR1 (forward) 5′-ACGTTGGAGAGTCATCCCGTAT-3′, murine AdipoR1 (reverse) 5′-CTCTGTGTGGATGCGGAAGAT-3′, murine hexokinase II (forward) 5′-GGCTAGGAGCTACCACACAC-3′, and murine hexokinase II (reverse) 5′-CCTTCCGGATCAGATCCACC-3′. The PCR conditions used were the following: human AdipoR1 mRNA −66°C annealing temperature, 45 cycles, 4 mmol/l $MgCl_2$; human AdipoR2 mRNA −64°C annealing temperature, 45 cycles, 4 mmol/l $MgCl_2$, and 5% (vol/vol) DMSO; murine AdipoR1 mRNA −61°C annealing temperature, 35 cycles, 4 mmol/l $MgCl_2$; and murine hexokinase II mRNA −64°C annealing temperature, 50 cycles, 3 mmol/l $MgCl_2$. Measurements were performed in triplicate. mRNA levels are given in femtograms per microgram total RNA or in arbitrary units (AUs), with 1 AU = 1 ag/100 ng total RNA.

Statistical analysis. Statistical analysis of data from the complete cohort (n = 40) was performed after log transformation. Data were log transformed to achieve normal distribution as verified by Shapiro-Wilk W test. Comparisons between two groups were then performed by Student's t test. For simple and multivariate linear regression analysis of individual data, ANOVA was used. Data obtained from muscle cell cultures of five arbitrarily selected donors (n = 5) or from four generations of C2C12 cells (n = 4) were analyzed by the nonparametric Mann-Whitney rank-sum test (two-group comparisons) or the Kruskal-Wallis ANOVA on ranks (many-group comparisons). For all tests, the statistical software package JMP 4.0 (SAS Institute, Cary, NC) was used. Generally, a P value of <0.05 was considered statistically significant.

RESULTS

Myotube adiponectin receptor mRNA expression. Cellular mRNA expression of AdipoR1 and AdipoR2 was determined in in vitro–differentiated myotube cultures from all donors (n = 40). AdipoR1 mRNA amounts reached a mean (±SE) value of 588 ± 35 fg/μg total RNA and individually varied between 239 and 1,248 fg/μg total RNA. AdipoR2 mRNA levels ranged at 321 ± 39 fg/μg total RNA and individually varied between 104 and 1,321 fg/μg total RNA. In comparison, human myotubes expressed 1.8-fold more AdipoR1 than AdipoR2 mRNA. Moreover, the mRNA expression levels of both receptors correlated with each other (Fig. 1).

Relationship between in vitro AdipoR mRNA expression and in vivo parameters. Associations between myotube AdipoR mRNA expression and in vivo parameters of the donors were studied by correlational analysis. As shown in Table 2, neither AdipoR1 nor AdipoR2 mRNA was correlated with any anthropometric parameter (sex, age, BMI, waist-to-hip ratio, and body fat). AdipoR1 mRNA

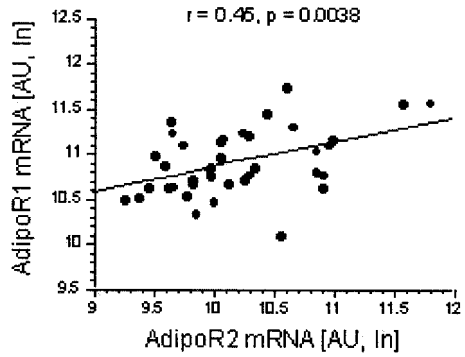

FIG. 1. Correlation between AdipoR1 and AdipoR2 mRNA expression in human myotubes (n = 40). Data were log transformed to achieve normal distribution (1 AU = 1 ag/100 ng total RNA). The correlation remained significant even after adjustment for sex, age, waist-to-hip ratio, and body fat (r = 0.47, P = 0.0022, ANOVA).

expression was positively correlated with serum insulin and C-peptide concentrations in the fasting state as well as during the OGTT (Table 2) and with first-phase insulin secretion as estimated from the OGTT (Fig. 2*A*). Furthermore, AdipoR1 mRNA was negatively correlated with the clamp-derived ISI and positively with fasting plasma concentrations of triglycerides and cholesterol (Table 2). AdipoR2 mRNA levels solely correlated positively with plasma triglyceride concentrations (Table 2).

The correlations found by simple linear regression were further analyzed in more detail. To this end, all parameters were adjusted for sex, age, waist-to-hip ratio, and percentage of body fat. Again, AdipoR1 mRNA expression was significantly associated with insulin and C-peptide concentrations as well as with first-phase insulin secretion (Table 3). Plasma triglyceride and cholesterol concentrations also remained associated with AdipoR1 mRNA (Table 3). However, ISI did not remain a significant correlate (Table 3). As to AdipoR2 mRNA expression, the association with plasma triglycerides remained significant after adjustment. Furthermore, insulin and C-peptide levels at 30 min of OGTT as well as first-phase insulin secretion were significantly correlated with AdipoR2 mRNA expression (Table 3). These correlations, however, were weaker than those seen with AdipoR1 mRNA (Table 3).

Based on these correlations, an unexpected relationship between myotube AdipoR1 mRNA levels and in vivo insulin secretion seemed to emerge. Therefore, we further assessed the role of AdipoR1 mRNA expression as a determinant of insulin secretion and its independence of insulin sensitivity by multivariate linear regression analysis (Table 4). First-phase insulin secretion was chosen as the dependent variable, and age and percentage of body fat were used as fixed covariates (Table 4, model 1). Inclusion of AdipoR2 mRNA expression as an additional covariate did not considerably elevate the r^2 of the model (0.13 and 0.19, respectively), and AdipoR2 mRNA did not independently determine first-phase insulin secretion (P = 0.10) (Table 4, model 2). By contrast, inclusion of AdipoR1 mRNA expression as a third covariate increased the r^2 of the model from 0.13 to 0.33, and AdipoR1 mRNA levels turned out to be an independent determinant of first-phase insulin secretion ($P < 0.01$) (Table 4, model 3). Addition of ISI or fasting plasma glucose concentration, respectively, as a fourth covariate raised the r^2 even more, without abolishing AdipoR1 mRNA expression as an independent determinant of insulin secretion (Table 4, models 4 and 5, respectively).

Regulation of myotube AdipoR1 mRNA expression by insulin. To examine whether myotube AdipoR1 mRNA expression is directly modulated by insulin, we treated the in vitro–differentiated myotube cultures from five arbi-

TABLE 2
Simple linear correlations with AdipoR1 and AdipoR2 mRNA expression levels

	AdipoR1 mRNA		AdipoR2 mRNA	
	r	P	r	P
Sex	—	0.9	—	0.23
Age	0.13	0.4	0.26	0.10
BMI	0.19	0.24	−0.12	0.5
Waist-to-hip ratio	0.04	0.8	−0.10	0.5
Body fat	0.16	0.3	0.01	0.9
Fasting plasma glucose	0.09	0.6	−0.20	0.22
Mean plasma glucose (OGTT)	0.04	0.8	−0.26	0.11
Fasting serum insulin	0.37	<0.05	0.03	0.8
30-min serum insulin (OGTT)	0.51	<0.001	0.30	0.06
Mean serum insulin (OGTT)	0.48	<0.01	0.13	0.4
Fasting serum C-peptide	0.33	<0.05	0.01	0.9
30-min serum C-peptide (OGTT)	0.51	0.001	0.31	0.05
Mean serum C-peptide (OGTT)	0.44	<0.01	0.10	0.5
Glucose infusion rate (clamp)	−0.21	0.22	0.06	0.7
ISI (clamp)	−0.35	<0.05	0.05	0.8
Fasting serum free fatty acids	0.09	0.6	−0.01	0.9
Fasting plasma triglycerides	0.33	<0.05	0.34	<0.05
Fasting plasma cholesterol	0.36	<0.05	0.27	0.09
Fasting plasma HDL cholesterol	0.09	0.6	0.27	0.09
Fasting plasma LDL cholesterol	0.31	0.06	0.18	0.3
Fasting serum leptin	0.22	0.18	0.17	0.3
Fasting serum adiponectin	−0.26	0.11	0.17	0.3

P values were determined by ANOVA on log-transformed data.

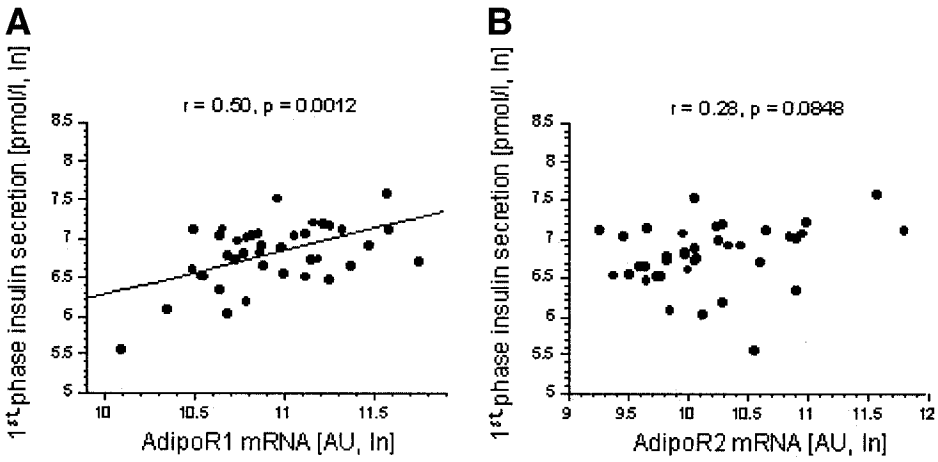

FIG. 2. Correlation of myotube AdipoR1 (*A*) and AdipoR2 (*B*) mRNA expression with insulin secretion (n = 40). Data were log transformed to achieve normal distribution (1 AU = 1 ag/100 ng total RNA). First-phase insulin secretion (OGTT) was calculated as described in RESEARCH DESIGN AND METHODS. After adjustment for AdipoR2, AdipoR1 mRNA still correlated with first-phase insulin secretion (r = 0.44, P = 0.0053, ANOVA). After adjustment for AdipoR1, AdipoR2 mRNA did not correlate with first-phase insulin secretion (r = 0.08, P = 0.6323, ANOVA).

trarily chosen donors (n = 5) for 48 h with increasing concentrations of insulin (0–100 nmol/l) and quantified AdipoR1 mRNA expression (data not shown). There was no significant effect of insulin on AdipoR1 mRNA expression at all concentrations tested (P = 0.11, Kruskal-Wallis ANOVA on ranks). To exclude that this lack of effect is due to variations in the myotube donors' genetic background, we performed analogous experiments with in vitro–differentiated murine C2C12 myotubes. In these cells, a trend toward lower AdipoR1 mRNA levels with increasing insulin concentrations could be observed, with a 15% reduction of AdipoR1 mRNA at 100 nmol/l insulin (Fig. 3*A*). This reduction, however, did not reach the level of significance (P = 0.10, Kruskal-Wallis ANOVA on ranks). To ascertain that AdipoR1 mRNA expression is not regulated by insulin, we also performed a time course with 100 nmol/l insulin (Fig. 3*B*). Again, no significant insulin effect could be observed at any time point tested (P = 0.4, Kruskal-Wallis ANOVA on ranks). To control for insulin's efficacy on gene expression in these cells, we quantified hexokinase II mRNA expression before and after 10 h of insulin treatment (100 nmol/l). As depicted in Fig. 3*B* (*inset*), insulin induced a significant 1.8-fold increase in hexokinase II mRNA (P = 0.03, Mann-Whitney rank-sum test).

DISCUSSION

On the assumption that insulin-sensitizing adiponectin signaling not only depends on the availability of adiponectin but also on the expression level of AdipoR, we asked whether AdipoR expression in human myotubes, if maintained in cell culture, correlates with in vivo measures of the insulin sensitivity of muscle cell donors.

In keeping with recent Northern blot data (17), we show here that human myotubes express mRNA of both receptors, with AdipoR1 being nearly twice as high as AdipoR2 mRNA expression. Intriguingly, both receptor mRNA levels positively correlated with each other. This interrelation points to a synchronous regulation of both genes, possibly due to the presence of regulatory sites within the gene promoters that are under the control of the same transcription factor(s). To further examine this, we performed initial in silico promoter analysis with MatInspector version 2.2 based on the transcription factor database Transfac 4.0 (see http://transfac.gbf.de and Wingender et al. [22]). In fact, we found that in the region ~2,000 bp upstream of the first exon, several bona fide binding sites for the following transcription factors are present in both genes: AP-1, GATA-1 and -2, SOX-5, TCF-11, S8, δ-EF1,

TABLE 3
Correlations with AdipoR1 and AdipoR2 mRNA expression levels after adjustment

	AdipoR1 mRNA		AdipoR2 mRNA	
	r	P	r	P
Fasting serum insulin	0.34	<0.05	0.02	0.9
30-min serum insulin (OGTT)	0.49	<0.01	0.37	<0.05
Mean serum insulin (OGTT)	0.48	<0.01	0.24	0.13
Fasting serum C-peptide	0.31	0.06	0.08	0.6
30-min serum C-peptide (OGTT)	0.51	0.001	0.37	<0.05
Mean serum C-peptide (OGTT)	0.47	<0.01	0.24	0.14
First-phase insulin secretion (OGTT)	0.47	<0.01	0.33	<0.05
Glucose infusion rate (clamp)	−0.15	0.4	−0.07	0.7
ISI (clamp)	−0.31	0.06	−0.11	0.5
Fasting plasma triglycerides	0.33	<0.05	0.50	<0.01
Fasting plasma cholesterol	0.39	<0.05	0.26	0.11

All metabolic parameters were adjusted for sex, age, waist-to-hip ratio, and body fat. P values were determined by ANOVA on log-transformed data.

TABLE 4
Multivariate linear regression models with first-phase insulin secretion (OGTT) as the dependent variable

Covariates	Estimate	SE	*P*
Model 1 (r^2 = 0.13)			
Age	0.34	0.35	0.3424
Body fat	0.29	0.16	0.0756
Model 2 (r^2 = 0.19)			
Age	0.18	0.35	0.6067
Body fat	0.31	0.15	0.0533
AdipoR2 mRNA	0.18	0.11	0.1000
Model 3 (r^2 = 0.33)			
Age	0.25	0.31	0.4276
Body fat	0.23	0.14	0.1060
AdipoR1 mRNA	0.51	0.16	0.0026
Model 4 (r^2 = 0.36)			
Age	−0.12	0.34	0.7120
Body fat	0.37	0.15	0.0221
ISI (clamp)	−0.04	0.12	0.7464
AdipoR1 mRNA	0.38	0.16	0.0282
Model 5 (r^2 = 0.40)			
Age	0.33	0.30	0.2799
Body fat	0.20	0.14	0.1453
Fasting plasma glucose	−1.18	0.59	0.0529
AdipoR1 mRNA	0.54	0.15	0.0011

LMO(2)COM, IK2, and MZF-1. Of course, detailed promoter studies are now needed to further support this coregulation.

The AdipoR mRNA expression levels in human myotubes varied several fold between individuals. When these were analyzed for correlation with in vivo metabolic parameters, we did not find an association with insulin sensitivity after rigorous adjustment. However, other rather unexpected correlations were detected. Fasting plasma triglycerides were a function of both receptors. This argues for an as yet unknown role of both AdipoRs in VLDL metabolism. More interestingly, AdipoR1 mRNA levels strongly associated with insulin secretion in all analytical settings, whereas AdipoR2 failed to do so in multivariate linear regression analysis. Since we demonstrated in vitro that chronic insulin treatment does not modulate AdipoR1 expression, the reasons for this unique link between myotube AdipoR1 expression and β-cell function are far from being obvious. However, the following points could be considered as possible explanations.

First, AdipoR1 expression levels in myotubes could mirror those in β-cells, where adiponectin via AdipoR1 could play a role in β-cell function. In keeping with this, distinct functions of adiponectin, such as induction of lipoprotein lipase and modulation of apoptosis, were recently reported in rat insulinoma cells (23,24). However, data on adiponectin functions in human β-cells are still lacking. We determined mRNA levels of AdipoR1 (and AdipoR2) in human pancreatic islets, which are composed of up to 80% β-cells. The cellular AdipoR1 mRNA amount ranged at ~600 fg/μg total RNA and is thereby comparable with that of human myotubes. Therefore, a linkage between the AdipoR1 expression levels of muscle and β-cells appears possible. In this case, it is conceivable that the common determinant of AdipoR1 gene transcription is genetics. The identification of polymorphisms within the AdipoR1 gene, especially those within the promoter region, may help to further clarify this issue.

Second, a hormone or metabolite could control both pancreatic insulin secretion and muscle cell AdipoR1 expression in parallel. To address this, we examined the relationship between glucose levels, AdipoR1 mRNA expression, and insulin secretion. Whereas blood glucose concentrations expectedly correlated with measures of insulin secretion, there was no association with AdipoR1

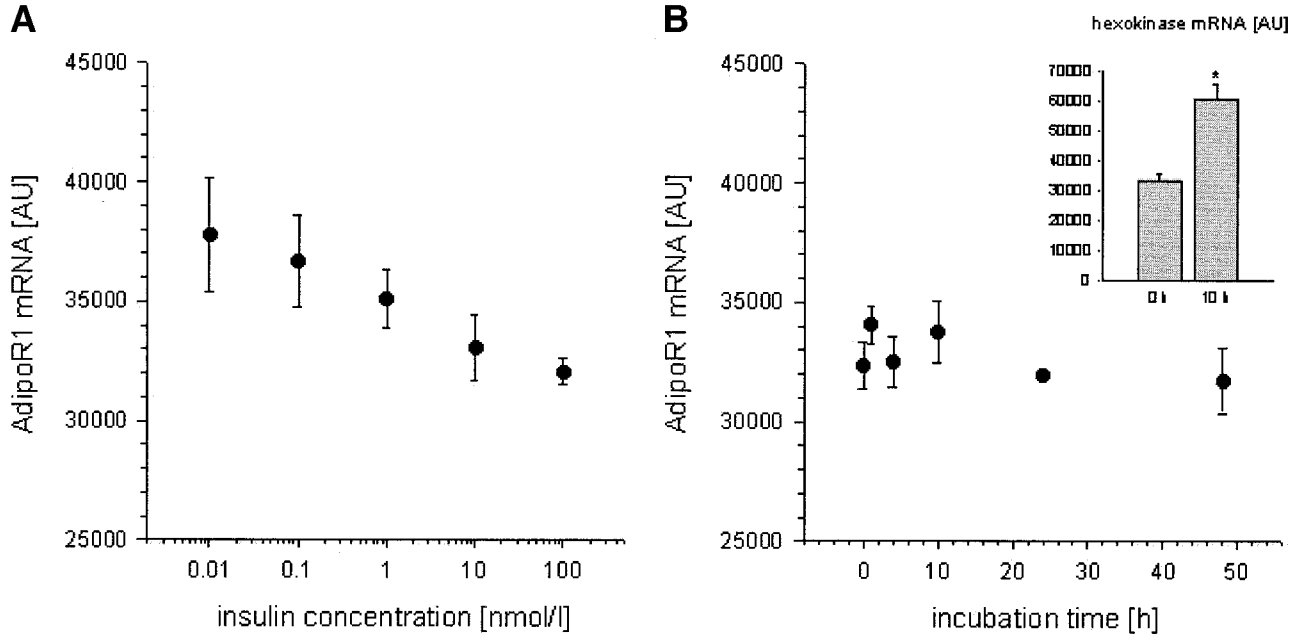

FIG. 3. Influence of insulin on AdipoR1 mRNA expression in murine C2C12 myotubes. Data are presented as means ± SE of four independent experiments (n = 4) (1 AU = 1 ag/100 ng total RNA). *A*: Dose-response curve: in vitro–differentiated myotubes were incubated for 48 h with different insulin concentrations (0–100 nmol/l). *B*: Time course: in vitro–differentiated myotubes were treated for different time intervals (0–48 h) with 100 nmol/l insulin. *Inset*: Hexokinase II mRNA expression before and after 10 h of insulin (100 nmol/l) treatment. *Significantly different from 0 h (P = 0.0286, Mann-Whitney rank-sum test).

mRNA expression in our dataset (data not shown). Therefore, other insulinotropic factors such as incretins may be better candidates. Alternatively, AdipoR1-dependent adiponectin signaling in muscle cells could alter the plasma concentrations of metabolites that affect β-cell function. Because, on the one hand, adiponectin was shown to increase fatty acid uptake and oxidation in muscle (7,8) and, on the other hand, fatty acids are well-known modulators of β-cell function (25,26), we tested whether there is a correlation between AdipoR1 mRNA expression and plasma free fatty concentrations. However, no association could be found by simple and multivariate linear regression analysis (data not shown).

Finally, it is tempting to speculate that muscular adiponectin signaling initiates some kind of communication between muscle and β-cells. Adiponectin could modulate the release of a humoral factor(s) that acts directly or indirectly (e.g., via the brain) on pancreatic β-cells. Our knowledge about muscle-derived hormones/cytokines is poor. However, interleukin-6 could be the missing link since it was shown to be secreted in substantial amounts during exercise (for review, see Febbraio and Pedersen [27]), possibly via AMPK (28), a pathway shared by adiponectin. Moreover, interleukin-6 is reported (29,30) to impair insulin secretion.

In summary, we show here that the expression levels of AdipoR1 and AdipoR2 in human myotubes correlate with distinct metabolic parameters but not with insulin sensitivity. Myotube mRNA expression of AdipoR1, but not AdipoR2, correlated with insulin secretion. The molecular mechanism, however, awaits further elucidation.

ACKNOWLEDGMENTS

The study was supported by the Deutsche Forschungsgemeinschaft (KFO 114/1-1).

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